Comparative Vertebrate and Human Anatomy: Ecology, Evolution, and Function

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INDIANAPOLIS, INDIANA



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Foreword

Comparative anatomy is an ancient field of science, with roots that stretch millennia into the past. But it is also a modern and dynamic discipline, with new techniques applied to make novel discoveries that could barely have been imagined mere years ago. Since its origins, the vertebrate lineage has evolved to produce an astonishing range of variations and new species—from placoderms to pangolins to people—which despite their variety are linked by the common components of their body plan. As vertebrates ourselves, understanding the structure, function, development, and evolution of this body plan holds inherent fascination as well as practical interest. By helping us understand the foundations of our own construction, comparative anatomy also provides keys to understanding our place in the history of life.

There has been a long tradition of well-written textbooks to help students navigate the concepts and content of comparative anatomy. But the advent of new knowledge, techniques, and perspectives makes a fresh entry into this tradition welcome. *Comparative Vertebrate and Human Anatomy* brings together an outstanding team of authors to update and refresh how the discipline is presented to students. Thoughtfully centered around five core concepts that emerged from a workshop held by scientists from the Society for Integrative and Comparative Biology (and later developed in a publication in its journal, *Integrative Organismal Biology*: Danos, Nicole, Katie Lynn Staab, and Lisa B. Whitenack, "The core concepts, competencies, and grand challenges of comparative vertebrate anatomy and morphology," *Integrative Organismal Biology* 4 (2022): obac019), the text begins with an outline of overarching themes in comparative anatomy and follows with detailed treatment of the specific organ systems and their variation across vertebrate groups. With Focus Questions to guide reading and numerous boxes highlighting recent studies, major

questions, and active learning opportunities, the structure of the text emphasizes engagement with this vibrant, modern field. The authors take care to acknowledge that the history of anatomy is rife with examples of exclusionary and ethically compromised practices. But with a care that shows their love of the discipline, the authors have devoted their efforts to make comparative anatomy a field that is widely accessible to students and welcomes inquiry. Comparative anatomy has a bright future, and we hope that the *Comparative Vertebrate and Human Anatomy* text will help students for years to come learn about this field and contribute to its future.

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January 30, 2025

Acknowledgments

We thank Amanda Hurford and Heather Myers of the Private Academic Library Network of Indiana (PALNI) for their guidance throughout the process of developing this text and promotion of the book. Many thanks also to Sue Wiegand (Saint Mary's College) for encouraging us to apply for the PALNI grant that enabled this project, serving as a liaison between our writing team and PALNI, and the many, many questions she helped us answer along the way.

We especially thank our illustrative team: Amy Cheu (senior illustrator), Kerry Stricker, and Allison Buck. Anatomy is an exceptionally visual field of study, and many of the figures in this book would not have been possible without their tremendous efforts. Thanks also to the contributing authors who poured their time, energy, and expertise into the chapters herein. This text has been a labor of love for many of these individuals, and we are grateful for their contributions.

This project was made possible by the generous support of PALSave: PALNI Affordable Learning, the Gans Foundation, and the Saint Mary's College Center for Women's Intercultural Leadership. We would also like to acknowledge the many funding agencies that have supported comparative vertebrate anatomy research through the years, some of which is highlighted in this book, and contributed to our ever-expanding understanding of vertebrate animal and human bodies. Special thanks goes to Saint Mary's College, Allegheny College, Cornell University, and the Society for Integrative and Comparative Biology Division of Vertebrate Morphology for supporting us in this endeavor.

We would like to thank Natalia Taft and Andie Ward for serving as reviewers for this text. Their valuable feedback has improved the clarity and quality of this book for faculty and student users.

Finally, we are grateful to our friends and families for their

unwavering support of the project. From indulging our excitement when we learned something new and cool that we just had to share, to cheering us to the finish line, you were with us every step of the way. “Thank you” isn’t enough, but we know you know.

1. Introduction: History and Core Concepts

LISA B. WHITENACK

Humans are not the pinnacle of evolutionary progress, but only an aberrant side branch of fish evolution.

—Peter Moyle and Joseph Cech, *Fishes: An Introduction to Ichthyology*

1.1 Introduction

Anatomy, the study of the structures of organisms' bodies, is one of the oldest subdisciplines in biology. To understand how organisms work, it helps to know what components they are made of. This is one of the reasons why anatomy is one of the first courses taken by medical and veterinary students. However, anatomy is not just for future health professionals. We can learn a lot about evolution by studying comparative anatomy. This is exactly what it sounds like: We compare the anatomy of different organisms to understand why there are similarities and differences in structure. Comparing the anatomy of different organisms can also tell us about the evolutionary history of the organisms we're studying.

Comparative vertebrate anatomy focuses specifically on vertebrates, which are animals that belong to the subphylum Vertebrata. These are animals that have a spinal column made of bone or cartilage, a distinct head with a brain, and an internal skeleton (we will define vertebrates more clearly in Chapter 3). This area is of specific interest because we, humans, are vertebrates. That means that comparing our anatomy to that of a fish, amphibian, or reptile can tell us something about our evolutionary history and why our bodies are the way they are. This is not to say that the other 78,000 species of vertebrates aren't interesting in their own right. Perhaps you have wondered to yourself: Why don't snakes have legs? How did whales and platypuses happen? Why can't pigs fly? Why do people seem to always have back problems? Comparative vertebrate anatomy can help answer these questions.

Despite how long the field of anatomy has been around, we are far from knowing everything. Comparative vertebrate anatomy is a vibrant and active field. New structures are still being discovered. Advances in imaging technology and analytical tools have led to breakthroughs in our understanding of the basic relationships between form (shape) and function (how it works). This has led in turn to a deeper understanding of how vertebrate anatomy and morphology (shape, size, texture, etc.) have evolved through time. In some cases, these discoveries and principles have led people to design new adhesives, filters, and other practical items. This is called **bioinspiration**, where scientists either are inspired by

or base their designs upon biological structures or processes. For example, understanding the form and function of manta ray gill structures has led to patents for new industrial filtering technology. Studying how geckos stick to vertical surfaces has led to the development of new adhesives.

We begin this chapter by taking a journey through the honest history of comparative anatomy. While there are a number of scientists who are typically celebrated for their accomplishments in summaries about history, many others are unsung—at least as this history is told in the Global North (countries with large amounts of wealth and technological development such as those in northern North America and Europe, Japan, South Korea, and Australia). History also often glosses over how scientific pursuits affect others, as science is not as objective as we may hope. The second part of this chapter will set up the organization of this book.

1.2 The History of Comparative Anatomy

Several large, heavy books have been written about the history of medicine, the history of anatomy, and the history of biology. We cannot hope to cover everyone and everything in one chapter. For example, numerous structures in our bodies are named for Western anatomists who described them (e.g., Eustachian tubes, Fallopian tubes, the bundle of His, the loop of Henle),

and readers can easily search for those individual histories online or in other texts. For readers who are intrigued by the history below, we have included several sources for further reading at the end of the chapter.

Anatomy Is an Ancient, Global Discipline

Anatomical study has been around for thousands of years, often in pursuit of understanding how human bodies work. While animal dissections were not performed for study, Mesopotamian clay tablets and a Babylonian clay model of a sheep's liver (including the segmentation marks; Figure 1.1) describe the structure of the organs of some of the sacrificial animals. These records date back to at least 2000 BCE.



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Figure 1.1—Clay model of a sheep's liver used for instruction in liver divination in a Babylonian Temple School, ca. 2000 BCE.

Records in Egypt date back to at least 3000 BCE, when Egyptians studied and documented the anatomical knowledge gleaned from surgery and mummification practices. The Edwin Smith Papyrus (3000–2500 BCE) contains the first known description of the brain, including gyri and meninges (Figure 1.2). The Ebers Papyrus (1550 BCE) contains information about the human cardiovascular system, while the Kahun Papyrus (1850 BCE) correctly describes the role of the placenta in fetal nourishment. Hieroglyphs show

that Egyptians' knowledge of anatomy extended beyond humans, particularly to bovines (cattle).



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Figure 1.2—The Edwin Smith Papyrus, the world's oldest surviving surgical document.

During this time period, anatomical study was also taking place in many other parts of the world. For example, in China, **Huangdi** (2600 BCE) wrote the *Nei Jing*, where he stated that blood continuously flows and is under the control of the heart and correctly linked the pulse to the heart (Figure 1.3). This is 4,000 years before William Harvey's "discovery." Anatomical study also occurred in Persia (currently Iran). During the Achaemenian Dynasty (sixth to fourth centuries BCE), the bodies of condemned criminals were used for medical research and dissection.

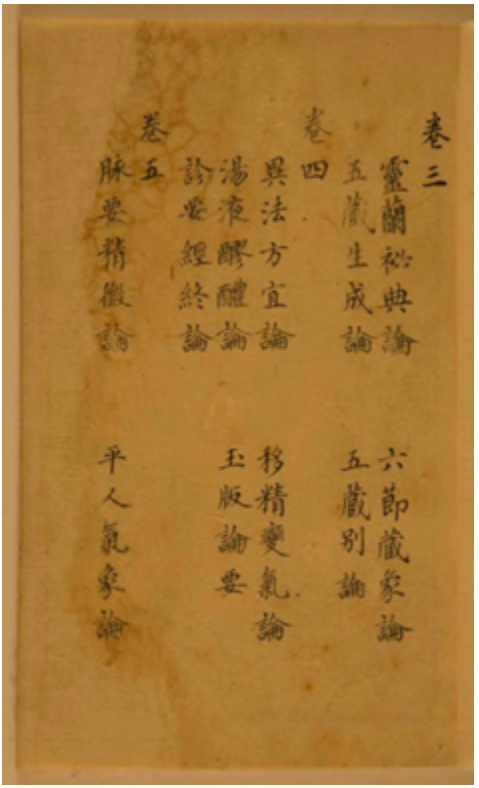

Figure 1.3—Scanned image of a page from the Huangdi Nei Jing.

An Indian physician named **Sushruta** (also written as Suśruta) wrote a surgical textbook, the *Susruta Samhita*, in the sixth century BCE, which described methods for dissecting human bodies in a systematic fashion and the anatomy of the eye, muscles,

joints, and ligaments (Figure 1.4). However, because Hindu law requires people to be cremated if they are older than two years old, Sushruta had to rely on the corpses of small children for the study of internal anatomy beyond what he could see in surgical procedures. Animal sacrifices were also used for the purpose of understanding human anatomy prior to Sushruta's time.



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Figure 1.4—Photo of a page from the Susruta Samhita, which is written on palm leaves.

Despite this rich history, most accounts of comparative anatomy history start in Greece. **Herophilus of Alexandria** (335–255 BCE), often called the "Father of Anatomy," is usually cited as the first person to perform dissection on human bodies for the express purpose of learning about anatomy. However, we have seen above that this is not true once we widen our view beyond the Western world. Herophilus's nickname is also in part due to the sheer number of humans he dissected—potentially 600 corpses of condemned criminals, with some reportedly vivisected (dissected while alive).

Even within Greece, anatomical history extends farther back than Herophilus. **Alcmaeon of Croton**, in the sixth century BCE, observed anatomical structures

in both humans and other animals, including sensory systems and blood vessels. Unlike many of the other anatomists discussed above, Alcmaeon has been recognized as the first anatomist to dissect animals for the express purpose of understanding their anatomy and not to use for understanding humans. **Aristotle** (384–322 BCE) is also known more for his contributions to human anatomy, but he was an outstanding comparative anatomist. He wrote several texts, including *De Partibus Animalium* (Parts of animals), *Historia Animalium* (History of animals), and *De Generatione Animalium* (Generation of animals). These texts cover several of our core concepts—structure, evolution, and development, respectively (Section 1.3). Within these texts, Aristotle also makes the case for comparative anatomy as a means to understand the human body; this is one of the core concepts of comparative anatomy.

Golden Ages of Anatomy

Comparative anatomy became an incredibly useful discipline as human dissection became illegal or taboo in cultures around the world, including in Greece, Rome, and China. Dissection of other animals was not necessarily forbidden. **Claudius Galenus** (usually referred to as **Galen**, 129–216 CE), a Greek physician, performed no human dissections. Instead, his anatomical knowledge was based not only on the surgeries he performed and the occasional human

remains from destroyed tombs but especially on the dissections of apes and pigs (Figure 1.5). Galen's texts influenced anatomists around the world for centuries, despite the fact that his texts contained several errors based on the differences between humans and the other mammals he dissected. However, he described a number of anatomical features correctly, such as features of the vertebral column, thoracic skeleton, several of the cranial nerves, and brain structures. He also correctly determined that ovaries and testes are **homologous structures** (sharing a common ancestry), although he did not correctly describe some of the functions of the ovaries. Galen also provided recommendations for dissection in his texts, pairing particular animals with particular organ systems—apes for the brain, bovines or equines (horses, donkeys) for reproductive anatomy, and pigs for the larynx.



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Figure 1.5—Illustration from Galen's Opera omnia showing the dissection of a pig.

As Galen's works were translated into other languages and distributed, scholars outside of Europe—especially in Egypt, Syria, and Persia—built upon his knowledge. For example, medicine flourished particularly between 800 and 1100 CE, and with that came anatomical study; this is the same period known as the Dark Ages in Europe. **Ibn al-Nafis** (1210–1288 CE; Figure 1.6) was the first to correctly determine the separation of blood in the right and left ventricles of the heart, to determine the path of pulmonary circulation, and to predict the presence of pulmonary capillaries. Pulmonary capillaries were discovered again in Europe by Marcello Malpighi 400 years later. Other scholars of this period who built upon and corrected Galen's work include **Abū Bakr al-Razi**, **Ali ibn al-Abbas al-Majusi**,

and **Ḥasan Ibn al-Haytham**. Some of their works were used by Western scholars in the Middle Ages; indeed, some of the anatomical terms we use today are based on Arabic terms (e.g., retina and sesamoid). During this same golden age, Persian physician **Yuhanna ibn Masawaih** (777–857 CE) dissected apes to better understand human anatomy and wrote several texts on anatomy and embryology.

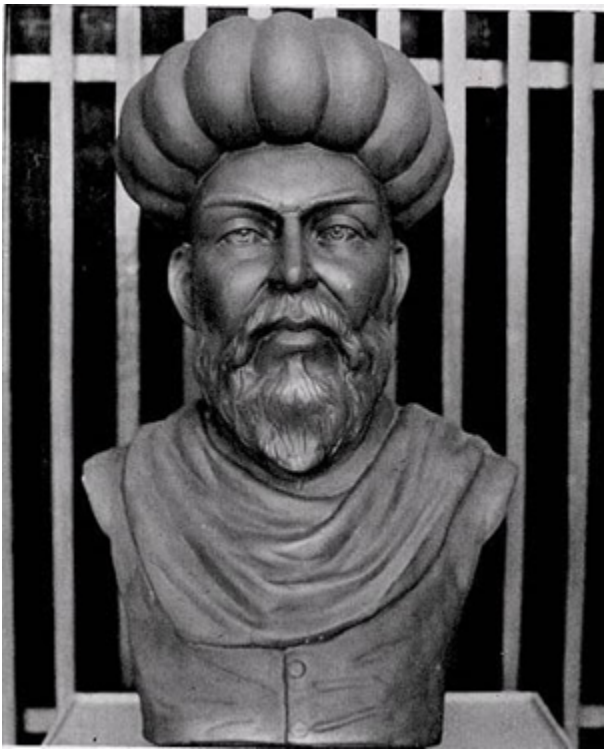

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Figure 1.6—Stone bust of Ibn al-Nafis.

Meanwhile, in the Western world, Galen's works were considered the definitive texts on anatomy for 12 centuries. However, early Renaissance artists also were anatomists, participating in dissections and studying the human body in service of their art. **Leonardo Da Vinci**'s (1452–1519 CE) relationship with anatomy is perhaps best

known; he was an observant anatomist who was also interested in form and function and produced incredibly detailed drawings of almost every human anatomical system.

Andreas Vesalius (1514–1564 CE) was the anatomist who started to shift Europe away from Galen. Based on his own writings, Vesalius initially was interested in medieval anatomical works. This led him to study anatomy through the dissection of local mammals such as rodents, cats, and dogs, followed by earning a medical degree from the University of Padua (Italy) and staying on as a professor. In 1543, Vesalius published *De Humani Corporis Fabrica* (*On the Structure of the Human Body*; see Figure 1.7); this tome is often marked as the beginning of "modern anatomy" and cites Galen hundreds of times. The book contained an array of beautiful illustrations by an unknown artist (some evidence points to Jan van Calcar) and refuted some of the errors in Galen's work. Several anatomists followed in Vesalius's footsteps studying anatomy and teaching at Padua, including **Hieronymus Fabricius** (1533–1619), who studied the anatomy of humans and other vertebrates and embryology. He went as far as to write a comparative anatomy treatise, *Totius Animalis Fabrkiae Theatrum*, but it was never published.



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Figure 1.7—Photograph of a page from Vesalius's De Humani Corporis Fabrica, Libri Septum (On the Structure of the Human Body, book 7); the page shows the muscular anatomy of a human body in profile view.

Anatomical study was also starting to flourish in other areas of Europe at this time. French anatomist **Pierre Belon** (1517–1564) was interested in not only humans but other vertebrates as well. He dissected and

studied cetaceans, fishes, and 200 different species of bird. He published a number of works, including *L'histoire naturelle des étranges poissons marins* (The natural history of strange marine fish), *La nature et diversité des poissons* (The nature and diversity of fish), and *Histoire des oiseaux* (History of birds). His 1555 publication on the history of birds contained an illustration of a human skeleton and a bird skeleton, side by side, demonstrating the **homology** (shared evolutionary origin) of the skeletons (although he called them archetypes rather than homologous structures; Figure 1.8).

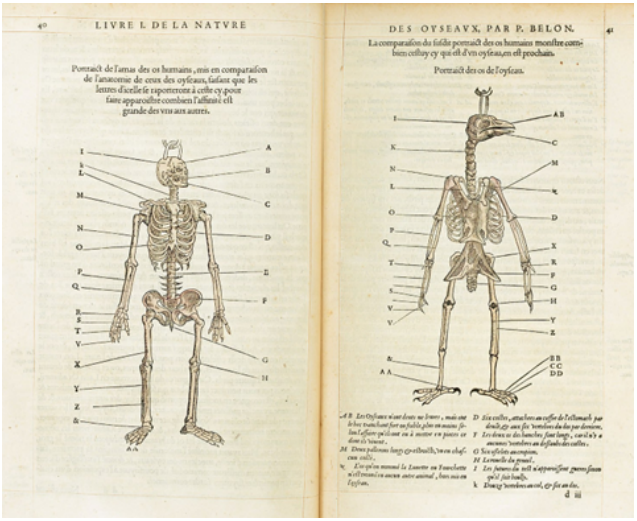

Figure 1.8—Illustration of a bird skeleton and a human skeleton side by side, published in Belon's Histoire des oiseaux.

British anatomist and physician **William Harvey** (1578–1657) earned his doctoral degree from the University of Padua in 1602 and was thus familiar with Vesalius's work. Through dissection, experiments, and observing his patients, Harvey determined how blood circulates around the body and the heart's role in circulation. While Harvey is perhaps as famous as Galen and Vesalius in the history of medicine because of that work, Harvey was a comparative anatomist at heart. *De Generatione Animalium* summarized many of his embryological studies, and he bonded with King Charles I over his scientific studies. Harvey, however, did not figure out how veins and arteries connected with each other. **Marcello Malpighi** (1628–1689), one of the first Europeans to study microscopic anatomy, used dissections of frog, hedgehog, and dog lungs to observe how blood flowed through capillary networks, which was the piece missing in Harvey's work. Neither the findings of Harvey nor those of Malpighi were accepted by their European colleagues during their lifetimes, as the works of Galen were still heavily influential. However, we would be remiss if we did not direct our readers back to Ibn al-Nafis and remind you that he figured it out 400 years before Harvey and Malpighi!

British physician **Thomas Willis** (1621–1675) is known as the "Father of Clinical Neuroscience," as he was one of the first Europeans to accurately describe the nervous system, including its blood supply, cranial nerves, and the autonomic nervous system. His *Cerebri Anatome* (1664) focused on humans but contains some illustrations of sheep brains, a staple of the comparative

anatomy teaching lab. Willis is also known for *De Anima Brutorum* (1672), a comparative anatomy text that he considered to be his most important work.

Nicholas Steno (1638–1686), also known as Niels Stensen, was a Danish physician and naturalist who made a number of observations of human anatomy that had not been recorded in the Western world. He described the duct of the parotid gland, how muscles contract, and ovary function. However, Steno is better known in comparative anatomy circles for his work on paleontology and geology, which would later help inform evolutionary theory. Steno, through his work on **extant** (not extinct) sharks, figured out that "glossopetra" (tongue stones; Figure 1.9) looked like shark teeth because they were indeed the teeth of sharks, although those species of sharks were no longer living (**extinct**). This and other observations eventually led him to the principles of original horizontality and superposition—sedimentary rock layers form horizontally, and higher layers are younger than lower layers, respectively. These principles then led to a better understanding of geologic time and the fossil record.


TAB. IV.
LAMIAE. PISCIS. CAPVT.
EIVSDEM LAMIAE DENTES

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Figure 1.9—Illustration from Steno's 1667 paper comparing the teeth of a shark head with a fossil tooth.

Cadavers and Crime

In Europe and elsewhere, cadavers in the late 1600s and 1700s were still scarce due to laws that limited their availability, which provided an obstacle to those wishing to learn anatomy. Anatomists continued to use animal dissections to further their knowledge as well as teach. In Scotland, **Alexander Monro Primus** (the first; 1697–1767) served as the chair of anatomy at the University of Edinburgh. Known as an outstanding teacher, he taught with mammal, bird, and fish dissections alongside the human cadavers. Mostly known for his teaching, **Alexander Monro Secundus** (1733–1817) followed in his dad's footsteps as a professor and anatomist and published on the neuroanatomy of humans, the anatomy of fishes, and the lymphatic system. Just as we rely on models in the teaching laboratory today, artists **Anna Morandi Manzolini** (1716–1774, Italy), **Marie Marguerite Bihéron** (1719–1795, France), and many others made extremely detailed wax models of human anatomy (Figure 1.10). Anna Manzolini is particularly interesting—upon the death of her husband, University of Bologna anatomy professor Giovanni Manzolini, she became a celebrated anatomy professor herself while continuing to do finely detailed wax sculptures of various anatomical structures. Anna

Manzolini even received commissions from Catherine the Great and the king of Poland.



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Figure 1.10—Detailed wax model of a human ear, plus muscles and glandular tissue, by Anna Morandi Manzolini.

To meet their needs, some physicians—and

anatomists in training—turned to anatomical schools, which were often privately owned. One of the first anatomical schools in London, the Windmill Street School, was established by the Hunter brothers. **William Hunter** (1718–1783) was a surgeon, obstetrician, and anatomist; he also was a student of Alexander Monro Primus. His younger brother, **John Hunter** (1728–1793), was also a surgeon as well as a comparative anatomist. William began the Windmill Street School in 1746, which functioned as both a school and a museum. The Hunters together amassed a diverse collection of specimens for teaching, including books, fossils, pathological specimens, and a variety of animal specimens. William eventually published *The Anatomy of the Human Gravid Uterus* in 1774 and acknowledged John as a major contributor. However, John wrote a paper on the structure of the placenta in 1780 and claimed he was the one who really did the work on the placenta in the 1774 book. The two brothers had a falling out and never spoke to each other again. However, their scientific work continued. John was a true comparative anatomist. He dissected and described more than 500 species of animals, including invertebrates and vertebrates. John was particularly interested in the link between form and function. In addition to a 1782 publication on fish auditory (hearing) organs, he wrote essays that classified various anatomical structures by function and linked these across animals (anatomical series). While the Windmill Street School is no more, John may have left another legacy. It is said his house in Leicester Square may have been the inspiration for Dr. Jekyll's

home in Robert Louis Stevenson's famous tale of Dr. Jekyll and Mr. Hyde (Figure 1.11).



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Figure 1.11—Photograph of a watercolor showing William Hunter's home and anatomy school.

The Parliamentary Act of 1752 enabled judges to send bodies of executed criminals from London to universities and even allowed for dissection to be a sentence that could be handed down. However, it left private anatomical schools in Scotland and England in need of a source of cadavers. Anatomical schools, including that of the Hunter brothers, instead had to obtain remains through other means and turned to resurrectionists (grave robbers; Figure 1.12). Scottish anatomist **Robert Knox** (1791–1862), a renowned anatomy teacher and scholar of comparative anatomy, is

not remembered for his work. Instead, he is best known for falling in with resurrectionists Burke and Hare. Burke and Hare kidnapped widows, orphans, and prostitutes, murdered them via suffocation, and then sold their corpses to Knox's anatomical school. When these and other similar murders were discovered, the public turned against anatomists. This led to the Anatomy Act of 1832, which recognized the need for human dissection to train physicians, made grave robbing illegal, and allowed unclaimed bodies in hospitals and workhouses to be given to schools for dissection. This Anatomy Act also allowed people to bequeath their remains for dissection and stated that dissected bodies must be buried.



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Figure 1.12—Engraving by Halbot Knight Browne showing five men removing a corpse from a grave. This engraving accompanied a story of John Holmes and Peter Williams, two resurrectionists.

Similar problems with resurrectionists were not limited to Britain. **Thomas Sewell**, who founded the George Washington University Medical School, was convicted of grave robbing for the purposes of

dissection in 1818. Starting in 1831, states within the United States passed legislation similar to the Anatomy Act, as did Canada in 1843. As for Burke, his body was later dissected, and his skeleton and wallets made from his skin are in the collections of the Royal College of Surgeons of Edinburgh.

Unfortunately, human dissection for anatomical study continued to be unethically sourced through much of our recent history as well. Unethically acquired remains, particularly those of African, African American, and Indigenous peoples, are examples of colonialism and the slave trade contributing to natural history collections at universities and museums. Victims of Nazi concentration camps also were used for anatomical study and research. **August Hirt** (1898–1945) was the director of the Institute of Anatomy at the Reich University in Strasbourg. He had concentration camp prisoners murdered to build his collection of skeletons from Jewish people. In addition, the bodies of people executed for high treason could not be claimed. These remains were given to universities in Germany, Austria, and Czechoslovakia (now known as the Czech Republic) for dissection. Thus, anatomical work from this period and region stems from the Nazi regime and the bodies of Jewish, Polish, and Russian people, among others.

Adding in the Why Behind Comparative Anatomy: Evolution

As we have seen so far, anatomists were using other vertebrates to help understand human anatomy throughout the history of anatomical study. However, why this worked—why there are similarities in structure and function across wildly different vertebrates—was not realized until evolutionary theory became accepted.

Carolus Linnaeus (1707–1778) and **Georges Louis Leclerc, Comte de Buffon** (often just referred to as Buffon, 1707–1788), laid the initial groundwork. Linnaeus is best known for establishing our current system of classification for living things (taxonomy), but he also boldly placed humans in the order Primates along with apes and monkeys. This implied that not only were humans not outside of nature, but they were more closely related to primates than other animals—a very controversial statement at the time. Buffon added another piece in his 44-volume series *Histoire Naturelle* (1749–1804), where he wrote that living things change through time and hypothesized it was due to environmental factors or chance (Figure 1.13). He also quietly supported Linnaeus's idea of humans and apes being closely related.



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Figure 1.13—Cover of volume 10 of Histoire Naturelle.

Jean-Baptiste, Chevalier de Lamarck (1744–1829), was even bolder than Linnaeus in his public support for biological change over time, which went against the widely accepted idea of divine creation and influence. Lamarck began his career as a botanist and later shifted to studying invertebrates. His focus on invertebrates led to his advocacy of a particular type of evolutionary change, the inheritance of acquired characteristics (also referred to as Lamarckian evolution

due to his fierce support for this idea, although it was not his original idea). Lamarck believed that how organisms use their body parts directly causes that body to change for its purpose during the organism's lifetime, and that new form would be inherited by offspring. His most cited example was concerning the long necks of giraffes, which he (incorrectly) believed evolved by the neck growing a little longer within each giraffe as they stretched to reach leaves, and those newly acquired neck lengths would be directly passed on to their offspring (Figure 1.14). Lamarck's ideas were supported by some, such as **Jean-Baptiste Bory de Saint-Vincent** (1778–1846) and **Geoffery Saint-Hilaire** (1772–1844). Saint-Hilaire thought all animals shared one body plan, and the diversity in form we see is due to modifications of the common plan. This led to a big push to find homologies across organisms.

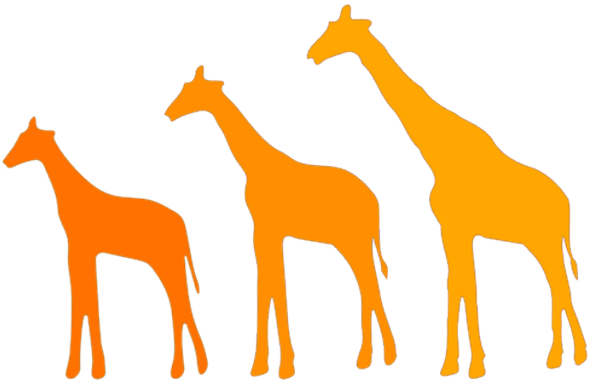

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Figure 1.14—Lamarck's most cited example of his incorrect idea of how evolution occurred. The giraffe with the shortest neck (left) would stretch to reach food. Lamarck believed that this new longer neck would be inherited by its offspring (middle giraffe), and this cycle would repeat across each generation (right giraffe).

French naturalist **Georges Cuvier** (1769–1832) did not agree with Lamarck in terms of inheritance, nor did he agree with Saint-Hilaire's idea of a single body plan. Cuvier was an advocate of catastrophism, where organisms living where natural disasters such as epic storms, floods, and mountain-building events occurred were rendered extinct by these disasters. While the idea of species going extinct held true, he did not think organisms changed through time. Instead, he believed that organisms were perfectly adapted to their environments and thus had no need to change. Although this idea was incorrect, Cuvier had another important role in the history of comparative anatomy. Not only was

he a renowned vertebrate paleontologist, particularly of dinosaurs and large mammals, but he published a nine-volume series (*Leçons d'anatomie comparée*, 1801–1805) in which he wrote that an anatomist must follow two principles: (1) the forms of one part of an organism will depend on the forms of other parts because they must work together, and (2) some physiological systems are more important than others, which in turn should determine how to classify organisms (Figure 1.15). In Cuvier's classification scheme, he separated vertebrates out from other animals (molluscs, radials, and jointed animals) based on their unique body plan.

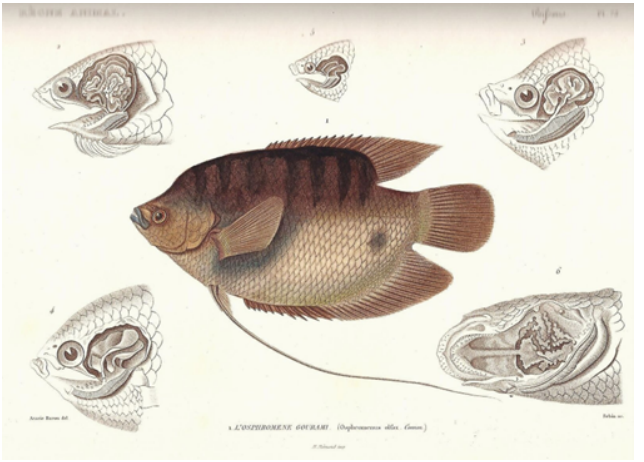

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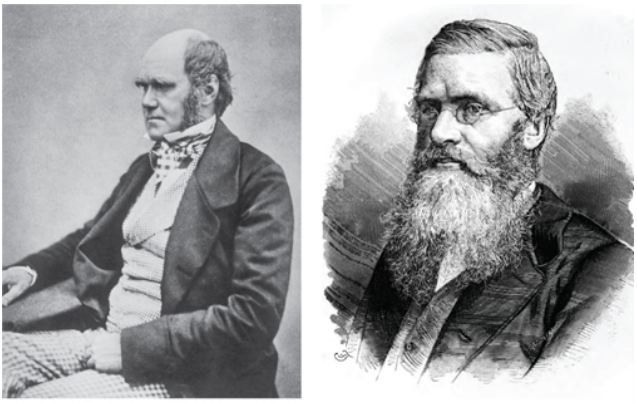
Figure 1.15—An illustration of the internal cranial anatomy of the fish Osphronemus alfax , as published in book eight of Cuvier's Leçons d'anatomie comparée.

Three more men helped push Charles Darwin

and Alfred Wallace toward natural selection (Figure 1.16). Geologists **James Hutton** (1726–1797) and **Charles Lyell** (1797–1875) moved geology away from catastrophism and recognized that the same processes of erosion, changes in sea level, and the formation of new rocks also have happened through the Earth's entire history and happen slowly. This became known as uniformitarianism and implied that the Earth was far older than what had been accepted until that time. In a completely different field, **Thomas Malthus** (1766–1834) published his *Essay on the Principle of Population* in 1798. Although his book dealt with political economics, in it he stated that human populations grow geometrically and, if left unchecked, would quickly outgrow the available food resources. Unfortunately, Malthus used this information to argue against improving life for those who were considered poor.



(a) (b)

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Figure 1.16—Charles Darwin (a) and Alfred Russel Wallace (b).

Charles Darwin (1809–1882) and **Alfred Russel Wallace** (1823–1913) independently arrived at the idea of natural selection through similar avenues. Darwin initially studied medicine at the University of Edinburgh but eventually left behind his medical studies to pursue natural history. Darwin was invited to sail on the HMS *Beagle*, a 10-gun ship belonging to the British Royal Navy that was used both for scientific expeditions and for taking control of the Falklands, from 1831 to 1836. While aboard, he worked with local people to collect fossils and living specimens, studied the geology of the places they visited, and meticulously kept a journal that would later be published. Darwin also read Lyell's *Principles of Geology* during the voyage and then read Malthus's essays upon his return. Darwin continued to quietly

collect evidence and ruminate on the theory of natural selection over the next 20 years. Importantly, much of Darwin's evidence from his voyages would not have been preserved if it hadn't been for **John Edmonstone**, a formerly enslaved person from Guyana who taught Darwin taxidermy prior to his voyage on the *Beagle*.

Meanwhile, Alfred Russel Wallace started a journey of his own. Wallace initially worked as a land surveyor but was more interested in natural history. He embarked on several trips abroad, traveling through the Amazon and Southeast Asia from 1848 to 1862. Like Darwin, Wallace worked with local people to collect specimens, read the same books, and independently arrived at the conclusion that natural selection explained much of what he saw.

Wallace and Darwin were correspondents prior to the publication of Darwin's most famous book. Wallace sent bird specimens to Darwin, but things took off in 1858 when Wallace described his ideas about natural selection to Darwin. Much to Darwin's surprise, Wallace described a similar idea to what Darwin had been working on for the last two decades. There were details that the two naturalists did not agree upon. For example, they disagreed on what taxonomic level competition can happen at (Darwin said that competition happens within the same species; Wallace thought it was between species or at a higher taxonomic level). They disagreed on the importance of competition for mates (sexual selection); Darwin later wrote an entire book on it in 1871 stating its importance, while Wallace thought only "male-male" competition

was important. They also disagreed on the role of artificial selection (Darwin thought it was a good analogy; Wallace thought it wasn't). Finally, Darwin thought evolution applied to humans, but Wallace didn't. Despite all these disagreements, Wallace and Darwin copresented the theory at a meeting of the Linnean Society in 1858. Darwin then published the book *On the Origin of Species by Means of Natural Selection* in 1859, plus three more from 1868 to 1872; thus he is the one often credited with having developed the theory of natural selection. There did not appear to be any hard feelings between the two naturalists. Wallace published a book titled *Darwinism* in 1889 that supported *Origin of Species* and natural selection and continued his other scientific work on zoogeography, which seeks to explain the geographic distribution of animal species.

While Darwin argued in *Origin of Species* that the branching version of evolution described in the text explained much of anatomy and paleontology, among other fields, many scientists did not immediately accept his ideas. The arguments against natural selection and *Origin of Species* were many. Some scientists held on to Lamarckian evolution, citing evidence from the fossil record and embryological development. Others did not see evidence of transitionary forms they would expect to see. Still others did not think that evolution could be linked to external forces, such as access to resources, and must be an act of the divine. British anatomist **Thomas Henry Huxley** (1825–1895) was one of the first to be convinced by Darwin and became such a fierce public advocate for Darwin's ideas that he was

nicknamed “Darwin’s Bulldog.” Huxley was also a fine comparative anatomist. He studied the embryology of vertebrates and determined that embryology was necessary to determine homology, and he was one of the first to realize that tunicates are in the same phylum as vertebrates (see Chapter 2). He also was particularly interested in the relationship between apes and humans, publishing “Note on the Resemblances and Differences in the Structure of and the Development in Man and Apes” as part of the second edition of Darwin’s *The Descent of Man and Selection in Relation to Sex*, and in 1863 he published *Evidence on Man’s Place in Nature*, which was one of the first books to link evolution to humans (Figure 1.17).

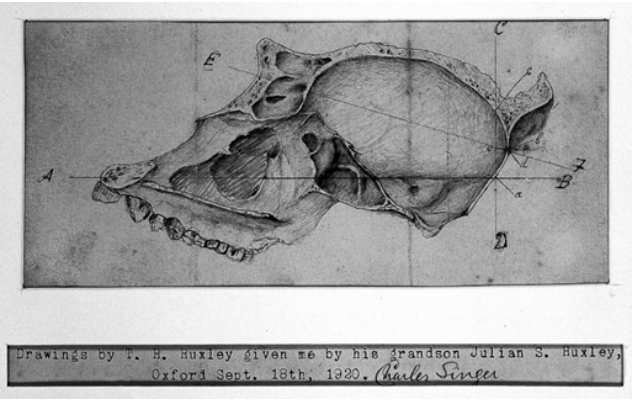

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Figure 1.17—Drawing of a lateral view of the internal anatomy of a primate skull by Thomas Henry Huxley.

At this point, the hows behind evolution were

also unknown. Darwin alluded to inheritance in *Origin of Species* but did not propose how traits are actually inherited. **Gregor Mendel** (1822–1884) laid out fundamental principles of inheritance in 1865 based on his many experiments with breeding and crossing common pea plants; however, it is thought that Darwin did not learn of them despite their overlap in time. Mendel's work was not brought back into the scientific community until roughly 1900, when botanists **Hugo De Vries** (1848–1935), **Carl Correns** (1864–1933), and **Erich von Tschermak-Seysenegg** (1871–1962) rediscovered Mendel's work while completing similar breeding experiments of their own. Similarly, naturalist **William Bateson** (1861–1926) found that Mendel's work supported his own ideas about mutation as a source for evolutionary innovation. It took until the 1920s for scientists to understand that natural selection could act on genes, thanks to the work of **Ronald Fisher**, **J. B. S. Haldane**, and **Sewall Wright**, among others. Later, American biologist **Thomas Hunt Morgan** (1866–1945) bred thousands of fruit flies and studied mutation, refining the work that Bateson started. His student **Thomas Dobzhansky** (1900–1975) later linked evolution, speciation, and genetics in his 1937 book *Genetics and the Origin of Species*. He determined that populations of the same species of fruit flies did not have identical genes and that separate species of fruit flies were reproductively incompatible. The variability in genes due to mutation could lead to variability in phenotype and the eventual loss of interbreeding between populations (thus becoming a new species).

However, it wasn't until 1944 that genes and inheritance would be linked to DNA (deoxyribonucleic acid), finally providing the how of evolution. DNA as a molecule was first described in 1869 by **Friedrich Miescher**, a Swiss chemist, who found it in human white blood cells. While work continued by others to describe the chemical properties and structure, **Oswald Avery**, **Colin Macleod**, and **Maclyn McCarty** of the Rockefeller Hospital in New York published a paper in 1944 that showed that genes and chromosomes were composed of DNA. **Alfred Hershey** and **Martha Chase**'s 1952 experiment further supported Avery and colleagues' work.

Modern Comparative Vertebrate Anatomy

While we have just spent a very long time discussing an abridged history of comparative anatomy through the 1950s, the field has continued to grow and move forward. As technology continues to evolve, so does our knowledge. For example, as scientists gain access to modern imaging techniques such as computed tomography (CT) scanning, magnetic resonance imaging (MRI), and X-ray reconstruction of moving morphology (XROMM), we can now see details that even fine-scale dissection did not reveal. Similarly, advances in genetic and genomic techniques have led to important discoveries about the roles of regulatory genes in body

patterning. As we move forward through this book, we will spend more time highlighting some of these incredible recent discoveries.

We are undoubtedly missing the contributions of many people to the field of comparative anatomy in this account. As we continue to study the history of any field, new artifacts emerge that add to or change the story we thought we knew. This history of science in general is starting to be examined through a more global lens, adding in the contributions of those who have been left out before. Understanding the honest history of this field is meant not to retroactively paint these scientists and physicians as bad people (and yes, some of them absolutely were) but to point to the issues inherent to our field and grapple with the implications of that history.

Box 1.1—Reflecting on and Discussing the History of Comparative Anatomy

Below, we have provided two prompts for reflection and discussion. Before engaging in discussion, whether you are writing a response or participating in a discussion in the classroom, it is important to establish some ground rules for discussing sensitive topics. Instructors and students should work together to establish mutually agreed-upon

norms prior to engaging with these prompts. If you are new to conversations like this and establishing classroom norms, there are many resources online for both instructors and students.

1. The history outlined in Section 1.2, while partial, contains a number of physicians and scientists who are typically not included in summaries of the history of this field in Western countries. Why do you think that is? As you think about this question, you should engage in one or more of the following activities:

- Read about the history of comparative anatomy on Wikipedia or other websites and compare that to the history outlined in this chapter. What is similar, and what is different?
- Research some of the names that you have not

heard of before and learn more about those people.

- Many of the people described in this chapter tend to focus on humans. Read about the history of human anatomy on Wikipedia or other websites. Which names are missing? Which appear here and in the other sources?

2. The history of science in general contains a number of atrocities that have been glossed over, and these stories are starting to be told. The history of comparative anatomy is no exception. However, it is not enough to just learn about these stories. Take some time to think about some of the potentially surprising things you learned about that are ethically unacceptable today, and consider the following questions:

- Many bodies that were used for anatomical study were the result of either grave robbing or murder (including genocide). If those remains still exist, what should be done with them? Should they remain available for study? If not, what steps should those institutions that hold those remains take?
- How should we acknowledge the source of scientific knowledge that comes on the back of murder and grave robbing?

1.3 Moving Forward: Core Concepts of Comparative Vertebrate Anatomy

If you were to open just about any anatomy textbook (such as this one) or lab manual and look at the table of contents, you would see that most chapters in these

kinds of books are arranged by system. There is a chapter on the digestive system, one or two on the skeletal system, one on the respiratory system, and so forth. We have followed a similar overall organizational scheme in this book. Within one of those chapters, you would probably see some generalized illustrations or a small number of representative vertebrates—a bony fish, a dogfish shark, a frog, some sort of reptile, a bird, and a cat. You would also see numerous figures with hundreds of structures labeled. This might give you the mistaken impression that anatomy is solely an exercise in memorization of names and locations of structures.

The field of comparative vertebrate anatomy is wide and rich. It covers vertebrates from the smallest fishes to the largest mammals (including humans!) and everything in between, whether it is extant or extinct. The field covers every anatomical system in these animals—its form, its function, its development, its evolution—and how these systems work together. The field also covers all levels of organization from cells to tissues, organs, body regions, and whole organisms. Given the scope of this field, we could easily write a textbook that is thousands of pages long, and we still wouldn't be able to cover everything that is weird, wonderful, and interesting.

Therefore, most chapters will be structured around the five core concepts that unite the field of comparative vertebrate anatomy. Core concepts are larger themes that unite the field, regardless of which anatomical system you are studying. In other words, it doesn't matter whether you're looking at the digestive

system or the nervous system—the same core concepts apply. These core concepts were developed by a group of scientists from the Society for Integrative and Comparative Biology who study and teach comparative vertebrate anatomy.

Below, we step through each of the five core concepts of comparative vertebrate anatomy. You may find them in a different order within the individual chapters, but we find the original order from Danos et al. (2022) to be useful for explaining each one.

1. Evolution: The diversity, variation, and unity of vertebrate anatomy are explained by descent with modification. Evolution is the unifying theory of biology and is applicable to every subfield. Comparative vertebrate anatomy is no exception. Natural selection acts on anatomical structures just like any other phenotype, so long as there is heritable variation in that structure. The versions of the phenotype that increase an animal's evolutionary fitness (an organism's ability to pass its genetic material on to the next generation) are more likely to be passed on to subsequent generations; these are adaptations. This is why we tend to look for connections between the form of the structure and its function. Something about the shape of the structure is making it work well. How the structure works is often correlated with ecology. Thus, as we learn about different groups of vertebrates and their anatomy, we can look for where natural selection favored variation on these

heritable traits and why. This also means that vertebrates share a common ancestry, and we see descent with modification as we move through evolutionary time. Natural selection only acts on existing structures.

2. Structure and function: The structure of vertebrate anatomy is under heavy selection to match its functional demands. However, because demands change over time and evolution acts on what already exists, an anatomical component at any given time may not have an optimal structure for its current function. The first core concept indicates that we should expect to see a solid correlation between form and function, as this will increase evolutionary fitness. We see this link across many levels of biological organization. After all, all bodies must obey the laws of physics. A structure with a form that doesn't work well may not survive to reproduce. However, we must also recognize that constraints on structures may prevent the highest level of fitness from being reached. Sometimes this is due to evolutionary history; the organisms may not have the genetic wherewithal to make it happen. Other times there are developmental or structural requirements that constrain evolution. For example, sometimes multiple structures interact and work together (integration, see Core Concept 4), so improving the function of one structure may make the integrated structure less fit. Some structures are used for multiple purposes that may be competing

with each other, leading to trade-offs—a "jack-of-all-trades, master of none" type of situation. It is also important to note that there can be more than one solution to a functional demand.

3. Morphological development: Vertebrate anatomy is expressed as phenotypes that are the result of genotypes executed through a developmental program. Major shifts in phenotype can be achieved through modularity, which allows certain aspects of the phenotype to undergo major variations yet remain integrated in other ways. Development is not as simple as having a genotype that codes for a particular phenotype. The way those genes are expressed determines phenotype. Therefore, changes in that developmental pathway can have effects at the cellular level, tissue level, and structural level of a particular anatomical feature. Natural selection can then act on those modified structures. Changing a development pathway that affects many structures can have effects on all those structures. However, we also see structures, genes, and developmental pathways that are independent or modular. If a single developmental module is modified, major changes to phenotype can be brought about without affecting other parts of the body and can perhaps lead to that structure being able to work with a structure that it couldn't before.
4. Integration: Anatomical structures develop, function, and evolve as integrated modules. These

processes occur across space, time, and biological levels of organization. Even if it has modularity in development, the animal must still function as a whole. For example, skeletal muscles and bones may develop via their own pathways, but those muscles must attach to the bones to move them. Muscle cells and those of the nervous system must work together for the muscle to contract and move the bones. Movement of bones happens at joints, where two bones interact. The development of these three components (bone, muscle, nerve) must come together to build a functional organism, as does the biochemistry, physiology, and mechanics of these structures. The study of anatomy is more than just pointing to structures; it requires the integration of fields like biochemistry, physiology, mechanics, ecology, and evolution because the structures themselves are integrated.

5. Human anatomy is a result of vertebrate evolution: As humans are vertebrate animals, their form has been constrained by phylogenetic ancestry. To the general public, vertebrate evolution is often thought of as a linear progression from fish to mammals, with humans as the pinnacle. After all, humans are unique in the ways that they have modified their environment and their possession of self-awareness. However, the tree of life has many branches; evolution is not a linear process, and there is no pinnacle. Humans are vertebrates. As such, we share a common

ancestor with all other vertebrates. We have features that are the same as other vertebrates, and understanding our shared evolutionary history explains why our bodies work the way they do as well as certain pathologies. This also means that the other four core concepts explained above also apply to humans. We will treat humans as a special case of comparative vertebrate anatomy because of the interest we have in how our own bodies work, not because they are separate from other vertebrates.

1.4 Summary

If you are feeling overwhelmed by the breadth of the field of comparative anatomy and the long history of the field, you are not alone! This is an interdisciplinary field of biology that incorporates concepts not only from other areas of biology but also from geology and physics. It is easy to get intimidated by everything that is encompassed by this field, and the authors of this book remember what it's like to get started in this course. We are going to lean heavily on those core concepts to make sure that you have the tools to explore any areas that interest you without getting lost in the particulars. The authors of this book are enthusiastic educators and scientists who love comparative vertebrate anatomy. We hope that you find it as fun and interesting as we do, and we will point out

some of our favorite things as we take this journey with you.

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2. The Nonvertebrate Chordates

LISA B. WHITENACK

Focus Questions—to Guide Your Reading of This Chapter

1. Which invertebrates are the closest relatives of the chordates? Of the vertebrates?
2. What are the three synapomorphies of the chordates? Do they need to be present in all life stages?
3. Which nonvertebrate chordate is most closely related to the vertebrates and why?

2.1 Introduction

If you took an introductory biology course that marched you through the animal phyla, you may have seen a figure that looks like Figure 2.1, which demonstrates where you fit into the larger animal kingdom. Humans, like other vertebrates, belong to the phylum Chordata.

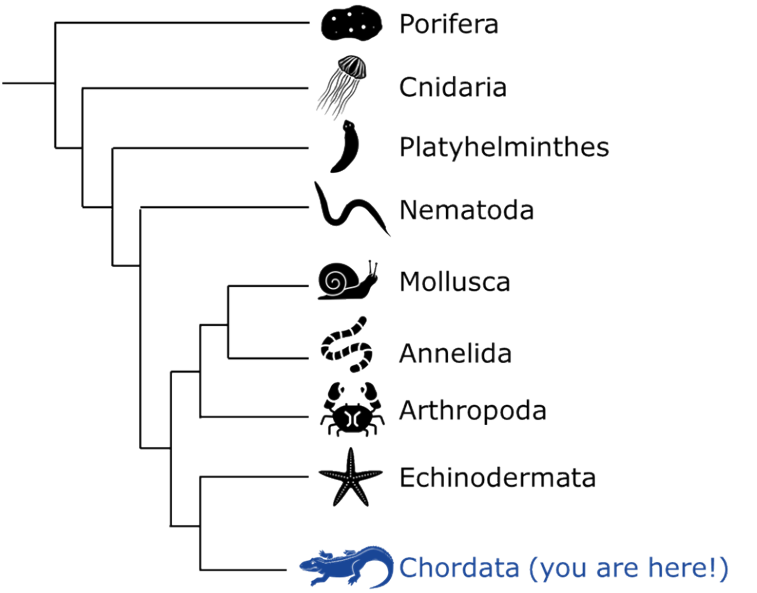

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Figure 2.1—A general phylogenetic (evolutionary) tree of animals. Note that not all animals are included in this diagram.

Chordates are united by a number of **synapomorphies**, or shared derived characteristics that were inherited from a common ancestor. *Shared* refers to characters or traits that the organisms have in common. *Derived* refers to when the trait evolved—sometime after the chordate lineage split from the common ancestor they shared with the echinoderms. The key with the chordate synapomorphies is that they possess them *at some point in their lifetimes* (Figure 2.2). Among these are

1. A *dorsal hollow nerve tube* (or *dorsal hollow nerve cord*)—**dorsal** indicates that a structure is opposite the belly (or ventral) side of the organism. In humans, this is our spinal cord.
2. A *notochord*—this stiff rod lies ventral to the nerve cord. In humans, the remnants of this are found in the intervertebral

discs of the spinal column.

3. A *postanal tail*—"postanal" indicates that the tail occurs posterior to (or away from the face) the anus. If you have a cat or a dog, you can think about where their tails are. Humans tend not to have a visible tail past the embryo stage.

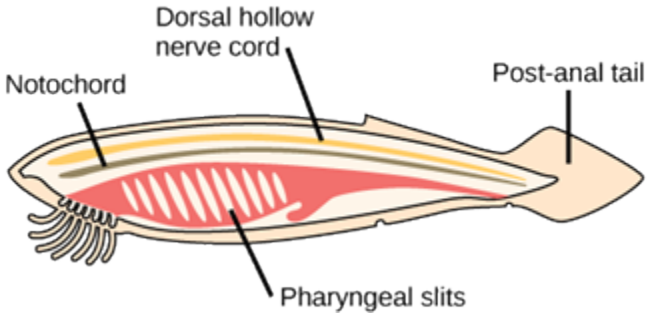

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Figure 2.2—The three chordate features (notochord, dorsal hollow nerve cord, and postanal tail), plus a more general deuterostome feature (pharyngeal gill slits) shown on a cephalochordate.

Box 2.1—Getting Oriented

We just used several important words—dorsal, ventral, and posterior. An important part of being able to discuss anatomy is to understand the road map of bodies. Just as we navigate a map by using compass directions (north, south, east, and west), we have specific terms that tell us where things are on animal bodies. We will first consider a quadrupedal animal, the kangaroo (Figure 2.3), as humans being bipedal and upright are a special case. However, when you finish this section,

practice finding and using these terms on yourself or a pet.

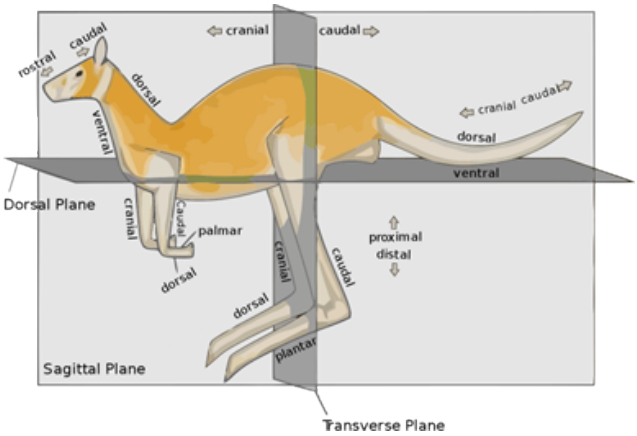

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Figure 2.3—Anatomical directions and planes of section on a kangaroo.

We can start with four basic directions: **Ventral** refers to the belly side of the body, whereas **dorsal** refers to the back (think of where a dorsal fin is on a shark—the iconic triangle that cuts above the water). **Anterior** refers to the head end of the animal and the face, whereas **posterior** refers to the tail end. You will also see these called **cranial** and **caudal**, respectively. If we're already on the head, we switch our terminology to **rostral** (toward the nose) and caudal.

Unlike two-dimensional paper or computer maps, kangaroos are three-dimensional. We have to add in another pair of directional terms: **Medial** refers to the

midline of the body, whereas **lateral** refers to sides (put your hands on your hips—those are lateral to your vertebral column, which is your midline).

We can also divide up our kangaroo into regions by planes, just as you would have an X-Y plane or X-Z plane in a 3D graph. If we split our kangaroo into two mirror images, the plane that divides them is called the **sagittal plane**, as it goes right down the midline. However, if we move that plane to divide our kangaroo into two unequal lateral halves, we call it a **parasagittal plane**. If we change directions and want to divide our kangaroo into dorsal and ventral halves, we do that with a **frontal plane** (sometimes called a dorsal plane). Similarly, we can divide our kangaroo into anterior and posterior halves with a **transverse plane** (also called a coronal plane).

What about humans? As we will see repeatedly, often human anatomy is given its own separate terminology even though things are homologous with other mammals. This is true for directions in the body as well (Figure 2.4). Because we're upright and bipedal, our faces are facing the same direction as our bodies, and the heads and tails of humans are no longer aligned with the face. The standard in human anatomy is to use superior (cranial), inferior (caudal), anterior, and posterior as our four cardinal directions. Dorsal and ventral are not often used to describe directions in human bodies.

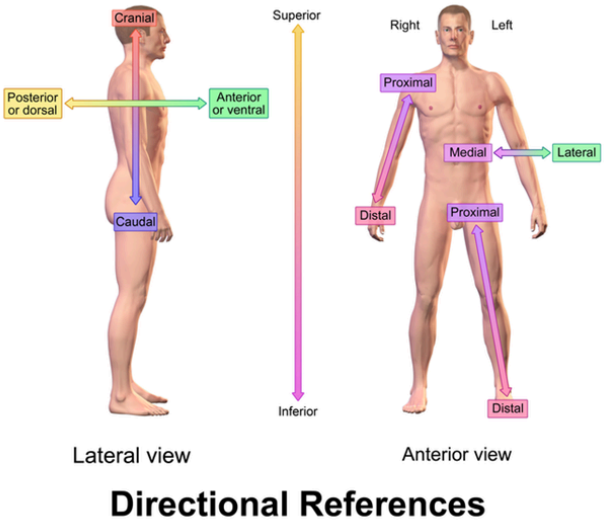

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Figure 2.4—Anatomical directions on a human.

We also have appendages that stick off both humans and our kangaroo. While it is true that if you move from the armpits to the fingers, you might be moving more laterally, we use special terms for the appendages: **Proximal** is closer to the trunk of the body, and **distal** is further out from it. In other words, your armpits are more proximal than your fingers, and your fingers are more distal than your armpits.

We have another important directional thing to discuss—left versus right. Consider where the left and right sides of your body are relative to you versus

someone standing across from you and facing you. Your left side is on your left side; however, the left side of the person standing across from you is on your right. When we use left and right in an anatomical context, we are always talking about the animal's or the subject's own left and right, not the left and right of the person examining the animal or subject.

To return to our map analogy, maps also name regions of land, such as towns or countries. We have names for body regions too, which are based on human anatomy (Figure 2.5). Some of these terms are also applied to other vertebrates. While there are too many to list here, you will see some of these come up again.

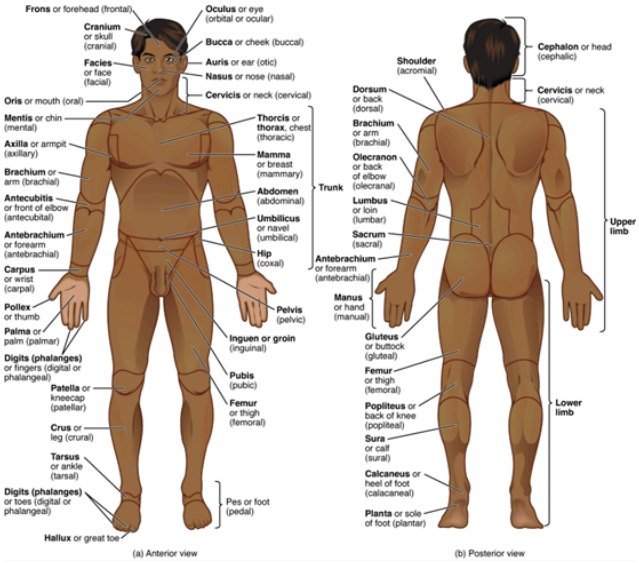

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Figure 2.5—Regional terms for the human body: (a) anterior view; (b) posterior view.

The chordates are also **deuterostomes** (superphylum Deuterostomia), as are the members of phylum Echinodermata (sea stars, sea urchins, sea cucumbers, sand dollars, and crinoids) and phylum Hemichordata (acorn worms and pterobranchs). We will briefly discuss these organisms more below, but it's worth asking why we are talking about sea stars in a book about vertebrates. Recall that our first core concept was evolution (see Chapter 1)—what we see in living organisms is due to descent with modification. Therefore, it is helpful to understand our closest living relatives, as different as they may seem, as a way to understand

how natural selection favored particular traits and why. That being said, we also have to remember that any **extant** (i.e., living and not extinct) representatives of these groups have had a very long time for natural selection to act and make changes. If we want to know what our earliest ancestors looked like, we cannot necessarily look to living taxa. The fossil record may not be much help either, particularly if these organisms had few hard parts to make it through the fossilization process. Thus, we need to understand the evolutionary relationships of all the deuterostomes and the features we use to construct hypotheses about those relationships.

Box 2.2—How to Read a Phylogenetic Tree

A **phylogenetic tree** (also referred to as an evolutionary tree or a cladogram) is a visual way of depicting a hypothesis about evolutionary relationships among the **taxa** (sing. = taxon, a group of organisms given a taxonomic name such as species or family) included in the tree. How the taxa are distributed on that tree depends on the data used (e.g., morphological characteristics or genetic information) and the algorithm chosen to build the tree; this is why trees represent evolutionary hypotheses and not definitive answers.

Understanding the hypotheses represented by these trees requires being able to read them properly. This is something that even PhDs can struggle with, so we're going to make sure everyone is on the same page. Figure 2.6A shows the anatomy of a tree, which is our first step. Taxa, like the shark and the frog, are typically at the tips of the **branches**, much like leaves on the branch of an actual living tree outside. As you move

down the branches away from the tips, branches converge at **nodes**. Nodes represent a hypothetical common ancestor from which the tip-taxa diverged. As you keep moving away from the branch tips and toward the **root** of the tree, it's similar to moving backward through time. Nodes higher up in the tree are more recent common ancestors than those in the lower parts of the tree, closer to the root.

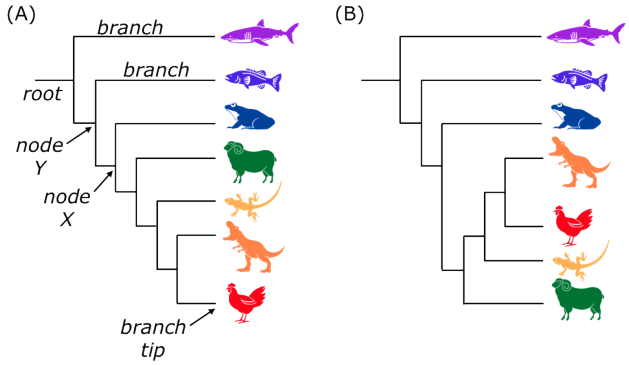

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Figure 2.6—(A) A generalized tree of the vertebrates with tree anatomy labeled. (B) This is the same tree as (A), but we have rearranged the branches by rotating them at particular nodes.

The trick to reading the tree, once we have a handle on the tree anatomy, is to read from the tips down toward the roots and to pay attention to the nodes (common ancestors). To practice, let's figure out which organism is more closely related to the frog in Figure 2.6A—the fish or the sheep? Begin by finding the frog and sheep on the tree, and literally put an index

finger on each taxon. Now trace their respective branches down the branches away from the tips until they meet at node X and make note of the node's location in the tree. Go back to the branch tips in Figure 2.6A and find the frog and fish. Repeat the same tracing motion; your fingers should have met at node Y. Node X is closer to the branch tips and farther from the root than node Y; thus, the frog and sheep have a more recent common ancestor than the frog and fish. This tells us that the frog and sheep are more closely related than the frog and fish, as they more recently diverged from their common ancestor.

The most common mistake people make is reading across the branch tips and thinking that how close taxa names are to each other tells you anything about evolutionary relationships. For example, in Figure 2.6A, one would make the erroneous conclusion that lizards are equally related to dinosaurs as to frogs. However, as you now know, it is the pattern of branching and nodes that indicates those evolutionary relationships. Because of this, we can rotate branches on the various nodes and get a tree that looks completely different from the original if we only read across the tips, but if we follow the branching and node pattern, we won't be fooled. Examine the trees in Figures 2.6A and 2.6B and take the time to follow the branches—these are the same exact evolutionary relationships.

We have one more piece of terminology to discuss—**clades**. Clades are groups that are **monophyletic**, which means they include an ancestral

lineage (which often is represented by a node and is sometimes called a **common ancestor**) and *all* its descendants. Looking at Figure 2.6A, if we wanted to make a clade that includes node X and all its descendants, we would have to include the frog, ram, lizard, *T. rex*, and chicken. Because clades are just monophyletic groups, they don't always match up with a taxonomic level (e.g., phylum, family). Ideally, all our taxonomic levels would be monophyletic clades because they reflect a complete evolutionary history of the taxa included in that clade. Unfortunately, many taxonomic groups were established prior to this idea of monophyletic groups. "Reptiles" are a great example. If we go back to Figure 2.6A, most folks would only include the lizard and *T. rex* as reptiles. However, if we are also going to include *all* the descendants of the common ancestor, we should also be including chickens! As you'll read in Chapter 3, birds evolved from a dinosaur ancestor.

2.2 The Deuterostomes

While genomic data supports the idea that the deuterostomes (echinoderms, hemichordates, chordates) are indeed closely related, their physical similarities are not at all apparent in their adult forms. After all, there are no chordates with pentaradial (five-sided) symmetry, which is a hallmark of the echinoderms. The Ambulacraria (Echinodermata + Hemichordata) haven't shared a common ancestor with the Chordata since about 660–550 million years ago (abbreviated as Ma or MYA) and thus have had a lot of

time for natural selection to work on these different evolutionary lineages.

However, if we examine the embryonic development of deuterostomes, it is easier to understand why these seemingly disparate groups are united (Figure 2.7).

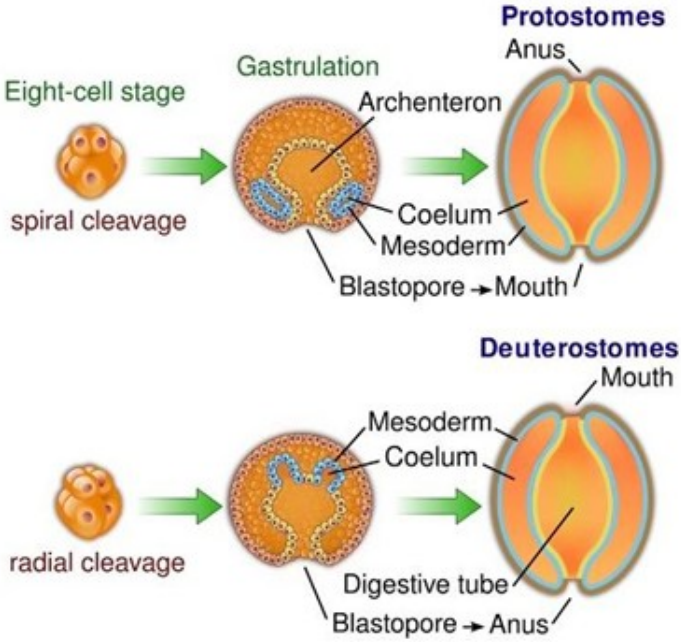

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Figure 2.7—Development of protostomes (top panel) versus deuterostomes (bottom panel). Note differences in cleavage patterns and the fate of the blastopore.

The similarity begins rather early in development, in those initial stages where the fertilized egg (**zygote**) is undergoing a series of cell division events (**cleavage**). In animals that have protostome development (generally called **protostomes**—e.g., annelids, arthropods, molluscs), cleavage follows a particular spiraling pattern

in which new cells are offset from their predecessors. The deuterostomes, on the other hand, do not follow the spiral pattern. Instead, they exhibit what's called radial cleavage, wherein new cells sit directly atop their predecessors. If we fast-forward a bit, eventually these embryonic cells continue to divide and arrange themselves into a hollow ball with three germ layers called a **gastrula** (see Chapter 4 for more detail on embryonic development). The hollow space is the start of the gut cavity, called an **archenteron**. A gut needs an opening for food to enter (a mouth) and another for waste to leave (an anus). This is where we see our next embryonic difference. *Protostomes* literally translates to "first mouth"—the first opening (the blastopore) that forms here becomes the mouth. *Deuterostome* translates to "second mouth"—the first opening instead becomes the anus, and the mouth forms later. There are other developmental characteristics that unite the deuterostomes as well, such as how the mesoderm forms. We must issue a word of warning here, though—some of these "deuterostome developmental traits" have been found to exist in at least one protostome species, so we cannot strictly say that only deuterostomes have these traits.

What other features might unite the deuterostomes? So far the only confirmed candidate is **pharyngeal gill slits**—the **pharynx** is the region between your oral cavity (mouth) and esophagus. Humans tend not to have pharyngeal gill slits past the embryonic stage, and we do see these in extinct adult echinoderms (although these have been lost in our extant echinoderms). Luckily, this is something that does get preserved in the fossil record occasionally. Some scientists have proposed that ***Hox* genes**, which help control anterior-posterior body patterning, are a good place to find genetic features that may unite the deuterostomes. In particular, within the deuterostomes examined so far, *Hox*9/10 appears to have split off from other *Hox* genes in its cluster and undergone some duplication events and changes. Alas, we generally cannot *Jurassic Park* our way into the fossil record and look at the genes of fossilized organisms. At the time of this writing, the oldest DNA recovered and sequenced

from fossilized remains comes from some mammoth specimens that are somewhere between 1 and 2 million years old.

Ambulacraria

The Ambulacraria includes two clades—the echinoderms and the hemichordates. Let's begin with the clade that you're probably more familiar with: phylum **Echinodermata** (Greek = "spiny skin"; Figure 2.8). These marine invertebrates come in a range of sizes and shapes and include sea stars, brittle stars, sea urchins, sand dollars, sea cucumbers, crinoids (sea lilies), and several extinct forms. Their wildly different morphologies come with different lifestyles: carnivores, detritivores, herbivores, and filter feeders, living on top of the substrate or underneath it (and sometimes swimming). Underlying this are a number of unifying characteristics—a calcite endoskeleton, larvae with bilateral (twofold) symmetry (Figure 2.9), and adults with pentaradial (fivefold) symmetry (Figure 2.8's sea star is a great example).



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Figure 2.8—Select representatives of Echinodermata. Clockwise, starting at the top left: sea star, brittle star, crinoid (sea lily), sea cucumber, and sea urchin.

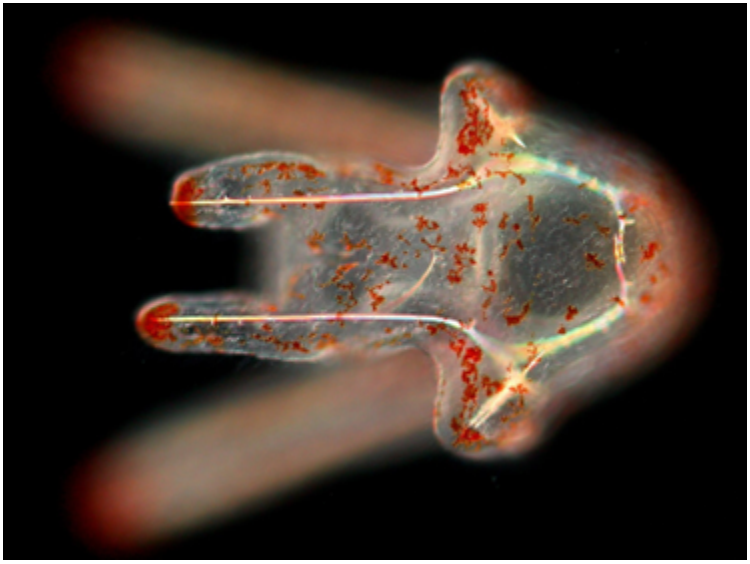

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Figure 2.9—Bilaterally symmetric echinoderm larva (Clypeaster subdepressus).

Phylum **Hemichordata** consists of two classes of marine invertebrates—Pterobranchia (Figure 2.10) and Enteropneusta (Figure 2.11)—that look wildly different from each other. The pterobranchs are small filter feeders (microscopic to 1 cm long) that build tubes of collagen that they live within. The enteropneusts are also known as "acorn worms." Most of these worm-shaped extant species live in or on top of the sediments of the sea floor and either filter feed or are detritivores. Unlike the pterobranchs, they tend to range from centimeters to 1.5 meters long. Just like we saw with the echinoderms, the hemichordates share some common features. Unlike the echinoderms, they do possess a dorsal nerve cord (Figure 2.12), although it is not necessarily hollow like we see in the chordates. Both classes also possess a muscular preoral organ that encloses the heart-kidney complex. In pterobranchs, this is the

cephalic shield, and in enteropneusts, this is the proboscis (Figure 2.11).

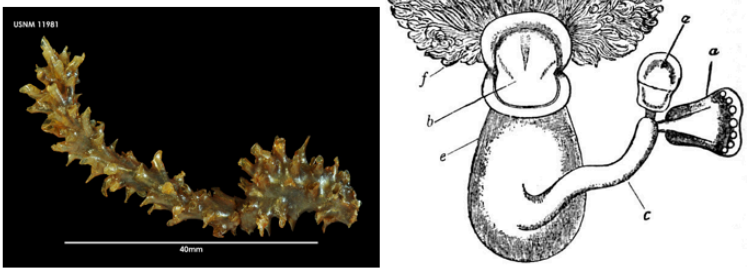

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Figure 2.10—Pterobranchia representatives (Cephalodiscus nigrescens, left; zooid of Cephalodiscus dodecalophus, right). For C. dodecalophus, see the following: (a) buds, (b) proboscis, (c) stalk, (d) arms and tentacles, (e) ventral edge of proboscis, (f) dorsal edge of the zooid.



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Figure 2.11—Enteropneusta representatives (i.e., acorn worms).

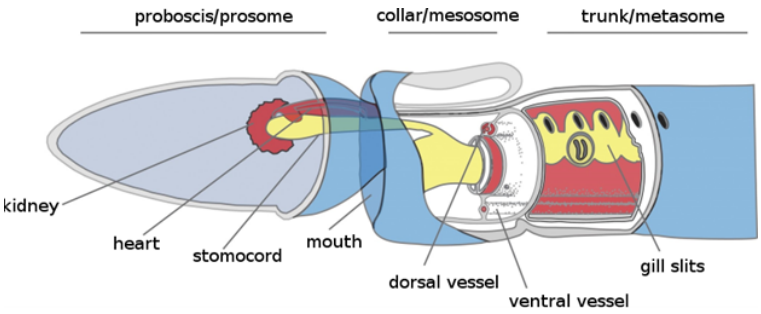

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Figure 2.12—The anatomy of an acorn worm Saccoglossus kowalevskii (Hemichordata, Enteropneusta, Harrimaniidae). The acorn worm is in the lateral view.

What unites these very different phyla, Echinodermata and Hemichordata, under the Ambulacraria? Molecular data support this grouping, particularly a set of microRNAs and a subset of *Hox* genes. There are also larval characteristics (Figure 2.13). Specifically, there are ciliated cells that form a preoral larval feeding band that creates a current to bring food to the larva's mouth, in addition to a perioral feeding band that moves food into the esophagus. The larvae of both phyla also have similar neuron structures that aren't seen in other taxa. Finally, despite outward appearances, there is some organizational similarity between the two phyla: The **coelom**, or body cavity, is divided into three sections by a series of coelomic sacs that run anterior to posterior.

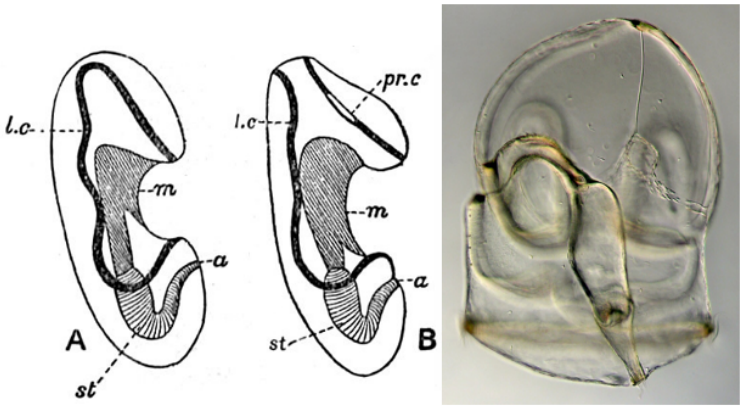

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Figure 2.13—Ambulacraria larvae. The drawn diagrams are the larvae of a sea cucumber (A) and sea star (B). In these drawings, a = anus, l.c. = longitudinal ciliary band, m = mouth, pr.c. = preoral ciliary band, and st = stomach. The photo on the right is a hemichordate larva for comparison to (A) and (B).

Phylum Chordata

There are three subphyla included in phylum Chordata: Urochordata (tunicates, sea squirts, and salps), Cephalochordata (lancelets), and Vertebrata (things with vertebrae). A glance at Figure 2.14 will once again show that this phylum contains animals that look wildly different from each other. Recall the start of this chapter, where we stated that there are three synapomorphies that the chordates demonstrate at some point in their lives: a dorsal hollow nerve tube, a stiff notochord, and a postanal tail.



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Figure 2.14—Representatives of phylum Chordata. Clockwise from top left: a lancelet, a tunicate, a tiger, and a tiger shark.

Let's first briefly introduce the Vertebrata so that you have them in mind as you consider the other chordates. Vertebrates have a head skeleton (often called the skull) that is cartilaginous at some point and surrounds a brain. Several neurological features are specific to vertebrates. These include unique motor and sensory nerves, embryonic neural crest cells, and cranial ectodermic placodes. **Neural crest cells**, which arise from the dorsal portion of the developing central nervous system, are particularly special. They are highly migratory, meaning that they don't necessarily stay where they originate during development. They are multipotent cells, which means that they can form a lot of different things. A partial list includes the cells that make up your skeletal tissues, various types of nerve cells, and the melanocytes (cells that produce the pigment melanin) in your skin. The **cranial ectodermic placodes**

(also known as **neurogenic placodes)**, which are associated with the formation of the head, form things like the sensory structure in your inner ear and nose, the lens of your eye, and sensory cells. During embryonic development, which we'll cover in more detail in Chapter 4, the internal organs of vertebrates develop from a different process than the rest of the deuterostomes. Finally, the Vertebrata have, well, vertebrae—skeletal elements associated with the dorsal nerve cord. We will see in Chapter 3 that the hagfishes do not have "true" vertebrae, but they have vertebrae-like elements that are homologous to the vertebrae in other vertebrates.

The subphylum Urochordata, sometimes referred to as the Tunicata as an alternate subphylum name, consists of roughly 3,000 living marine species. Some adults look like potatoes, others look like clumps of vases, and still others look like long chains of jellylike structures (Figure 2.15). Most urochordates are filter feeders. We will use tunicates as our representative urochordate here. Water enters the **branchial siphon**, which leads to the mouth and then the **pharyngeal basket**. Food is caught in mucous sheets within the basket and passed along to the stomach. Water leaves via the **atrial siphon**.

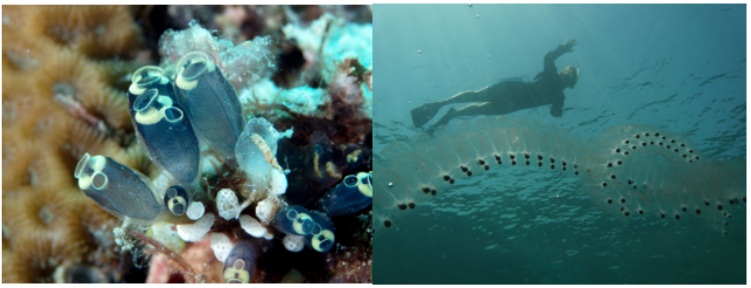

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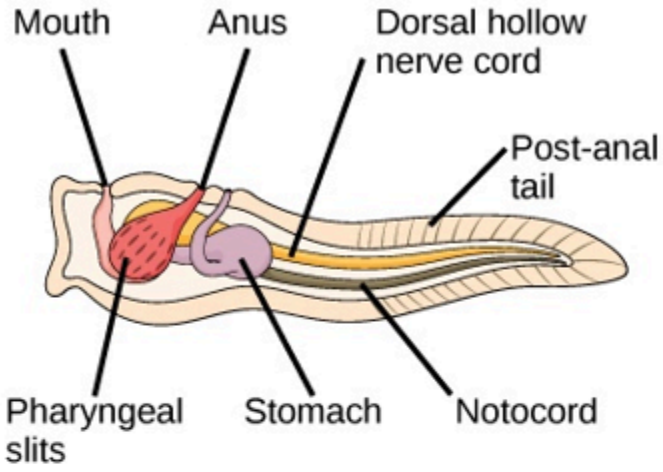
Figure 2.15—Tunicates (left) and salps (right).

None of the adult urochordates are recognizable as chordates because they do not retain the three chordate

synapomorphies as adults. As we saw with the ambulacrarians, a lot of evolutionary time has passed, and the adult forms we see now are highly modified compared to their early chordate ancestors. However, urochordates do have those synapomorphies as larvae! These are easiest to see in the tunicates, where the larvae resemble tadpoles (Figure 2.16). The features we saw in the adult tunicate are largely present in what looks like a head region, superficially—siphons, digestive organs, and other organs are all present. However, there is also a muscular tail, complete with a dorsal hollow nerve cord and notochord. When it is time for the tunicate larva to settle, it undergoes metamorphosis and resorbs its tail, nerve cord, and notochord. The cerebral ganglion, which is homologous (sharing a common ancestry) to the vertebrate brain and is responsible for controlling larval movement, also regresses.



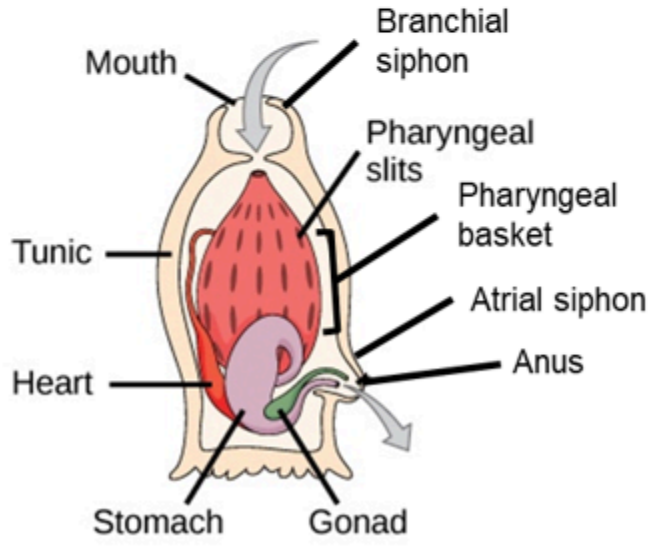


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Figure 2.16—Tunicate anatomy (larva, top; adult, bottom). Note that the pharyngeal basket and both siphons are labeled in the bottom figure (adult) but also exist in larvae (top figure).

The cephalochordates, or lancelets, consist of about 20 extant species that live in marine or brackish environments. Unlike with the urochordates, there isn't much variability across species—they look vaguely fishlike and are under 5 cm long. They tend to spend most of their time buried in the sediment, except for their heads, and are capable of swimming. The lancelets demonstrate the three chordate synapomorphies as both larvae and adults. They also have characteristics that seem very vertebrate-like. For example, the anterior portion of the nerve cord is somewhat enlarged, and at least part of it is considered homologous to parts of the vertebrate brain. The muscles in the body wall are arranged in chevron-shaped blocks called myotomes (also known as myomeres), and we see a similar arrangement in fishes (Figure 2.17).

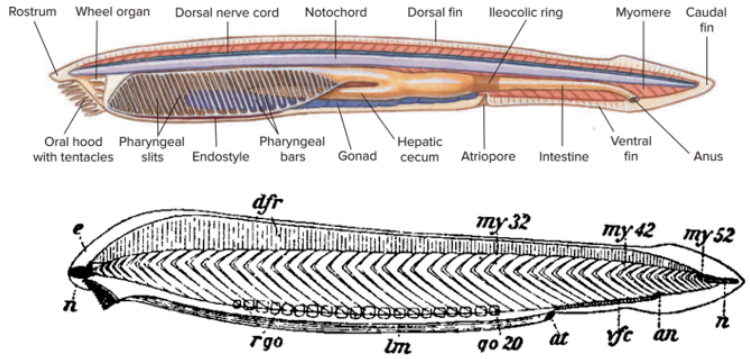

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Figure 2.17—Cephalochordate anatomy. The lower photo shows the chevron-shaped myotomes with specific myotomes labeled as "my." At = atriopore, an = anus, e = eyespot, n = notochord, rgo and go = gonads, dfr = dorsal fin, vfc = ventral fin chambers, rm and lm = right and left metapleur (which are similar to fin folds).

The fossil record of cephalochordates and urochordates is almost nonexistent. There is a fossil urochordate from the Cambrian (543 million years ago) that looks very similar to modern tunicates. However, similarly aged fossils that can be confidently assigned to

Cephalochordata have not been found. Thus, the fossil record is not much help in figuring out chordate relationships. Given the anatomy of the extant nonvertebrate chordates, we probably would expect that the vertebrates are more closely related to the cephalochordates than the urochordates. Alas, this is not the case! Molecular data indicate that urochordates are more closely related to vertebrates than cephalochordates (Figure 2.18). This is why it's also important to look at the larval urochordates—they actually share more characteristics with vertebrates than the cephalochordates do. For example, motor neurons connect to muscles in the same way in both vertebrates and urochordates but in a different way in cephalochordates. The microscopic structure of the notochord is also more similar in the larval urochordates and vertebrates. Last, it is possible that urochordates have neural-crest-like cells and ectodermal placodes like vertebrates do. However, this is still being explored.

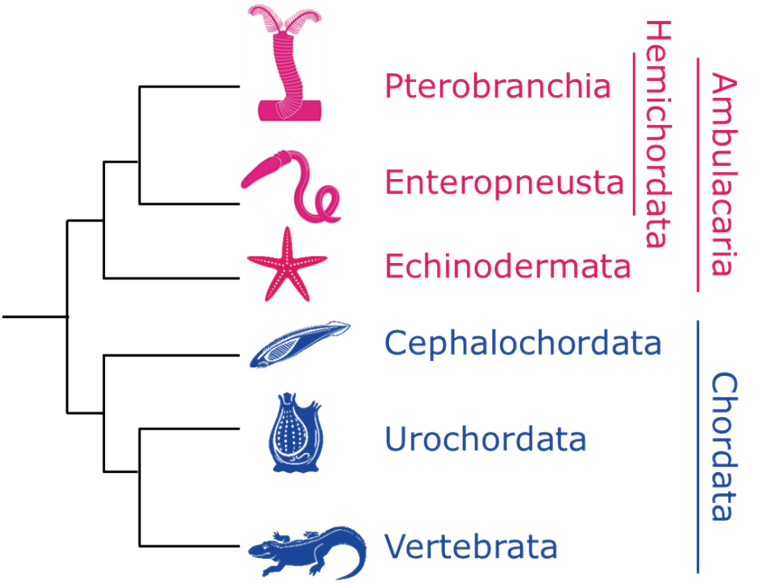

Figure 2.18—Chordata and Ambulacraria phylogeny.

2.3 What Did the Early Vertebrates Look Like?

While we learn about most of our vertebrate groups in Chapter 3, let's pause in the Cambrian period for a moment. We just mentioned that we don't have much in the way of early urochordates or cephalochordates in the fossil record. What about the Vertebrata? Some wonderful fossils from the Chengjiang formation in China that are about 516 million years old look very "fishy" in body shape and have fins (Figure 2.19). They also have all the chordate characteristics we've discussed, so there's no doubt that they are chordates. However, they also have fairly well-defined heads that include branchial bars (supports for the gills) and a pair of eyes. These fossils do not show evidence of jaws, but as we will see in Chapter 3, vertebrates did not always have jaws (and some still don't).



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Figure 2.19—Early fossil vertebrates from the Chengjiang formation (~516 MYA). The upper image is the actual Myllokunmingia fossil, and the lower drawing is a reconstruction of how this fossil may have looked in life.

2.4 Summary

The chordates, which include the vertebrates, are united by three synapomorphies (dorsal hollow nerve cord, notochord, and postanal tail). They share developmental characteristics with both

echinoderms and hemichordates. The vertebrates are united by neural crest cells, cranial ectodermal placodes, and vertebrae.

Application Questions

1. Since humans are vertebrates, and therefore chordates, we also possess the three chordate synapomorphies. Where is/was our notochord located, and what happened to it? Dorsal hollow nerve cord? Postanal tail?
2. Humans are also deuterostomes. Recall that one feature of deuterostomes is pharyngeal gill slits. Where are/were our gill slits, and what happened to them?

2.5 Further Reading

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3. Vertebrate Origins

LISA B. WHITENACK

Focus Questions—to Guide Your Reading of This Chapter

1. What are some of the defining characteristics of vertebrates? Gnathostomes? Eugnathostomes?
2. What familiar vertebrates fall into each taxonomic group of vertebrates?

3.1 Introduction—What Is a Vertebrate, Anyway?

In Chapter 2, we introduced you to deuterostomes and, more specifically, the chordates. We also introduced you to the vertebrates in general, including what early vertebrates may have looked like. Let's dive deeper into some features that vertebrates share, and we'll add some new ones to your list.

Neural crest cells have been regarded as one of the most important unique vertebrate features. The cells develop along either side of the **neural plate**, which makes up the embryonic nerve cord (neural tube) and embryonic brain (Figure 3.1). You will read more about these and what they become in Chapter 4, but they have two

important properties. The first is that they are migratory. Across vertebrates, we see these cells develop at the neural plate from anterior to posterior, and then they distribute themselves around the body. Once they arrive at their destination, we see their second important property: They are multipotent. That means that they can become many different things. They can make the structural tissues in your head (bone, cartilage, dentine in your teeth), nervous system cells, or pigment cells, depending on which genes are expressed and when. Altogether, neural crest cells are responsible for many vertebrate features.

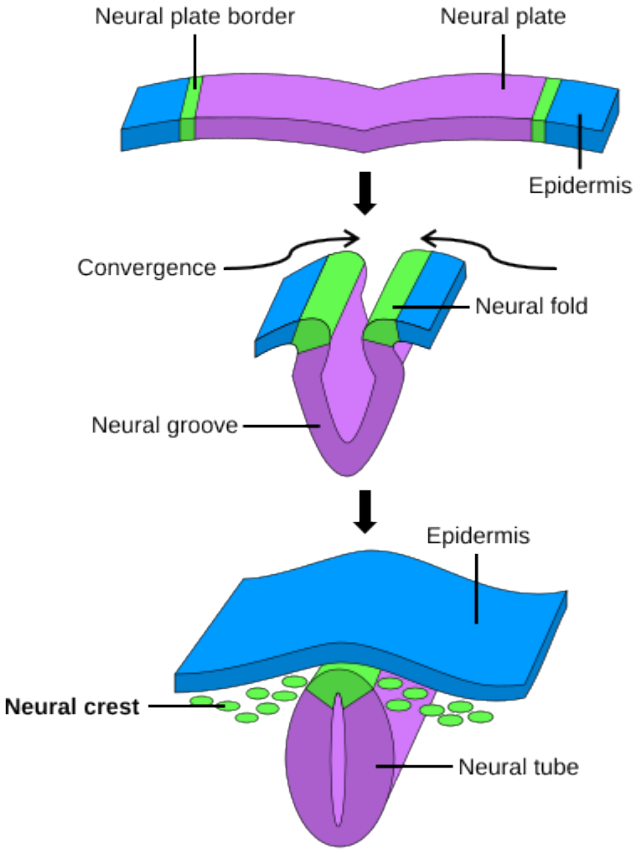

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Figure 3.1—Neural crest cell formation during the process of neurulation. Note that the neural tube develops into the dorsal nerve cord.

Let's now imagine a vertebrate—it might be you, your cat, or your goldfish. Regardless, whichever vertebrate you've imagined has a head. If we had to define what a head is, we would say something like "it's the part of the body where the mouth, brain, skull, and sensory organs are located." Note that we didn't say there

was a jaw or teeth—not all vertebrates have these (more on that later). It's also worth noting that many invertebrates have heads (albeit without skulls) too—insects are great examples.

Considering the vertebrate head leads us to more vertebrate features. The first was mentioned in the previous chapter, **cranial ectodermal placodes**, also called **neurogenic placodes** (Figure 3.2). These embryonic features are a thickening of tissue at various places in the head region. Based on interactions between cells and tissues, placodes can differentiate into a number of different structures depending on their location. These include the lens of the vertebrate eye, portions of various cranial nerves (nerves that come directly off the brain), structures related to olfaction (sense of smell), and more. You will read more about these placodes in Chapter 4 and about some of their derivatives in the chapters about the nervous and sensory system (Chapters 18, 19, and 20).

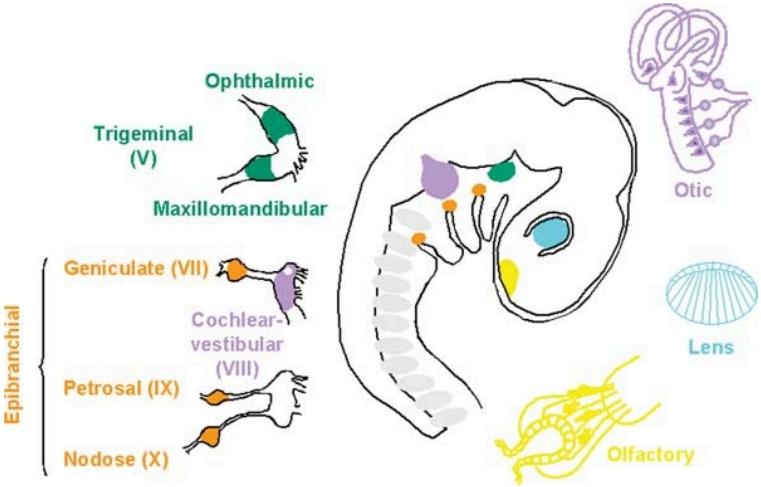

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Figure 3.2—The olfactory (yellow), lens (blue), trigeminal (green), otic (purple), and epibranchial (orange) cranial ectodermal (neurogenic) placodes locate to characteristic positions, here shown in a side view of a three-day-old chick embryo. You will learn much more about these names and structures in later chapters.

Speaking of nervous systems, vertebrates are also known for their fairly complex brains. If the vertebrate you thought of earlier was a goldfish, you may not categorize its brain as "complex." However, it's all relative—if we compare vertebrate brains to those of other chordates, for example, even that goldfish brain is pretty complex. Vertebrate brains are divided into three regions: a **forebrain**, a **midbrain**, and a **hindbrain** (Figure 3.3). These are very distinct regions that have fairly distinct functions, even in goldfish. Cephalochordates, on the other hand, do not have any obvious divisions at all. They have a slightly larger mass of neural tissue in their head region called the cerebral vesicle. There is some difference in gene expression as you move anterior to posterior in the cerebral vesicle, but that's it. Urochordates have no brain at all. Neural crest cells are not responsible for making any vertebrate brain tissue itself. However, they do help make some of the cranial nerves coming off the brain. You'll learn more about these in Chapter 19.

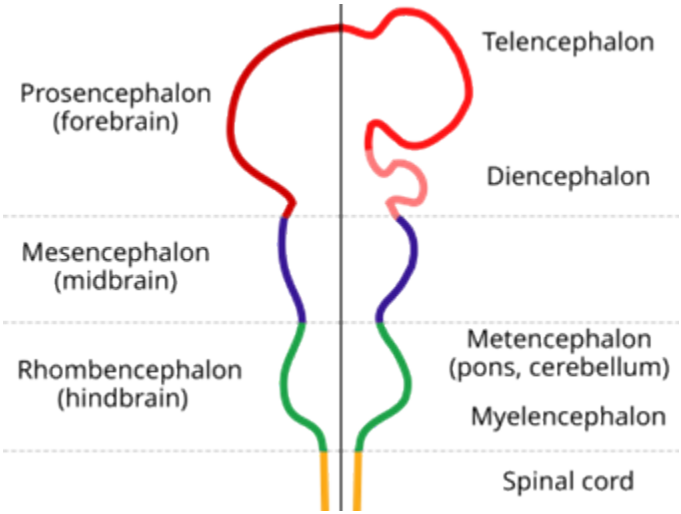

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Figure 3.3—Divisions of the embryonic vertebrate brain during different periods of development.

Vertebrates protect their brains with a **skull**, also called the **cranium**. This is typically made up of either cartilage or bone, which means that the skull is a feature that is derived from those very important neural crest cells. Skulls are fascinating parts of the body that can tell you about sensory systems, the brain itself, posture, feeding mode, and much more. In fact, we've devoted an entire chapter just to the skull (Chapter 8).

If we could summarize everything we've just discussed, we could say that vertebrates are defined by their heads. Indeed, as scientists began to understand the importance of neural crest cells and the neurogenic placodes, Carl Gans and Glenn Northcutt proposed the "New Head Hypothesis" in 1983. The hypothesis contains several different strands that concern the origin of unique vertebrate tissues, homology, and much more. The general idea is this:

- Neural crest cells and placodes, their resulting features, and some innovations in the pharynx all resulted in a change from filter feeding to active predation.
- This switch in feeding mode opened up several new niches and allowed vertebrates to flourish.

The various strands of the New Head Hypothesis have undergone extensive testing. The importance of neural crest cells and neurogenic placodes to vertebrate evolution has held true. However, as technology continues to advance and allow new ways to test these ideas, some have not been supported. This includes the trend of more active predation through early vertebrate evolution.

Let's move posteriorly along the vertebrate body. As we leave the head, we encounter skeletal elements called **vertebrae** (sing. = vertebra), which are informally called a backbone and are part of the **axial skeleton** (which also includes the skull, ribs, and parts of the pharynx). Each vertebra is composed of either bone, cartilage, or other connective tissue, depending on which vertebrate you're examining. These supportive structures usually

surround the notochord, and in many vertebrates, the notochord is incorporated into the vertebra or its nearby structures to varying degrees. Most vertebrae are composed of two distinct structures, a **centrum** (the body of the vertebra) and **vertebral arches** (located dorsal and ventral to the centrum), although several vertebrate groups only have one or the other (Figure 3.4). Vertebral morphology varies greatly across taxa and regionally within a species, making it useful for understanding locomotion, taxonomy (classification), and the transition of vertebrates from aquatic to terrestrial environments. Once again, we have an entire chapter devoted to the noncranial parts of the axial skeleton (Chapter 9).

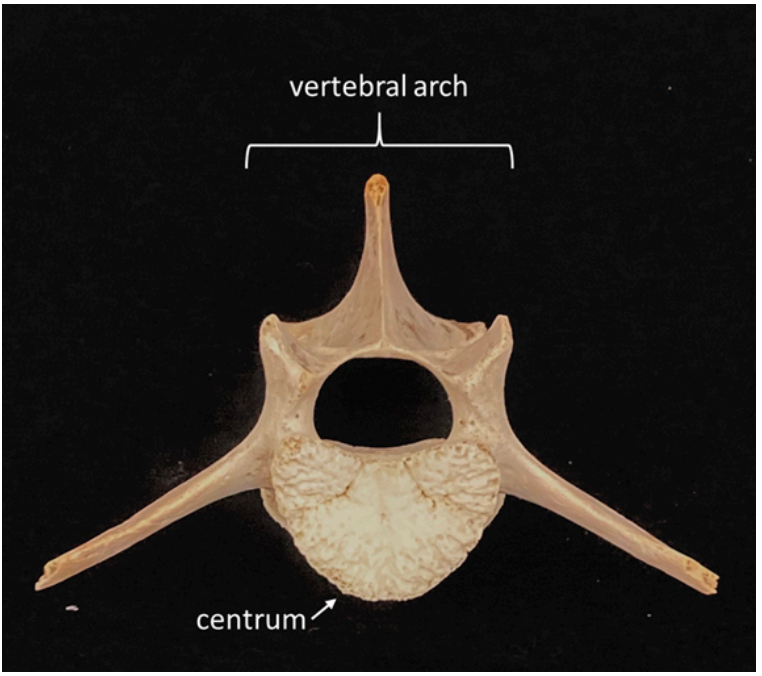

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Figure 3.4—Mammalian thoracic vertebra. Note that this vertebra only has a vertebral arch dorsal to the centrum.

For our final vertebrate feature, we are going to move from structures to genes. *Hox* genes, which we introduced in the previous chapter, control anterior-posterior body patterning in bilateral animals (both invertebrates and vertebrates). In the vertebrates, they are also associated with the cranial neural crest cells and appendages, among other things. How did this happen? If we go back to the invertebrates, the *Hox* genes are located within a single, compact cluster. However, in vertebrates, these clusters have gone through several rounds of duplication (Figure 3.5). As a result, vertebrates have somewhere between three and eight clusters of *Hox* genes, depending on which group of vertebrates you're considering. Once important genes are duplicated, constraints on some of those copies can be lifted, allowing natural selection to act on one copy without detrimental effects. What do the nonvertebrate chordates have? *Amphioxus*, one of our living cephalochordates, has just one *Hox* cluster. Interestingly, extant urochordates that have been studied have no *Hox* clusters, although they do have *Hox* genes. We have to once again remember that our living nonvertebrate chordates diverged from the vertebrates over 500 million years ago, and their genome has had a lot of time to change. It is likely that urochordates probably had at least a single *Hox* cluster at some point and have lost it over time.

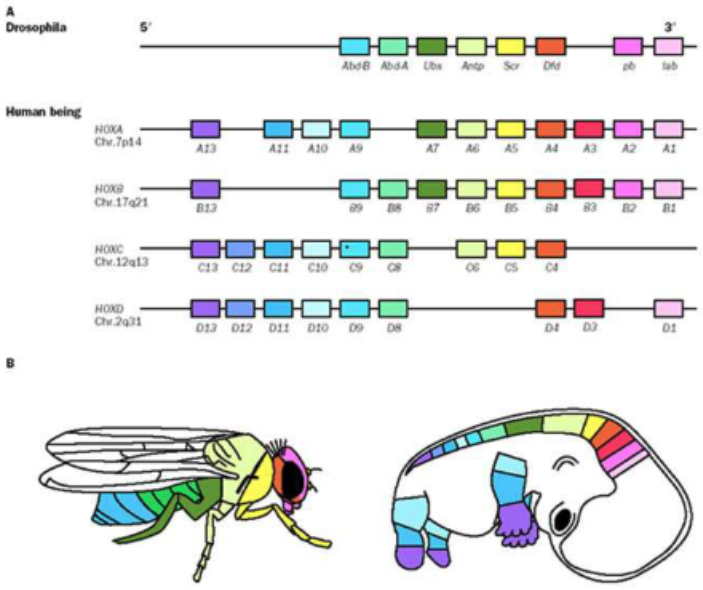

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Figure 3.5—Despite rearrangements, inversions, and duplications, all bilaterally symmetrical animals contain recognizably comparable sets of Hox genes. Mammals, such as mice and humans, have four clusters of Hox genes (labeled HOXA–HOXD) that are expressed along the anterior–posterior axis.

3.2 Let's Meet the Vertebrates

So far, we've determined that the vertebrates have a head and vertebrae, as well as embryonic and genetic similarities. Which living taxa have these? You do, as do other mammals. So do birds, reptiles, amphibians, and fishes of all kinds. We also have a lot of extinct vertebrates to account for across their 500+ million years of existence. All these vertebrate groups have their own suite of characteristics that define them, and we use these characteristics to figure out vertebrate evolutionary relationships (Box 3.1). As we dive into the different groups of vertebrates, keep in mind that

the evolutionary relationships we discuss here may change in the future as scientists continue to study the various vertebrate groups. Similarly, you may find phylogenetic trees with other branching patterns if you look at other sources of information, depending on when those trees were generated and what kind of data they used to build them.

Box 3.1—Phylogenetic Trees Are Hypotheses

When we introduced you to phylogenetic trees in the last chapter, we were concerned with how to read them. However, it's also important to understand how those trees are generated in the first place—Why is one taxon linked more closely to another taxon, compared to a third? Why are the nodes placed the way they are? Why do phylogenetic trees with the exact same taxa differ in branching pattern depending on what source you're looking at?

Two general components are important in making phylogenetic trees—the data that you input and the evolutionary model that you chose to apply those data to. The choices that scientists make for each of those components come with particular assumptions, strengths, and weaknesses. Not all data types or evolutionary models are appropriate for all evolutionary questions. In other words, scientists need to choose the most applicable data and models for the evolutionary questions they are seeking to answer.

The data that are collected to generate these trees can be either morphological or molecular. Morphological data are essentially anything you can

measure on the organism, whether that is a feature (e.g., presence/absence or counting something), a shape (e.g., length, width, oval), or physiological characteristics (e.g., the ability to break down lactose). When incorporating fossil data into phylogenies, you are limited to morphological characteristics. One also must consider if the characteristics they want to measure are actually informative for the evolutionary question or not. For example, if one were interested in the evolutionary relationships of sharks, the presence or absence of an eye is not informative because all sharks have eyes!

Molecular data generally concern the sequence of genes in some way. We can use DNA or RNA, genetic information from the nucleus or mitochondria, genetic or amino acid sequences, whole or partial gene sequences, and more. Which of these things we choose matters just as much as which morphological characters we choose. For example, mutation rates vary across genes, between mitochondrial and nuclear genes, and even within a single RNA strand. If our research question concerns evolution in a very short time frame, we would want to focus on a gene that has a high mutation rate; otherwise, we may not see any differences in sequence. Similarly, if our research question concerns a very long time frame (such as all of vertebrate evolution), we may want a gene that has a very slow mutation rate so that any differences due to evolutionary relationships are not swamped by noise from many mutations over 500 million years.

Once we have the data, we have to apply evolutionary models to it. For morphological data, this

means making decisions about which traits are more ancestral than others and coding them accordingly (or making a decision to not code the data this way). We then have to decide how we want the computer to apply evolutionary processes to these data—Do we allow evolutionary reversals? What do we do when branching patterns are equally likely? Do we apply statistical analyses to help figure that out? Do we want random evolution or more directed evolutionary processes? We have similar decisions to make for molecular evolution models, but we have to add in other considerations, such as assumptions about mutation rates and what kinds of mutations are more likely than others.

There are entire graduate school courses on the data and models that we use to make these trees, and our goal is only to give you a taste of this here. The point we want you to take from this is that there are a lot of human-generated decisions that go into making phylogenetic trees. Even if we stick with molecular trees, changing which gene you use can result in a different branching pattern. **Thus, phylogenetic trees are evolutionary hypotheses, not definitive answers.** When we see the same patterns coming up with multiple types of data and models, we have strong evidence that the relationships we see in the trees—those hypotheses—are the most likely scenario. The phylogenetic trees in this book, and the hypotheses they represent, fall into that category as of the writing of this textbook in 2025.

We can divide the Vertebrata into two broad groups,

Cyclostomata (hagfishes and lampreys) and **Gnathostomata** (everything else; Figure 3.6).

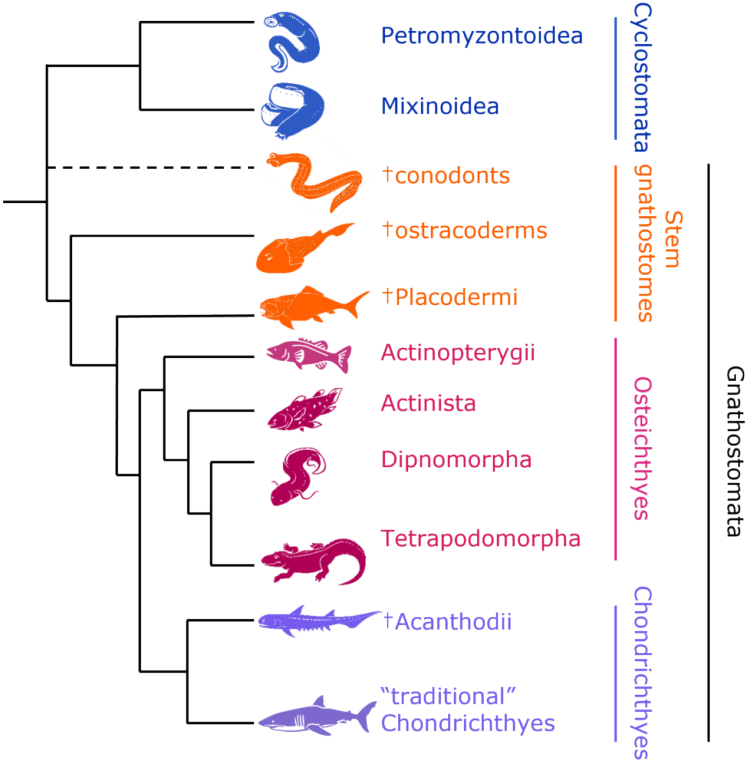

Figure 3.6—Phylogenetic tree of the Vertebrata. Dotted lines indicate that the placement of the branch is uncertain. The dagger (†) indicates that all members of that taxonomic group are extinct.

Cyclostomata

The cyclostomes include extant hagfishes (Mixinoidea, Figure 3.7) and lampreys (Petromyzontidae, Figure 3.8) and their extinct relatives. The word *Cyclostomata* translates to "circle mouth," which

aptly describes the most prominent feature of lampreys. Hagfishes and lampreys have other morphological features in common as well. Both are jawless and have teeth made of keratin (a protein that your nails and hair are also made of). Both groups have a single nostril, gill pouches, and evolutionary precursors to vertebrae called **arcualia**. Both are long and don't have scales. Beyond that, the morphological similarities are few. Most of the support for placing these two taxa into one group is based on molecular data sources, including both nuclear DNA, mitochondrial DNA, and RNA. A key thing to remember is that the cyclostomes diverged from the gnathostome line in the Early Cambrian period, leaving a lot of evolutionary time for hagfishes and lampreys to accumulate phenotypic changes that may mask their relatedness.

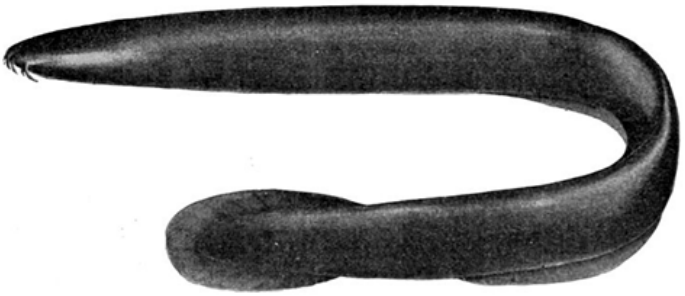

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Figure 3.7—The black hagfish, Eptatretus deani.



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Figure 3.8—An aquarium full of sea lampreys, Petromyzon marinus.

There are about 76 extant species of hagfish, which are also known as "slime eels." Hagfishes, which are strictly marine, can produce several liters of slime from glands in their skin when attacked by a predator—this process only takes roughly 100 milliseconds. This mucus-like slime quickly adheres and clogs fish gills, making it an effective deterrent (Figures 3.9 and 3.10). They are also known for performing a unique knotting behavior when they encounter a particularly tough piece of carcass: They wrap themselves into a knot, then pass this knot down the body, creating some extra force to leverage the chunk of carcass off. This is helpful, given that they lack jaws. These knots are also used to escape, remove mucus, and occasionally pull live prey (worms and crustaceans) from burrows. Some hagfishes may also absorb nutrients from their surrounding environment through their skin.



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Figure 3.9—A hagfish that has tied itself into a knot.

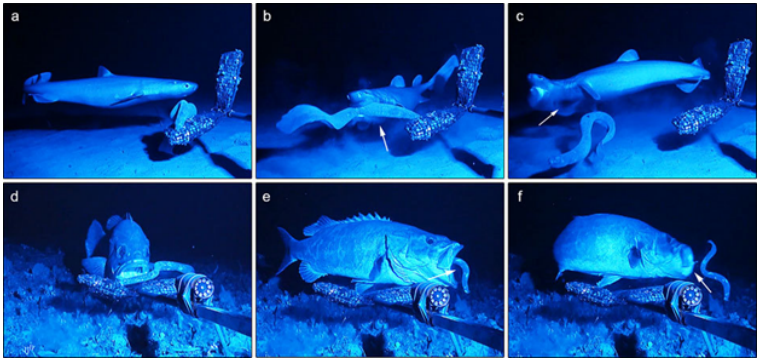

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Figure 3.10—The seal shark Dalatias licha (a–c) and the wreckfish Polyprion americanus (d–f) attempt to prey on the hagfishes. First, the predators approach their potential prey. Predators bite or try to swallow the hagfishes, but hagfishes have already projected jets of slime (arrows) into the predators' mouths. Choking, the predators release the hagfishes and gag in an attempt to remove slime from their mouths and gill chambers.

The lamprey, of which there are about 38 extant species, can be found in both fresh and salt water. Most lampreys spend the majority of their lives in the larval stage, called an **ammocoetes larva**. The larvae eventually metamorphose into a juvenile or adult, depending on the species. The ammocoete and adult lamprey are extraordinarily different from each other. Ammocoetes hatch in freshwater, live within the substrate at the bottom of the water body, and are filter feeders. Adult lampreys are not filter feeders. Some species, referred to as brook lamprey, do not actually eat as adults and stay in their original bodies of freshwater. They mature into a sexually mature adult from metamorphosis, **spawn** (reproduce), and die. The remaining species are categorized as parasitic lamprey. They metamorphose into sexually immature juveniles, start parasitizing other fishes, and then continue to grow and mature into sexually mature adults (Figure 3.11). While some species of parasitic juveniles stay in freshwater, other species will migrate to salt water. However, both will return back to freshwater to spawn and then die.

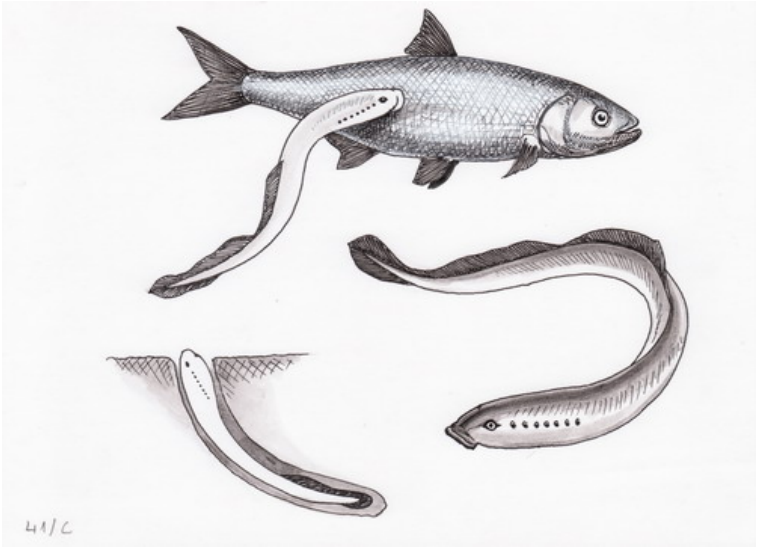

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Figure 3.11—The life stages of a parasitic lamprey. Bottom left: filter-feeding ammocoetes larvae; top: parasitic juvenile; bottom right: parasitic adult body form.

Gnathostomata

All of the other vertebrates fall under the Gnathostomata, which translates to "jaw mouth" (Figure 3.6). Historically, the only vertebrates that were included in this group were vertebrates with jaws—bony and cartilaginous fishes, two groups of fossil fishes (placoderms and acanthodians), amphibians, reptiles (including birds), and mammals. The cyclostomes plus extinct jawless fishes were previously united under the group "Agnatha" ("without jaws"). However, in the last few decades, new technologies and techniques for studying fossils and evolutionary relationships have added two extinct jawless vertebrate groups (conodonts and ostracoderms) to our "jawed vertebrates." These two groups, plus the placoderm fishes, are collectively referred to as **stem gnathostomes**, with the

"stem" designation reflecting that they are some of the earliest members of the Gnathostomata. As we move through the gnathostomes, you will see that some taxa will have a dagger icon (†) in front of the name. This symbol is used in biology to indicate that that taxon is completely extinct.

Box 3.2—Understanding Geologic and Evolutionary Time

Think about the oldest person you know and how long ago they were born. It probably seems pretty far away. Now think about how long ago World War II feels (1939–1945). What about the Napoleonic Wars (1803–1815)? The American Revolution (1765–1783)? Egypt's Golden Empire (1560–1080 BCE)? The events we talked about in Chapter 1 of this book? All of those probably feel incredibly long ago. For humans, a handful of decades, let alone centuries, seems like an incredibly long time ago.

When we're talking about the evolutionary history of the vertebrates, the history of life, or even the full history of the Earth, the number of years ago shifts by several orders of magnitude: millions and billions of years ago. Given how humans think about time, this is really difficult for people to wrap their minds around. If you read that the Earth is approximately 4.5 billion years old, can you really fathom that amount of time? Or, what if we add a new fact: Life is 3.7 billion years old? We can add another fact that often messes with people's minds: We are closer in time to *Tyrannosaurus rex* (extinct 65 million years ago) than *T. rex* was to *Stegosaurus* (extinct 145 million years ago).

One way that we deal with trying to reconcile geologic and evolutionary time in our heads is to talk about geologic periods and eras instead of how many millions of years ago. For example, "Jurassic" is a geologic period from 201 to 145 million years ago (abbreviated as Ma or MYA)—this is the period that *Stegosaurus* lived in. The Jurassic period, along with the Triassic and Cretaceous periods, make up the Mesozoic era. We can similarly divide up the time before and after the Mesozoic era into various periods and eras. Working with the names of periods and eras, and knowing the relationship of those periods and eras to one another, is a useful way of keeping the timeline straight. We call this system the **geologic time scale** (Figure 3.12). Look at Figure 3.12 and find the Mesozoic era. Which of the three periods in this era did *T. rex* live in? You will see some of these periods and the associated times they cover throughout this book.

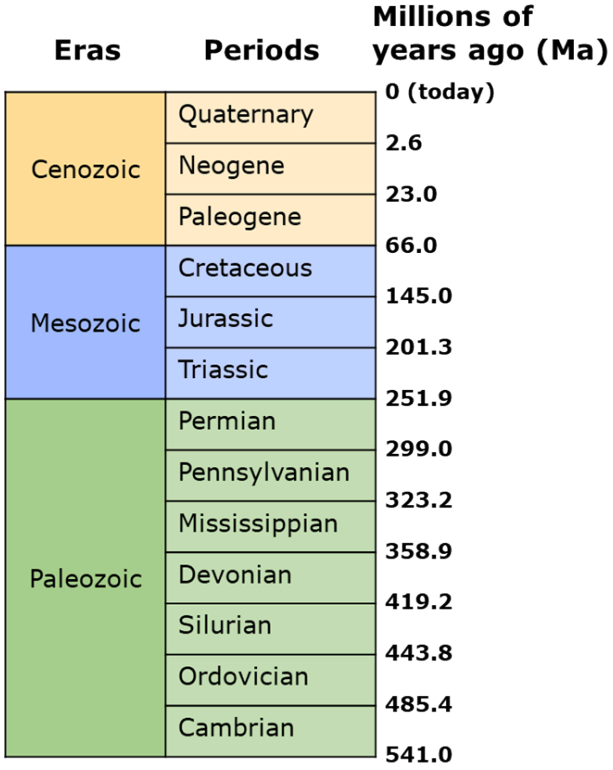

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Figure 3.12—The geological time scale for the Phanerozoic Eon. Oldest is at the bottom; youngest is at the top. Dates are based on the Geological Society of America's geologic time scale, V. 6.0 (2022). Note that the Phanerozoic is only the most recent eon in geologic history.

This still doesn't solve the problem of scale, though—how much a million and a billion are. For that,

we usually turn to analogy. In this case, we are going to think about the face of a clock (Figure 3.13). Assume that the Earth's origin (4.5 billion years ago, abbreviated as 4.5 Ga) is at 12:00. Life originates around 2:00. Eukaryotes (organisms with a nucleus and membrane-bound organelles in their cells) don't show up until 6:30. Animals appear at around 10:00, mammals sometime after 11:00, and hominins (a group that includes humans) at 11:59:59—in the last second.

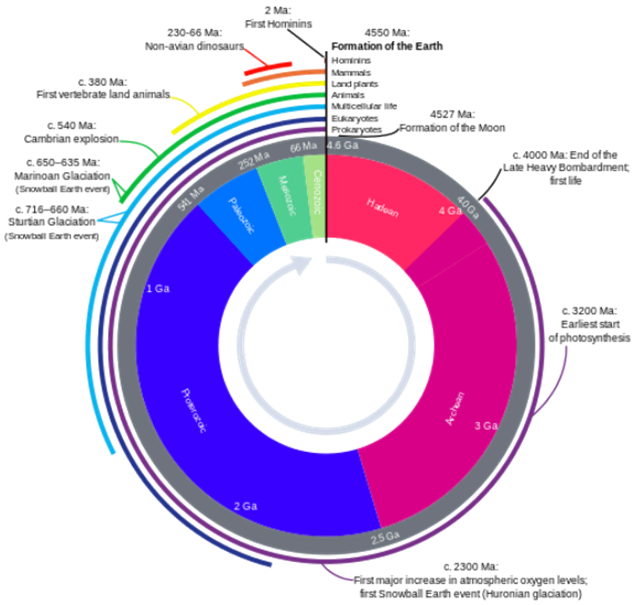

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Figure 3.13—A projection of Earth's 4.5-billion-year history onto a clock. Ma = millions of years ago, Ga = billions of years ago. Notice that the Phanerozoic Eon (which we saw in Figure 3.12) doesn't begin until 541 Ma.

The gnathostomes have several features that unite them. These include

1. The presence of a **sclerotic ring**—a bony element near the eye that forms due to ossification of the sclerotic capsule. You can see this clearly in the eye region of *Dunkleosteus* in Figure 3.18.
2. **Paired pectoral appendages** that have supporting skeletal structures—your arms are a great example, but so are the

pectoral fins in fishes.

3. **Dermal bone**—a specific type of bone that forms via **intramembranous ossification**, a fancy way of saying bone that forms within a membrane instead of replacing a cartilage template.
4. **Dental tissues**—those that make up true teeth, such as dentin and enamel.
5. **Perichondral ossification** (or some sort of calcification) of the endoskeleton—unlike intramembranous ossification, this type of bone formation starts with cartilage acting as a template for the bone tissue to replace.
6. **Cellular bone**—this is a particular type of bone that is formed by **osteoblasts** (bone-forming cells) within a bony matrix. Acellular bone (without osteoblasts) currently exists in some bony fishes, but your bones are decidedly cellular.

Note that this is only a partial list of gnathostome characteristics. There are other specific organs and skeletal structures as well, but these six will come into play shortly.

†*Conodonts*

The conodonts, which lived in the marine realm from the Cambrian through the Late Triassic, are a bit of a paleontological mystery. Very few whole-body fossils exist, but those rare body fossils reveal more chordate and vertebrate features: notochords, segmented trunk muscles, paired sensory organs, a caudal fin, and gill pouches (Figure 3.14). The vast majority of the fossil record of these extinct vertebrates is toothlike elements made of **apatite**, a mineral composed of phosphate and calcium, which are no larger than 1 mm long (Figure 3.15). Using apatite as a skeletal component is another vertebrate feature, and the array of tissues in these tooth elements is very similar to dental tissues in other gnathostomes (see gnathostome characteristic 4; Figure 3.16). However, it is not clear

whether these are truly homologous with other gnathostome tooth tissues, and thus their relationship to other vertebrates is cloudy. For now, conodonts are considered to be stem gnathostomes.



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Figure 3.14—A reconstruction of a conodont.



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Figure 3.15—Tooth elements from the Ordovician conodont Iowagnathus grandis.

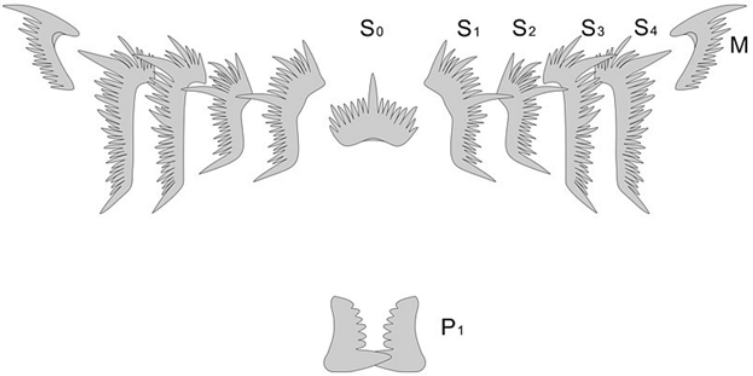

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Figure 3.16—Tooth elements from the conodont Hindeodus parvus arranged in the order that they occur in the mouth. Elements at the top of the diagram occur anteriorly, while those at the bottom are posterior to those at the top of the diagram. Tooth element M is the most lateral tooth element, while tooth element S0 is the most central.

†*The "Ostracoderms"*

The "**ostracoderms**" are a variable group of jawless fishes that lived from the Ordovician through the Devonian (Figure 3.17). We've put their name in quotes because they represent several evolutionary lines, as opposed to one particular taxonomic group. All the "ostracoderms" have some sort of bony armor, but its shape and size vary across the groups. For example, there are two groups, the Cephalaspidomorphi ("head shield form") and Pteraspidomorphi ("wing shield form") that have extensive head shields made out of bone. The Thelodonti and Anaspida are two other groups that have small scales instead of large bony shields. This group of largely jawless fishes seems to be an odd thing to include in the Gnathostomata, a taxon named for having jaws. However, when one considers characteristics beyond jaws, the inclusion makes more sense. All ostracoderms have some sort of mineralized, dermal bone skeleton. Some ostracoderms have all six of the gnathostome

characteristics highlighted above, plus a few more, indicating that these particular gnathostome features appeared prior to jaws! For example, two groups of cephalaspids, the Galeaspida and Osteostraci, are the closest to the jawed gnathostomes. The galeaspids have a **neurocranium** (braincase) composed of mineralized cartilage, while the osteostracans have one composed of perichondral bone (gnathostome characteristics 5). The osteostracans also have paired pectoral fins and cellular bone (gnathostome characteristics 2 and 6), making them the closest jawless fish group to the jawed vertebrates (Figure 3.6).

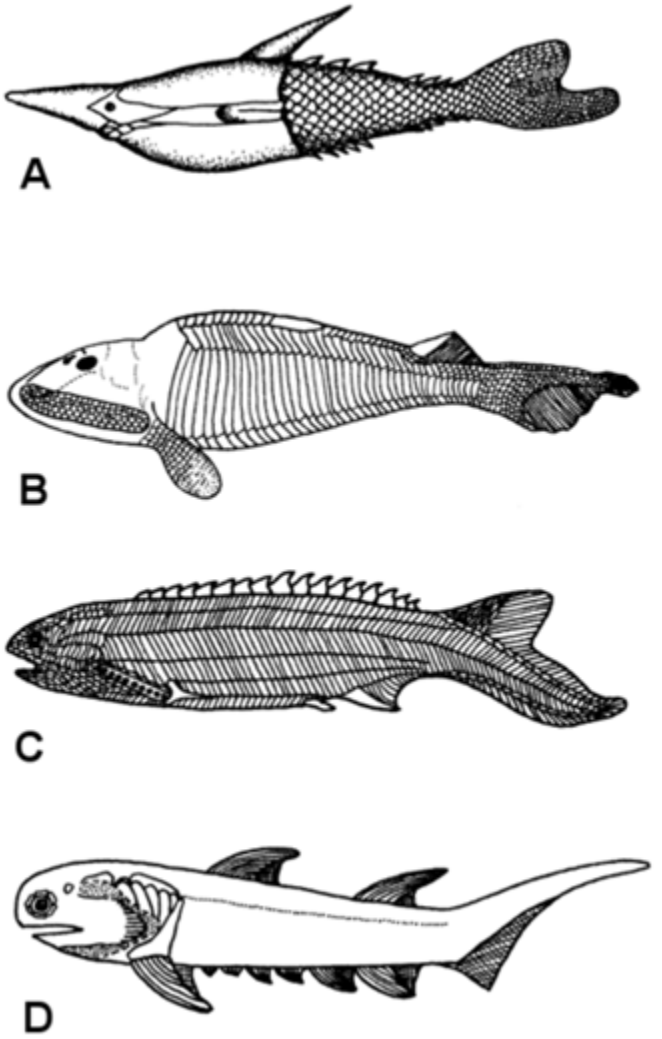

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Figure 3.17—Fossil jawless and jawed fish. (A) Pteraspis, a pteraspidomorphan. Two Cephalaspidomorphans: (B) Hemicyclaspis (Osteostraci) and (C) Pterygolepis (Anaspida). A, B, and C are all ostracoderms (jawless, armored fish). (D) Climatius is a fossil acanthodian with a jaw.

†The "Placoderms"

The "**placoderms**," which lived from the early Silurian to the late Devonian, are another group of fishes with extensive head armor and varying morphologies. The most well-known placoderm group (at least to those who frequent natural history museums or are fans of the movie *Ponyo*) is the arthrodires, specifically the very large *Dunkleosteus* (Figures 3.18 and 3.19). Most arthrodires were active predators with sharp dermal plates on the jaws. However, most orders of placoderms were **benthic** (bottom-dwelling) forms such as *Bothriolepis* (Figure 3.20). They differ from the ostracoderms due to several characteristics that they share with other gnathostomes:

1. Paired **pelvic appendages** that have supporting skeletal structures—your legs are a great example, as are the pelvic fins of many fishes.
2. Five or fewer **branchial arches**—in fishes, these are the elements that support the gills. You will see in later chapters where these gill arches have gone in terrestrial vertebrates.
3. Jaws that operate dorsoventrally—this is different from the mouths of some conodonts and the cyclostomes, which move laterally. As you will read in Chapter 8, there are two main elements, the **palatoquadrate** (upper jaw) and **Meckelian element** (lower jaw). These elements will be greatly modified as we move through evolutionary time, including one of those elements being incorporated into the mammalian ear.
4. Gnathostome jaws also bear **dermal denticle plates**, which act as teeth. The question of what defines something as a true tooth is currently being debated by scientists, as much of the helpful soft tissue and developmental data are not available in the fossil record; thus we cannot say whether placoderm teeth are truly homologous with other gnathostome teeth.



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Figure 3.18—Dunkleosteus terrelli, an arthrodire placoderm from the Devonian of Cleveland, Ohio. This is a cast of one of the most complete fossils we have for this fish.

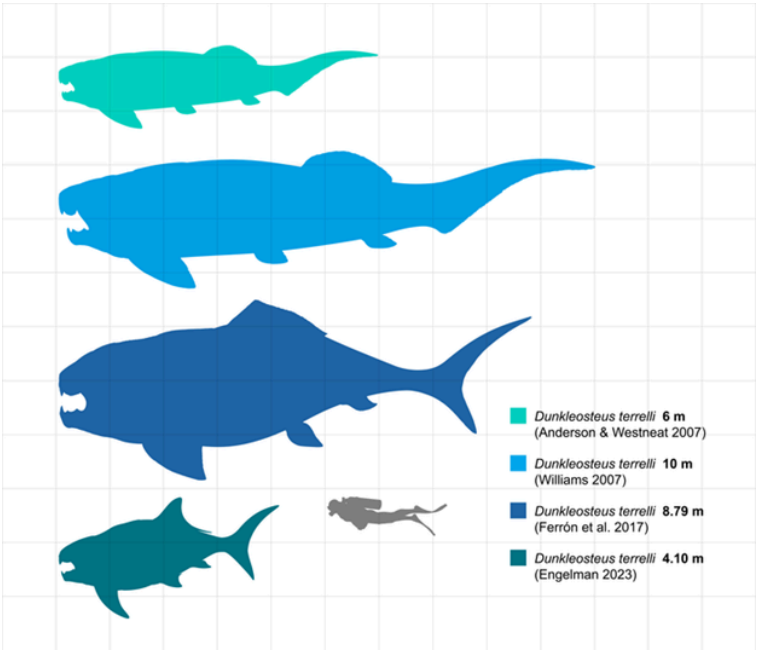

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Figure 3.19—Various size estimates for Dunkleosteus terrelli, with a human shown for scale. Because we have only found the head and part of the shoulder girdle, we have to hypothesize the size and shape of the body.



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Figure 3.20—A reconstruction of the Devonian antiarch placoderm Bothriolepis canadensis.

We've placed the name "placoderms" in quotes because it's not clear whether the placoderms should be put together into one group or if they represent several evolutionary lineages. Historically, these fishes were grouped together because they are where we first see jaws in the fossil record. We also know that all placoderms share particular features of the neurocranium that other gnathostomes do not and that they may share a unique version of dentine not seen in other gnathostomes. However, recent studies have also shown that variations in other neurocranium features, the articulation of the **hyoid arch** with the neurocranium, and inner ear features support multiple evolutionary lineages. There is also a group of placoderms that are called "maxillate," as they have a **premaxilla**, **maxilla**, and **dentary** instead of just a palatoquadrate and Meckelian element. More simply put: We have not figured out what to do with the placoderms other than agreeing they are stem gnathostomes.

Eugnathostomata

The remainder of our gnathostomes fall under the **Eugnathostomata** ("true gnathostomes"), reflecting that these organisms all have the features we've already discussed as gnathostome characteristics, plus true teeth and several cranial characteristics related to the skull and eye. While teeth will be discussed more in Chapter 8 and we mentioned some of the issues defining teeth above, we will point out that among our extant eugnathostomes, true teeth are in part defined by developing within a **dental lamina**—an epidermal soft tissue that does not fossilize readily—in a particular pattern.

The eugnathostomes include the **Chondrichthyes** (cartilaginous fishes), **Osteichthyes** (bony fishes, which technically include amphibians, reptiles, birds, and mammals), and an enigmatic group of extinct fishes called **acanthodians** (see Figure 3.6 if you need a reminder of what the vertebrate phylogenetic tree looks like).

Chondrichthyes

The Chondrichthyes ("cartilage fish") have been around for over 400 million years, meaning that they have been here longer than trees, insects, and the rings of Saturn. The chondrichthyans are divided into two groups: the Elasmobranchii ("plate gills"), which includes sharks, skates, and rays; and the Holocephali ("complete heads"), which includes the chimeras, rat fishes, and ghost sharks (which are not sharks; Figure 3.21). Across their evolutionary history, the Chondrichthyes have varied considerably in morphology and lifestyle, including everything from the six-inch extant dwarf lantern shark *Etmopterus perryi* to the extinct "buzzsaw shark" *Helicoprion* (which is actually a holocephalan that was potentially 20 ft. long) to the massive extinct *Otodus megalodon* that was at least 18 meters long (Figures 3.21 and 3.22). The biggest extant fish, the

whale shark, can be up to 40 feet long and feeds on krill and other small organisms (Figure 3.21).

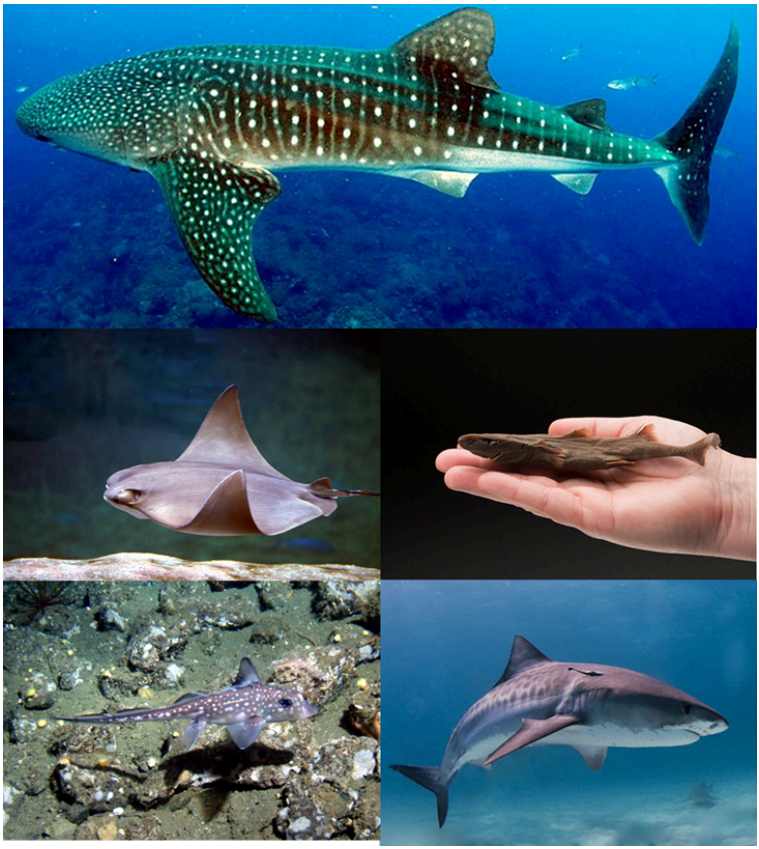

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Figure 3.21—Various extant chondrichthyans. Top: Rhincodon typus, the whale shark. Middle: (left) Rhinoptera bonasus, the cownose ray; (right) Etmopterus perryi, the dwarf lantern shark. Bottom: (left) Hydrolagus colliei, the spotted ratfish; (right) Galeocerdo cuvier, the tiger shark.

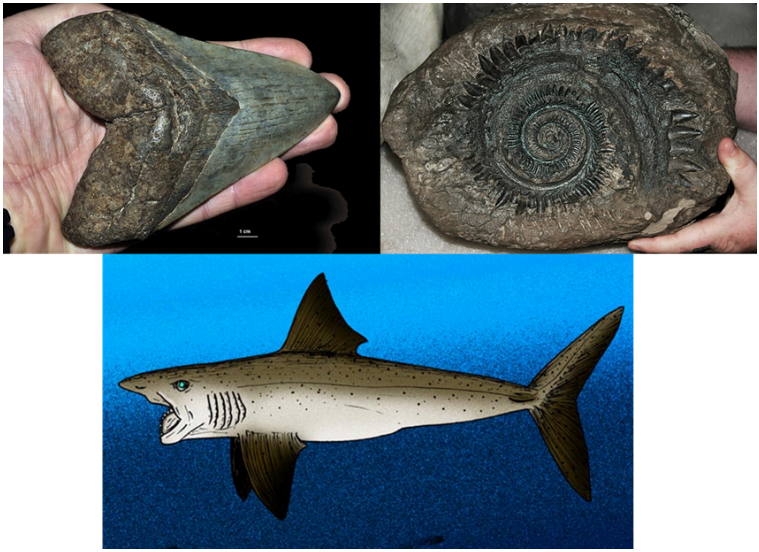

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Figure 3.22—Top: (left) a tooth from Otodus megalodon; (right) a tooth whorl from Permian-aged Helicoprion. Bottom: a reconstruction of Helicoprion.

As the name implies, the chondrichthyans have endoskeletons made of cartilage, not bone. This specific cartilage is unique to the chondrichthyans in that it is **tessellated prismatic calcified cartilage** (Figure 3.23). *Calcified* refers to the addition of calcium phosphate hydroxyapatite to the cartilage. **Tesserae** are small blocks of calcified cartilage that overlie an unmineralized cartilage core (thus we say the cartilage is "tessellated"), whereas **prismatic** refers to the pattern of calcification that allows the cartilage to refract light when sliced thinly and examined under a microscope. While this unique cartilage is more durable than the cartilage that is, for example, in your nose, it still is not as durable as bone when it comes to surviving the fossilization process. Thus, the vast majority of the evolutionary history of chondrichthyans is a story told largely in teeth and **denticles** (scales), which are composed of more durable **enameloid** and **dentine**, as well as

vertebrate and fin spines, which are more heavily mineralized than other cartilaginous elements. This means that the earliest chondrichthyan fossils may be isolated denticles from 450 million years ago. The earliest chondrichthyan teeth are about 420 million years old, and the oldest body fossil is 394 million years old.

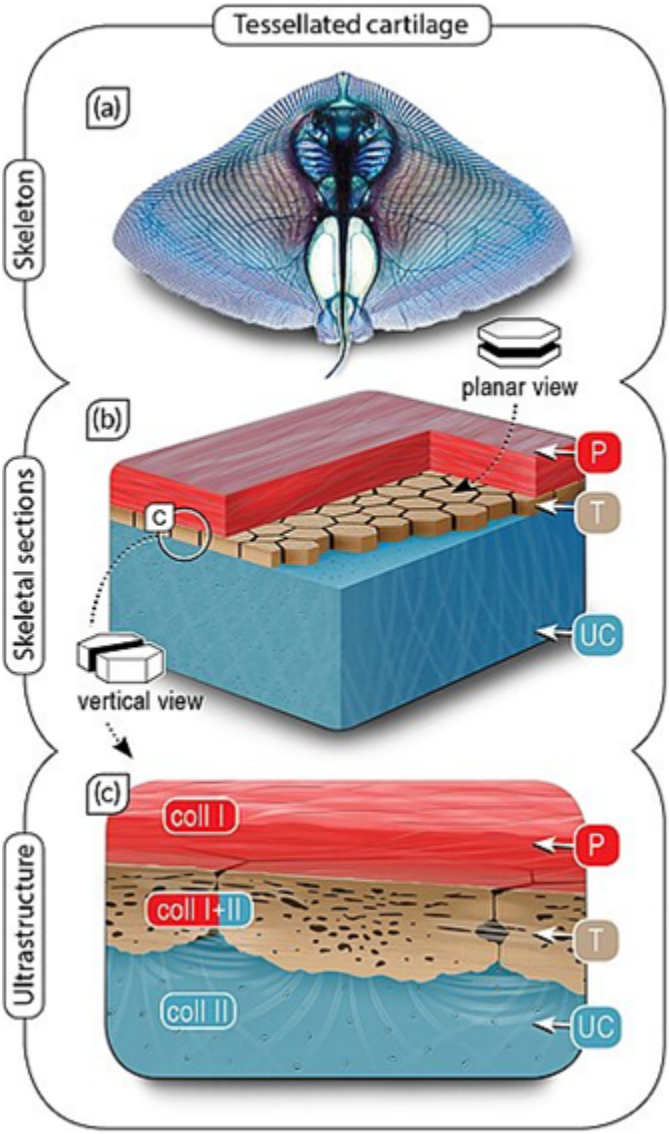

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Figure 3.23—General organization of the primary mineralized skeletal tissue in sharks and rays, tessellated cartilage. (a) Cleared and stained butterfly ray skeleton (Gymnura sp.). Specimen is young and not yet fully mineralized; blue color shows the cartilage of the skeleton, which will form a mineralized layer later in life. (b, c) Structure of tessellated cartilage of sharks and rays, comprising

mineralized tiles (tesserae) covering the skeletal cartilage. coll = collagen; P = perichondrium; T = tesserae; UC = unmineralized cartilage.

†The Acanthodians

The acanthodians ("spiny" or "prickly"), which lived from the Silurian through the Permian, have often been referred to as the "spiny sharks" due to their characteristic ventral fin spines (Figure 3.17). While most recent studies indicate that they represent multiple evolutionary lineages, studies do not agree on where they should be placed with regard to their relationships with the other eugnathostomes. They seem to share some features specific to the chondrichthyans, such as a largely cartilaginous skeleton, tesserae, and a few braincase features. However, other features are more typical of the osteichthyans, such as the bony bases of their fins, their scale structure, and having **enamel** (which has an epidermal origin) instead of enameloid (which has a dermal origin). As we saw with the chondrichthyans, a cartilaginous skeleton is not conducive to fossil preservation; as such, the fossil record of this group is sparse, which makes it harder to resolve their relationship with other eugnathostomes. When all these characteristics are taken together and subjected to phylogenetic analyses, the consensus seems to be that the acanthodians are stem chondrichthyans, even though how those acanthodians are related to each other is still unclear.



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Figure 3.24—A Devonian-aged acanthodian, Diplacanthus striatus.

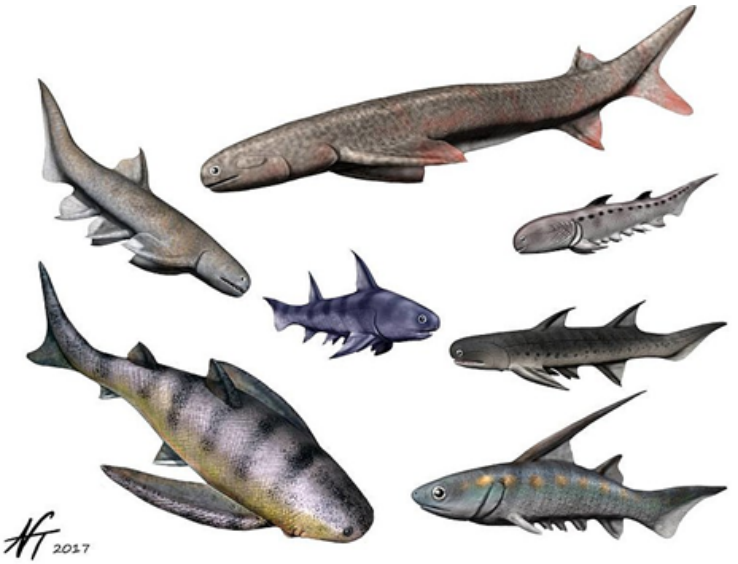

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Figure 3.25—Reconstructions of various acanthodians clockwise from top center: Acanthodes bronni, Climatius reticulatus, Ischnacanthus gracilis, Parexus recurves, Gyracanthus formosus, Cheiracanthus murchisoni, and (center) Diplacanthus crassissimus.

Osteichthyes

The Osteichthyes ("bony fish") have been around for almost as long as the chondrichthyans, first appearing in the fossil record in the Silurian period (roughly 425 million years ago). As the name implies, the osteichthyan skeleton is composed mostly of bone, and many of the features that characterize the osteichthyans are indeed bony. For example, the braincase and jaws are surrounded by a particular set of dermal bones. Likewise, the palate and a large component of the pectoral bones are also composed of dermal bones. Fin rays, called **lepidotrichia**, are composed of bone too. There is a long list of other characteristics involving the braincase, other skeletal elements, and nerves that we will not go into here. The take-home

message is that there are many characteristics that define osteichthyans, and most of them revolve around the skeleton.

The osteichthyans include two groups (Figure 3.6). The first, the Actinopterygii ("ray fins," named for their fin structure, as the fin is mostly composed of the long-fin rays called lepidotrichia), are what you probably would think of if we asked you to think of a fish (Figures 3.26 and 3.27). These include over 50% of the extant vertebrates. They include minnows, goldfish, gar, paddlefish, eels, flounders, and seahorses. Extant actinopterygians range in size from the 8-mm-long carp-like fish *Paedocrypris* to the giant oarfish *Regalecus glesne* at up to 36 feet long.



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Figure 3.26—The morphological diversity of extant actinopterygians.

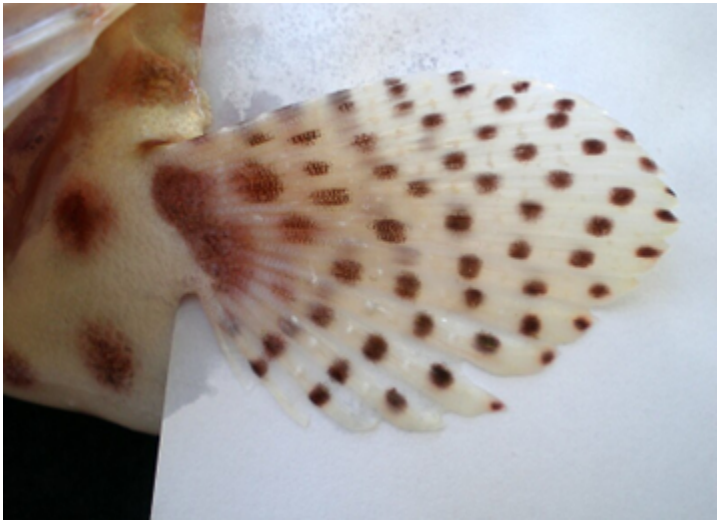

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Figure 3.27—The pectoral fin of the actinopterygian Epinephelus merra, the honeycomb grouper.

The actinopterygians are typically divided into three groups based on both genetic and morphological data. The first is the Cladista, composed of the bichirs and reedfish (Figure 3.28). The extant members of this freshwater group live in Africa. The second group is the Acipenseriformes ("sturgeon form"), which includes sturgeons and paddlefish (bottom left of Figures 3.26 and 3.29, respectively). All other actinopterygians fall under the Neopterygii ("new fin"). Unlike the other groups of actinopterygians, the neopterygians have a caudal fin that has generally symmetrical lobes as well as forty other common features that are beyond our introduction here. The neopterygians are further divided into the Holostei ("whole bone," gars and bowfin; Figures 3.26 [bottom right] and 3.30, respectively) and the Teleostei (everything else, Figure 3.26 except bottom row). The holosteians, while looking very different from one another, are united by having two vertebral centra fused to the occipital condyle of the skull and 15 other

features. The identity of those 15 features is not important for our purposes, but it demonstrates that these fishes have more in common than external appearances imply.



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Figure 3.28—Polypterus senegalus, the Senegal bichir, is a member of Cladista.



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Figure 3.29—Polydon spatula, the American Paddlefish, is a member of the Acipenseriformes. Another acipenseriform, a sturgeon, can be seen in the bottom left corner of Figure 3.26.



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Figure 3.30—Amia calva, the bowfin, is a member of the Holostei. Another holosteian, a gar, can be seen in the bottom right corner of Figure 3.26.

The **Teleostei** ("complete bone") encompass the majority

of the extant actinopterygian species, approximately 35,000, and are united as a group by an assortment of skeletal and soft-tissue features. A number of innovations have led to their massive evolutionary success. They are the only group of fishes that has duplicated their entire genome, which, if you recall from the section on *Hox* genes, can release constraints and allow for evolutionary experimentation. In particular, the evolution of oral jaw protrusion (Figure 3.31) and **pharyngeal jaws** (an independent set of jaws derived from the gill arches; Figure 3.32) opened up new feeding niches that were previously unavailable. Changes to the fin shapes and locations along the body further allowed teleosts to exploit a variety of new habitats.



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Figure 3.31—A largemouth bass, Micropterus salmoides, showing jaw protrusion as the fisher opens the bass's mouth.

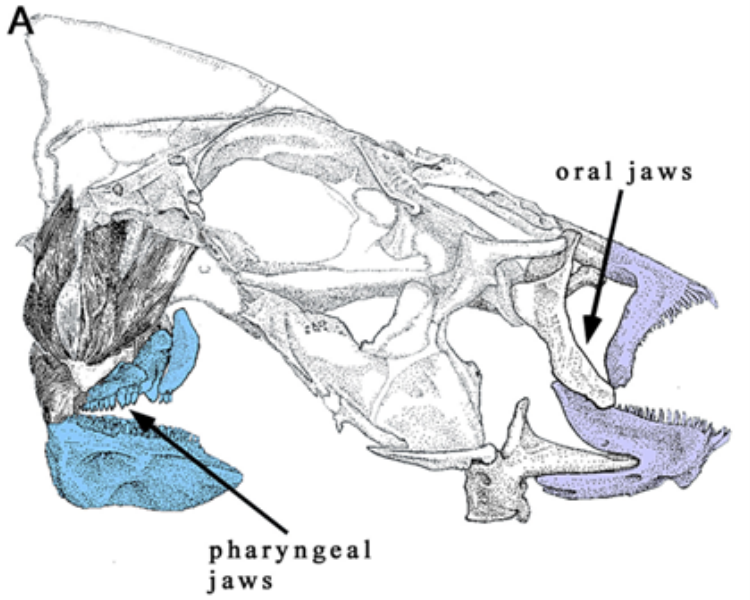

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Figure 3.32—Schematic drawing (lateral view) of a generalized cichlid (teleost) cranial skeleton, showing the relative location of the oral jaws (purple) and the pharyngeal jaws of the most posterior arch (blue).

The second osteichthyan group is the Sarcopterygii ("flesh fins"). Unlike the actinopterygians, only a small portion of their pectoral and pelvic fins are composed of lepidotrichia. The base of the sarcopterygian pelvic and pectoral fins are composed of only one bone (the **metapterygium**), whereas in actinopterygians there are four (collectively called **basals**). The sarcopterygian fins are also fleshy with muscle and connective tissue, unlike the actinopterygians. Together, these fin features give the sarcopterygians their common name of "lobe-finned fishes."

The living sarcopterygians include three groups (Figure 3.34). The first is the Actinistia, which comprises the coelacanths (Figures 3.33 and 3.35). The extant coelacanths were only brought

to light in 1938 by Marjorie Courtenay-Latimer, a South African museum naturalist who received a call from a fisherman who brought in an unusual fish he thought she would be interested in. This was particularly noteworthy because coelacanths had been known from the fossil record for much longer and were thought to have been extinct for 80 million years. The genus of extant coelacanths is named *Latimeria* in her honor. The two extant species, which can be up to 6.5 feet long, live in the deep sea of the West Indian Ocean and Indonesia and can live for almost a century. However, the actinistians were far more species-rich in the Paleozoic era and showed far more morphological diversity than we've seen since then. The extant coelacanths superficially resemble some of the extinct forms but have their own particular bone pattern in their pelvic and pectoral fins.

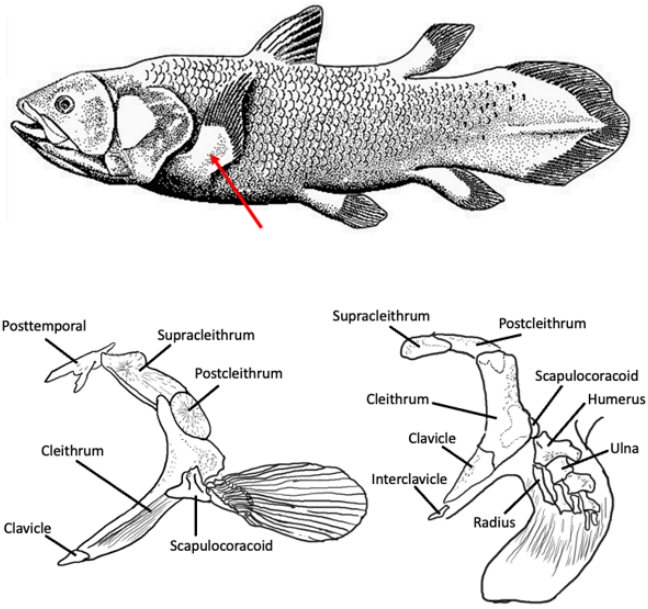

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Figure 3.33—Top: A sarcopterygian fish, an extant coelacanth. Note the fleshy lobed pectoral fins (red arrow), and compare that to the actinopterygian ray-fin in Figure 3.27. Bottom left: The skeletal elements of an actinopterygian pectoral fin (from the bowfin Amia). Bottom right: The skeletal elements of a sarcopterygian pectoral fin.

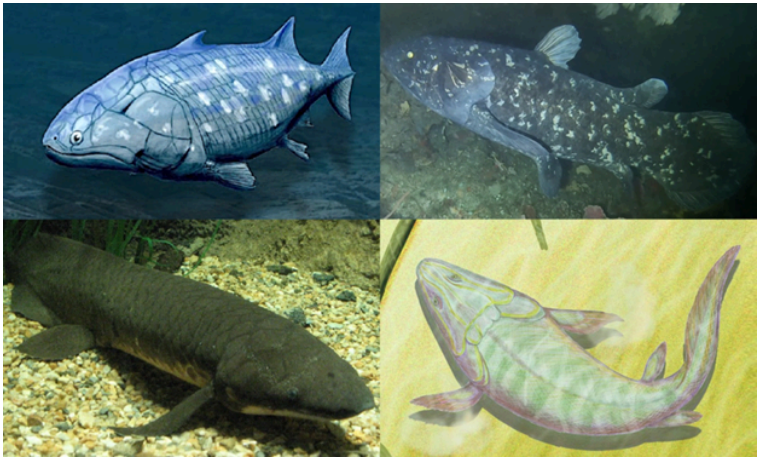

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Figure 3.34—From top to bottom and left to right, examples of sarcopterygians: †Guiyu oneiros, Latimeria chalumnae, Neoceratodus forsteri, and †Panderichthys rhombolepis.



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Figure 3.35—Marjorie Courtenay-Latimer and coelacanth after its discovery.

The second group is the Dipnoi ("two lungs"), or lungfishes; they are named for their paired lungs, which are modified swim bladders that can indeed absorb oxygen (Figure 3.36). They also share a unique arrangement of tooth plates, which are only located on the palate, and firmly joined halves of the mandible. Like the coelacanths, lungfishes were far more diverse in the past than they are today. Only three genera are still extant—one each in Africa, South America, and Australia—and it's important to remember that they have changed quite a bit since their lineage split away from the other sarcopterygians.



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Figure 3.36—A marbled lungfish, Protopterus aethiopicus, which lives in Africa.

The final group of sarcopterygians is the **Tetrapodomorpha**. The Tetrapodomorpha includes a line of fishes that existed from the Devonian through the Early Permian but also includes the evolutionary lineage of the Tetrapoda, the terrestrial vertebrates (amphibians, reptiles, birds, and mammals). We will talk about the fishes here and then treat the Tetrapoda separately in the next section. The "fish contingent" of the Tetrapodomorpha contains four evolutionary lineages that cover the transition from fully aquatic fishes to the "fishapod" transitionary forms such as *Eustenopteron* and *Tiktaalik* (Figures 3.37 and 3.38). You will learn about these fishes in greater detail in subsequent chapters, as they've played an important role in understanding one of the biggest evolutionary shifts in vertebrate history: from aquatic to fully terrestrial environments. For now, we know that the tetrapodomorphs, including the fully terrestrial forms like us, are united as a group because of the presence of internal nostrils called **choanae**. However, it seems that some lungfishes have choanae as well, supporting the idea that lungfishes are more closely related to tetrapodomorphs than the coelacanths but with the problem of not having a uniting feature for all the tetrapodomorphs.



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Figure 3.37—A cast of the "fishapod" Tiktaalik roseae at the Field Museum of Natural History.

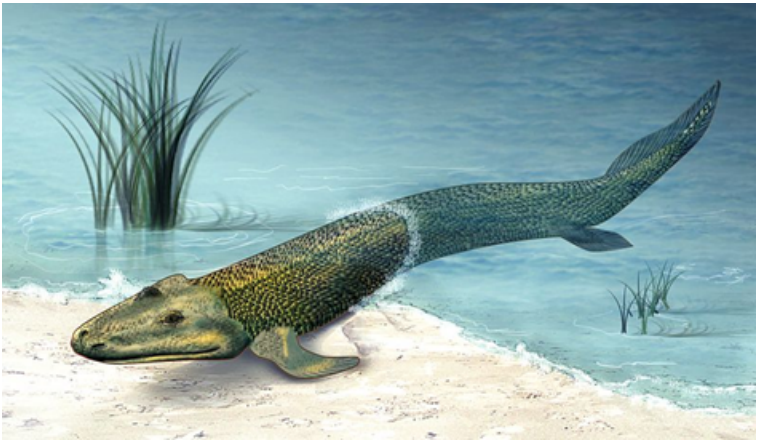

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Figure 3.38—A reconstruction of Tiktaalik roseae.

3.3 Tetrapoda

The rest of the vertebrates fall under the Tetrapoda. This includes amphibians, reptiles, birds, and mammals (Figure 3.39). *Tetrapoda* translates to "four feet," pointing to their defining characteristic: The front and hind limbs have **digits** (i.e., fingers and toes). The tetrapodomorph "fishapods" don't have digits on the end of their fins. There are, of course, exceptions among our living tetrapods, including the limbless snakes and marine mammals such as dolphins and whales. But as we've said before, we have to remember that these extant tetrapods have had a lot of time for evolutionary changes. Other defining characteristics include specialized articulations between vertebrae to support the backbone against gravity and modifications to the **ulna** (a bone in the forearm) to support muscle attachment.

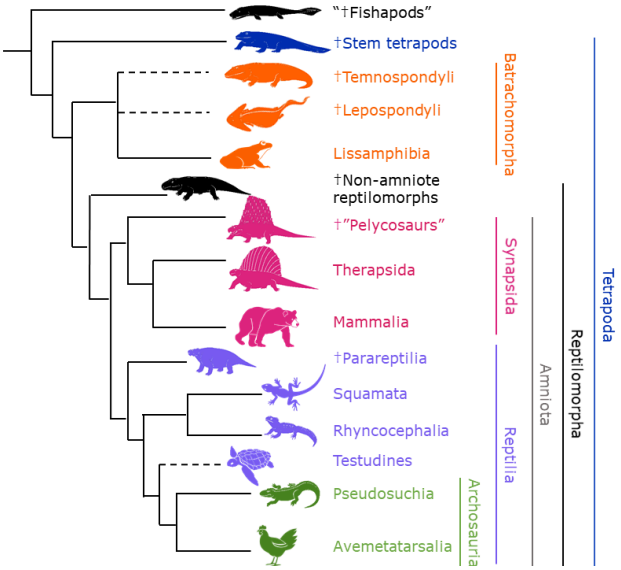

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Figure 3.39—A phylogenetic tree of the Tetrapodomorpha. Dotted lines indicate that the placement of the branch is uncertain. The dagger (†) indicates that all members of that taxonomic group are extinct.

The earliest tetrapods (stem tetrapods), such as *Ichthyostega*, made their appearance in the late Devonian period and looked superficially very similar to the "fishapods" like *Tiktaalik* but with feet (which are really fins with digits) instead of fins without digits. They retained several fish characteristics, such as their gills and **lateral line canals** (a sensory system that only works in water; see Chapter 20). As time progressed, the stem tetrapods lost more and more of their fishy features as adaptations allowed them to become more terrestrial.

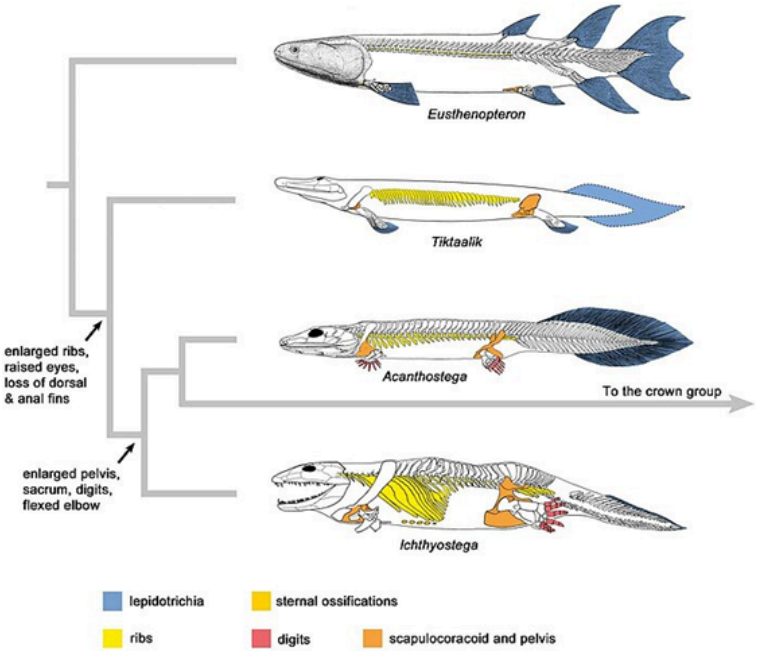

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Figure 3.40—A simplified phylogenetic tree showing the fish-tetrapod transition, illustrating some of the major anatomical changes.

Batrachomorpha

The Batrachomorpha ("frog forms") encompasses two extinct groups of tetrapods, called the temnospondyls and lepospondyls, and the group that includes the extant amphibians and their extinct relatives, the Lissamphibia. All groups have four or fewer digits on their hands. The †Temnospondyli ("cut vertebra") have been found in fossiliferous deposits from the Mississippian period through the Early Cretaceous around the world (Figures 3.41 and 3.42). They were quite successful and diverse in size and shape, and they comprised over 160 genera across both land and water. Most temnospondyls were carnivores. The †Lepospondyli ("husk vertebra") lived from the Mississippian to the late Permian (Figure 3.43). These were not quite as diverse as the temnospondyls, tended to be relatively small and elongated, and included some burrowers with reduced limbs. It is not clear whether the Lissamphibia lineage came from one of the temnospondyl evolutionary lines, if it came from one of the lepospondyl lines, or if the individual lissamphibian lineages arose from multiple temnospondyl and lepospondyl lines. There are some questions about the timing of lissamphibian fossils versus likely temnospondyl ancestors, as the earliest lissamphibian fossils are also from the Mississippian period.

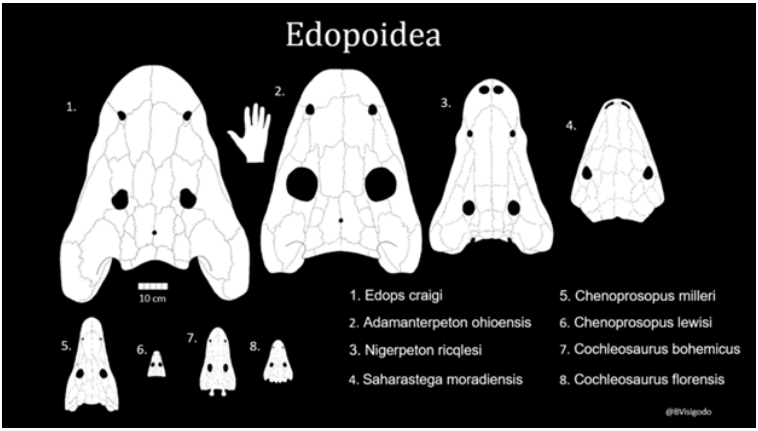

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Figure 3.41—A variety of early temnospondyl skulls from the clade Edopoidea, showing some of the diversity in size and shape within the †Temnospondyli.





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Figure 3.42—The temnospondyl Eryops. The top image is the full skeleton, and the bottom image is a close-up of the snout and teeth.



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Figure 3.43—A skeleton cast and reconstruction of the lepospondyl Diplocaulus.

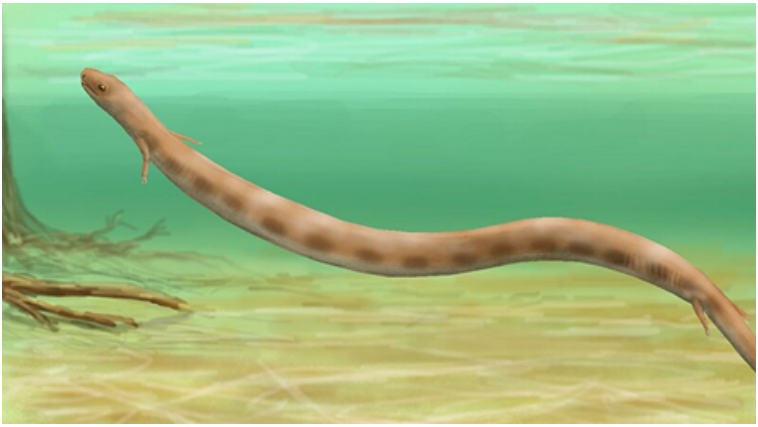

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Figure 3.44—A reconstruction of the lepospondyl Brachydectes newberryi.

The Lissamphibia include the extant amphibians and their extinct relatives. The extant Lissamphibia, representing approximately 20% of extant tetrapods, is composed of three diverse groups: the Caudata (salamanders), Gymnophiona (legless amphibians also known as caecilians), and Urodela (frogs), and these extant groups all originate in the Jurassic period (Figure 3.45). Both molecular and morphological data support the idea that the urodeles and caudates are more closely related to each other than to the gymnophionans. Even though extant lissamphibians have very different morphologies, they are united by the shape of their teeth (two-cusped and with a base composed of dentine between the jaw bone and tooth crown), the presence of a particular eye muscle called the **levator bulbi** that serves to elevate the eye, having particular skin glands (see Chapter 6), and many skeletal features. As the name "amphibian" indicates, these animals are tied to both water and land ("amphi" = both).



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Figure 3.45—Extant lissamphibians. Top left: Australian green tree frog Litoria caerulea. Bottom left: Mexican burrowing caecilian Dermophis mexicanus. Right: Eastern newt Notophthalmus viridescens in the red eft phase.

Reptilomorpha

The Reptilomorpha ("reptile shape") includes the remaining vertebrates, the amniotes, as well as a few reptile-like tetrapods that aren't amniotes. We will treat the amniotes in their own section. The nonamniote reptilomorphs (Figure 3.46), which lived from the Mississippian through Permian periods, share a handful of features such as narrow **premaxillae** (part of the upper jaw) and a particular pattern of joint numbers as you move across the toes (we are 2-3-3-3-3, but the nonamniote reptilomorphs are 2-3-4-5-4). As one might expect, many of the nonamniote reptilomorphs look like a cross between an amphibian and a reptile and include aquatic, semiaquatic, and terrestrial forms.

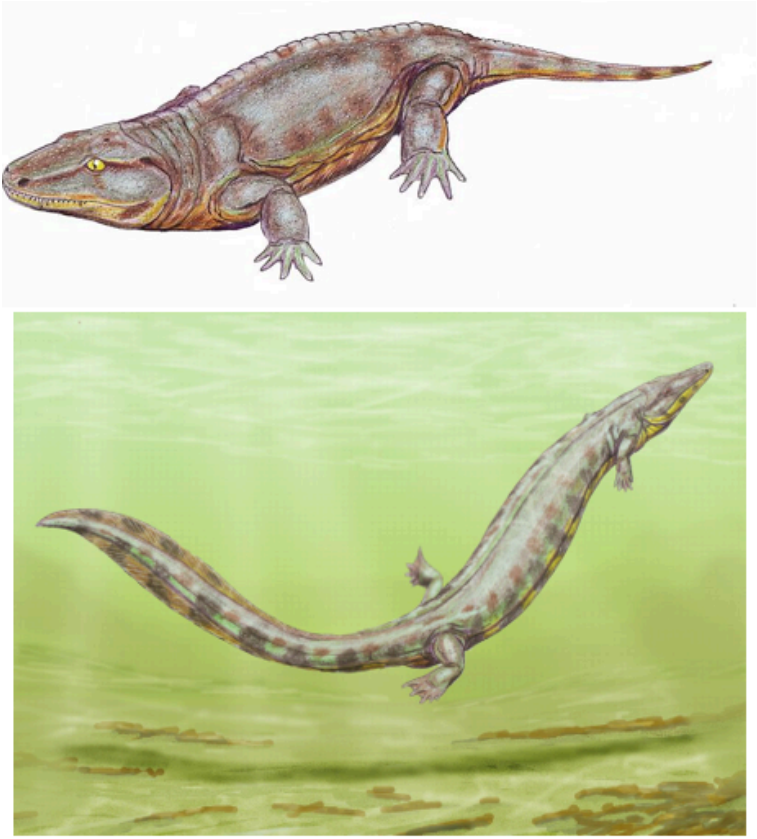

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Figure 3.46—Reconstructions of nonamniote reptilomorphs. Top: Seymouria baylorensis. Bottom: Archeria crassidiscus.

Amniota

The Amniota are named after their most characteristic shared feature: the **amniotic egg**. This special egg has four **extraembryonic layers** ("outside of the embryo"), the amnion, chorion, yolk sac, and allantois. You will learn a great deal more about this egg and why

it helped the amniotes conquer terrestrial environments in Chapter 17. The amniotes are divided into two groups, the Synapsida and the Reptilia (sometimes referred to as the Sauropsida). These two groups diverged from each other during the Carboniferous period. Historically, the amniotes were separated into various groups based on the location of various openings called **temporal fenestrae** ("windows") in the skull that are used for the attachment of jaw-closing muscles: Synapsida possessed a single temporal fenestra on each side of the skull (the lateral temporal fenestra), Anapsida possessed none, and Diapsida possessed two (upper and lower temporal fenestra). However, the use of molecular data and data from other skeletal features has reminded us that organisms change over evolutionary time, and many amniotes secondarily lost or regained various fenestra. While the Synapsida and Diapsida remain valid groupings, the anapsids have been distributed across various other groups.

Synapsida

The Synapsida include a large number of extinct reptiles, including the sail-backed Dimetrodon (which is not a dinosaur!) and several other "mammal-like reptiles," as well as the Mammalia. These groups are generally united by possessing a single temporal fenestra on each side of the skull, although there are several other skull features as well. The Synapsida arose about 318 million years ago, during the Pennsylvanian period. The synapsids can be split into two groups: the Therapsida, which gave rise to the mammals, and a group of basal synapsids that are generally called the "†pelycosaurs" but are not a particular evolutionary grouping. The pelycosaurs (Figure 3.48) were the main terrestrial vertebrates from the Pennsylvanian through the Early Permian period and were extinct before the end of the Triassic period. The pelycosaurs were more than just Dimetrodon—they included herbivores, carnivores, and piscivores (fish eaters), and many did not have sails. Their body

plans were fairly similar to some of the nonamniote tetrapods, with sprawling limbs, long tails, and a **homodont** dentition (all teeth were roughly the same shape).

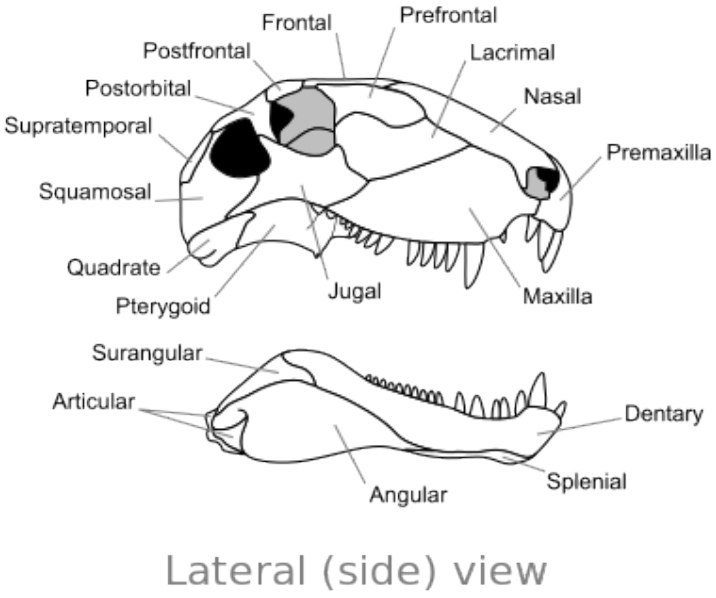

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Figure 3.47—The bones in the skull and jaws of the synapsid Dimetrodon, shown in lateral view. You will learn a lot more about these bones in Chapter 8.

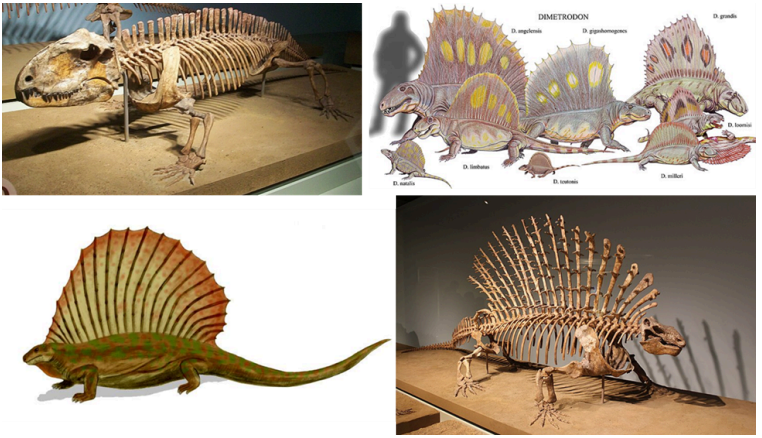

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Figure 3.48—Various pelycosaurs. Top left: Spenacodon ferox. Top right: various Dimetrodon species. Bottom row: reconstruction and skeleton of Edaphosaurus pogonias.

The therapsids (Figure 3.49) were the dominant terrestrial vertebrates from the Middle Permian until the mass extinction event at the end of the Permian period. There are approximately six different groups of therapsids, and they span a wide range of morphologies and sizes. Unlike the pelycosaurs, most therapsids were herbivores, although there were several carnivorous species, such as the gorgonopsians, which had very large canines and could open their mouths more than 90 degrees (Figure 3.50). The therapsids also show evidence of more active lifestyles, indicating a potential shift toward **endothermy** (body temperature is internally generated and regulated). Cynodontia is the particular group of therapsids that led to the mammals (and technically includes the Mammalia). Cynodonts first appeared in the fossil record toward the end of the Permian period and persisted until the Triassic period. We see a few mammalian features in the cynodonts, such as particular muscular markers on the skull and a **heterodont** (Greek for "different teeth") dentition. However, it's worth noting that

various groups of therapsids that are *not* cynodonts independently evolved characteristics that we consider "mammal-like" as well, including a complete **secondary palate**, precise occlusion of teeth in the upper and lower jaws, and particular bone patterns in the hands and feet.



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Figure 3.49—Reconstructions of five therapsids that illustrate the diversity of body forms. Clockwise from top left: Inostrancevia, Alopecognathus, Ostehria, Moschops, and Castorocoda.



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Figure 3.50—Skull of the gorgonopsid Gorgonops.



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Figure 3.51—The cynodont Procynosuchus. Note that the limbs are under the body instead of a more reptilian sprawling posture.

The Mammalia first appeared in the fossil record during

the Late Triassic period. These tended to be small, shrew-sized insectivores at first but started diversifying in the Late Jurassic period. Mammals, including you, are united as a group by the presence of milk-producing glands called **mammary glands** and hair. Unfortunately, these are difficult to preserve in the fossil record, so we often look for shared skeletal features as well. Specifically, these are the presence of three bones (**incus**, **malleus**, and **stapes**) in the middle ear, a lower jaw composed of only one bone (dentary), and the formation of the jaw joint between the dentary and a bone in the skull called the **squamosal** (see Figure 3.47). We will follow these skeletal transitions in more detail in several other chapters.

Extant mammals have diversified into over 6,600 species, from the bumblebee bat (1 inch long) to the largest animal on Earth, the blue whale (110 ft. long). All extant mammals are **endothermic**, meaning they produce their own body heat, and they are found in just about every available habitat. We divide extant mammals into three groups: the Monotremata (egg-laying mammals), Metatheria (marsupials), and eutherians (all other mammals, including you); the latter two are united together as the Theria. Each group is generally defined by a particular reproductive strategy, which you learn more about in Chapter 17.

Extant monotremes (Figure 3.52), the egg-laying mammals, are currently restricted to Australia, Tasmania, and New Guinea. They include only the platypus (*Ornithorhynchus anatinus*) and echidnas (four species); however, they were more diverse during the Late Cretaceous and included the largest mammals at that time (weighing up to 20 kg). One fossil has been found in Patagonia, indicating that they may have lived in Antarctica as well (there was no other way for them to get to South America, as these continents were united as the supercontinent Gondwana at that time). The Monotremata diverged from the lineage leading to the Theria (Metatheria + Eutheria) sometime between the Triassic and Early Jurassic periods, but studies using various molecular techniques do

not agree on the specific timing. The earliest monotreme fossils are from the Early Cretaceous.



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Figure 3.52—Extant monotremes. Clockwise from top left: platypus, short-beaked echidna, eastern long-beaked echidna, western long-beaked echidna.

The extant 399 species of metatherians are represented by the marsupials (Figure 3.53), most of which are restricted to Australia, the islands near Australia (collectively called Wallacea after Alfred Russel Wallace), and South America. These include kangaroos, koalas, opossums, bandicoots, quokkas, and Tasmanian devils. North America only boasts one extant species of marsupial, the Virginia opossum *Didelphis virginiana*, a recent immigrant that only arrived 3 million years ago when the isthmus of Panama formed. The restriction of marsupials to the Southern Hemisphere reflects the geological history of the Gondwana and its isolation from Laurasia (another supercontinent, composed of North America and Eurasia). During this period of isolation, marsupials formed communities as diverse as we see in today's eutherian-dominated

mammalian communities. These included large predators, insectivores, browsers, and more.



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Figure 3.53—Extant metatherians. Clockwise from left: kangaroo, Virginia opossum, long-nosed bandicoot, monito del monte, Tasmanian devil.

The eutherians (often called "placental mammals," which misleadingly suggests that the marsupials don't have a placenta) diverged from the marsupials in the middle of the Jurassic period. The extant eutherians include most of the living mammals, roughly 6,200 species, including us (Table 3.1; Figure 3.54). The earliest fossils come from Mongolia and Montana and are about 105–110 million years old. They tended to be rather small, but once the

nonavian dinosaurs went extinct at the end of the Cretaceous period, most of the extant orders had made an appearance within 15 million years. These were by no means the only orders present at that time; another 16 or so orders also have come and gone. Most of our extant orders of mammals originated in the Northern Hemisphere, with the exception of the Xenarthra (anteaters and their kin in South America).

Table 3.1—A list of the various extant eutherian orders, plus an estimate of the number of extant species
Data from the American Society of Mammalogists Mammal Diversity Database

Order name	# of extant species	What is in that order
Afrosoricida	55	Golden moles, otter shrews, tenrecs
Artiodactyla	360	Even-toed hoofed mammals: deer, bovines, antelope, dolphins, whales, hippos
Carnivora	310	Cats, canines, raccoons, otters, weasels, hyenas, bears, pinnipeds, mongooses
Chiroptera	1,466	Bats, flying foxes
Cingulata	22	Armadillos
Dermoptera	2	Colugos
Eulipophyla	584	Hedgehogs, shrews, moles
Hyracoidea	6	Hyraxes
Lagomorpha	111	Rabbits, hares, pikas
Macroscelidea	20	Elephant shrews
Perissodactyla	18	Horses, zebras, rhinos, tapirs
Pilosa	17	Anteaters, sloths
Pholidota	8	Pangolins
Primates	517	Apes, monkeys, lemurs, humans
Proboscidea	3	Elephants
Rodentia	2,680	Mice, rats, squirrels, beavers, capybara, chinchillas, hamsters

Order name	# of extant species	What is in that order
Scandentia	23	Treeshrews
Sirenia	4	Manatees, dugongs
Tubulindentata	1	Aardvark



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Figure 3.54—A variety of extant eutherian mammals. Left column, from top to bottom: common vampire bat Desmodus rotundus, eastern gray squirrel Sciurus carolinensis, plains zebra Equus quagga, aardvark Orycteropus afer, humpback whale Megaptera novaeangliae, and black and rufous elephant shrew Rhynchocyon petersi. Middle column: humans Homo sapiens, ground pangolin Manis temminckii, Sunda flying lemur Galeopterus variegatus, West Indian manatee Trichechus manatus, European hedgehog Erinaceus europaeus, nine-banded armadillo Dasypus novemcinctus. Right column: southern elephant seal Mirounga leonina, Asian elephant Elephas maximus,

reindeer Rangifer tarandus, giant anteater Myrmecophaga, giant panda Ailuropoda melanoleuca, and American pika Ochotona princeps.

Reptilia

The Reptilia (also known as the Sauropsida) contain what we typically think of as reptiles (snakes, lizards, crocodylians), as well as dinosaurs, pteranodons, marine reptiles like ichthyosaurs, and birds. Despite diverging from the synapsids approximately 315–330 million years ago, the Reptilia did not become the dominant terrestrial fauna until the Mesozoic era. The Reptilia are often split into two main groups, which do not include a small number of stem Reptilia: the †Parareptilia, which were the dominant group of reptiles in the Permian and the Early Triassic periods, and the Eureptiles, which contain our extant reptiles and were the dominant reptiles of the Mesozoic. Both of these main groups are considered part of the Diapsida. The parareptiles included a wide variety of reptiles that occupied several ecological niches across the globe, including small carnivores, aquatic predators, and large herbivores (Figure 3.55).



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Figure 3.55—Parareptilia. Top: Bradysaurus baini. Bottom left: Mesosaurus. Bottom right: Sclerosaurus armatus.

In addition to some stem eureptiles, the Eureptilia include all the extant reptiles (including birds) and several notable extinct reptiles (nonavian dinosaurs, pteranodons, and several marine reptile groups). Many of the familiar extant reptiles fall into the Lepidosauria, which has over 11,000 extant species; these are divided into the Squamata (snakes, lizards, and a different group of legless reptiles called amphisbaenians) and Rhynchocephalia (one extant species, the tuatara, which lives in New Zealand). These two groups likely diverged sometime in the Early Triassic. The lepidosaurs share particular lower limb structures, including a unique structure of the knee joint, the ability to self-amputate their tails (**caudal autonomy**) as a self-defense mechanism, and some soft-tissue features that you will learn about in the chapters on urogenital structures (Chapters 16 and 17).

Despite only having one living species, the Rhynchocephalia ("beak heads") were globally abundant during the Late Triassic and Jurassic periods (Figure 3.56). Most of these were small, terrestrial, and lizard-like, as is the extant tuatara *Sphenodon*. However, there were also aquatic versions, called pleurosaurs, that were elongated and had reduced limbs. At their most abundant, the Rhynchocephalia included herbivores, omnivores, carnivores, and insectivores.



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Figure 3.56—Rhynchocephalia. Top: Pleurosaurus. Bottom: Henry, the world's oldest Sphenodon.

Even though the extant Squamata (snakes, lizards, and their relatives; Figure 3.57) are largely restricted to terrestrial habitats with a few notable exceptions, their evolutionary history includes a

global distribution (including the Arctic and Antarctic) and almost every terrestrial and aquatic environment. The finer details of the evolutionary tree and taxonomy of squamates are highly contested, as molecular, morphological, and developmental data are at odds with each other. However, the taxonomic groups generally include the Serpentes (snakes), Iguania (iguanas, *Anolis* lizards, chameleons, agamas, and more), Anguimorpha (varanids like monitor lizards and Komodo dragons, Gila monsters, glass lizards, and a few legless lizards), Scincoidea (heavily scaled lizards including the skinks), Lacertoidea (spectacled lizards, legless amphisbaenids, and some other lizards), and Gekkota (geckos). These are generally united by a **pleurodont** dentition (teeth are on the lingual, or tongue side, of the jaw), how **kinetic** (movable) their skulls are, and their foot structure. The extinct mosasaurs (Figure 3.58), large Mesozoic marine reptiles such as the *Mosasaurus* seen in the movie *Jurassic World*, are technically squamates, as they belong to the Anguimorpha.



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Figure 3.57—A sampling of extant squamates. Top row, from left to right: Mexican blind lizard Anelytropsis papillosus, common flat-tail gecko Uroplatus fimbriatus, common chameleon Chamaeleo chamaeleon. Middle row: Sand lizard Lacerta agilis, rock monitor Varanus albigularis, grass snake Natrix natrix. Bottom row: Proctoporus chasqui (which doesn't have a common name), red worm lizard Amphisbaena alba, common blue-tongued skink Tiliqua scincoides.

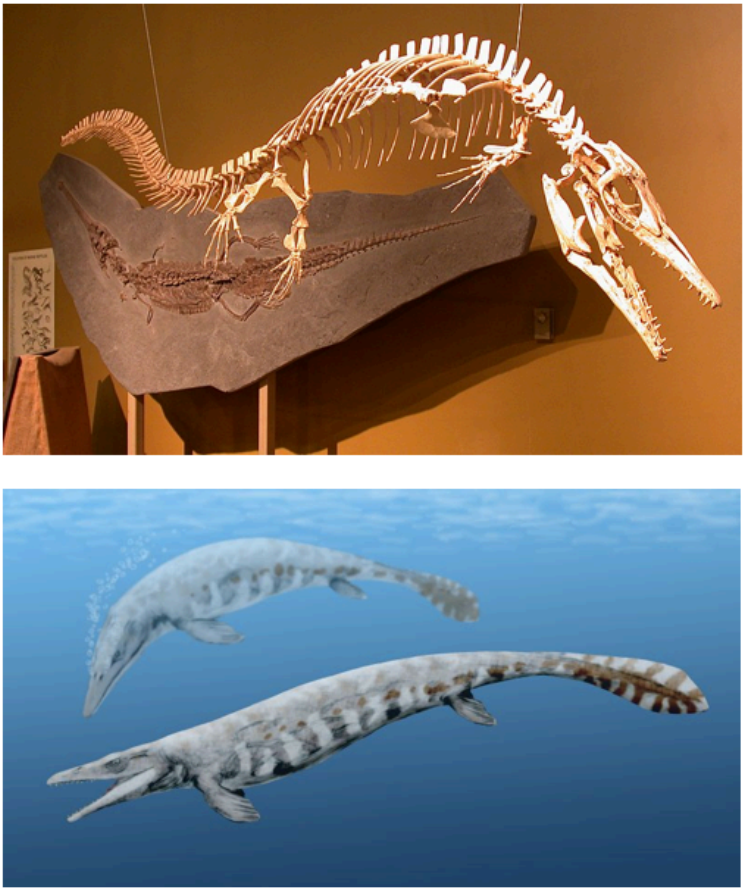

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Figure 3.58—The skeleton and a reconstruction of the mosasaur Halisaurus.

We now leave the lepidosaurs to learn about a few more eureptile groups. The turtles and tortoises (Figure 3.59), or Testudines, are somewhat of an enigma in that their anatomy is so specialized that it makes understanding their evolutionary history difficult. Their most prominent feature is their shell, which is composed of a dorsal **carapace** and ventral **plastron**. Contrary to

what cartoons may have led you to believe, the carapace and plastron are part of the skeleton, and turtles cannot exit their shells. The ribs and vertebrae are fused with several other bones (about 50 individual bones in total), and the pelvis and shoulders are highly modified to work with the shell. Extant turtles have no temporal fenestrae, nor do they have teeth. In other words, all the skeletal features we would normally use to figure out the relationship between turtles and other reptiles have been highly modified. As such, turtles have moved around the evolutionary tree of amniotes since people started to study them. The fossil record is not terribly helpful either; all of a sudden, there are recognizable turtles and no obvious transitional turtles. Molecular studies, paired with developmental data and more fossil work, have all helped us understand that turtles are indeed diapsids and have secondarily lost their temporal fenestrae. However, we're not sure where in the Diapsida they fit, though the current accepted placement is as sister to the Archosauria (crocodylians and birds).



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Figure 3.59—Extant Testudines. Clockwise from upper left: Red-bellied short-necked turtle (Emydura subglobosa), Indian flapshell turtle (Lissemys punctata), Hawksbill sea turtle (Eretmochelys imbricata), and Galápagos tortoise (Chelonoidis nigra). Note the diversity of body forms.

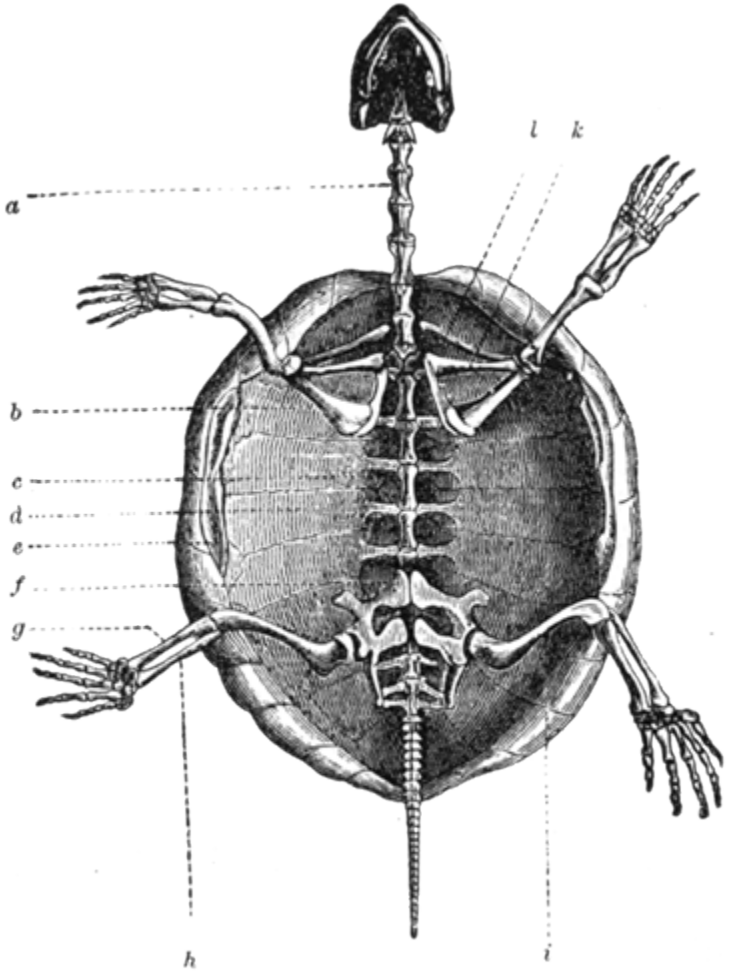

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Figure 3.60—Skeleton of a tortoise with the plastron removed. (a), cervical vertebrae; (b), carapace; (c), vertebrae; (d), ribs; (e), marginal bones of the carapace; (f), pelvis; (g), tibia; (h), fibula; (i), femur; (k), scapula, and (l) acromion. Note how the ribs and vertebrae are fused to the carapace. See Chapter 9 for more information.

The last group of reptiles to consider is the Archosauria (Figure 3.61). Not only do they have the two temporal fenestrae, but they also have an additional **anteorbital fenestra** ("before eye window") located rostrally (toward the nose) from the orbit (eye socket) and a **mandibular fenestra** on the lower jaw (Figure 3.62). In addition, they have a **thecodont** dentition, with the teeth in sockets. The archosaurs include the crocodylians, pterosaurs, nonavian dinosaurs, and birds—in other words, a wildly diverse group of vertebrates! Archosaurs first appeared in the fossil record in the Permian period and greatly diversified in the Late Triassic period, remaining the dominant vertebrate for the Jurassic and Cretaceous periods.



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Figure 3.61—A variety of extant and extinct archosaurs. Left column, top to bottom: mallard ducks Anas platyrhynchos, the pterosaur Dimorphodon macronyx, the rauisuchian Saurosuchus galilei, and the theropod dinosaur Deinonychus. Right column: Tyrannosaurus rex, Triceratops horridus, and the Nile crocodile Crocodylus niloticus (with fellow archosaurs in the background!).

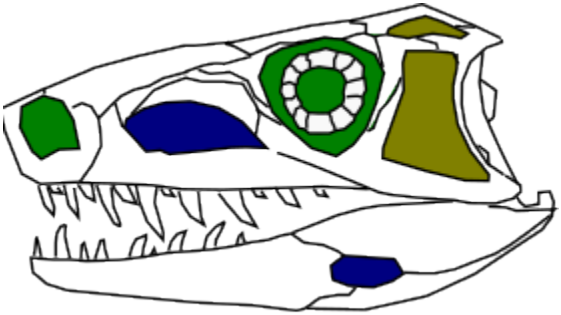

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Figure 3.62—An archosaur showing the various openings of the skull. Temporal fenestrae are in brown (right of image), the orbit is green (with sclerotic ring inside of it), the nostril is green (left of image), and the anteorbital fenestra (between orbit and nostril) and mandibular fenestra (on lower jaw) are blue.

Archosaurs are divided into stem-archosaurs plus two groups: the Pseudosuchia (sometimes called the Crurotarsi) and Avemetatarsalia (Figure 3.63). The Pseudosuchia ("false crocodile") include the Crocodylomorpha and a host of extinct relatives, including aetosaurs, ornithosuchids, and rauisuchians (Figure 3.64). The extant crocodylomorphs have 27 species, including crocodylians, alligators, and gharials. Extant crocodylomorphs are generally large, semiaquatic carnivores. While the crocs might be our go-to image for the Pseudosuchia, the extinct pseudosuchians were wildly diverse, including both bipedal and quadrupedal herbivores, giant carnivores, heavily armored forms, and even some toothless forms.

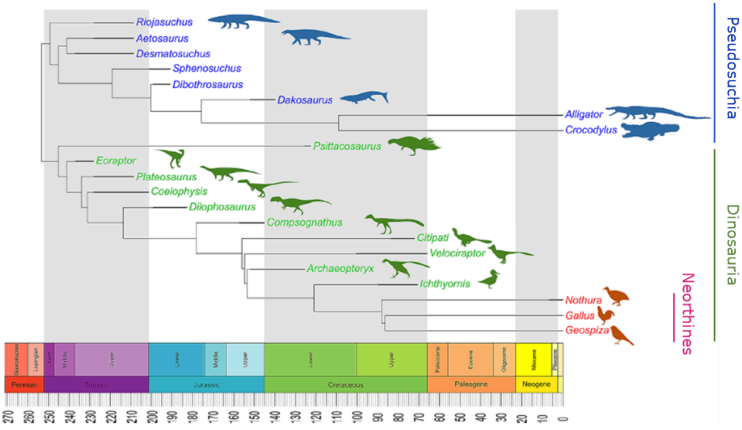
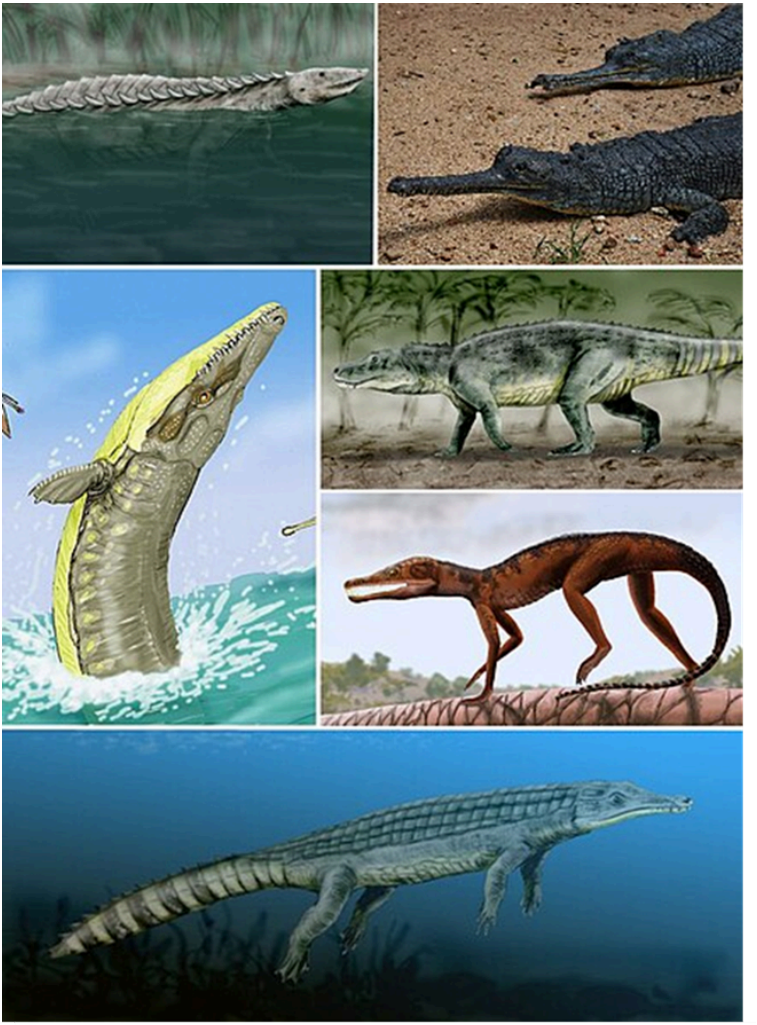


Figure 3.63—Phylogenetic tree of the Archosauria, modified from Lee et al., 2020.



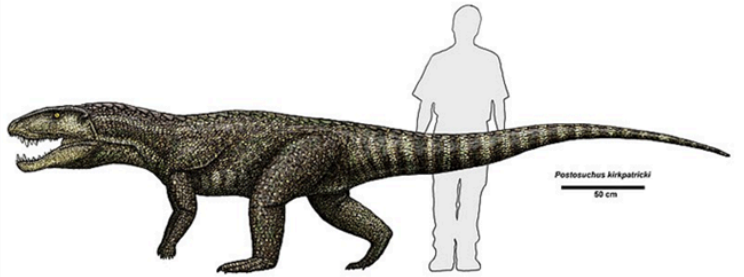

Postosuchus kirkpatricki
50 cm

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Figure 3.64—Various pseudosuchians. First four rows, clockwise from top right: Gavialis gangeticus, an extant crocodilian; Saurosuchus galilei, a rauisuchian from the Late Triassic of Argentina; Litargosuchus leptorhynchus, crocodylomorph from the Early Jurassic of South Africa; Chenanisuchus lateroculi, a dyrosaurid crocodylomorph from the Late Paleocene of Morocco; Dakosaurus maximus from the Late Jurassic of Western Europe; Longosuchus meani, an aetosaur from the Late Triassic of North America. Bottom image: A reconstruction of the Triassic Postosuchus kirkpatricki with a human for scale.

The Pseudosuchia lineage and Avemetatarsalia lineage split from each other approximately 250 million years ago and are largely differentiated by their different ankle structures (Figure 3.65). The Avemetatarsalia include the Pterosauria (flying reptiles like Pteranodon) and the Dinosauria (nonavian dinosaurs and birds).

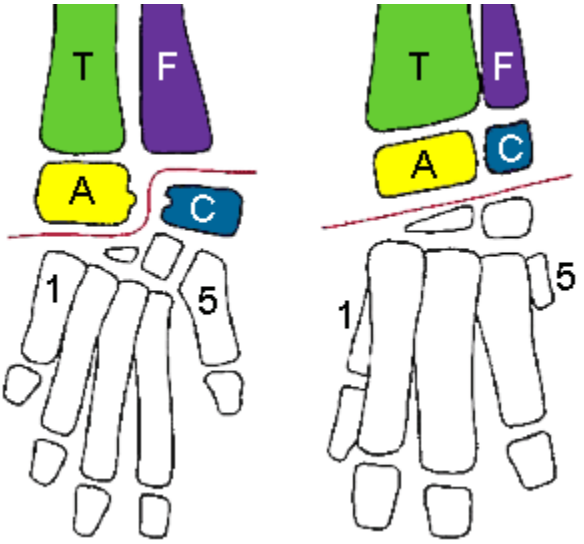

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Figure 3.65—Left: a typical crurotarsal ankle found in Pseudosuchia. Right: a typical mesotarsal ankle found in Avemetatarsalia. T = tibia, F = fibula, A = astragalus, C = calcaneum.

The pterosaurs last shared a common ancestor with the dinosaurs about 216 million years ago and lasted until the end of the Cretaceous period (Figure 3.66). Pterosaurs were the first vertebrate group to achieve active, flapping flight and have a unique wing anatomy compared to birds and bats. This is particularly impressive when one considers that the largest pterosaur, *Quetzalcoatlus*, weighed more than 70 kg and had wingspans of 12 meters! Even though their basic body plans are quite similar, pterosaurs likely had a variety of feeding modes that overlap with several groups of modern birds, such as insectivory, piscivory, carnivory, and filter feeding. However, this is an area of active study as technological advances have created new possibilities for studying pterosaur diets and feeding mechanics.

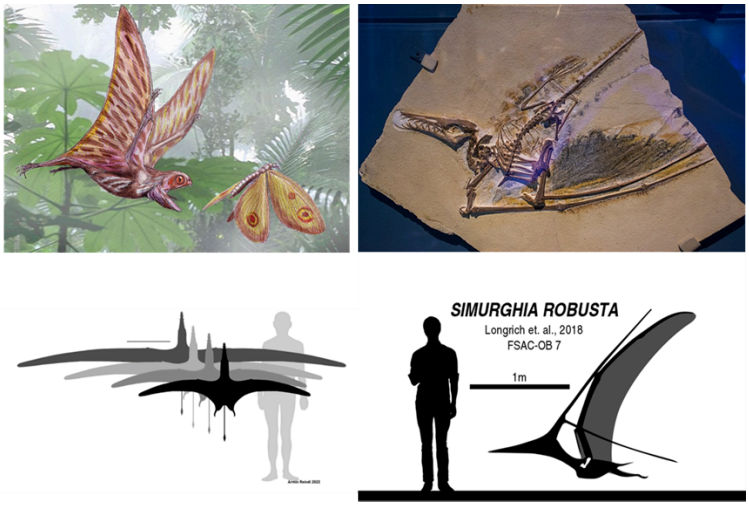

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Figure 3.66—Examples of Pterosauria. Top left: Anurognathus ammoni; Top right: cast of Rhamphorhynchus muensteri; Bottom left: Wingspan estimates of Dearc sgiathanach; Bottom right: Size estimate of Simurghia robusta.

The Dinosauria historically have been divided into two groups, the Saurischia ("lizard-hipped") and Ornithischia ("bird-

hipped"; Figure 3.67). As the names imply, the two groups have different pelvic girdle arrangements. Saurischians have a **pubis** that points anteriorly, while ornithischians have a pubis that points posteriorly.

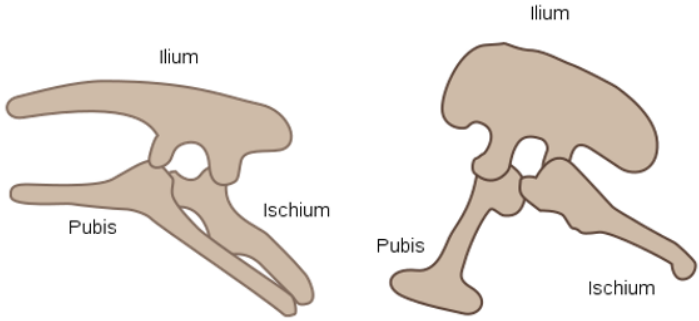

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Figure 3.67—Dinosaur hip structure. For both images, anterior is to the left of the image. Left: ornithischian hip; Right: saurischian hip.

The ornithischian dinosaurs include primarily herbivorous dinosaurs, including the ceratopsians (e.g., *Triceratops*), ankylosaurs, pachycephalosaurs, hadrosaurs (sometimes called "duck-billed dinosaurs"), and stegosaurs (Figure 3.68). Note that despite the name, the birds are not included in the Ornithischia, and their current hip arrangement is a derived feature!

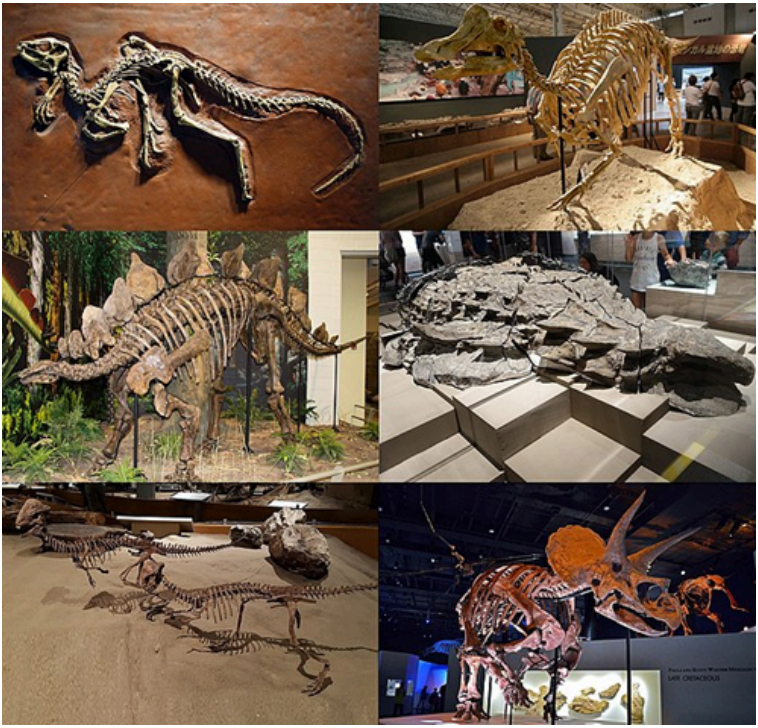

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Figure 3.68—Ornithischian dinosaurs. Clockwise, starting with the top left: Heterodontosaurus, Nipponosaurus, Borealopelta, Triceratops, Stegoceras, Stegosaurus.

The saurischian dinosaurs also include some herbivores, such as the Sauropodomorpha (e.g., *Diplodocus*, *Brontosaurus*; Figure 3.69), as well as the bipedal, mostly carnivorous Theropoda (e.g., *Allosaurus*, *Tyrannosaurus*, *Velociraptor*; Figure 3.70) and the Aves (birds, nested within the Theropoda).

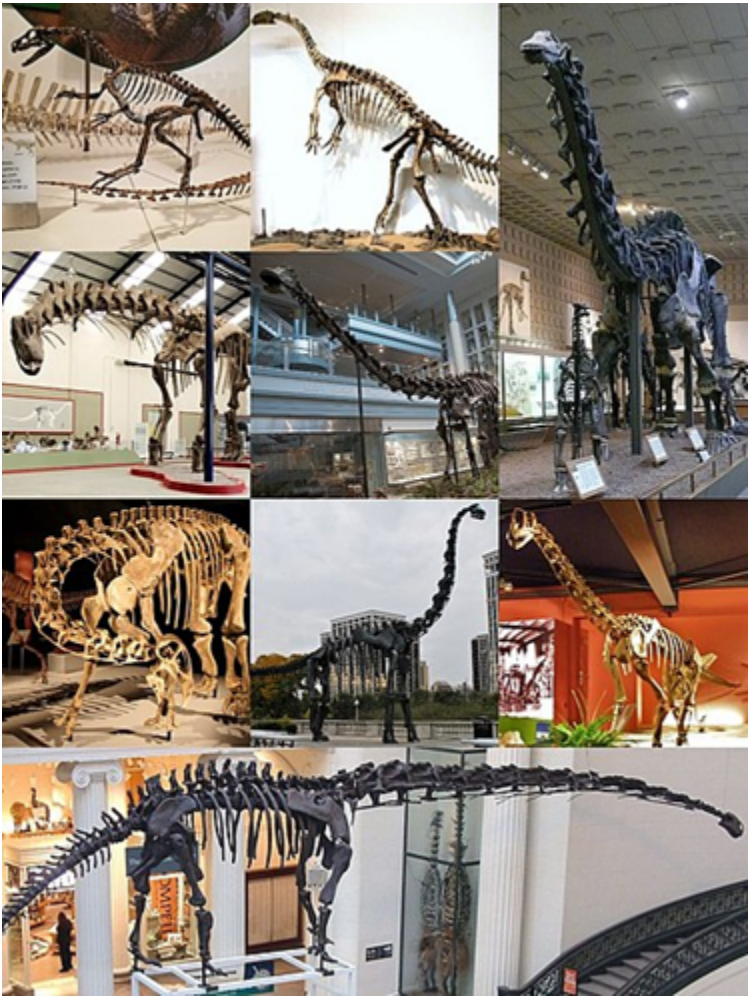

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Figure 3.69—A variety of sauropodomorph dinosaurs. Clockwise from upper left: Eoraptor lunensis, Plateosaurus engelhardti, Brontosaurus excelsus, Europasaurus holgeri, Mamenchisaurus hochuanensis, Nigersaurus taqueti, Argentinosaurus huinculensis, Diplodocus carnegii, Brachiosaurus altithorax.

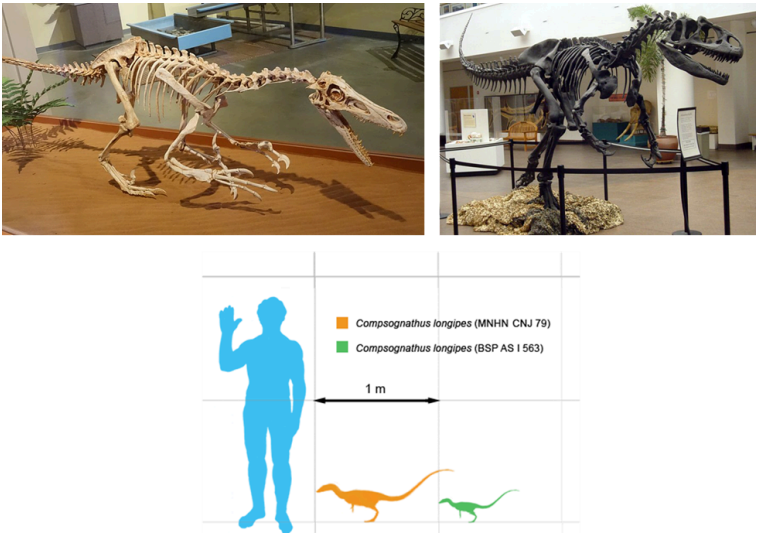

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Figure 3.70—Theropod dinosaurs. Top left: Velociraptor; Top right: Allosaurus fragilis; Bottom: size estimates for two species of Compsognathus.

Ideally, every clade includes a common ancestor and all their descendants sharing synapomorphies. We call this a **monophyletic** grouping. In 2017, a study found evidence that the Saurischia is not a monophyletic clade, although the Sauropodomorpha and Theropoda both are individually. Since then, paleontologists and evolutionary biologists have found support for several different distributions of these three groups on phylogenetic trees and are still working on sorting out these relationships (Figure 3.71).

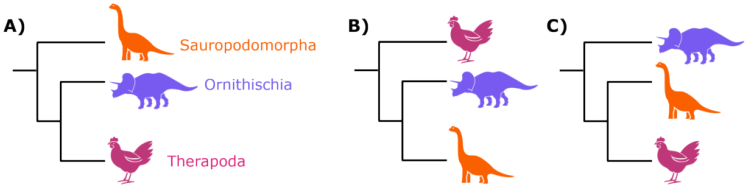

Figure 3.71—Three equally plausible phylogenetic trees illustrating the relationships between theropods, ornithischians, and sauropodomorphs.

That being said, the fact that birds (Aves) evolved from a line of theropod dinosaurs is not under dispute. While the most famous fossil showing both dinosaur and avian features may be *Archaeopteryx*, there is a suite of outstanding feathered dinosaur fossils from China that have further supported the dinosaur-bird hypothesis (Figures 3.72 and 3.73). There are several lines of evidence for this hypothesis, including similarities in integumentary structures such as particular feather structures, various skeletal features (e.g., a **keeled sternum**, **wishbones**), egg roosting behaviors, and egg structure. The first definitive bird fossils, about the size of a chicken, appear in rocks that are somewhere in the Middle or Late Jurassic period. It is difficult to point to a particular fossil as being the first bird, as the transition from dinosaur to bird was quite gradual. The evolution of flight is complex and does not just belong to the Aves. Based on what we currently know, wings and the types of asymmetrical feathers that are necessary for flight show up in some of the paravian dinosaurs (the dromaeosaurs—*Velociraptor* is one of these—and another similar-looking group called troodontids; Figures 3.72) and were likely for display or other nonflight activities. We also know that those same paravian dinosaurs evolved the ability for gliding flight independently of the birds. Finally, powered flight, as we see in extant birds, is something that evolved sometime in or after the Early Cretaceous period. Early Aves, such as *Archaeopteryx* (Figure 3.73) were missing some of the skeletal features that would support

flight muscles. Interestingly, some of the other requirements for flight, such as highly derived brains and potentially birdlike lungs, appeared in theropods first.



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Figure 3.72—Examples of paravian dinosaurs. Clockwise from top left: Confuciusornis sanctus from the Cretaceous of China, a dromaeosaur fossil, Microraptor gui from the Cretaceous of China, Anchiornis huxleyi from the Jurassic of China, an extant raven, and the troodontid from the Mesozoic era.



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Figure 3.73—Top left: Confuciusornis sanctus, from the Lower Cretaceous of China. Top right and bottom: Archaeopteryx.

We do know that birds first diversified in the Early Cretaceous period, filling some niches we see extant birds in, including semiaquatic birds, large generalists, and small tree-dwelling birds. However, the Neornithes ("new birds"), the group our extant birds belong to, did not diversify until after the Cretaceous period (Figure 3.74). Almost every extant neorthine order appears

in the 15 million years after the end-Cretaceous mass extinction event. The radiation of the Neornithes is thanks to a number of adaptations, including those related to flight, perching, and brain development. Today, over 10,000 species of extant birds extend across all seven continents, covering a huge range of niches. They range in size from the tiny bee hummingbird at 2.6 grams and 6 cm long to the largest extant bird, the ostrich at up to 2.8 meters tall and over 150 kg. However, the ostrich is not the largest bird of all time—the giant waterfowl *Dromornis stirtoni* (263 kg) and the elephant bird *Aepyornis maximus* (406 kg) get those honors (Figure 3.75).

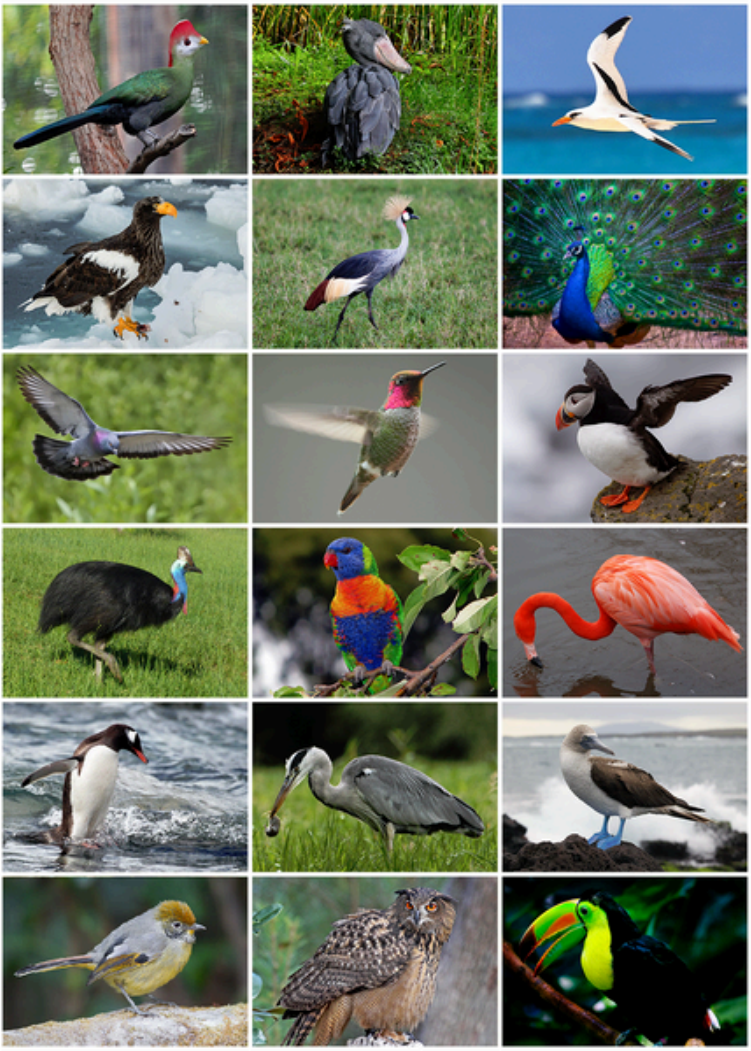

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Figure 3.74—A variety of extant birds illustrating different body, beak, and feather morphologies. Top row (row 1): Red-crested turaco Tauraco erythrolophus, shoebill Balaeniceps rex, and white-tailed tropicbird Phaethon lepturus. Row 2: Steller's sea eagle Haliaeetus pelagicus, gray crowned crane Balearica regulorum, and Indian peafowl Pavo cristatus. Row 3: Common pigeon Columba livia, Anna's hummingbird Calypte anna, and Atlantic puffin Fratercula arctica. Row 4: Southern cassowary Casuarius casuarius, rainbow lorikeet Trichoglossus moluccanus, American flamingo Phoenicopterus ruber. Row 5: Gentoo penguin Pygoscelis papua, gray heron Ardea cinerea,

blue-footed booby Sula nebouxii. Row 6: Bar-throated minla Actinodura strigula, Eurasian eagle-owl Bubo bubo, and keel-billed toucan Rhamphastos sulfuratus.



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Figure 3.75—Top: Aepyornis, a dodo, and an extant ostrich. Bottom: Dromornis stirtoni.

3.4 Summary

The vertebrates are characterized by several embryological features, as well as the presence of a well-developed brain, skull, and vertebrae. The vertebrates include a diverse range of extinct and extant organisms that span all ecosystems across the globe.

Application Questions

1. What features classify you (a human) as an osteichthyan? A tetrapod? A reptile? A synapsid? A mammal?
2. Is it accurate to say that dinosaurs are extinct? Why or why not?

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4. Embryology

BRADLEY M. HERSH

> It is not birth, marriage, or death, but gastrulation which is truly the most important time of your life.
>
> —Lewis Wolpert, developmental biologist

Focus Questions—to Guide Your Reading of This Chapter

1. How does a single-celled zygote generate the variety of cell and tissue types present in a mature multicellular vertebrate?
2. How do interactions between cells and tissues contribute to anatomical features?
3. How might changes in developmental processes over evolutionary time lead to observable differences in vertebrate groups?

4.1 Introduction

For a time of such staggering importance that it eclipses birth or death, "gastrulation" certainly doesn't have the same universal recognition. What is this process that commands such esteem, and how is it relevant to anatomy and physiology? Fundamentally, any study of vertebrate anatomy and physiology must consider vertebrate embryology, encompassing the processes that produce organismal shape and function—that is, the set of developmental events, including gastrulation, that an embryo undergoes as it proceeds from a unicellular fertilized zygote to a distinct, complete, multicellular organism. In addition, differences in form and function between related organisms necessarily involve evolutionary changes in these embryological processes. Thus, we will introduce a few key concepts of developmental biology and then examine how those concepts are illustrated in vertebrate biology and evolution.

4.2 Major Concepts of Developmental Biology

The progression of a fertilized **zygote**, the single-celled result of the fusion of egg and sperm, into a fully formed multicellular individual requires coordination between complex developmental processes that can differ in their specifics in different organisms. However, these mechanisms share fundamental similarities that operate across animal groups.

Differentiation

Although muscle cells and nerve cells, for example, appear obviously distinct in their shape and properties, vertebrate cells typically share the same full set of genetic information (that is, the genome

of all cells is identical—with some interesting exceptions when it comes to cells of the immune system!). Therefore, distinctions between cell types must arise from differences in which portions of the genetic information are switched on or off (i.e., which genes are actively expressed and which are not). **Differentiation** is the process cells take on the shape and properties of their defined cell type, also referred to as their **cell fate** (Figure 4.1). Cell fates can be considered broadly (e.g., nervous system) or narrowly (e.g., pyramidal neurons). An important cell fate defying our typical expectations for what cells do is **apoptosis** (also known as programmed cell death), in which the fate of the cell is to self-destruct in a carefully controlled manner, helping to sculpt the shape of limbs and digits (Box 4.1—Cell Death in the Feet of Waterfowl) or to eliminate self-reactive cells in the immune system.

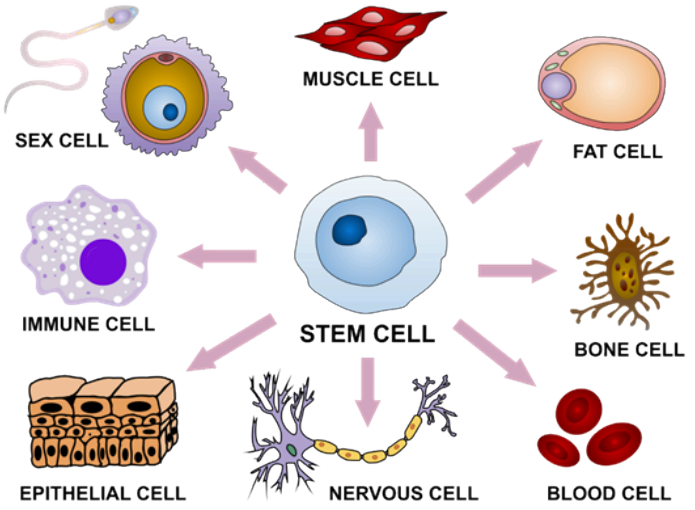

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Figure 4.1—A stem cell (middle) is simply a cell that retains the ability to become many different types of cells with different physical properties and appearances, as shown in the outer ring of examples.

Determination

The fate of most cells is already established well before those cells actually take on the physical properties of their final cell fate. **Determination** is the set of mechanisms cells commit to a cell fate. Determination can be thought of as a progressive narrowing of the fates available to a given cell. Cells that can achieve any fate, of either the embryonic body or extraembryonic supporting tissues, are referred to as **totipotent**. Cells of the very early embryo are totipotent but typically lose this extreme flexibility after only a few cell divisions. Similarly, **pluripotent** cells have the ability to produce any embryonic cell type but not extraembryonic tissues (such as the placenta and amnion), which provide physical support and nutrient access for the developing embryo. Embryonic stem (ES) cells derived from mammalian embryos can be maintained in petri dishes in an undifferentiated state but, when returned to an embryo or stimulated with growth factors, can generate all types of embryonic cells, demonstrating their pluripotency (Figure 4.1). Determination is affected by the internal status of the cells, such as the activation state of specific genes, and by external conditions, which may include either nonbiotic environmental cues or communication between cells in an individual.

Induction

The cells, tissues, and organs of an organism must be carefully organized and regulated to ensure proper interaction and function. For example, the alignment of structures within the vertebrate eye is important for effective vision, and this critical alignment is achieved by a series of reciprocal interactions between cells that regulate the process (Figure 4.2). This specific case illustrates the more general concept of **induction**, by which one set of cells or

tissue can trigger changes in the determination or differentiation of neighboring cells. The communication of these inductive signals can be via direct contact or by transmission of soluble signaling molecules, such as sonic hedgehog or decapentaplegic (many of these molecules were originally discovered in fruit flies or other **model organisms**, and their discoverers got to choose their odd, funny, or whimsical names).

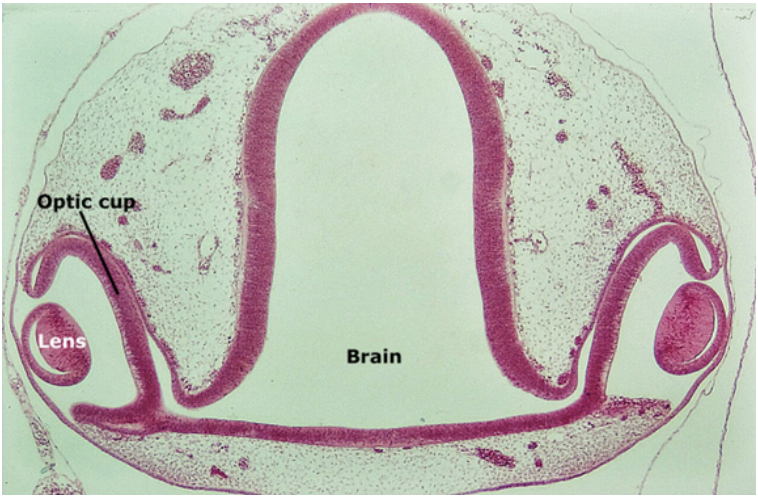

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Figure 4.2—A cross section through the developing brain and eyes of a chicken embryo. Inductive interactions between the brain, optic cup, and lens ensure proper alignment as the eyes develop. The curved and layered tissues also serve as examples of morphogenesis.

Morphogenesis

Morphogenesis comprises the processes at the levels of cells and tissues that generate anatomically relevant shapes, including tubes, branches, and layers (Figure 4.2). These processes involve changes in cell growth, motility, adhesion, and division that together can create the emergent properties of tissues and organs. For example,

if the cells lining the intestine failed to adhere to each other properly, the leaky gut structure would be significantly disruptive. Conversely, if cells on opposite sides of the intestine adhered to each other inappropriately, the tube would collapse and be blocked. The proper formation of a tube, whether in the nervous system, the intestines, or the lungs, requires specific cell movements and interactions.

Modularity

Modules, broadly considered, describe independent or distinguishable sets of parts that can interact with other modules to build more complex structures. Because organisms typically display distinct physiological, behavioral, or anatomical traits, and many of these traits can vary independently (or mostly independently) from one another, we can assert that organisms are modular in nature. This **modularity**, or the distinguishability and independence of complex traits, enhances evolutionary change by allowing variation of isolated traits without detrimental effects on other traits. Modularity can occur at multiple levels, including the morphological (e.g., different bones within the vertebrate limb have evolved independent size and shape changes, such as the elongated finger bones of bats or the shortened arm bones of whales and dolphins) or the developmental (e.g., shifting boundaries of *Hox* gene expression in nervous and skeletal system leading to differences in brain and axial skeleton development).

4.3 Fertilization and Early Development

Gametes and Fertilization

A convenient place to start in our consideration of vertebrate development is the formation of the **zygote** via fertilization of a haploid ovum (or egg cell) by a haploid spermatozoon (or sperm cell) to reconstitute a complete diploid genome. Fertilization can occur externally (outside of the reproductive tract) or internally (within the reproductive tract). Even animals with internal fertilization may then develop externally (for example, birds).

Both egg and sperm are **gametes**, haploid products of meiosis that carry just half of the genetic material of the parent. Gametes are cells of the **germ line**, which are the only cells of the organism to pass along their hereditary information. By contrast, the cells of the **soma** (also called somatic cells) form the structures of the organism but do not transmit information to subsequent generations. Thus, gametes represent a specialized cell fate within an organism, and developing sperm and eggs undergo specific differentiation events to generate cells that are, respectively, small, elongated, and motile or large, spherical, and nonmotile.

In different species of vertebrates, sperm shape (such as the hook-shaped heads of rodent sperm) and size (from 28 μm for a porcupine to 349 μm for a honey possum) can vary significantly. Sperm consist of three segments—head, midpiece, and flagellum (tail or terminal piece)—and the length of each component can vary independently of the others (Figure 4.3). Tail length appears to have an evolutionary relationship to fertilization mode (internal or external), but other variations in shape and size do not always have obvious evolutionary correlates.

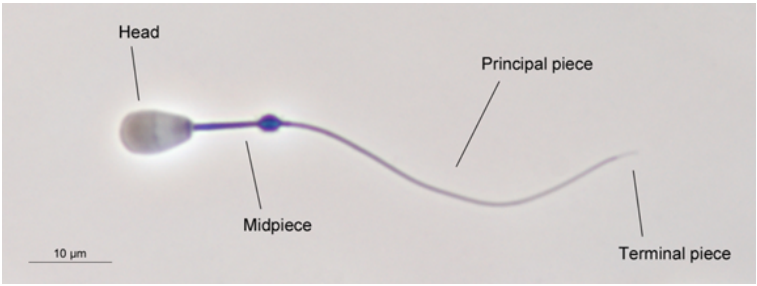

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Figure 4.3—Red deer spermatozoon, with major regions labeled.

Within a given species, the egg is always larger than the sperm cell, but across different groups, egg size also can vary widely. The egg contains all the nutrients and other components (such as RNAs, transcription factors that switch on or off RNA production, and signaling molecules that regulate physiological responses or transcription factor activity) for the initial developmental steps. Embryos that develop externally must have sufficient energy reserves and materials to complete development to a stage where the individual can obtain its own resources. Most of the resources are contained within the yolk, which consists of proteins and energy-rich lipid molecules, and the amount of yolk will affect how long an embryo can survive before reaching a feeding stage. Eggs with small amounts of yolk are termed **microlecithal**, whereas **macrolecithal** eggs have substantial quantities of yolk (Figure 4.4). By contrast, internally developing embryos are less dependent on yolk because they typically obtain nutrients from their parental host.

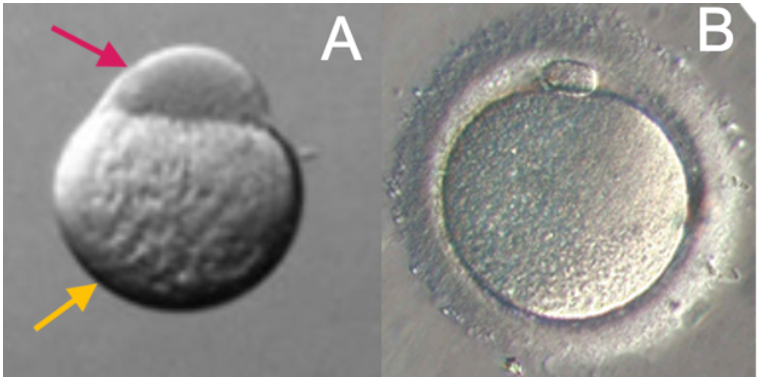

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Figure 4.4—(A) Macrolecithal zebrafish embryo with single cell (red arrow) on top of large yolk (yellow arrow); (B) Human oocyte (large cell surrounded by the slightly fuzzy zona pellucida) beside the smaller polar body cell (top center) but lacking any yolk.

Fertilization requires the entry of sperm into the egg, fusion of the sperm and egg nuclei, and activation of cellular processes within the newly generated zygote. Sperm entry, sometimes envisioned as the culminating event of a single "victorious" sperm, may in many cases actually require the action of multiple sperm in order to penetrate the various external support cells and cell membranes of the egg. Eggs and sperm use various mechanisms to ensure that only sperm of the same species can undergo fusion with an egg, and evolutionary changes in the components of these systems can act as isolating mechanisms that may lead to speciation.

Sperm entry triggers several responses within the egg. First is the cortical reaction, which prevents entry of additional sperm. In addition, some eggs, including those of humans, do not finish their meiotic divisions until sperm entry stimulates completion. The specific location of sperm entry may also establish the embryonic head-to-tail (anterior-posterior) and back-to-belly (dorsal-ventral) axes by promoting the rearrangement of intracellular components.

Cleavage

Once the fertilization process is complete, the zygote undergoes a series of rapid cell divisions, known as **cleavage**, in which the divisions are not separated by any periods of cell growth. At the end of this process, the multicellular embryo, now called a **blastula**, will be approximately the same size as the zygote but now composed of many smaller cells called **blastomeres**. A small amount of growth and minor cell movements may occur at this stage, but the blastula is largely setting the stage for the major set of cell movements that will occur in the next developmental period.

The shape and organization of the blastula differ across vertebrate groups, ranging from spherical (amphibians and fishes, Figure 4.5A, B) to discoidal (a flattened, circular shape in birds, Figure 4.5E, F). The blastula often, but not always, has an interior cavity called the **blastocoel**. In eutherian mammals, the blastomeres form two distinct sets of cells—the outer layer of the **trophectoderm**, which will generate extraembryonic tissues like the placenta, and the internal cluster of the **inner cell mass**, from which the embryo proper will arise. Pluripotent embryonic stem cells can be derived from this inner cell mass. Overall, this multilayered embryo, referred to as a **blastocyst** (Figure 4.5C, D), will implant into the uterine wall and grow slowly into a cylindrical shape. The inner cell mass is itself divided into the **hypoblast** (primitive endoderm) and the **epiblast**, which form additional support structures, like the yolk sac, and the primary layers of the embryo, respectively.

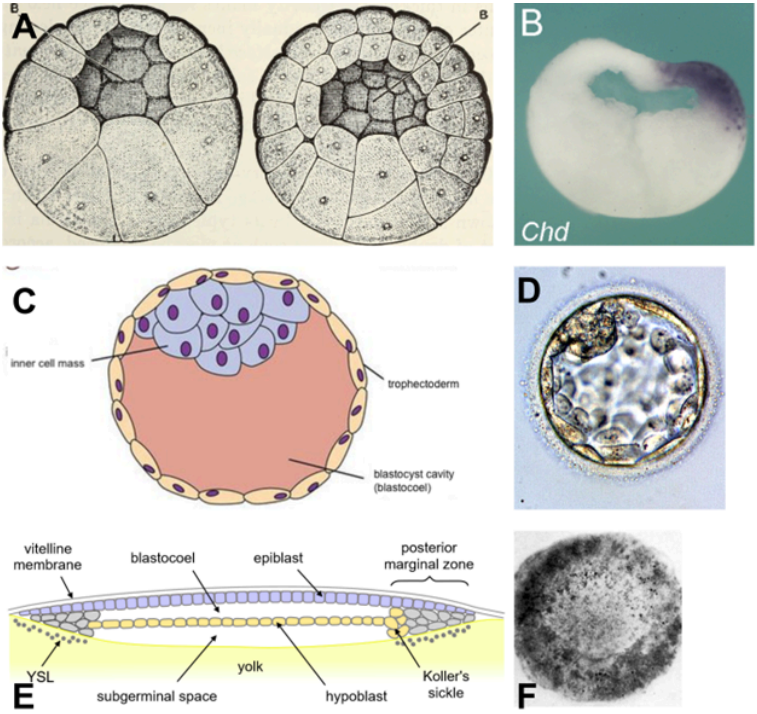

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Figure 4.5—(A) Sketch of Xenopus frog embryos at blastula stage, with open blastocoel space visible. (B) Microscope image of Xenopus blastula cross section. Expression of the Chordin gene is shown in blue. The cells above the blastocoel have been pushed in, slightly compressing the blastocoel space. (C) Diagram of a mammalian blastocyst, showing the inner cell mass and trophectoderm regions. (D) Microscope image of human blastocyst. (E) Cross-section diagram of the flattened chicken blastula atop the yolk. (F) Microscope image of the discoidal chicken blastula (lighter cells in the center), top view.

Gastrulation

And so we finally arrive at that stage of promised importance, **gastrulation**, which involves extensive cell rearrangements that establish the three layers of all vertebrate embryos (for videos of

these movements in *Xenopus* embryos, check out a cross-section view and this animation). These three layers, from outside to inside, are the **ectoderm**, **mesoderm**, and **endoderm**, and each will produce distinct tissue types in the mature animal. The three layers can be formed by different modes of cell movement, which largely depend on the type of blastula present at the start of gastrulation.

In amphibians and fish, gastrulation initiates at a small inward fold called the **blastopore** (Figure 4.6). The region located just dorsally of the blastopore is known as the Spemann-Mangold organizer, and transplanting this region into another embryo can trigger a second site of gastrulation and an entire second axis of development in the recipient embryo (Box 4.2—Ethel Browne, Hilde Mangold, and the Organizer Concept). Cells alongside the blastopore move inward in coherent sheets by the process of **involution**, spreading into the interior surface of the embryos. These cells will form the internal endoderm and mesoderm layers. The cells at the top of the blastula spread downward and around the outside of the embryo via **epiboly** (which is a bit like how caramel dripped onto the top of an apple might flow around the outside to cover it), forming the initial ectoderm tissue.

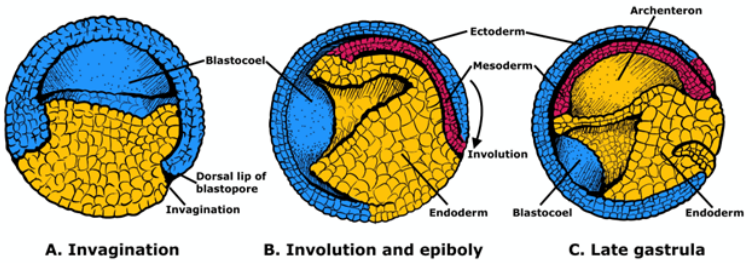

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Figure 4.6—Gastrulation in a Xenopus embryo starts (A) as an invagination of cells at the dorsal lip of the blastopore. (B) Involution then moves cells internally, and epiboly brings the external cells down around the internal cell layers. (C) Together the cell movements generate the three layers of the embryo: ectoderm (blue), mesoderm (red), and endoderm (yellow).

Gastrulation in birds and mammals starts at a structure called the **primitive streak**, which starts forming at the posterior pole and then extends anteriorly and will reflect the main body axis of the developing vertebrate. Cells converge at the primitive streak and then move inward at a condensation of cells, called Hensen's node in birds, which has equivalent organizing properties to the Spemann organizer in amphibians. Instead of the coherent sheets of cells (**epithelia**) during involution, cells from the discoidal bird blastula move individually via **ingression** (Figure 4.7). The loose aggregates of cells (**mesenchyme**) that have moved internally will again form mesoderm and endoderm, whereas the cells that remained external to the primitive streak will contribute to the ectoderm. Mechanisms of epithelial-mesenchymal transition (EMT), critical here for proper development, can be disrupted in cancer cells, allowing them to detach from their originating tissue and move elsewhere in the body to form metastases. In the mammalian egg cylinder blastula, the primitive streak movements also involve **convergent extension**, wherein cells move toward and then past each other, displacing into adjacent layers and extending the length of the overall axis.

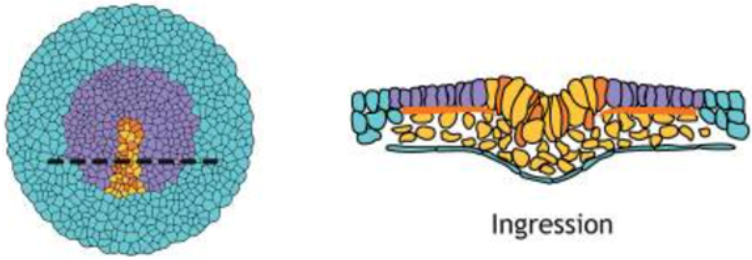

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Figure 4.7—Gastrulation in a chicken embryo. The primitive streak is visible in the discoidal embryo (left, top view) as mesendodermal cells (yellow and red) detach and move inward (right; cross-section view at dotted line).

Regardless of the specific cell movements that produce

the layers, the fates generated by the layers are remarkably similar (Figure 4.8). The endoderm forms the lining of the digestive tract, as well as structures evolutionarily derived from it, such as the lungs. The ectoderm forms the obviously externally facing structures, such as skin (epidermis), and also the nervous system, which administers the animal's interface with the outside world. Sensory structures, like eyes, nose, ears, and lateral line organs, are derived from this layer, often via interactions between epidermal and neural ectoderm cells. Finally, mesoderm derivatives include the notochord, muscles, bone, heart, kidneys, and blood.

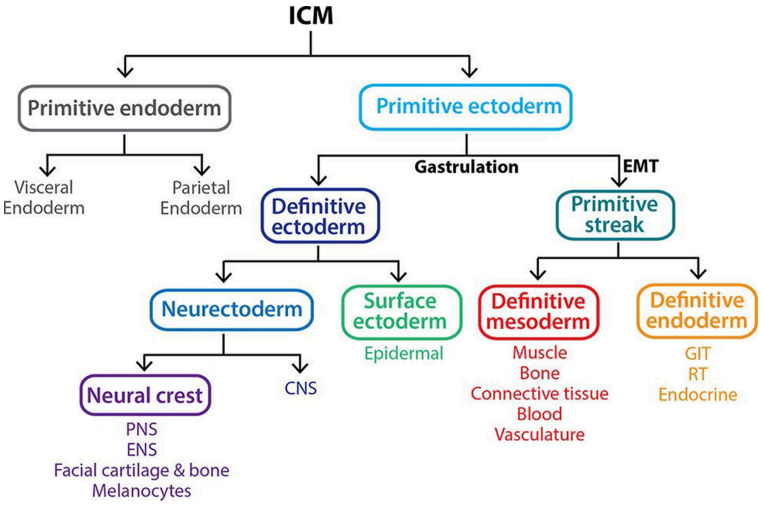

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Figure 4.8—Derivatives of the mammalian inner cell mass (ICM). Though the mammalian case is shown here, ectoderm, mesoderm, and endoderm derivatives are similar across all vertebrates. Cell movement at the primitive streak requires epithelial-mesenchymal transition (EMT).

4.4 Neurulation

Once the three cell layers have been established, the work of building the distinct structures of the body can begin in earnest.

Of critical importance because of its role in regulating the function of many other systems is the early development of the nervous system. Following directly from the dramatic tissue reorganization of gastrulation, the process of **neurulation** folds up portions of the ectoderm to generate the neural tube, which will form the developmental basis for the brain and spinal cord. Because the specifics of gastrulation differ between vertebrates, so too must the details of neurulation. Despite these differences, the outcome is a generalized nervous system with similar major structures.

The nervous system arises from the ectoderm via inductive interactions with the underlying mesoderm tissue. A crucial inductive signal comes from the **notochord**, which is the defining taxonomic characteristic of chordates, a condensed rod of tissue that runs along the anterior-posterior axis, providing both structural support and inductive instructions. The notochord signals to the overlying ectoderm cells, causing them to elongate and to form the thickened **neural plate**. The various changes in cell shape ultimately cause the edge of the neural plate to rise up, generating the **neural folds** with the lower **neural groove** in the region in between (Figure 4.9). The neural folds move toward each other before meeting at the dorsal midline of the embryo, where they fuse to form the hollow neural tube. The neural tube becomes separate from the ectoderm above it, which will become epidermal, and the cells of the **neural crest** will derive from the neural fold regions near the conjunction of the neural and nonneural ectoderm. This process occurs toward the anterior end of most vertebrate embryos and is referred to as **primary neurulation**. Toward the posterior of vertebrate embryos (and all along the axis in some, such as teleost fishes), **secondary neurulation** forms the neural tube from a solid rod of cells, similar to the notochord. Small openings form in this **medullary cord**, and the progressive fusion of these small cavities forms a hollow tube via the process of **cavitation**.

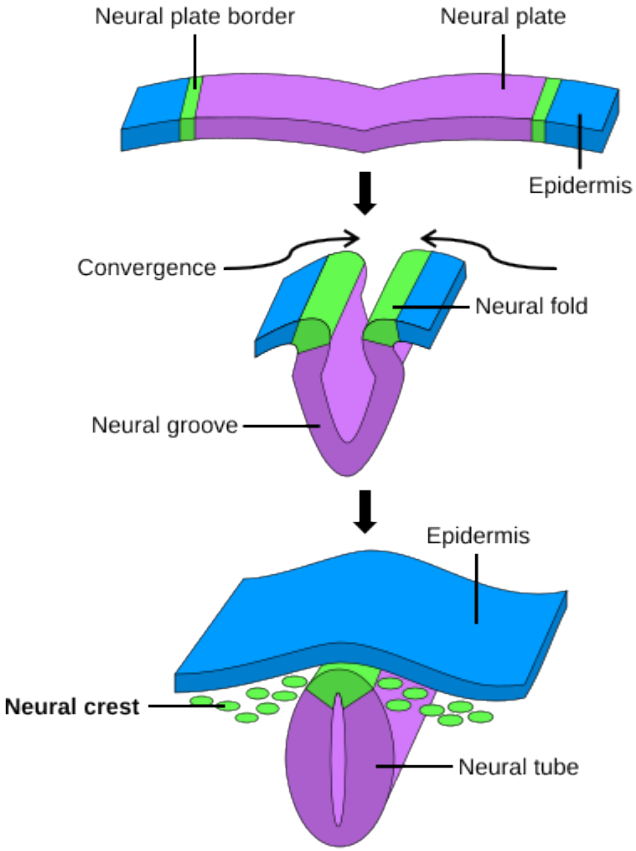

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Figure 4.9—Neurulation forms the neural tube and neural crest. Epidermis and neural tissue are both ectodermal in origin, but the neural tissue becomes distinct during neurulation. The neural plate region (top) begins to buckle inward, forming the neural folds and neural groove (middle). The tightly structured neural tube and loosely aggregated neural crest cells form when the neural folds meet each other and separate from the overlying epidermis (bottom).

The proper formation of the neural tube is critical, and defects in the process can lead to severe defects in the embryo.

Failure of primary neurulation at the anterior may result in anencephaly, where the brain and other head structures fail to develop entirely. Improper secondary neurulation at the posterior end of the neural tube may contribute to spina bifida, the severity of which depends on how much of the neural tube is defective. Collectively, neural tube defects are among the most common during human development, appearing in roughly 1 in 500 live births and additionally contributing to some significant proportion of spontaneous miscarriages.

4.5 Early Mesoderm Development and Somite Formation

Nearly simultaneously with the process of neurulation in the ectoderm, the vertebrate mesoderm begins to form primordia for the various mesodermal tissues and organs. Within the mesoderm, three main regions emerge on each side of the dorsal midline. The **paraxial** ("beside the axis") **mesoderm** lies alongside the neural tube, whereas the **lateral plate mesoderm** forms a slightly flattened region farthest from the midline, with the **intermediate mesoderm** sandwiched between the two other regions (Figure 4.10).

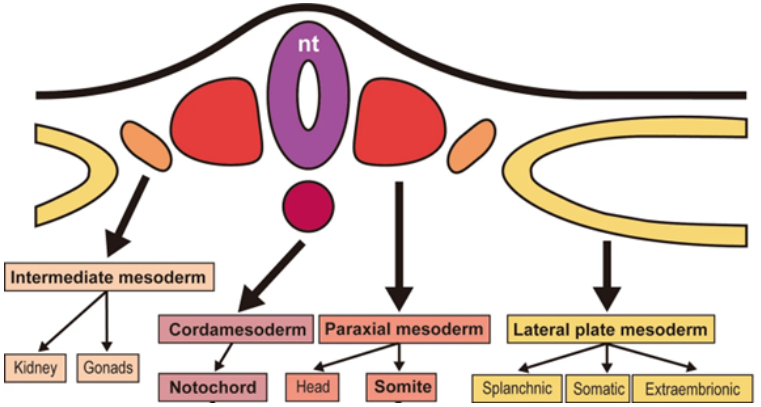

Figure 4.10—Mesoderm regions and their major derivatives. The notochord, a mesoderm structure, forms below the neural tube (nt; purple). Alongside the neural tube, the mesoderm separates into paraxial (red), intermediate (orange), and lateral plate (yellow) regions.

As the lateral plate mesoderm splits into two layers, the definitive **coelom**, or main body cavity, will form and expand in the space between the two layers. The inner, or **visceral**, layer will support and surround the internal organs, such as the gut, heart, and lungs. The outer, or **somatic**, layer will contribute to the muscles of the body wall. From the internal layer, the **splanchnic mesoderm** forms the muscles surrounding the gut, as well as the arteries, veins, heart muscle, and blood cells of the circulatory system. The intermediate mesoderm generates the kidneys as well as other portions of the excretory and reproductive systems.

Although we don't think of vertebrates as obviously segmented (unlike, say, arthropods or annelids), we can observe within the paraxial mesoderm a clear indication of vertebrate segmentation in the form of **somites**. Somites are paired blocks of tissue that arise alongside the notochord and will form the muscles and bones of the trunk (Figure 4.11).

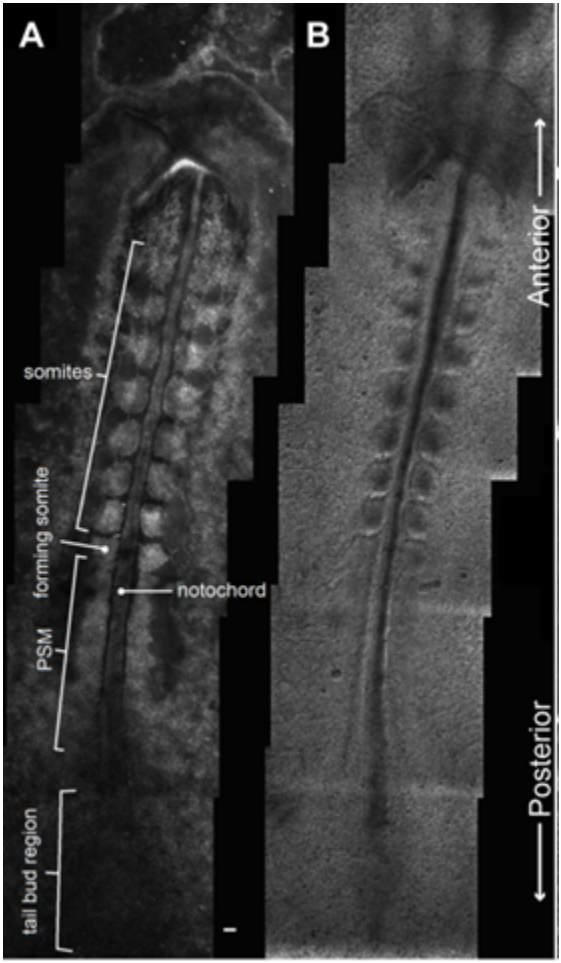

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Figure 4.11—Fluorescent (A) and light (B) microscopy views of developing somites. Somites are condensed blocks of tissue along the notochord and form at regular intervals from anterior to posterior.

Humans, mice, and birds all have ~50 somites, whereas snakes can have many hundreds. Much of the process of somite formation has been described in chick embryos because of the ease

of observation and manipulation (including transplantations, dye injections, and gene expression labeling). Between Hensen's node (the site of active gastrulation movements) and the most recently formed somite is the presomitic mesoderm, from which the next pair of somites will arise. New somite pairs are formed every 90 minutes within the chick embryo; the highly regular interval is determined by a complex interaction of signals within the somites and presomitic mesoderm described as the "clock-and-wavefront" model. Waves of expression of the transcription factor c-hairy1 and components of the Delta-Notch signaling pathway, including the amusingly named Lunatic Fringe protein, start at the tail end of presomitic mesoderm and move anteriorly until they reach the site of the next somite, where they persist through the visible formation of the condensing somite.

4.6 Nervous System Formation

The nervous system in adult vertebrates consists of both central and peripheral components, most of which are ultimately derived from the tissue divisions formed during neurulation. In general terms, the central nervous system arises from the neural tube, whereas the peripheral nervous system arises from the neural crest. In addition, various sensory systems, such as the nose and ear, will develop from **neurogenic placodes**, ectodermal thickenings lateral to the forming neural tube (Figure 4.12).

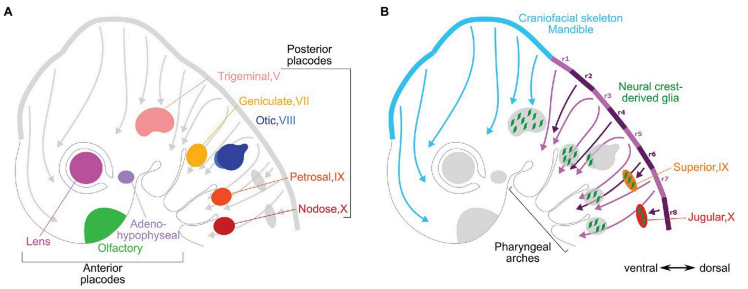

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Figure 4.12—Diagram of 10-day-old mouse embryo indicating approximate locations of the (A) sensory (neurogenic) placodes and (B) neural crest descendants. Arrows indicate migration of neural crest cells. Rhombomeres (B) are labeled in purple, and midbrain and diencephalon are labeled in blue. Roman numerals indicate the associated cranial nerves.

The anterior portion of the closed neural tube will develop into the brain, which is divided during development into the forebrain (**prosencephalon**), midbrain (**mesencephalon**), and hindbrain (**rhombencephalon**; Figure 4.13).

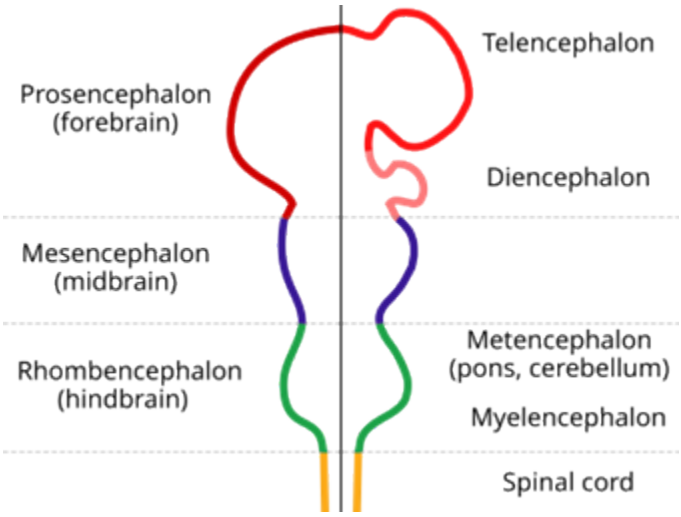

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Figure 4.13—Subdivisions of the brain early (left) and later (right) during development.

The forebrain and midbrain are unsegmented, but the hindbrain is divided into eight segmented regions (r1–r8) called **rhombomeres** (Figure 4.12). Cells within one rhombomere remain separate and distinct from cells of neighboring rhombomeres, and different cranial nerves arise in each rhombomere before extending into the distinct **pharyngeal arches** (also known as branchial arches or branchiomeres) from which the facial skeleton will arise. All developing vertebrates display branchial arches, and gill openings do form between pairs of branchial arches in embryonic fishes (both cartilaginous and teleost). These openings are the source of the inaccurate statement that humans have gills during development, but the gill openings either are transitory or never open at all in amniotes and never resemble anything close to functional gills.

The closed neural tube also gives rise to different populations of neurons across its dorsal-ventral axis. This dorsal-ventral pattern is established by complex, competing interactions

between concentration gradients of the sonic hedgehog (Shh) signal released from the ventral notochord and bone morphogenetic protein (BMP) signals induced by epidermal ectoderm in the dorsal neural tube (Figure 4.14). The signals stimulate transcription factor responses in cells, based on the high-to-low concentration of each signal, resulting in different gene expressions in different cells based on where they are physically located within the neural tube. Cells closest to the notochord form the **floor plate**, which serves as a ventral organizing structure influencing neuronal patterning and migration (in part by its own production and release of Shh). Spinal motor neurons and interneurons develop from ventral regions near the floor plate, with different subtypes depending on local concentration of Shh. By contrast, commissural neurons, which send their axons across the neuronal midline in order to connect distant regions within the neural tube, arise dorsally.

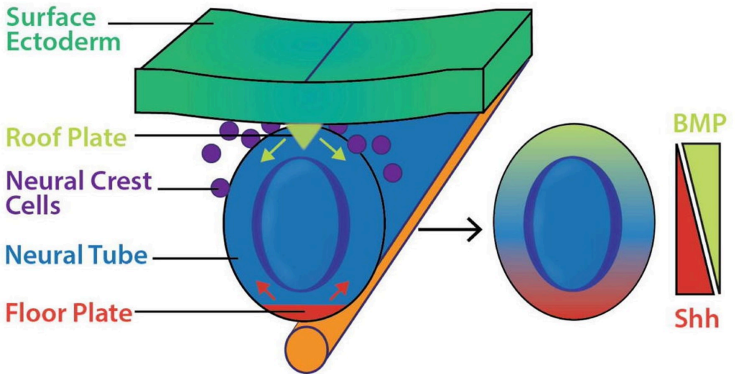

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Figure 4.14—Sonic hedgehog signaling from the floor plate and BMP signaling from the roof plate and epidermal ectoderm form opposing concentration gradients (differences in concentration range from high to low across tissue) that establish different neuronal fates within the neural tube. High concentrations of BMP present in the dorsal region trigger dorsal fates, such as roof plate and commissural neurons, but the lower concentrations of BMP in the ventral region do not induce these fates. Similarly, high concentrations of Shh in the ventral region trigger floor plate, motor neuron, and interneuron fates.

The sensory neurons of the peripheral nervous system develop from neural crest cells outside of the neural tube but can make connections in the dorsal region of the dorsal neural tube. Neural crest derivatives also include pigment-forming cells, facial nerves, sensory and autonomic ganglia, and some head skeletal structures.

The proper function of the entire nervous system is dependent on the formation of appropriate connections between neurons in both the central and peripheral nervous systems. These specialized connections within the nervous system are called **synapses**, which occur where neurons come into close proximity to each other or target muscle cells but are still physically separated by a short distance, called the synaptic cleft (Figure 4.15). Chemical, rather than electrical, signals are passed across the synapse, and so proper patterning of neuronal connections is absolutely essential for transmission of neuronal messages throughout the body.

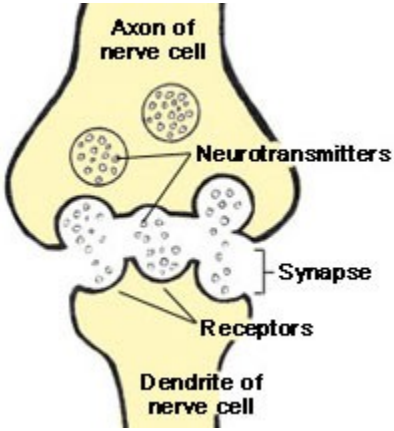

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Figure 4.15—Two neurons communicate chemically at a synapse, with the presynaptic cell releasing neurotransmitters and the postsynaptic cell responding based on binding of neurotransmitters to cell-surface receptors.

As neurons develop, they extend long, thin **axons** that will eventually form one-half of a synaptic connection. Axons lengthen toward their targets by the dynamic extension and retraction of cytoplasmic filaments from the **growth cone** region, often appearing to be searching their environment for the correct path. This search behavior occurs by the growth cone responding to chemically attractive or repulsive signals, including netrins, semaphorins, cadherins, and ephrins. The signals may be diffusible long-range molecules or contact-dependent short-range molecules, but in both cases they will interact with receptors in the growth cones to influence the extension and retraction movements.

The other major source of sensory structures is the neurogenic placodes, which are initially formed in the head region but may migrate to trunk locations (Figure 4.12). Examples include the nasal placode, which will form the odorant-responsive cells of the olfactory epithelium; the otic placode, which will form the fluid-filled ducts of the inner ear; and several lateral line placodes, from which the tactile and electroreceptors of the lateral line systems of fishes and amphibians are derived.

4.7 *Hox* Genes and Evolution

Some major questions we have been avoiding up to now are, How are the limbs correctly placed along the body, and how do the head and tail form at the proper ends of the animal? Narrowing further to the developing brain, how are the fore-, mid-, and hindbrain regions organized in the appropriate order? To a large extent, the answer to these questions of regional specification involves the action of the *Hox* gene family.

Hox genes were originally identified in the fruit fly but have since been identified in virtually all animals and share functions in anterior-posterior specification in all bilaterally symmetrical animals, including vertebrates (Figure 4.16). The *Hox* genes all

encode transcription factors that can switch other genes on or off, thereby regulating cell fates. Curiously, the *Hox* genes form clusters of closely spaced genes, and a gene's position within the cluster parallels its expression along the anterior-posterior axis. Although fruit flies possess only a single *Hox* cluster with eight genes, vertebrates have at least three clusters (in jawless cyclostome fishes) up to a maximum of eight clusters (in teleost fishes), with all tetrapods possessing four clusters. The cluster duplications in teleosts have been hypothesized as a trigger facilitating the adaptive radiation of morphological diversity within this largest vertebrate group. The clusters in vertebrates have both overlapping and separable functions, with major roles in the development of the mesodermally derived axial skeleton and ectodermal derivatives in the nervous system.

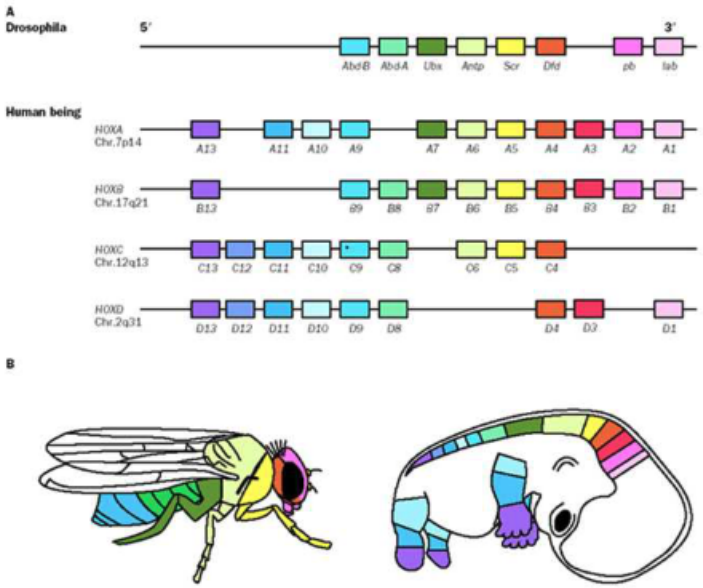

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Figure 4.16—Despite rearrangements, inversions, and duplications, all bilaterally symmetrical animals contain recognizably comparable sets of Hox genes. Whereas the fruit fly, Drosophila, has only a single cluster, mammals, such as mice and humans, have four clusters of Hox genes expressed along the anterior-posterior axis.

One of the most visible aspects of *Hox* function in the nervous system is in the patterning of the r1–r8 rhombomeres (Figure 4.17). Expression boundaries for genes of the *Hoxa* and *Hoxb* clusters correspond to the boundaries between rhombomeres, and experimental manipulation of *Hox* expression can change the fate of the cranial nerves emanating from the altered rhombomeres. In the mesoderm, misexpression or loss of *Hox6* and *Hox10* paralogs can transform cervical and lumbar vertebrae into thoracic-like vertebrae with ectopic rib structures.

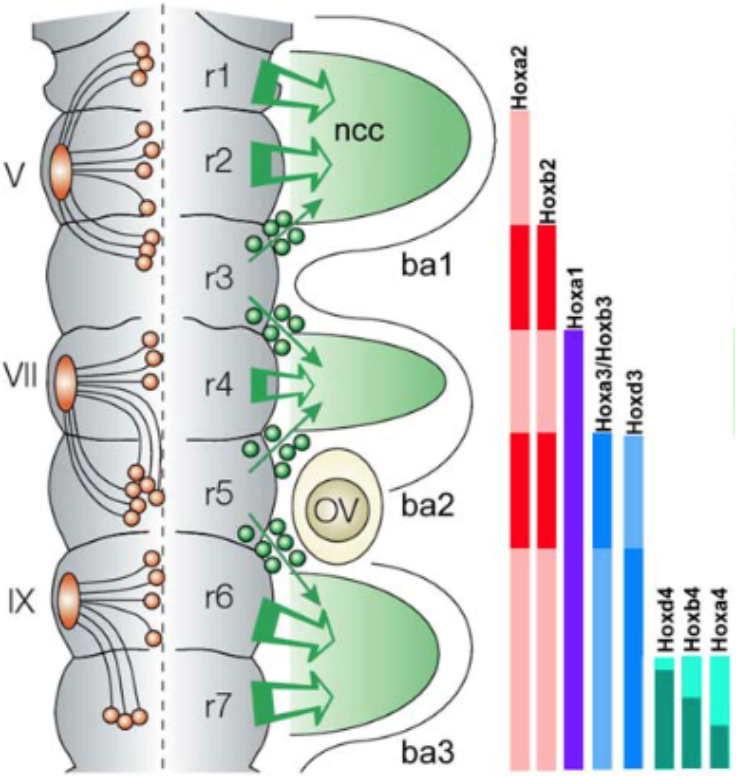

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Figure 4.17—Boundaries of Hox gene expression (colored bars) in the hindbrain correspond to rhombomere (r) boundaries, and each rhombomere expresses a different set of Hox genes to give its distinct identity. Positions of neural crest cells (ncc), branchial arches (ba), branchiomotor nerves (V, VII, IX), and otic vesicle (OV) are indicated.

Despite the largely similar complements of *Hox* genes across vertebrates, changes in precisely where, when, and in what combinations they are expressed may account for some of the observable variation in anterior-posterior morphology. These differences in expression pattern and resulting morphology are a good example of developmental modularity. For example, the

pectoral girdle where bird forelimbs articulate always forms at the boundary between cervical and thoracic vertebrae, but different birds have different numbers of cervical vertebrae and therefore different forelimb positions. Zebrafinch, chicken, and ostrich embryos develop limbs at different axial positions. Zebrafinches, which have the fewest cervical vertebrae, express *Hoxb4* in the smallest domain and for the shortest duration, whereas ostrich embryos (with the most cervical vertebrae) express *Hoxb4* for longer and in a larger region than either zebrafinches or chickens. Furthermore, changing of *Hox* expression on just one side of the body in chicken embryos can shift the limb position with respect to the unmanipulated side, suggesting a causal role for *Hox* expression in limb positioning. Another example comes in the secondary loss of limbs in snakes. Snakes lack forelimbs entirely, though some groups of snakes develop rudimentary hindlimb buds during development, which in some cases are retained as vestigial adult structures. *Hox* expression associated with thoracic development in other groups is expanded in python embryos, suggesting that the snake axial skeleton takes on a riblike thoracic identity, eliminating the domains from which limbs would arise, preventing forelimb development entirely and minimizing the hindlimb development that can occur.

4.8 Limb Development

The diversity of functions and morphologies for limb structures within the vertebrates makes limb development a fascinating system for the study of pattern formation, development, and evolution. We have already seen how changes in *Hox* expression are correlated with evolutionary changes in limb position along the body in tetrapods. We will consider in this section major mechanisms for patterning within the limbs and look at an example of how variations in limb developmental mechanisms contribute to evolutionary variation.

Most vertebrate limbs have differences along three discrete developmental axes: proximal-distal (from closest to furthest from the trunk), anterior-posterior (digit 1 [e.g., thumb] to digit 5 [e.g., little finger]), and dorsal-ventral (from top to bottom [e.g., paw pads on a dog or cat]). The specific mechanisms for each axis are distinct, but all follow the familiar model of inductive signaling interactions and changes in gene expression that produce different outcomes in other body regions. And although we will focus on one axis at a time, they are highly integrated in the ways they interact with one another to coordinate the final shape of the vertebrate limb.

Proximal-Distal

The close-to-far differentiation of limbs is easily observed in tetrapods, which have a general skeletal pattern of "one bone, two bones, many bones" (corresponding to the zeugopod [humerus], stylopod [radius/ulna], and autopod [wrist/digits] regions) that itself is presaged in limbs of transitional Devonian-era tetrapods like *Tiktaalik* (Figure 4.18).

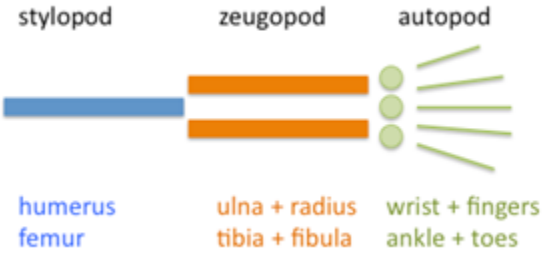

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Figure 4.18—Vertebrate limbs have a standard, three-part arrangement.

Initial development of the limb bud (Figure 4.19) occurs as an outgrowth of the somatic mesoderm, covered by a thin layer of ectoderm. A raised portion of the ectoderm, referred to as the apical ectodermal ridge (AER), is essential for continued outgrowth of the limb bud. Removal of the AER early results in truncation of the entire limb, but removal at progressively later stages allows formation of progressively more distal limb structures. Replacement of the AER with a bead releasing the growth factor FGF (fibroblast growth factor) can restore limb development, indicating a key role for this signaling molecule in the process of proximal-distal patterning.

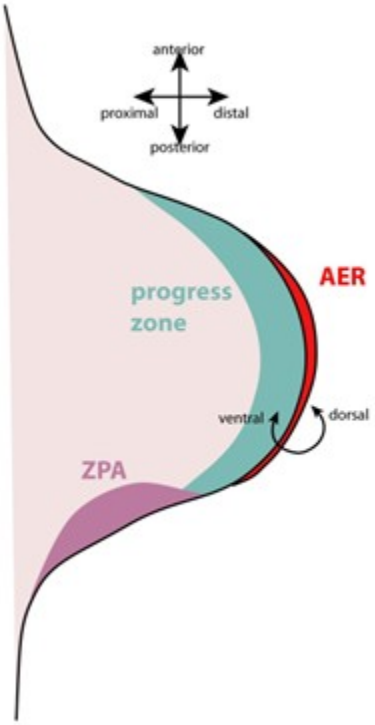

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Figure 4.19—Developing vertebrate limb bud, with apical ectodermal ridge (red), zone of polarizing activity (purple), and progress zone (blue-green) indicated.

One model, called the "progress zone model," for how this FGF signal might be translated into distinct limb elements suggested that the length of time a cell resided in the population of rapidly dividing mesoderm near the AER (and therefore, responding to the FGF signal) would establish its proximal-distal (P-D) fate. However, more recent evidence suggests that a combination of a proximal retinoic acid signal and the distal FGF signal from the AER may establish P-D fate early, followed by outgrowth of the limb bud tissue to form the different P-D regions.

A reciprocal signaling interaction from the mesoderm to the ectoderm is also critical, as removing the mesodermal progress zone stops limb development, and transplanting the leg progress zone into a developing wing bud in chickens will cause the bud to produce leg structures.

Anterior-Posterior

The proliferation of acronyms continues as we consider the anterior-posterior (A-P) patterning of the limbs. A small region of mesodermal tissue at the posterior margin of the limb bud, called the zone of polarizing activity (ZPA), releases the sonic hedgehog (Shh) signal (Figure 4.19). Either transplanting a second ZPA into the anterior of the limb bud or adding Shh can cause a mirror-image duplication of the digits. Genetic mutations associated with polydactyly in cats, mice, and human families cause Shh to be switched on inappropriately at the anterior margin of the limb bud, replicating the digit duplications observed by experimental manipulation.

Positive feedback between the AER and ZPA is critical to maintain proper growth of the limb. The BMP proteins we saw earlier during nervous system patterning also act here, signaling to inhibit FGF in the AER. However, Shh from the ZPA blocks the action of the BMPs, allowing continued FGF expression; that FGF, in

turn, enhances expression of Shh. This type of feedback loop helps coordinate proper formation of the limb. In fact, in both pythons and dolphins, hindlimb buds begin to develop, but one or more aspects of the AER-ZPA signaling loop appear to be defective, leading to adult animals that completely lack hindlimbs.

Dorsal-Ventral

The AER forms at the border between dorsal and ventral ectoderm in the developing limb bud, so the establishment of the dorsal and ventral compartments is also critical for proximal-distal patterning and outgrowth of the limb. The ventral ectoderm expresses a gene called *en1*, which is induced by signaling from the neighboring lateral somatopleuric mesoderm. This gene is not activated in the dorsal ectoderm, and the populations of expressing and nonexpressing cells are present and separate even before the limb bud itself is visible. Beyond positioning the AER, *en1* also acts in later portions of dorsal-ventral patterning by regulating the expression of later target genes that are directly involved in aspects of limb determination.

Evolutionary Modification of Limbs

Vertebrate limbs are highly variable—the range of forelimb structures, for example, includes the "arm wings" of birds and the "finger wings" of bats, the paddle-like flippers of whales and dolphins, and the shovel-like paws of moles, as well as our own finely articulated grasping hands. Given the shared similarity in the underlying bones and the underlying developmental axes, the vertebrate limb provides an intriguing model for examining how

changes in development can generate differences in final limb structures.

Some of the most stunning limb modifications in mammals are the dramatic elongation of the fingers and expansion of interdigital membranes that allow powered flight in Chiroptera (bats). Comparing development in bats and mice has been fruitful in identifying processes that vary in the two groups and likely contribute to the formation of the specialized bat wing. Importantly, limb buds in both groups initiate their development virtually identically, and the gene sequences of the limb development factors in both groups are either nearly or absolutely identical. These observations further support a model in which relatively subtle changes during development, often in when, where, and at what level a gene is active, are capable of producing substantial differences in final outcomes.

For example, levels of the BMPs, which we have seen in multiple contexts, are higher in developing bat limbs than developing mouse limbs (Figure 4.20). Growing limbs from either animal in artificial culture media with added BMP also causes greater limb length, adding a causative effect to the correlation observed for expression. Similarly, the gene *Prx1* has a higher expression level in bat limbs than mouse limbs. The DNA regulatory sequences that control this gene expression have differences between bats and mice, and substituting the bat sequence into the mouse genome causes those mice to develop longer limbs, suggesting that the DNA differences may have contributed to bat forelimb elongation. Beyond the elongated bones of the fingers, another notable modification in bats is the retention of the membranous webbing between the digits. Our good friend BMP makes another appearance here, though in this instance, BMP causes the interdigital cells to undergo apoptotic cell death, and it is the novel presence of the Gremlin BMP-inhibitor protein in bats that allows the webbing to survive.

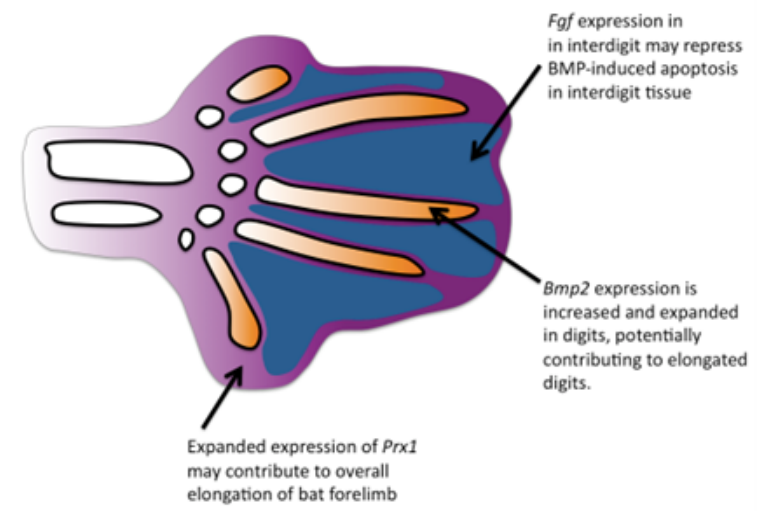

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Figure 4.20—Gene expression in the developing bat wing, indicating key expression changes that are thought to contribute to the elongation of forelimb, digits, and expansion of webbing.

4.9 Summary

Vertebrate development is a highly dynamic process involving dramatic cell movements and careful coordination between regions via signaling pathways that are used repeatedly across different contexts, ultimately resulting in cells, tissues, and organs with very different physical properties from one another. This chapter has emphasized the shared developmental processes that generate the different basic regions and structures of vertebrates, and we will see throughout the remainder of the book how those physical properties affect overall structure and function of vertebrate anatomical systems.

Application Questions

1. In Figure 4.8, we see a diagram of the fates of cells within the inner cell mass. Beyond simple observation, what other approaches might we use to identify the developmental fates of these cells?
2. Signaling molecules like Shh are important at multiple stages and in various tissues during vertebrate development. Describe how a single molecule such as Shh might be able to have so many different effects in different contexts.
3. We introduced Chapter 4 with Lewis Wolpert's quote about gastrulation. Based on the information from this chapter, why do you think Wolpert made this statement? In what ways do you agree or disagree with this statement?

Box 4.1—Cell Death in the Feet of Waterfowl

One common adaptation in waterbirds is the presence of partial or full webbing in the foot to aid in water-based locomotion, as we might commonly envision in ducks. But birds not adapted to water do not typically have this feature, and the lack of webbing appears to be

the ancestral character state for birds. How did the webbing get retained in particular lineages of birds, and how do we think of this difference in terms of cell fates? In other vertebrates without webbing, interdigital cells undergo programmed cell death, and so well-separated fingers and toes are partially sculpted not just by growth and bone condensation but also by the self-destruction of the cells in between digits. In birds with webbed feet, the fate of those cells is changed—they must survive instead of dying. Across birds with various levels of webbing, we see one common feature that may help cells opt for this new fate of surviving instead of dying. During development, cells in the feet of birds with webbing express a gene called *Gremlin1* that is not switched on in the feet of birds without webbing, such as quail and chickens. The activation of this new factor appears to be enough for the cell to choose to live instead of to self-destruct.

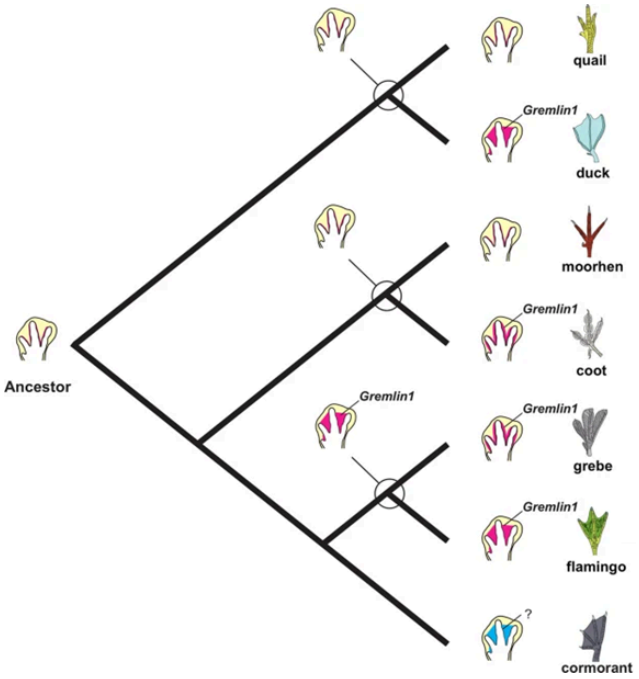

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Figure 4.21—Higher expression of the Gremlin1 gene correlates with greater amounts of webbing in waterfowl.

Box 4.2—Ethel Browne, Hilde Mangold, and the Organizer Concept

The history of the organizer concept in

developmental biology demonstrates how circumstances and personalities can puncture the myth of scientific objectivity that often accompanies tales of scientific discovery. As we will see, the conventional narrative of the organizer importantly leaves out critical individuals and experiments.

In the standard narrative, Hans Spemann, a prominent developmental biologist at the University of Freiberg in Germany, was awarded a Nobel Prize in 1935 for the discovery of the organizer, the tiny chunk of tissue (subsequently named after him) that promotes formation of a duplicate axis when transplanted from one embryo into another. In 1918, Spemann had published experiments describing transplantations from early and late gastrula stages, demonstrating that early cells were not yet determined in their fates but that during gastrulation cells did commit to particular fates. These experiments set the stage for the organizer experiments, but Spemann himself actually did not perform those experiments for which the Nobel Prize was awarded.

Instead, his graduate student Hilde Mangold used transplantation techniques originally developed by Spemann (including using a tiny glass needle to carefully carve cells from a developing embryo and a loop of human hair to gently move the embryos and transplants from dish to dish) to transfer the dorsal blastopore lip region between pigmented and unpigmented newts to be able to distinguish native from transplanted tissue. Over two years of experiments, she completed over 200 transplantations, but only results from six embryos were

ever published to demonstrate the stunning outcome of a newt embryo with two distinct and properly patterned axes, involving both transplanted and host tissue (Figure 4.22).

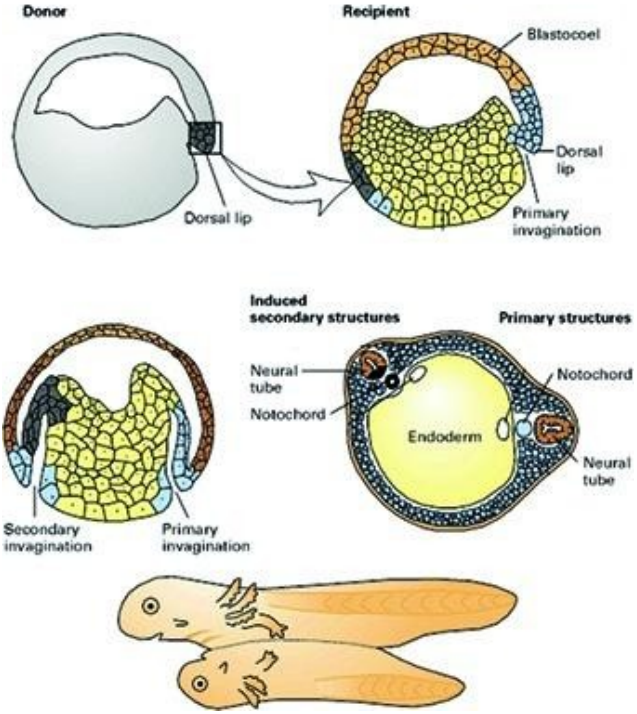

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Figure 4.22—Mangold's transplantation experiments generated newt embryos with duplicated axes, demonstrating the concept of induction.

The first successful embryo was added as a

postscript to a 1921 publication of Spemann that also introduced and defined the "organizer" terminology. The full set of experiments was submitted in 1923 and published in 1924, with Spemann's name listed first. Viktor Hamburger, a graduate student who joined Spemann's laboratory at the same time as Mangold and who himself later became one of the most remarkable developmental biologists of the 20th century, described in his memoirs Mangold's reaction to that arrangement: "[Mangold] was not happy that Spemann had added his name to her thesis publication, while Holtfreter and I and all the rest of us saw ourselves proudly in print as sole authors. Moreover, Spemann had insisted on having his name precede hers! But Spemann was perfectly right in claiming precedence, while she apparently did not fully realize the significance of her results." The basis on which Hamburger makes these claims is not obvious, and clearly Spemann had not insisted on primary authorship (or any form of coauthorship) for the work of any of his other graduate students, who were all men. Tragically, the question of whether Mangold should have shared in the Nobel Prize never arose because Mangold died from severe burns suffered from an oven fire in 1924, and the Nobel committee does not award posthumous prizes.

But the trail of omissions in the organizer story appears to have yet another link to consider. Before Mangold began the newt experiments, Spemann had asked her to replicate the 18th-century experiments of French naturalist Arthur Tremblay on inverting the *Hydra* freshwater cnidarian to investigate the

interchangeability of inner and outer layers of cells. Ultimately, neither Mangold nor Spemann could repeat this technically challenging experiment (the *Hydra* would inevitably uncurl itself, undoing the inversion), so Spemann eventually let Mangold shift to her work on the newts. But if all the other students in Spemann's lab were focused on newts, why might he assign a student to instead pursue work in a completely different (and nonvertebrate!) system?

An intriguing possibility is the potential influence of a 1909 paper by Ethel Browne on induction in *Hydra*. Browne transplanted unpigmented hypostome tissue (from the mouth region of the *Hydra* polyp) to the flank of a pigmented host individual. She then observed that the unpigmented tissue induced the host tissue to form a completely new secondary axis. The similarities between Browne's and Mangold's experiments are striking: the use of pigmented and unpigmented tissues; the formation of a new axis following transplantation; and the reorganization of host tissue, not just growth of the transplant, to generate the new axis.

Browne did not use the term *organizer* in her publication, but her experiments clearly describe a phenomenon closely resembling the organizer from the Spemann and Mangold paper. Curiously, that paper does not cite Browne's work (nor do any other of Spemann's published papers). Did Spemann perhaps not know Browne's work with *Hydra*? Notably, developmental biologist Howard Lenhoff described in 1991 a reprint of Browne's work in a collection of Spemann's papers—a reprint with only the single phrase "induced the

formation of" underlined and an exclamation point in the margin. While it cannot be definitively established that it was Spemann who read and marked the paper, its presence in Spemann's papers coupled with his directive that Mangold start her thesis work with *Hydra* suggests he likely had some familiarity with the topic.

Instead of a narrative throughline from Ethel Browne to Hilde Mangold to the organizer concept of Hans Spemann's Nobel Prize, we instead typically hear a much-truncated version that emphasizes solitary discovery and obscures the experimental and conceptual complexity contributed by two often overlooked women researchers. When considering the history of scientific knowledge, it's critical that we endeavor to look beyond narrow, simplified narratives to better appreciate the full spectrum of contributors, who may have deliberately or inadvertently been left out of those narratives.

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5. Form and Function

KELLY M. DIAMOND AND VANESSA K HILLIARD

Focus Questions—to Guide Your Reading of This Chapter

1. What are the most basic metrics of anatomical forms?
2. How do we quantify function?
3. Why do some anatomical structures grow to different proportions compared to others in the same organism? In different organisms?
4. What are Newton's three laws of motion? How are they relevant to our consideration of functional anatomy?
5. Does form always relate to function?

5.1 Introduction

Oftentimes, learning anatomy is approached with a memorization mindset: There are lists of structures, and one just needs to memorize their names and where they are. However, we find that such an approach robs the study of anatomy of its richness, because

it reduces complex, integrated bodies into stagnant, stand-alone "parts," with little acknowledgment of their roles within the body. By extension, this lack of functional recognition also leaves us with little explanation of the "why" behind anatomical diversity across vertebrate taxa, which (in our opinion) is one of the most fascinating questions in all of biology.

In this chapter, we're going to consider the relationship between form and function and some of the factors that influence this dynamic. We'll first define what we as anatomists mean by *form* and how we measure it. Then we will move on to the important question: What is function? And in addressing this broad question, we'll take a peek into the fields of functional morphology and comparative biomechanics, which aim to explain how bodies work and are deeply rooted in the study of anatomy. Generally speaking, these fields of study seek to understand things like why we switch from walking to running at certain speeds, how flying critters actually fly, and how the structure of different anatomical parts makes things like feeding and locomotion possible in different types of environments. In other words, we start with normal activities of ordinary organisms and ask "how?" and "why?" And in order to answer those questions, we need to draw on aspects of the physical world. In short, this is a field of biology that uses principles of physics and engineering in order to understand how bodies work. There are both advantages and disadvantages when approaching biomechanics and functional morphology. One distinct advantage is our familiarity with the phenomena that we're trying to explain—we experience things like gravity every day—though we may not always be consciously aware of it. And this everyday experience gives us an innate sense of reality when addressing functional questions. However, the downside is that explaining and quantifying the physical world requires us to use tools and draw on concepts that are not often well used (or even present, in some cases) in the standard biologist's intellectual toolbox. So in this chapter, we're going to introduce you to some of those tools and concepts so that you are well equipped to think about anatomy in a functional

context, and we will answer the "how" and "why" questions that naturally arise in the study of comparative anatomy.

5.2 What Is Form and How Do We Measure It?

It might seem obvious that **form** is the anatomy that you are aiming to learn with this book. However, before we dive into our discussion of *function*, we first must discuss how we, as anatomists, define the term *form* and how we measure it. For each of these forms, you will learn more detail in later chapters. Our goal for this section is to introduce the basic building blocks and how they relate to each other.

Anatomical Forms

Vertebrates experience forces that their structural systems, or **anatomical forms**, must withstand. Some of these forces are produced internally by an organism, while other forces are produced outside of the organism, by environmental factors. There are many different types of form-function relationships. For this chapter, we will mainly focus on two categories of form-function relationships: those associated with **locomotion** and those associated with **feeding**.

For both categories, the underlying anatomical form, upon which forces act, is the vertebrate **skeleton**. Since skeletal elements are relatively stiff structures, movement of the skeleton occurs at **joints**, where different skeletal elements come together. Specialized connective tissues, called **ligaments**, connect the skeletal elements together across a joint. The points at which two skeletal elements interact with each other are called **points of articulation**. Normally

you can observe these points on the skeleton because the cartilage or bone is smoother where they **articulate**, or rub together.

Another anatomical element that we need to consider as part of our anatomical form is the **skeletal muscles**, which contract when stimulated by **motor neurons**. Skeletal muscles are connected to skeletal elements via other specialized connective tissues, called **tendons**. As these muscles contract, they will produce forces that act on the tendons, which in turn act on the skeletal elements, causing movement at the joints.

Anatomical Materials

How does the material that a critter is made of impact the way it works? Anatomical materials, or **biomaterials**, differ from architectural materials due to the requirements for anatomical structures to change in size and shape over time while still maintaining their durability.

The beauty of, and the trouble with, bodies is that classifying the stuff an organism is made of can be somewhat difficult. One way to approach this idea is hierarchically, based on chemical composition. But the difficulty with this approach is that chemical composition doesn't necessarily correspond with mechanical properties—and for our purposes, that's not super useful. So instead of classifying things chemically, we'll classify solid materials based on their **mechanical behavior**, which describes how well a material maintains its shape. This way of classifying materials puts biomaterials in one of three categories:

1. **Tensile materials**, which are good at resisting stretching;
2. **Pliant materials**, which are relatively easy to deform but return to their original shape when the load is removed; and
3. **Rigid materials**, which strongly resist deformation and don't return to their original shape after deformation.

But this is still a little abstract. What types of anatomical forms actually represent these three categories of materials? Let's begin with tensile materials. **Tensile materials** function by resisting pulling forces or **tensile stress**. They are, in essence, biological ropes. But even though we can think of these as biological ropes, they aren't always used as ropes and are often found as part of other materials. When we look at living organisms, we find four distinct kinds of tensile materials: silk, collagen, cellulose, and chitin. And since we're concerned with vertebrates and human bodies in the context of this book, collagen is the major one on this list that you'll want to pay attention to. **Collagen** is a protein, and it can be found in fairly pure form in our own tendons (Figure 5.1A). But collagen can be found in lots of other structures as well, often as one element of a **composite material**, materials made up of more than one thing. Some examples of composites that contain collagen include things like skin, bone, muscle, and the walls of blood vessels (most notably arteries).

Pliant materials will deform somewhat when stressed, meaning that they'll stretch or squeeze some, and will return to their original form after the stress is removed. Examples of pliant materials include rubber-like proteins and pliant composites. **Rubber-like proteins** can be drastically distorted and then rapidly return to their original shape. The most familiar of these is probably **elastin** (Figure 5.1B), which can be found in your own skin and arterial walls. Rubber-like proteins are all functionally similar in that they are stretchier than tensile materials. **Pliant composites** are materials that only exist as, well, composites (hence the name), and this is a pretty varied group of stuff. Within the classification of pliant composites, you'll find such diverse materials as the jelly of jellyfish and sea anemones, the cartilages of our ears, nose, and intervertebral disks, our skin and arterial walls, and even the mucus that provides traction for snails and slugs!

The third class of materials is **rigid materials**. These are materials that strongly resist any kind of deformation, and all rigid

materials are composites. Examples of familiar rigid materials include things like bone (Figure 5.1C), keratin, and enamel.

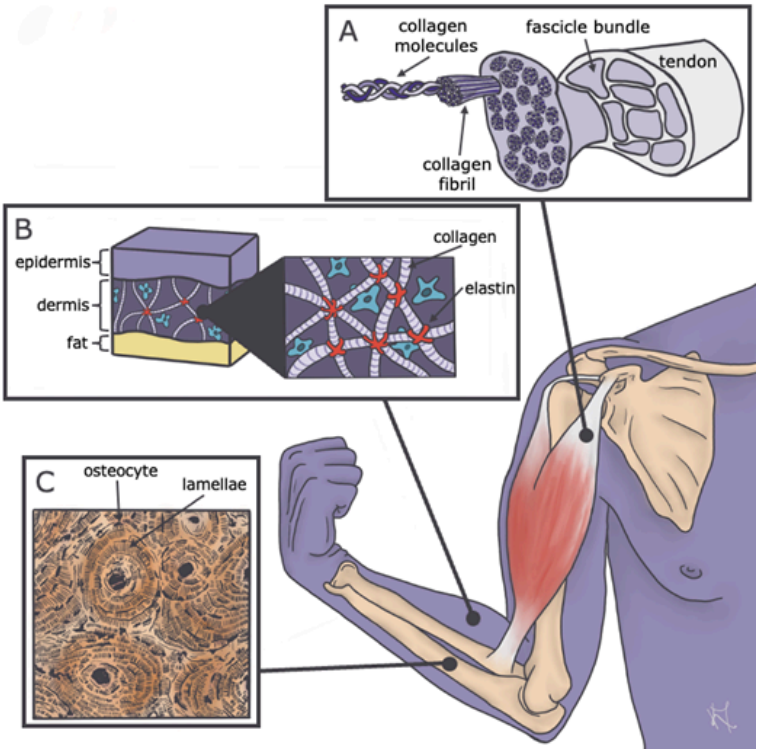

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Figure 5.1—Anatomical examples of the three different categories of biomaterials. Tensile materials (A) resist pulling forces, as seen in the collagen fibers in a muscle tendon. Pliant materials (B) such as the elastin in your skin are easy to deform but bounce back to their original shape when a force is removed. Rigid materials (C) strongly resist any kind of deformation, as seen in bone tissue.

Measuring Form

If we want to quantify the form an organism has, we first need to define some basic physical quantities. We will then use those basic

physical quantities to derive additional physical quantities that tell us more about the form itself and how that form could be expected to function. Finally, we will discuss how we use different reference systems to quantify and compare these different metrics.

Basic Physical Quantities

The two most basic physical qualities that we as anatomists use to define form are length and mass. **Length** (l) refers to the distance between two anatomically defined points. Our second basic physical quantity is **mass** (m), which is the total amount of stuff that makes up an organism. A third basic physical quantity that is not directly related to form, but is used to quantify how forms move, is the quantity of **time** (t), which is used to quantify the duration of a particular event. These three quantities are used to derive additional physical quantities that describe form and associated metrics of function.

Derived Physical Quantities

Our first set of derived physical quantities, which are defined relative to the length of a form, will help us further quantify the form of an organism. We will use a square or cube, with length (l) equal to the length of one side of the cube, as our example form to show how length is used to define each quantity (Figure 5.2). **Area** ($A = l^2$) is the amount of space a two-dimensional object takes up. Moving into three-dimensional space, **volume** ($V = l^3$) is the amount of space that a three-dimensional object takes up. **Surface area** ($SA = 6\ l^2$) is the amount of space covering the outside of an object. The derived quantity that quantifies how much mass is in each unit of volume is **density** ($D = m/V$).

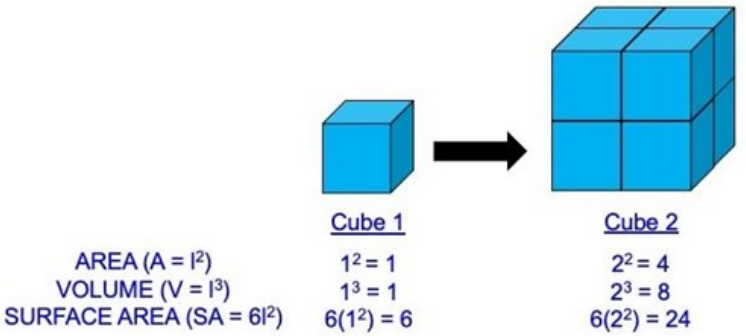

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Figure 5.2—Area, volume, and surface area of two cubes. In this example, the length (l) of Cube 1 has been set to 1, which yields an area of 1, a volume of 1, and a surface area of 6. When we double the size of the cube (l = 2), we see how area increases 4-fold, volume increases 8-fold, and surface area increases 24-fold. So a simple change in length can have a radical effect on other derived physical quantities.

Our next set of physical quantities help us understand the forces associated with movement of an organism or parts of an organism. We can start to get a sense of these relationships by asking questions that help us describe motion. For example, some of the questions we can ask include (Note: This is not an exhaustive list):

- *How hard is it to move the organism*? This is described by **force** (F), calculated as mass (m) times acceleration (a) (F = ma). Force is related to **weight** (w), because weight is simply mass times acceleration due to gravity (often abbreviated as g). In Figure 5.3A, the weight of the sitting cat is the mass of the sitting cat times the acceleration due to gravity. If you wanted to pick up the cat, it would take a force opposing the weight of the cat.
- OK, we have a force, but *how intensely is the force being applied*? We can describe this as either **stress** (σ) if the force is internal, as it would be on a bone when a muscle pulls on it, or

a **pressure** (p) if the force is applied from an external source outside of the structure, such as when the atmospheric pressure changes depending on your altitude. Regardless of how force is applied, stress and pressure both essentially describe the relationship between force and area (force divided by area, F/A, to be precise). Stresses and pressures both cause structures to deform to some degree (Figure 5.3B), and this measure of deformation as a result of stress or pressure is what we call a **strain** (e). Strain is calculated by taking the change in length of the structure and dividing it by the structure's original length.

- *How far does the force move the organism?* This question asks about **work** (W), which is force times distance. The capacity to do work is **energy**!
- *How fast is the organism moving?* Once a strong enough force is applied to an organism, we can start to talk about motion. First we can measure **speed**, which is determined by taking the distance traveled (which is another length) and dividing it by time. In Figure 5.3C, this would be the amount of time it takes the tortoise and the hare to move from the starting line to the finish line, divided by the distance between the starting line and the finish line. We can even take this a step further and describe the direction the organism is moving if we have that information, which would give us the organism's **velocity** (v)—that is, speed in a specific direction. Since the race in Figure 5.3C is only from the starting line to the finish line, we could describe the movement of the tortoise and the hare as their velocity.
- *How fast is velocity changing?* The answer to this question is **acceleration** ($a = v/t$), which is determined by dividing velocity by time. In Figure 5.3C, the tortoise accelerates at the start of the race and decelerates at the end of the race, but in between it maintains a constant speed, or has no acceleration, while the hare accelerates and decelerates with each jump it takes during the race.

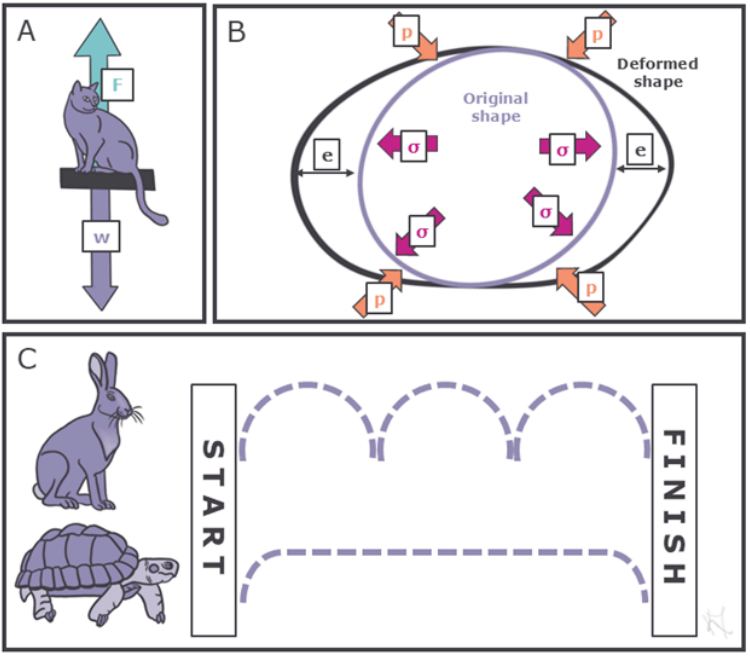

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Figure 5.3—How forces work. Forces must overcome the weight of an object in order to move an object (A). Forces can be applied from internal stressors (σ) or external pressures (p) to cause a deformation of the shape, measured as the strain (e) on the object (B). Forces can cause objects to move, or do work, such as when the tortoise and the hare use energy to race (C), both animals use different velocities (dotted lines) and accelerations as they move from the start to the finish line.

Reference Systems

Now that we've defined different physical quantities, we need to discuss how we compare those physical quantities. For each quantity, there is a unit that is used to standardize how each of these measures is compared. Table 5.1 provides some examples of such standardized units. Many of these units can be plotted to visualize differences between groups or changes over time. To make

these plots, we often use a **Cartesian coordinate system**, where points are plotted in a horizontal (x) axis, vertical (y) axis, and for three-dimensional objects, a third (z) axis that is perpendicular to both the x and y axes.

Table 5.1—Standardized units for form-function quantities

Metric	Abbreviation	Unit
length	l	meter
mass	m	kilogram
time	t	seconds
force	F	Newtons
pressure and stress	P and σ, respectively	Pascal

Before moving on to function, you may have noticed that force keeps emerging as a recurring theme in many of the derived physical quantities listed above. This is because forces are absolutely critical to understanding the motion of organisms. Forces are analyzed as **vectors**, meaning that they have both magnitude and direction. To visualize this, think about how we describe a force: If I try to pick up the cat in Figure 5.3A, I'm exerting a force that must be larger than the weight of the cat in the opposite direction of the weight of the cat. The amount of force I use to pick up the cat is the magnitude or size of the force I'm exerting, and up is the direction in which the force is being applied. But what does this mean if we're trying to understand how organisms or their parts move? How do we figure out how a force will affect a structure? These are the concepts we will cover in our next section on function.

5.3 What Is Function and How Do We Measure It?

So what is "function"? In general, **function** can be defined as how things work, and in the context of comparative anatomy, we can frame this definition specifically as how *organisms* work. And oftentimes function revolves around motion: either making things move or resisting movement. There are four primary, overarching factors that affect organismal function:

1. Size and shape
2. Mechanics
3. Environment
4. Phylogeny/evolution

The last of these—phylogeny—is largely covered elsewhere in this book (e.g., in the nonvertebrate chordate in Chapter 2 and to a certain degree in each of the anatomical systems chapters), so we won't spend much time on that particular factor here. For the remaining three factors, we will explain basic concepts here, and then in our final section of this chapter, we will discuss how these concepts are used to study form-function relationships in the real world.

Consequences of Size and Shape

For every type of animal there is a most

> convenient size, and a large change in size
> inevitably carries with it a change of form.
>
> —Haldane, 1927

The quote above eloquently describes how as the size of an organism changes, the demands on different body parts change disproportionately. In anatomy, we describe this as differences in **scaling**. All vertebrates are three-dimensional beings, meaning that they have length, width, and height.

All vertebrates also change body size, either through individual growth or as changes among species through the course of evolution.

Isometric Growth

Let's consider growth first, which involves dimensional change. As the individual grows, each of the dimensions (i.e., length, width, height) can change in equal proportion. This type of increase would produce animals that are **geometrically similar** and represent what we refer to as **isometric growth** ("iso" = same, "metre" = measure or, in this case, dimension).

This can be a little bit abstract to wrap one's mind around, so to help illustrate this concept in a simplified manner, we're going to examine a cube-shaped organism and see how things change as it undergoes isometric growth (Figure 5.4). So let's begin with our cube-shaped organism, and we'll define its anatomy as 1 cm in length, width, and height. Now if our animal grows twice as large, it will double each of these dimensions, so length, width, and height

each become 2 cm. So far, so good. *But what does this change in length do to the organism's surface area and volume*? Well, length is twice as large after growth, but area is four times larger and volume is eight times larger! What this demonstrates is that a doubling in length doesn't just double the area and volume—it actually produces a fourfold increase in area and an eightfold increase in volume (which can be equated to an eightfold increase in mass). These are *very big* changes. But aside from a cat sitting in a cube-shaped box, are there really any cube-critters out there? How does this translate to living organisms? Well, this pattern holds true for other shapes as well, not just cubes; as you can see in Figure 5.4, dimensions change for spheres and cylinders (which are much more realistic approximations of vertebrate body shapes—most mammal bodies tend to be spherical, whereas fish, amphibian, and reptile body shapes tend to be cylindrical).

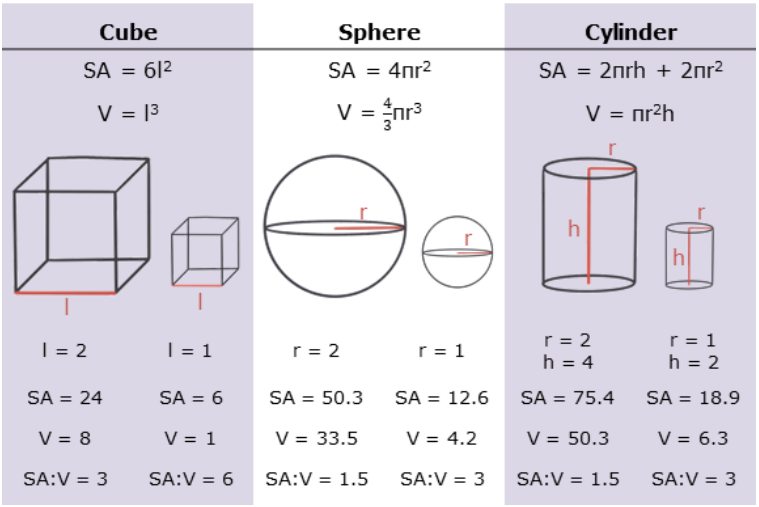

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Figure 5.4—Scaling relationships between size (l = length, r = radius, h = height), surface area (SA), and volume (V), in 3D cubes, spheres, and cylinders.

Even in these simple forms, the **surface-area-to-volume**

ratio as depicted in Figure 5.4 has some pretty major consequences for function. For example, in endothermic (self-heating) vertebrates, heat loss scales with surface area, but heat generation scales with volume. What this means is that smaller animals need a relatively higher metabolism and to eat relatively more food, to maintain their body temperature compared to larger animals, because their surface-area-to-volume ratio means they lose more heat to their environment. We can also see how the surface-area-to-volume ratio interacts with environmental variables by looking at body shapes of animals that live in cold versus warm climates. In cold habitats, endotherms want to maximize heat production (volume) and minimize heat loss (surface area), so they tend to be more sphere-shaped with shorter extremities to maximize their surface-area-to-volume ratio—for example, the snowshoe hare (*Lepus americanus*) seen in Figure 5.5. Whereas in warmer climates, endothermic animals tend to be less sphere-shaped with longer extremities to maximize heat loss. Note the relatively longer limbs and ears in the jackrabbit (*Lepus californicus*) compared to the snowshoe hare in Figure 5.5.



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Figure 5.5—Surface-area-to-volume ratios in the wild. Snowshoe hares (left) live in colder climates and are more spherical to maximize their volume and minimize their surface area, while jackrabbits (right) live in warmer climates and have proportionally longer limbs to maximize their surface area, allowing for greater heat loss.

Allometric Growth

The surface-area-to-volume ratio is one driver that can cause different parts of an animal to grow at proportionally different rates through development or over evolutionary time. Now we're going to focus on an example that shows how changes in proportion affect the way in which the skeleton supports body mass. Consider a bone in the leg. If this bone is loaded and the stress of that load becomes too high, the bone will break. If an animal increases in size isometrically, how will the stress that results from an increase in size change as animals get bigger? We know that force is proportional to mass (F = ma) and that the acceleration in this equation is acceleration due to gravity. So limb bone stress should increase as animals get bigger, because stress = force divided by area (stress = F/A).

In fact, there was a time when researchers used this relationship to predict a maximum size for animals using limbs. However, organisms have found a way around the maximum size limitations imposed by isometric growth. Indeed, animals can also change shape as they grow or through the course of evolution—and this change in shape alongside a change in size is called **allometry**. In other words, if isometry is growth *with* geometric similarity, then allometry is growth *without* geometric similarity.

For example, if we were to consider our leg bone example again, what if the diameter of the leg bone increased twice as quickly as the length? If this happened, the bone would become relatively thicker with increasing size, thereby increasing the cross-sectional area of the bone. Because we know that stress = F/A, an increase in cross-section area actually helps limit increases in stress as the animals get bigger. And we can evaluate whether animals show allometry by simply measuring their dimensions, plotting the relationship, and calculating the slope of the plot. For isometric length-mass relationships, we'd expect the slope of the line to be 1/3. If the slope of the line is greater than 1/3, that tells us that

the diameter of the bone is increasing faster than body mass and therefore is helping to accommodate increases in stress. In contrast, if the slope is less than 1/3, that means that body mass is increasing at a faster rate than cross-sectional area, and in this situation, an animal can be in pretty big trouble (because mass will quickly exceed the limits of the bone and lead to structural failure). These relationships help to explain why people don't grow indefinitely and why we don't see humans 50 feet tall walking around and terrorizing major cities (as depicted in some 1950s horror flicks and even the 1990s movie *Honey, I Blew Up the Kid*; see Box 5.1).

Box 5.1—Allometry of Movie Monsters

Why can't 50-foot-tall movie monsters exist? Thankfully, the reason we don't have to avoid the Godzillas and King Kongs of the movies is because for each unit increase in length or height, an animal's or human's body volume increases eightfold (Figure 5.4). But recall that bone cross-sectional area increases like an area, so for each increase in length, it only increases fourfold (assuming no change in shape). What this means is that if we take a 5-ft-tall person and turn them into a 50-ft-tall person with no other change in body proportions, that individual will be 10 times taller, their bone cross-sectional area will be 100 times larger, and their mass will be 1,000 times larger. And on the surface, these values may appear OK, but what this really means is that this kind of growth would put 10 times more stress on the person's bones than the bones are actually capable of withstanding! In essence, as animals get bigger, forces on bones increase faster than the body's

ability to resist forces, *unless proportions of the bones change* (i.e., unless there's allometric growth).

Question to think on: Using the graph provided in Figure 5.6, what do you think could be the maximum size of our hypothetical "movie monster"?

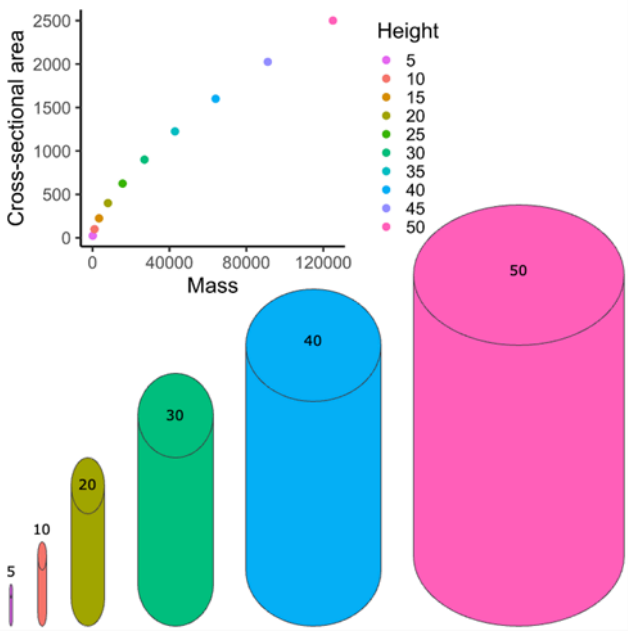

Figure 5.6—Allometry of movie monster bones. The graph shows the relationship between body mass and bone cross-sectional area. The points in the graph are color coded for movie monster height. The cylinders below the graph show a visual relationship between height and diameter for several of the color-coded points on the graph. The numbers above each cylinder represent the represented height.

Consequences of Mechanics

The field of **biomechanics** applies the physical principles of mechanics to the living world. We will start off this section by reviewing **Newton's laws of motion**. Then we will use those laws to describe the simplest type of machine, the **lever system**. We will then discuss how lever systems are **loaded** with different forces to cause movements, a field of biomechanics called **kinematics**.

Newton's Laws of Motion

- **The law of unbalanced forces**. This law tells us that a body at rest stays at rest or a body in uniform motion in a straight line stays in uniform motion in a straight line unless a force is applied to it. In other words, things that are still stay still, and things that are moving stay moving unless an outside force acts upon them.
    - In Figure 5.7, if neither the triceps muscle nor biceps muscle is shortening, then the arm will remain at rest. If either muscle is shortening, then the forearm will move in the direction that the muscle pulls it until the muscle stops shortening.
- **The law of acceleration (F = ma)**. This one should look familiar—it's the same definition we provided for force earlier in this chapter! What this law tells us is that acceleration is proportional and in the same direction as the applied force.
    - In Figure 5.7, the force that either the triceps or biceps muscle can produce is equal to the mass of the muscle and the rate at which that muscle can shorten to cause the extension or flexion of the forearm.
- **The law of action and reaction**. This law tells us that when one body exerts a force on another, the second always exerts a

force back on the first in the form of a **reaction force**. What's more—these two forces are equal in magnitude, opposite in direction, and act along the same line.

- This law explains how the musculoskeletal system works to allow our bodies to move. When the biceps muscle contracts (Figure 5.7), the muscle is exerting a force on the radius bone in the forearm. The radius and the rest of the connected forearm react to that force by flexing the forearm at a rate proportional to the force produced by the biceps.
- It's worth mentioning here that this third law tells us that forces are still exerted on an object even if the object doesn't move! If in Figure 5.7 the person was trying to pick something up that was extremely heavy—say, a 1,000-lb dumbbell—the person's bicep would still be exerting a force (F_b) on the dumbbell, and the dumbbell would still be exerting a force on the muscle, even though neither would be moving! In this situation, the forces are in balance, and the system is said to be in **equilibrium**.

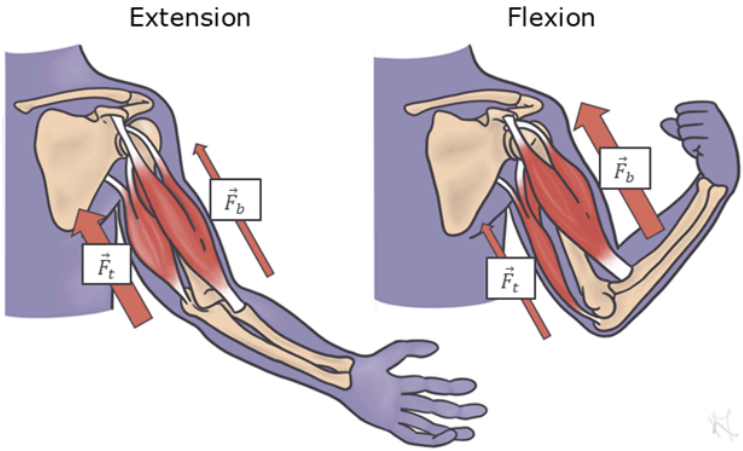

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Figure 5.7—Newton's laws of motion applied to muscle movements. The force of the triceps muscle (Ft) controls the extension of the forearm, and the force of the biceps muscle (Fb) controls the flexion of the forearm.

Lever Systems

In order to apply these laws of motion, we need to next think about how anatomical forms work together to create motion. In moving parts of the body, what we often observe are bones and muscles working together as **lever systems**. Now thinking of parts of the body in terms of simple machines may seem a little strange at first, but in fact, relating biological systems to familiar, simple machines can actually be quite helpful in understanding how they work. So before getting into specific lever examples of the vertebrate musculoskeletal system, we first need to introduce a basic lever, its parts, and how it works.

We'll begin by considering a teeter-totter (or seesaw, if you will). This familiar playground toy is the perfect reference, because it is simply a big lever (Figure 5.8A). The two benches are the **lever**

arms, or the components of the lever where force enters the system and exits the system (in the form of motion); the hinge, or the point about which the lever arms pivot, is called the **fulcrum**. On one side of the fulcrum, force will be loaded via the **in-lever arm** (L_{in}), and on the other side of the fulcrum, force will be output via the **out-lever arm** (L_{out}). The force that the movement of the L_{out} in general works to move an opposing force called a **load**, but more on that in the next section.

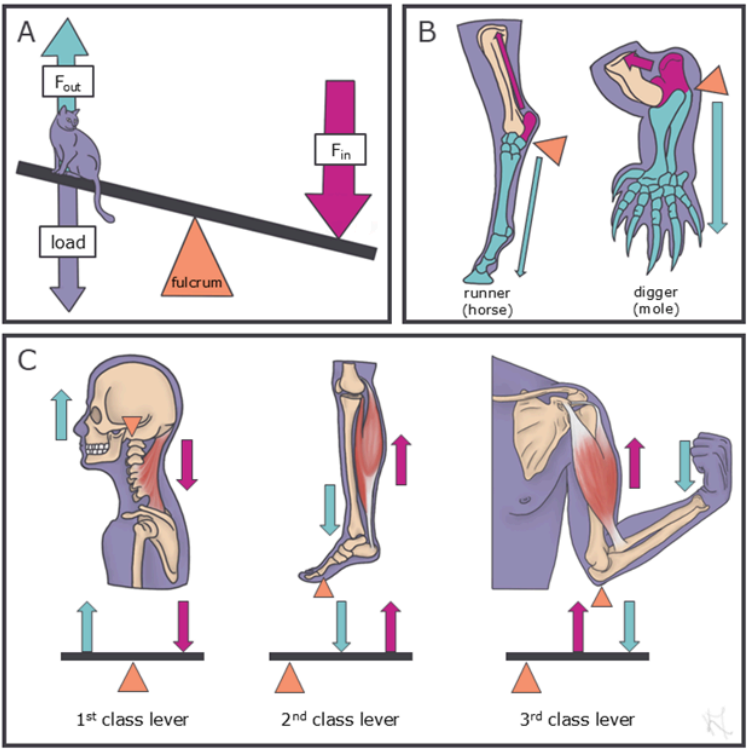

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Figure 5.8—Lever systems. A basic lever system (A) shows the fulcrum (orange), separating the two lever arms, with the in-lever on the same side as the input force (Fin in pink), the out-lever on the same side as the output force (Fout in blue), and the loading force represented by a cat here in purple. Colors are consistent throughout the figure. A comparison of the lever system of a runner versus digger forelimb (B). The three classes of lever systems with biological and basic diagram examples (C).

Let's bring back Newton's third law here—for every action, there is an equal and opposite reaction. What this means is that the forces (F) transmitted through lever arms (L) can be expressed as

$$F_{in} \times L_{in} = F_{out} \times L_{out}$$

This also means the relative lengths of the lever arms of the lever system can impact whether a lever is built for strength or built for speed. When the L_{in} is relatively long compared to the L_{out}, like it is in a crowbar, a lever can move a large load with relatively little force input; these levers have a **mechanical advantage** (Figure 5.8B). When the L_{in} is relatively short compared to the L_{out}, a lever will require more force, but it can move a load a given distance quickly; these levers have a **velocity advantage** (Figure 5.8B). So velocity (V) and lever arm length (L) have an inverse relationship, which can be expressed as

$$L_{in} \times V_{out} = L_{out} \times V_{in}.$$

But why are levers important in the body? Let's consider our lower leg and the calf muscle as an example (Figure 5.8C). The muscle that pulls on the back of the foot when it contracts (i.e., gastrocnemius) is inputting force (F_{in}), causing the foot to extend. Notice here that the foot actually has a small posterior extension—this is the heel bone (i.e., calcaneus) where the tendon for the calf muscle attaches. What this means is that the force from the muscle is actually acting at a distance from the ankle joint rather than on the ankle joint directly. Any force acting a given distance from a joint will tend to cause **rotation** about that joint, so the joint becomes a **point of rotation**. The distance between where the force is acting and the joint that is rotating is called the **moment arm** (r), which is the same thing as a lever arm. The process of a force acting at a distance from a point is called a **moment** and can be calculated by $M = r \times F$. So when the calf muscle contracts, it will pull on the heel and cause rotation about the ankle—this is what's happening in your lower leg when you point your toes (or really any time you extend your ankle)!

Animals that move in different ways through their habits tend to have skeletons with different lengths of lever arms. For

instance, if we compare the elbow joints of animals that dig versus animals that run, we can see how the lever system of the elbow links form and function (Figure 5.8B). Looking first at diggers, they tend to have relatively long in-levers at the elbow (i.e., the in-lever is long compared to the length of the out-lever) in order to confer mechanical advantage, whereas runners have relatively long out-levers in order to confer velocity advantage (i.e., the in-lever is short compared to the length of the out-lever). This is because digging limbs need force, whereas running limbs need velocity.

There are a few different classes of levers, each distinguished by the relative position of the fulcrum and the load (Figure 5.8C). **First-class levers** are those in which the fulcrum is located between the point of applied force and the load to be moved; as such, the direction in which force is applied is opposite to the direction of motion produced. Your neck joint, when involved in lifting the chin, can be classified as a first-class lever, because the Splenius capitis muscle originates on the vertebrae and functions to pull the occipital region of the skull dorsally. In a **second-class lever**, the force application and direction of motion are on the same side of the fulcrum, which is located far from the applied force, with motion produced between the force and fulcrum. An example of a second-class lever would be your foot as you rise up on tiptoe. **Third-class levers** also have force application and produce motion on the same side of the fulcrum, but this lever is distinguishable from second-class levers in that the force is closer to the fulcrum that the movement produced. An example of a third-class lever in the body is the action of a biceps curl (i.e., elbow flexion).

Loads

At this point, we've mentioned the concept of "loading" a couple times, but we haven't really explained what loading is yet. A **loading regime** (Figure 5.9) is the way in which a force or set of forces is

applied to a structure. Bringing Newton back into the discussion, in general, when a load is applied to a structure, the structure will either resist movement (Law 1) or move (Laws 1–3). Loads can be applied to structures in several different ways. Tensile loads, or when a load is applied in **tension**, are loads that result in pulling along the long axis of a structure. This will result in the lengthening and narrowing of the structure's material (to help you visualize, think about what happens when you stretch a rubber band). **Compression** is the opposite of tension; this is a loading regime in which forces applied in opposite directions produce a squashing load along the axis of a structure; this results in a shortening and widening of the material. **Shear** is a loading regime produced when there are two opposing parallel forces, but they are not directly opposite each other. This causes one part of the material to slide relative to another part and separate. Shear is produced, for example, when cutting with scissors: The two blades slide past one another, generating parallel, opposing forces that cause the paper to separate. **Torsion**, or twisting, is caused by rotational forces that cause the material to twist. **Bending** is the result of the interaction of tensile and compressive loads. If tension is applied on one side and compression on the other, the structure will bend.

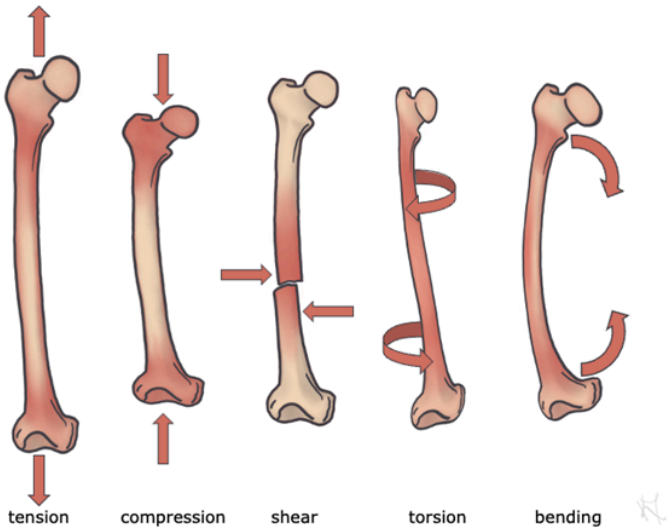

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Figure 5.9—Loading regimes of femur bone. Arrows represent the direction of force for each loading regime listed below each bone. Red highlighting shows where the force is highest on the bone for each loading regime.

Now we can discuss in more detail how the material and shape an anatomical structure has can determine what type of loads it can resist. For example, support structures such as limb bones are often good at resisting axial tension (i.e., tension applied along the long axis of the bone) and compression but are less strong in bending. Materials may be **isotropic**, where mechanical properties do not depend on the direction of the load, or **anisotropic**, where the material properties do depend on the direction of the load. We bring this up now, because rigid biological materials (in contrast to human-manufactured materials) are all anisotropic, meaning that their mechanical responses depend very much on the directions in which they're loaded, as you can see in Figure 5.9.

When we apply loads to solids, this produces **deformation**. And if we apply a known force and measure the resulting

deformation, this allows us to quantify the biological properties of a given material. As you already know, size can impact the way forces affect a structure, so to standardize for differences in size among specimens, forces are converted to stress (recall: **stress** = force / area), and deformation is converted to strain (recall: **strain** = change in length/original length). And if we were to plot these values, the resulting **stress-strain curve** allows measurement of several properties.

Let's take a look at a generic stress-strain curve as an example to see what kinds of information we can get from it. The maximum stress that an object can withstand before failing is what is formally defined as the **strength** of the object; similarly, the maximum amount an object can be deformed (e.g., stretched, squashed, etc.) is the **extensibility** or **failure strain** of the object. But this raises the issue of how we define "failure." If you look at Figure 5.10A, you'll see that there are two potential failure events. At the far end is **fracture**; this is where the item breaks, and we can pretty confidently identify that as an obvious failure of the structure. But notice the shape of the curve—there is an earlier change in slope that occurs well before fracture (in this example). Along the initial linear portion of the plot, when the load is removed, the material will rebound to its original shape. After the point at which the line's slope changes, though, the structure has been damaged and is no longer able to rebound to its original shape when the load is removed. This change in slope represents something called **yield**, and since yield indicates substantial damage (i.e., an inability to return to the original shape), it is also considered to be an indication of structural failure. If we look again at the slope of the initial linear part of the plot, we find that it shows how much a structure deforms for a given amount of stress, thereby indicating the **stiffness** of the material. Essentially, stiffness represents how well the material resists deformation. Finally, we can use the stress-strain curve to measure **toughness**, which we can think of as the amount of work an object will absorb before it breaks. Toughness is represented by the area under the stress-strain curve.

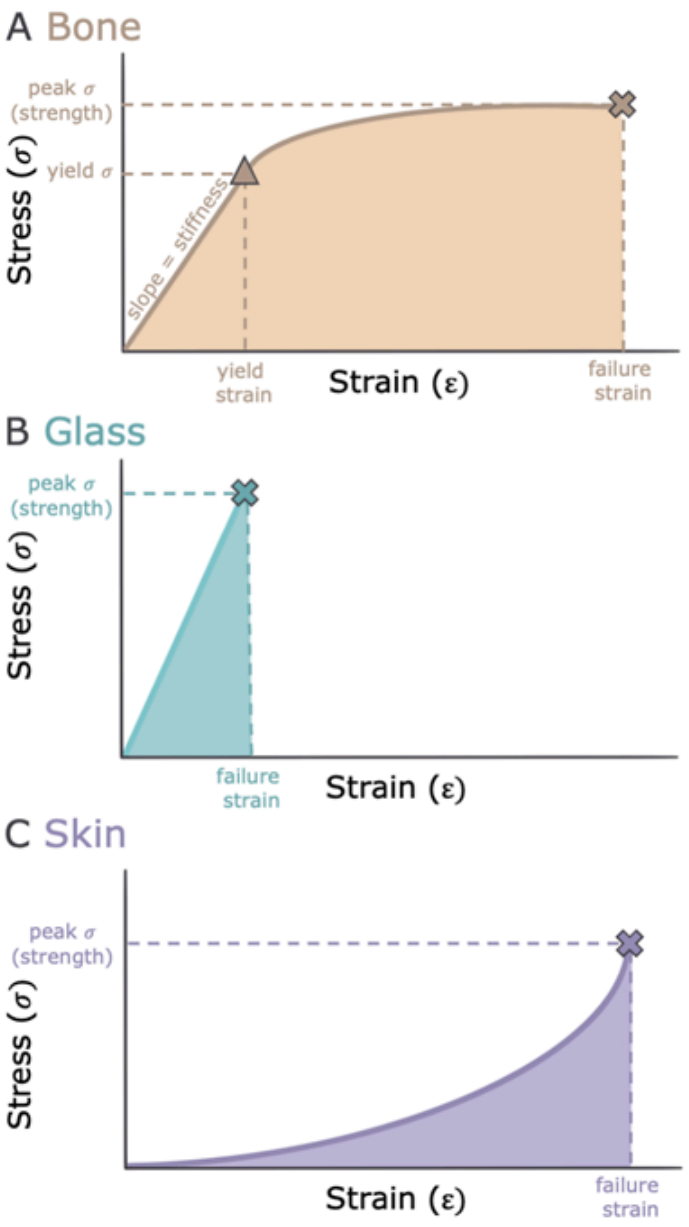

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Figure 5.10—Stress-strain curves of bone (A), glass (B), and skin (C). Stress is a

metric for material strength, and strain is a metric for deformation. The X's represent peak failure (fracture), and the triangle on the bone graph represents the yield point. Shaded areas under each curve represent material toughness.

Now if you want to think about what differences in these properties mean for the behavior of materials, let's compare stress-strain curves for two different materials. Our sample in Figure 5.10A is a pretty good representation of the stress-strain curve for bone: You can see it has fairly high strength, large extensibility, and a high toughness. If we were to compare that to another type of material—glass, for example—we'd see that both materials have similar strength, but compared to bone, glass has higher stiffness (Figure 5.10B). This higher stiffness results in less work required to fracture, meaning that this material cannot absorb energy very well, thus making it **brittle**. That's why it's easier to produce a fracture in glass than it is in bone and why bone makes a better material for building skeletons than glass does!

Let's take another look at our standard stress-strain curve. Rigid and many tensile materials—things like horn, bone, and keratin—have **concave-down stress-strain curves**, such as Figure 5.10A. This means that as the material nears its breaking point, it requires less and less stress to deform. Pliant materials, in contrast, usually have a **concave-up** or **J-shaped stress-strain curve**, such as Figure 5.10C. Thus, as pliant materials are deformed, it takes more and more stress to keep deforming them. But how do we get this J-shaped curve? J-shaped curves can be produced in materials if they have tensile fibers embedded in a more pliant matrix. As the material is stretched, fibers are aligned (as we see occurring in skin) and kinky fibers straighten out (as happens in arterial walls)—both of these changes increase the material's resistance. This type of concave-up stress-strain relationship has some advantages:

1. It offers built-in protection against breaking as the risk of

breaking gets closer. For example, the pliant nature of our arteries is really valuable because it follows this rule and thereby helps prevent aneurysms (i.e., burst blood vessels).
2. It also helps impede the spread of cracks through the material. J-shaped curves have less area under the curve compared to concave-down curves—this means that they absorb less energy. Energy release drives the spread of cracks through a material, which can ultimately cause a failure of the entire structure. So by absorbing less energy, pliant materials have less energy to release; this helps prevent cracks, which helps keep failures local (if they were to occur).

But why do we care about crack formation and mitigating it? Well, cracks are bad for materials, because they act as **stress concentrators**, meaning they focus the application of force on a very small area (specifically, the tip or sharp edge of the crack). This concentration results in an increase in the intensity of the force on a small area and thereby increases the stress on the material. If stress is increased beyond the material's capacity, it can result in failure of the structure. Living organisms very much benefit from avoiding cracks in their materials, because cracks can lead to a major failure of structures; such structural failures can result in the organism's death or at the very least remove an individual from the reproductive pool for a while, which is just about as bad as dying from an evolutionary fitness perspective. So it's advantageous for organisms to avoid cracks or to be able to mitigate crack formation when they do form, and they do so in two major ways:

1. Organisms mix pliant materials with rigid materials in composite structures. When cracks develop in composites, the cracks are prevented from spreading when they encounter pliant portions of the composite because pliant materials have that J-shaped curve and are really resistant to crack formation. For example, the pliant elastin in your skin (Figure 5.1B) prevents cuts from expanding.

2. Organisms also have rounded cavities throughout rigid materials. When cracks spread and encounter these cavities, the stress that was concentrated at the tip of the crack is spread out along the wall of the cavity. By spreading the stress along the cavity wall, the area over which the force is applied increases, and therefore the stress is decreased. We can see an example of such rounded cavities when we look at the structure of compact bone in vertebrate bones (Figure 5.1C).

At this point, we've spent a lot of time on measuring the loads that materials encounter and how the type or structure of the material influences the way in which the load affects a structure. But why is this important? One major reason is that if structures cannot handle the loads they encounter, they'll fail, and this could be very bad for the organism—again, either leading to death or taking it out of the reproductive pool for a while. So typically organismal structures are at least slightly "overdesigned"—in other words, they can withstand higher forces than they usually encounter. This degree of "overdesign" is called a **safety factor**, which is determined by taking the ratio **failure load / usual load**. This ratio should always be greater than one; otherwise, the structure will always be broken. Let's consider bone again, as an example. If a bone breaks at a strength of 200 MegaPascals (MPa) but during running bone typically only experiences 50 MPa, then we could take the ratio 200 MPa / 50 MPa and it would show us that bone has a safety factor of 4. This means that bone is capable of withstanding loads up to four times greater than the typical maximum load experienced during normal activity. And the advantage of having high safety factors is that it confers the ability to withstand unexpected high loads! However, safety factors cannot be indefinitely high. There are limits to the degree of "overdesign" that the body can accommodate because it is expensive (energetically speaking) to build and carry around extra material. This is why we see differences in safety factors across organisms and even across the specific parts of an organism. For example, if

we were to consider the bones of the mammalian leg, the femur has a higher safety factor than the bones of the lower leg (i.e., the tibia and fibula). This is because more material farther from the body costs more energy to move around, so extensive overbuilding of the tibia and fibula will result in a huge energetic expense every time we walk around.

Now, there are trade-offs when we consider mechanical properties, and this is because single materials usually cannot maximize all potentially advantageous biological properties. We can think of this as a bit of a "jack-of-all-trades, master of none" problem. So, for example, stiff materials aren't great at absorbing energy, and strong materials tend to have limited extensibility. As a result, organisms tend to be composed of many different materials with different properties that correspond with the performance of different functions. Consider our own bodies: We have stiff bones to transmit forces, but our tendons, which are made of collagen, allow elastic energy storage and recoil during locomotion. What's more, a single material can actually vary in its properties due to differences in components or organization, and these variations also often relate to differences in function. John Currey examined the mechanical properties of bone and found that bone (which we can think of as a pretty standard and invariable biological material) actually varies substantially across species. And this variation actually reflects differences in mechanical properties related to function. In his work, when Currey compared a deer antler to a cow femur (both of which are mammalian bones), he found that the antler absorbed more energy than the femur and as a result was better able to withstand impacts (Currey, 1970). This makes a certain degree of sense if you think about the role of an antler in deer ecology (often as a combat tool during territorial disputes) compared to the role of the femur in cows (to support body weight and for locomotion). Further, when he looked at the tympanic bullae of whales (part of the skull, near the ear), he found that whale tympanic bullae are stiffer than normal bone (i.e., a cow femur), thereby making the bullae more effective in transmitting sound

waves to the middle ear bones. What this work demonstrates is that even the same material can show differences in its mechanical properties, depending on what structure it contributes to and the function of that structure.

Consequences of Environmental Factors

While there are many different aspects of the environment that can influence form-function relationships, one of the most influential aspects is the type of fluid that an animal is moving through. **Fluids** are materials that have no fixed shape and yield easily to external forces. By this definition, fluids include all gases and liquids. Bringing Newton's third law back one more time for this section, even though fluids easily deform, they also exert an equal and opposite force back on the objects that are pushing on them.

The study of the consequences of how fluids impact motion is called **fluid dynamics** and is broken into two categories depending on whether the fluid surrounding the object is still, **static fluids** or moving, **dynamic fluids**.

For static fluids, the main forces acting on an animal are the pressure and buoyancy. **Pressure** is our metric for measuring forces applied in multiple directions. For terrestrial vertebrates, our main pressure is air pressure, which changes with weather conditions, temperature, and altitude. This is because air is a mixture of several different gases, including nitrogen, oxygen, and carbon dioxide. Each of these gases independently produces its own pressure, and the sum of all these pressures is what we call the **total pressure**. As the relative amounts of gases change, the **partial pressures** of each gas change, changing the total pressure. For example, as you drive up a mountain, the relative amount of oxygen present in the air decreases, which decreases the partial pressure of oxygen as well as the total air pressure. You've probably felt this as you feel the need to "pop" your ears to equalize the changing pressure inside your

body compared to outside your body. A similar concept applies to aquatic vertebrates, but here the surrounding pressure is from the water pushing on the object, and typically, water pressure increases with increased depth.

An additional static metric that we need to consider in the aquatic environment is that of **buoyancy**, or the force exerted by a fluid that opposes the weight of an object. Buoyancy is closely tied to the relative density of the object relative to the density of the fluid that an object is moving in. If the density of the object is less than the density of the fluid, then the object will float. If an object has a greater density than the surrounding fluid, it will sink. For fully aquatic vertebrates, the most energetically efficient strategy for moving through a static fluid is to be **neutrally buoyant**, meaning that the density of the organism is equal to the density of the surrounding fluid. Many aquatic vertebrates have specialized structures, such as the swim bladder of teleost fishes or the oily liver of sharks, that enable them to adjust and maintain neutral buoyancy as they move through water of different depths.

5.4 Form-Function Relationships in the Real World

In this section we are going to apply the concepts covered above in two areas of biomechanics: locomotion and feeding. We have incorporated the discussion of the ecological and evolutionary implications of these types of form-function relationships.

Locomotion

The way a vertebrate moves, its **locomotion**, is dependent on the environments in which it must move through. The three main types

of locomotion include **terrestrial locomotion**, or methods of moving on land; **aquatic locomotion**, or moving through water; and **aerial locomotion**, also known as flying.

Fluid Locomotion

Both aquatic and aerial methods of locomotion are dependent on an organism's ability to push against the fluid they are trying to move through.

One way this can be accomplished is by using **drag-based locomotion**. A force that acts in an opposing parallel direction to the motion of a moving object with respect to the surrounding fluid is called **drag** (Figure 5.8). So, for example, if a fish is trying to swim upstream, then the drag is the force that is working against the fish's aquatic locomotion. If we dive a little deeper (pun intended), then we can look at why so many aquatic organisms have a similar body form. There are two major components to drag. **Friction drag** is the resistance of a fluid moving over the surface of an object. Meaning that as a fish is swimming through water or a bird is flying through air, the fluid that is directly touching the moving organism will be slowing down the longer it interacts with the surface of the organism (Figure 5.8). Now remember Newton's third law, *for every action there is an equal and opposite reaction*. Thus, the fluid is also applying a force to the fish or bird trying to swim or fly. If an animal that moves through a fluid wants to decrease friction drag, then they would need to decrease their surface area. The second component of drag is **pressure drag**, or the resistance an object incurs as it moves itself through a fluid (Figure 5.11). In order to minimize pressure drag, organisms need to minimize the size of the parts of their bodies that hit the fluid first. This is why we see many aquatic and aerial animals that have a lemon-shaped, or **fusiform**, body shape. Anatomists are using what they know about these two components of drag along with experimental studies to

explore better ways to design underwater vehicles (i.e., Wainwright and Lauder, 2020). This intersection of biology and engineering is called biomechanics or bioinspired design.

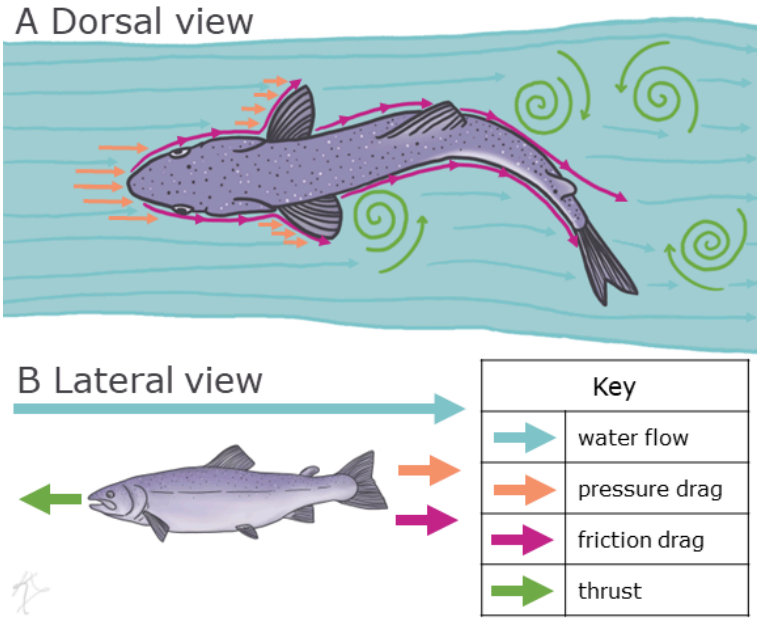

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Figure 5.11—Drag-based locomotion from the dorsal view (A) and lateral view (B). Arrows show the vectors of different forces acting in drag-based locomotion, with blue arrows representing water flow, orange arrows representing pressure drag, pink arrows representing friction drag, and green arrows representing thrust.

In order to propel themselves forward in a fluid environment, organisms must produce a force in the opposite direction of drag; this force is known as **thrust** (Figure 5.11). We can tell quite a lot about how an organism produces thrust by looking at their body shape. The thrust-producing parts of aquatic animals will aim to reduce drag while maximizing thrust. So, for example, when you think of a fish (Figure 5.11), they use their deep (tall)

bodies and caudal fins to produce thrust in a side-to-side direction, but otherwise they are fairly thin to minimize drag. Whereas most fishes swim by moving their bodies side to side, marine mammals, like whales and dolphins, have tails that are rotated 90 degrees compared to the fishes. This means that instead of pushing on the water by moving side to side, or *laterally*, these vertebrates will produce thrust by moving their bodies up and down, or *dorsoventrally*. This difference reflects the constraints placed on animal form based on evolutionary history. The ancestors of marine mammals were terrestrial and had limbs. Therefore, their ability to swim is constrained by the anatomy they started with from their terrestrial ancestors. We can actually measure the amount of thrust an organism produces using a method called digital particle image velocimetry (DPIV) developed by Willert and Gharib (1991). To use this method, researchers use high-speed video cameras and neutrally buoyant particles to measure the velocity of the fluid that an animal moves. Bringing Newton's third law back again, by measuring how fast the fluid around the animal is moving, we can calculate how much thrust the animal is using to push that fluid in order to achieve locomotion.

The other form of fluid locomotion is **lift-based locomotion**. When force is perpendicular to the oncoming flow direction, we call this force lift. By angling the leading edge of the wing or fin, the edge that hits oncoming flow first, swimming and flying animals can control how much fluid is flowing above versus below the limb (Figure 5.12). As an animal flaps its wing or fin, it changes the angle of the **leading edge** (the edge that interacts with oncoming flow first) so that there is always a positive, lifting pressure below the limb that pushes the animal in the opposite direction of gravity and perpendicular to oncoming flow.

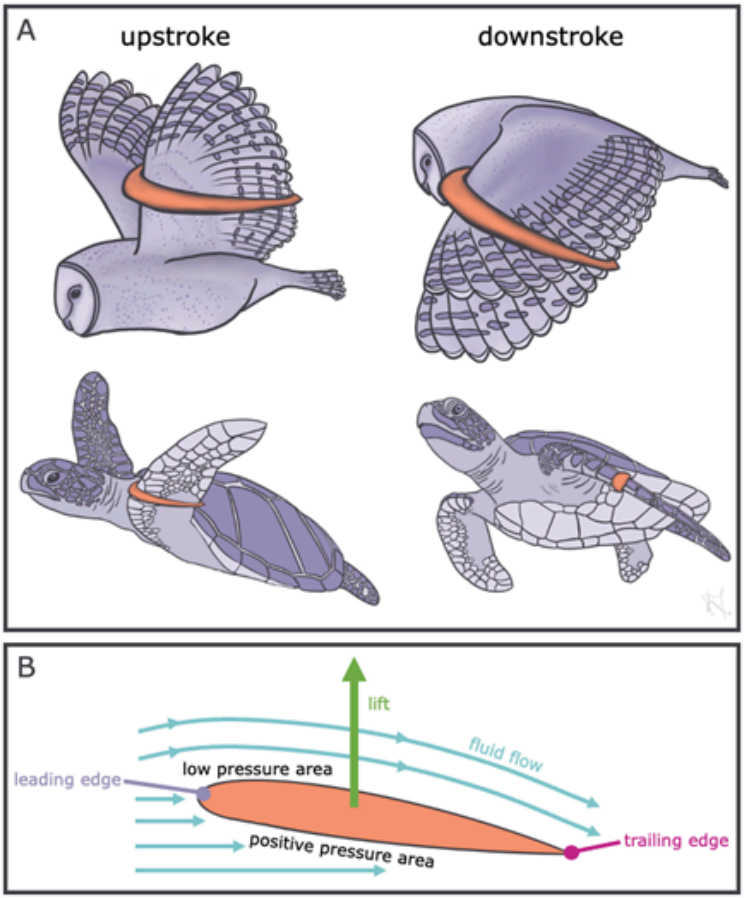

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Figure 5.12—Lift-based locomotion. Examples of animals using lift-based locomotion (A) show how the angle of the limb changes during the upstroke and downstroke in an owl and sea turtle. The orange shape represents the limb cross section and highlights how the limb angle changes. A schematic of the limb cross section (B) shows how the fluid flowing around the limb provides positive pressure areas under the limb and low-pressure areas above the limb, generating lift perpendicular to fluid flow.

Terrestrial Locomotion

When vertebrates moved from the aquatic to the terrestrial environment, the forces required for locomotion shifted. As seen in the previous section, in the aquatic environment, animals primarily rely on thrust and lift-based locomotion. On land, instead of pushing against the surrounding fluid, locomotion requires that animals push off the ground, and the reacting force propels the animal forward. We call this force the **ground reaction force** (**GRF**), and the resting GRF, when the animal is not moving, is the weight of the animal.

As vertebrates moved onto land, they benefited from lower viscosity than moving through water but also lost their buoyancy to counteract gravity. So terrestrial vertebrates needed adaptations to their bones, muscles, and postures to move effectively on land. As the force of gravity became a larger factor in locomotion, vertebrate limbs moved more ventrally to what is called a **sprawling posture** (Figure 5.13). Early vertebrates and modern amphibians who use this sprawling posture rely on lateral motions, similar to how fish swim. What is different is that instead of pushing off the fluid with lateral undulations, vertebrates with sprawling postures use the opposite arm and opposite leg to produce GRFs that propel them forward. As vertebrates became more terrestrial, many taxa furthered this pattern by moving their limbs underneath their body in an **upright posture** (Figure 5.13). Upright postures allow animals to produce more efficient GRFs, because forces are applied directly through the limb rather than laterally, enabling faster locomotor speeds.

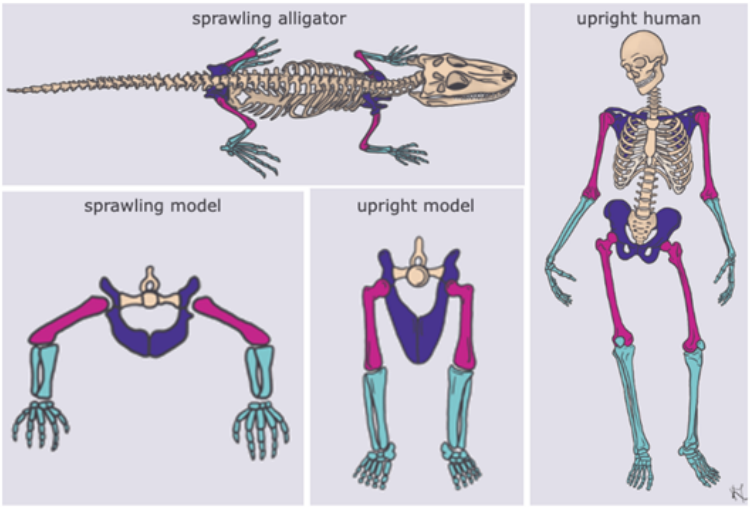

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Figure 5.13—Sprawling versus upright posture. Model diagrams are provided for each posture as well as an alligator and human example. All models show the axial skeleton in tan, the pectoral/pelvic girdles in purple, the upper limb bones in pink, and the lower limb bones in blue.

As vertebrates became more specialized for living in different terrestrial environments, we began to see additional locomotor specializations. For example, vertebrates that use **arboreal locomotion** tend to have longer forelimbs with extra surfaces for improving gripping, while animals that specialize in jumping, or **saltatory locomotion**, tend to have the opposite body proportions, with longer hindlimbs and proportionally shorter forelimbs.

Human Locomotion

Of course, humans fall into this broad category of "organisms" we've been talking about here, and it should come as no surprise that

locomotion is important for us too. Movement is essential for individual psychological and physical well-being. In fact, regular physical activity helps prevent a whole host of diseases, including things like heart disease, cancer, osteoporosis, and diabetes, among others. Furthermore, it helps with the prevention and management of psychological disorders like depression, anxiety, and others. And yet less than half of the world's population has sufficient activity levels to maintain their physical and mental health (that's 4 *billion* people who aren't moving around enough!).

By integrating our understanding of anatomical structures and the physical principles that govern movement, we can gain a more complete understanding of human locomotion and apply that understanding to the development of new tools, technologies, and training to help improve mobility, physical activity, and ultimately human health. This is a primary focus of the fields of human biomechanics and kinesiology. In these disciplines, anatomic and physical principles can be employed in the study of human locomotor disorders, such as those observed in individuals with cerebral palsy, and outcomes of this work can inform health-care decisions related to surgical and other types of medical interventions intended to improve a person's locomotor ability and quality of life. Indeed, the impacts of this type of research go beyond just the scientific realm: It is used for athletic training and equipment development, by the film and gaming industry in the production of realistic CGI, by engineers and manufacturers for the development of ergonomic tools and furniture, and in medicine in novel approaches to joint surgery and the engineering of powered exoskeletons for people with paralysis.

Box 5.2—When Form Doesn't Match Function

Earlier in this chapter, we talked about how the

vertebrate skeleton can be thought of as a collection of levers. In many cases, such as the example in Figure 5.8, the function that an animal uses matches the lever system that is observed in their skeleton. In Figure 5.14A, we have the forelimb of several different vertebrates as well as their main method of locomotion. For each of these limbs, we've color coded the parts of the limb that represent the lever system for forelimb extension (a.k.a. what happens when the triceps muscle flexes). Some of these forelimb levers are built for strength, such as the diggers noted in the figure, while other forelimb levers are built for speed, such as runners. Now let's look at the sloth forelimb in Figure 5.14B. Sloths are relatively slow creatures that spend most of their time with their forelimbs extended hanging from the branches of trees. Look at their limb structure and think through the following questions:

- Is the sloth forelimb built for strength or for speed based on the lengths of the lever arms?
- What evolutionary advantage would being fast or strong have for the sloth?
- If there is no strong evolutionary selection for being fast or strong, what happens to the anatomy of the skeletal system?

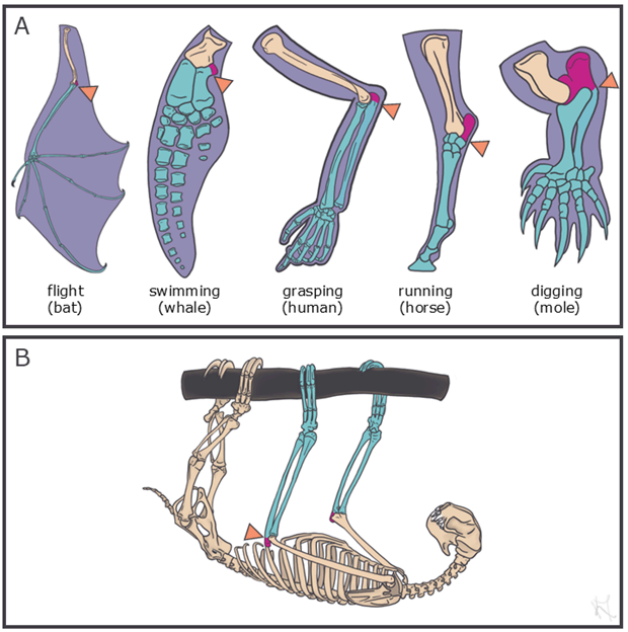

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Figure 5.14—When form doesn't match function. The forelimbs of several vertebrates are provided (A) *to compare with the sloth* (B)*. Each limb is color coded to show the fulcrum (orange), in-lever (pink), and out-lever (blue) for forearm extension. Under each limb, the animal and its primary mode of limb movements are provided.*

Feeding

Feeding can be broken down into three major categories: searching,

acquisition, and ingestion. You will learn more about how animals sense their environment in later chapters, and you have learned how animals move in the previous section of this chapter, so here we will focus on food ingestion. The anatomy a vertebrate has and the environment in which it lives can tell us a lot about how an animal ingests their food without ever needing to see them feeding in action. In terrestrial vertebrates, looking at skull and tooth shape can tell us if a vertebrate is likely going to have a primarily herbivorous, carnivorous, or omnivorous diet. Skull shape will tell us where muscles meant for chewing attach, with larger attachment sites relating to relatively more/stronger muscles. Tooth shape can tell us if a tooth is meant for grinding (e.g., molars) or slicing (e.g., incisors, canines). You will learn more about tooth shape when we discuss the skull in Chapter 8, so for now we will focus more on function than on the specific names of the different types of teeth. Herbivores, who have a mostly plant-based diet, have more grinding teeth, fewer slicing teeth, and muscles that allow for side-to-side movements for grinding of plant materials (Figure 5.15). Carnivores, who rely mainly on eating meat, have more teeth meant for slicing and have more muscles for stronger bite forces (Figure 5.15). Omnivores, such as humans, who eat a combination of plant and animal diets, have more generalized tooth structures and muscles meant for both slicing and grinding (Figure 5.15).

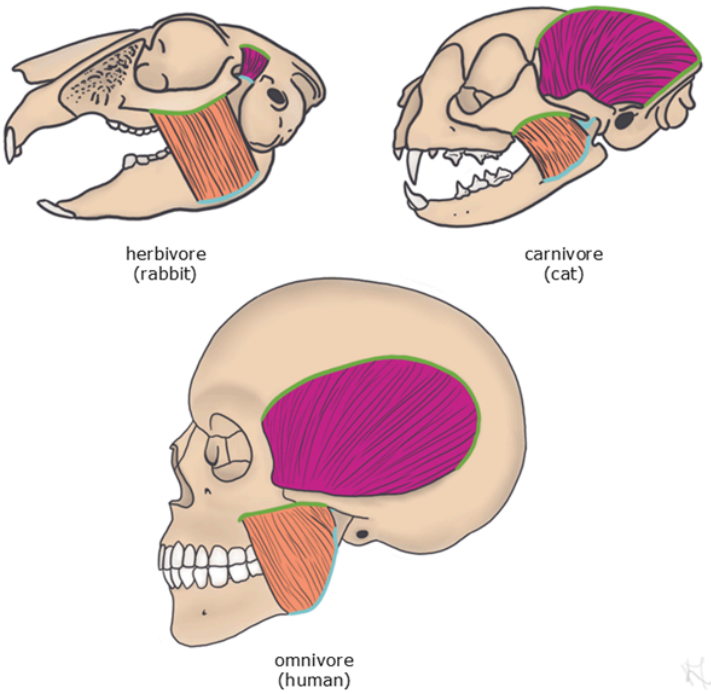

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Figure 5.15—Feeding morphology in an herbivore (rabbit), carnivore (cat), and omnivore (human). The chewing muscles are color coded to show the importance of bite force from the temporalis muscle (pink) compared to the importance of grinding from the masseter muscle (orange). For both muscles, the areas of attachment for each muscle's origin (green) and insertion (blue) are outlined.

Box 5.3—Try It Yourself! Using Open-Source Platforms to Measure Form-Function Relationships

In addition to observing the form-function relationships in the real world, many repositories and software programs have been developed over the past 20 years to aid in the collection of digital morphological data.

There are several repositories where you can download scans of specimens. One such repository is MorphoSource.org (Boyer et al., 2016). Funded by the National Science Foundation, MorphoSource allows users to share and download anatomical images, such as computed tomography (CT) scans, models, magnetic resonance imaging (MRI) scans, photogrammetry, and more. MorphoSource accounts are free, but often you will need to request permission from the data owner in order to download or use the files of interest. This allows museums and other institutions to keep track of how the data are being used.

After you've found a digital specimen or specimens you want to collect measurements on, there are also free, open-source software tools to allow you to collect data without expensive software packages. One example is 3D Slicer (Fedorov et al., 2012), which is used heavily in the medical industry for quantifying and modeling anatomy from medical images (CT and MRI). There are many plugins for 3D Slicer; one that is particularly useful for measuring anatomical structures is SlicerMorph (Rolfe et al., 2021). The developers of SlicerMorph have a repository of tutorials if you'd like to test out these tools yourself.

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6. Integument

BILL RYERSON

Focus Questions—to Guide Your Reading of This Chapter

1. What is the primary function of the integument across vertebrates? How does achieving this function change through time and environment?
2. What key elements helped the tetrapods "break free" from a life in water?
3. What do the derivatives of the skin (nails, claws, hair, feathers, etc.) have in common, how do they differ, and what can they tell you about the evolution and natural history of vertebrates?

6.1 Introduction

Picture a vertebrate in your head. What do you see? Do you see brightly colored feathers? Glistening fur? Smooth scales? Almost everything you see, covering the external surfaces of that animal, is a part of the integument. The **integument** is not just the skin but everything found within and derived from the skin. This includes

all those structures you probably envisioned: feathers, scales, and hair. It even includes structures like talons, nails, claws, and antlers (Figure 6.1). The integument serves many functions in vertebrates, and while they share a common overall makeup, even tiny changes in structure have implications for the various functions of the integument.



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Figure 6.1—Examples of derivatives of the integument. Starting in the top left and moving clockwise: hair on a European brown bear, scales on a goldfish, the feathers of an eagle-owl, the claws of a Komodo dragon, and the antlers of a moose.

The integument serves many functions, as we will discover in this chapter. However, the primary function of the integument is to *isolate the organism from its external environment*. Isolation occurs at multiple levels in our case. For example, shells and scales serve as armor, protecting an individual from the teeth and jaws of a potential predator. Thick layers of an **epidermis** full of **keratin** (like yours) help keep out infectious microbes and parasites. Keratin,

acting with a class of molecules called glycolipids, helps prevent the loss of water from cells. Without the integument, predation, infection, and dehydration are just three of a host of problems that an organism would face. We will see that the basic structure of the integument is well suited to this task and is structured consistently across the groups of vertebrates. Our generalized structure in place, we see how changes in the structure of the integument allow for the many secondary functions to occur and are modified in individual groups. In addition, unlike many of the other systems discussed in this textbook, the integument is full of structures that provide for a plethora of different functions, many of which have been modified from their original function to best suit the current needs of that species/group. Hair, for example, is thought to have evolved originally in mammals for the purposes of thermoregulation. As we look across mammals, we can find examples of hair performing a multitude of tasks: Whiskers (vibrissae) are incredibly sensitive touch organs. The quills of porcupines are effective deterrents to predators. The stripes of a tiger allow it to disappear in tall grass. These are but a few examples of how modified structures of and within the integument contribute to the overall biology of vertebrates.

6.2 General Structure of the Integument

Across the vertebrates, we will find that the integument has the same basic components/layers, and it is the variation within the layers that allows for the diversity of functions we observe. The three major layers of the integument are the **epidermis**, **dermis**, and **hypodermis**. Note that the root of all these layers is "dermis," which originates from the Greek "derma," conveniently referring to skin and/or leather. We can think of the dermis as the base of the integument. Above that, closer to the surface/external environment (i.e., superficially), is the epidermis. Below that, deep to the dermis,

is the hypodermis. Figure 6.2 illustrates these three layers as they are structured in the human. Broadly, these layers will look similar across the vertebrates. However, it is the thickness of these layers, and what they contain, that will provide insight into each group's environment, ecology, and evolutionary history.

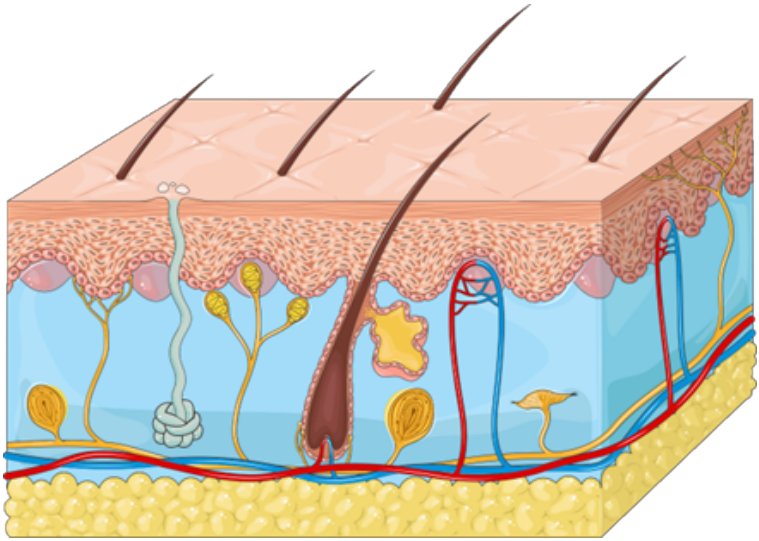

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Figure 6.2—General illustration of the human skin layers. The pink is the epidermis, the blue is the dermis, and the yellow is subcutaneous fat in the hypodermis.

The epidermis is the topmost layer of the integument and is by definition the layer that separates the organism from its environment. As such, we will see that many of the adaptations that help an organism interact with its environment are contained within the epidermis. We will also see that the epidermis varies greatly in thickness and constituency across our groups. The epidermis is primarily epithelial tissue, of a type we refer to as **stratified squamous epithelium**. "Squamous" tells us that the cells are often flattened, and "stratified" tells us that there are many layers to this

tissue. In many vertebrates, particularly our terrestrial vertebrates, the layers of the epidermis closest to the environment are dead and serve only as a physical barrier to the outside world. Figure 6.2 is a close-up of a piece of the human epidermis. The structures beginning with "stratum" are the individual layers of the epidermis. The stratum basale links the epidermis to the underlying dermis via a basement membrane, a characteristic of most epithelial tissues and where many cells are rapidly dividing. The stratum spinosum consists of cells that are rapidly undergoing mitosis, diving and pushing the layers above closer to the surface. At the level of stratum germinativum (granulosum, in humans), the differences between our terrestrial and aquatic vertebrates start to become obvious. In this layer, cells we ultimately call **keratinocytes** begin producing keratin within themselves. Keratin is an important constituent of the integument. In addition to being within the epidermis, keratin is the biggest component of hair, claws, and many of the derivatives of the integument. When a snake "sheds" its skin, it is actually multiple layers of keratin that are being replaced. Your fingernails and the scutes of a turtle shell are also keratin, albeit slightly different versions of keratin. It is the bird's beak and simultaneously the feathers. Keratin is a key component of the integument. As cells move closer to the surface, more and more keratin is produced, becoming the major component of the cell. At the outermost region of the stratum germinativum, the cells have died, leaving only some organelles, the cell membrane, and the accumulated keratin. Above this point, there are no living cells in the epidermis, only layers of keratin linked together by glycolipids, the same molecules mentioned earlier. This is the stratum corneum, named for the remaining keratin. Within the epidermis, there are structures passing through from the dermis (see the hair follicle in Figure 6.2), but there are also structures unique to the epidermis.

In most vertebrates, the epithelial tissue is dominant within the epidermis, and there are few other cell or tissue types found. Connective tissue adheres the bottom layers of the epidermis to the dermis, and there is no skeletal muscle. Capillaries of the

cardiovascular system do not extend into the epidermis, only reaching the upper layers of the dermis. There are a few types of cells and tissues that do extend into the epidermis, and their importance should not be understated. Some we will leave for other chapters, but it is worth noting their role within the integument. Sensory receptors abound in the integument, and many are rooted in the epidermis or run through the epidermis. Pressure and touch receptors, temperature receptors, the lateral line of fishes, and electroreceptors all are integrated into the epidermis. Most of the specialized receptors, like the lateral line and electroreceptors, may be found deep in the epidermis/dermis, with an opening in the epidermis that allows for interaction with the environment. The touch and pressure receptors are embedded within the epidermis, at the levels of the stratum basale and germinativum. Chapter 20 on the special senses will expand on these receptors in more detail. In addition to the sensory receptors, the epidermis is home to structures that give the integument its color. Structures like chromatophores, melanocytes, and other pigment-producing cells are found in the epidermis. Some of these elements are structural. As light passes through a structure, it can be reflected or refracted, which we (and other animals) interpret as a color. Blue is the most common structural color, while others such as reds, greens, and yellows are the products of the pigment-producing cells. As the integument is the first level of defense against outside pathogens and disease, it should not come as a surprise that cells of the immune system may be found in the epidermis as well. Specialized macrophages move through the epidermis and dermis.

The dermis can be found deep to the epidermis. In most vertebrates, the dermis is the larger of the two layers. The dermis can be composed of many different types of tissue, including representatives from the circulatory and nervous systems. In certain vertebrates, smooth muscle may also be present in the dermis to interact with hair, feathers, or glands. The major component of the dermis is connective tissue, which provides strength and flexibility to the integument. The most abundant

component of that connective tissue is a molecule known as collagen. **Collagen** is a protein, the most common protein in the animal body. Collagen is a key component of the dermis, but it is also similarly important in bones, tendons, ligaments, joints, and many other places. The arrangement of collagen in the dermis is almost as important as the amount. Collagen is strong, but only strong in the direction of its fibers. To resist forces in multiple directions, the collagen must be found in multiple directions. We see this most obviously in the fishes. Collagen fibers are bundled together and wound around the body in a cross-helical fashion (Figure 6.3).

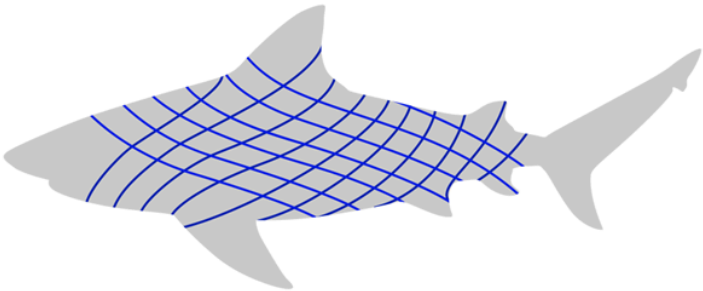

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Figure 6.3—The outline of a shark showing the cross-helical arrangement of collagen in the skin.

This gives the dermis strength and some flexibility to deal with all the bending that occurs in the body. How well suited is this arrangement? The passive stretching and relaxing of collagen in the dermis allows a dead fish to swim! There are other types of connective tissue found throughout the dermis; all help in anchoring the epidermis to the dermis and the dermis to the hypodermis, and all provide a combination of flexibility and strength that is unique to the integument.

The hypodermis sits below the dermis (Figure 6.2) and primarily anchors the dermis to the underlying tissue. In many organisms, this collection of connective tissue, blood vessels, and nerves is where we would meet the other systems of the body, such as the major nerves of the peripheral nervous system, the skeletal muscles, and the major arteries and veins. Absent are many of the elements of the sensory system that we would have seen in both the epidermis and dermis. We will not spend a considerable amount of time on the hypodermis in this chapter, except in the cases where it provides us a better understanding of an organism's biology (the blubber in marine mammals, for example). In fact, this layer is crucial for human and veterinary medicine. Hypodermic needles are used to inject medication into this layer of the integument, where it can be taken up into the larger blood vessels for faster transportation around the body.

In our brief discussion of the general layers of the integument, we neglected to introduce some of the derivatives mentioned in the introduction, including antlers, claws, and so on. That is because these elements are not restricted to a single layer. Instead, these elements often arise from the interactions of the layers, typically the epidermis and dermis. Many of these will protrude from the integument (nails, claws, feathers), while some may reach through and empty their contents onto a layer (sweat glands, mucus glands). There are some notable structures arising from the integument that we should discuss now, as they have a drastic impact on the evolutionary history of vertebrates, while others will naturally come up as we work through the diversity of vertebrates and their integument.

Arguably, the most important element that arises from the integument is **dermal bone**. While not a feature you see frequently, dermal bone has played an outsized role in vertebrate evolution. Dermal bone is exactly what it sounds like, bone that arises from the dermal layer of the integument. Dermal bone arises in sheets via a process known as intramembranous ossification (discussed in more detail in Chapter 7—Bone and Cartilage). Because it develops

in between these layers, dermal bone is often flat or has a slight curve to it. Dermal bone will make appearances in this chapter as well as the skull chapter (Chapter 8). The evolution of dermal bone was important for the fossil fishes and remains important for reptiles and mammals.

6.3 Development

While it is easy to assume that all of the integument is derived from **ectoderm**, the reality is more complex than that. Only the epidermis itself is derived from purely ectoderm, while the specialized cells and tissues found throughout the integument have different developmental origins. Like many other connective tissues, the dermis and hypodermis are primarily **mesoderm**, and it is their interactions with the ectodermal cells that help produce the underlying structures. All the glands embedded in the integument are derived from ectoderm. Blood vessels in the dermis are derived from mesoderm.

After gastrulation, that very early stage in development, something takes place that is relevant to the integument. The ectoderm, which will eventually become the epidermis, folds over a part of itself, forming the neural tube (neurulation). While the neural tube will eventually become the spinal cord and brain, there are other cells cast off at this stage that have widespread impact. These cells, which are derived from the interaction of the ectoderm and the neural plate (what folds to become the neural tube), are known as **neural crest cells**. Neural crest cells can be classified as mesenchymal cells, as they are extremely mobile and will migrate throughout the body. Neural crest cells will play a key role in many parts of the body: nerves for the integument and digestive tract, cartilage and bone development of the face, and regions of smooth muscle. Neural crest cells in the integument, in addition to some of the nervous system structure, become the smooth cells that

move hair as well as melanocytes that provide coloration and UV protection.

6.4 Evolution of the Integument—a Phylogenetic Approach

As we explore the major groups of vertebrates, we will highlight structures unique to that group, what their function is, and how it relates to overall evolution and functional biology of that group. Remember that the ultimate purpose of the integument is to isolate the body from the external environment and protect the body from microscopic pathogens to large predators.

Hagfishes and Lampreys

While often neglected in discussions of vertebrate evolution, structure, and function, hagfishes and lampreys are considered to be highly modified representatives of the ancestral vertebrates. Hagfish skin is remarkably variable across species and is truly distinct from other vertebrates. Rather than muck about in the details of all the different varieties of hagfish integument, we will instead focus on two key elements of the skin that are important to the whole group. The first is that hagfish skin is only loosely connected to the underlying tissue. The dermis of the hagfish is separated from the hypodermis by a series of blood sinuses and only has a tight connection to the hypodermis at its basal membrane. In effect, the skin can move freely relative to the underlying body structures. The function of this adaptation is still unclear. Several researchers have attributed the hagfish's unique ability to tie itself into knots to the loose skin, while others have suggested that it makes it very difficult for predators—for example, sharks—to

securely bite and grasp the hagfish, as the skin slips loosely under their teeth. The other element of the hagfish integument worth exploring is their famous slime glands. Hagfish skin is full of glands with a special type of cell known as a **thread cell**. Thread cells produce a complex fiber, which reacts with water. The reaction is immediate, and hagfishes are able to produce an impressive volume of slime (Video 01). The purpose of this slime is obvious. Secreted in response to disturbance or harassment, the slime quickly fills the space around the hagfish, absorbing water rapidly. Any potential predator receives a mouthful of slime for its trouble.

Lamprey integument follows the generalized pattern of all vertebrate integuments. While not as remarkable as the hagfish, there are some elements to the lamprey that are worth noting. The first is that lampreys have a very large epidermis, larger than that of the dermis. This is worth noting because in other fishes, the opposite is the case. The second is that we start to see specialized glands embedded in the epidermis. These glands consist of singular cells, secretory granular cells, whose purpose is to produce mucus. Not quite the same thing as the thread cells in hagfishes, these granular cells cover the entirety of the epidermis in mucus. Mucus is extremely important to the integument in most fishes (in addition to many other systems in the body). Mucus helps reduce friction between a swimming fish and its watery environment. It can also trap and prevent infectious pathogens from crossing from the environment into the body. For the lamprey (and other fishes) the mucus helps the isolating function of the integument.

Ancestral Fishes

It is important to highlight a few groups of ancestral fishes to help our understanding of the evolution of the integument. This is because unlike other fossil vertebrates, where the skeleton is what was fossilized, in these groups it is the dermal bone that remains.

The phylogenetic relationships of these groups to our extant species are a bit fishy, and so we will treat them as a separate entity to understand how the integument played a large role in the evolution of the vertebrates.

Placoderms are the most famous of these ancestral fishes. Their name comes from Greek, meaning "plate-skinned," and they lived from the Silurian to the late Devonian (about 440 to 360 MYA). Placoderms not only had dermal bone but had no other type of bone throughout their body. Like in many of the other groups of ancestral fishes alive at this time, this dermal bone served as armor around the head region. This armor was also a key element to their jaws, and we currently consider the placoderms to be among the first jawed fishes. The dermal armor surrounded just about every available surface on the head of our placoderms, in species big and small. The largest known placoderms, the genus *Dunkleosteus*, have some well-preserved specimens that show the extent of the dermal bone (Figure 6.4). This dermal bone embedded in the integument serves as both armor and a feeding apparatus and appeared many millions of years prior to the evolution of bone as an internal skeletal feature. In fact, dermal armor appears in the evolutionary record before jaws do in many ancestral fishes, where the dermal bony armor is quite apparent but the jaws are clearly missing. These include such fascinating groups as the ostracoderms (a paraphyletic group of jawless fishes) and the Pteraspidomorphi ("wing-formed"), heavily armored jawless fishes that are currently considered to be the closest known relatives to the jawed fishes.



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Figure 6.4—CMNH 5768, the largest complete individual of Dunkleosteus terrelli in oblique left lateral view. Some of the thoracic plates are reconstructed, but the skull and ventral armor are entirely real. The proportions of the reconstructed plates closely resemble other specimens of Dunkleosteus.

Chondrichthyes

The Chondrichthyes—sharks, rays, and chimeras—will serve as our first real introduction to vertebrate integument. An epidermis and dermis are present, with a strong anchoring to the hypodermis. The collagen fibers in the dermis are wound cross-helically across the body, which provides elastic energy back to a shark when it swims,

through the stretching and relaxing of the collagen. The epidermis of sharks and rays is extremely thin—many times thinner than the dermis. There are few glands in the integument, but there are two key sensory structures that are embedded in the integument. On the rostrum of sharks, rays, and chimeras are a multitude of small pores. These small pores are part of the electroreceptive apparatus of chondrichthyans. Chondrichthyans can detect the electrical fields generated by muscle contractions of other animals in the water through small jelly-filled pores (see Chapter 20). These pores open through the epidermis. The receptors themselves are found below the dermis. Similarly, along the sides of sharks (and other fish), we find the openings to the lateral line, a special type of mechanical receptor that also has openings in the epidermis.

The most unique feature of the skin of chondrichthyan fishes is the denticle. **Denticles** are small, toothlike structures that completely cover the skin of cartilaginous fish (Figure 6.5). Also known as **placoid scales**, denticles strongly resemble teeth in structure and shape. In fact, they are currently considered to be homologous to all vertebrate teeth. Denticles have an inner pulp cavity, with blood vessels and nerves, and a thicker layer of dentine covered by a thin layer of enamel. The denticles form within the dermis but then "punch" through the epidermis, exposing them to the outside environment. Which developed first, teeth or denticles, is still being debated. One of the consequences of this similarity is clear. Divers who have brushed up against a shark, and even students in a dissection lab, may end up with a slight burn or abrasion as a result of the denticles. A lesser-known consequence of this structure has only been uncovered in the last 30 years. The shape of the denticles as well as their spacing and arrangement work to reduce the drag of a swimming shark. This means that it takes less energy to keep moving the shark forward. Despite performing similarly across sharks, denticles are species specific, greatly vary in shape, and have been used to identify fossil shark species.

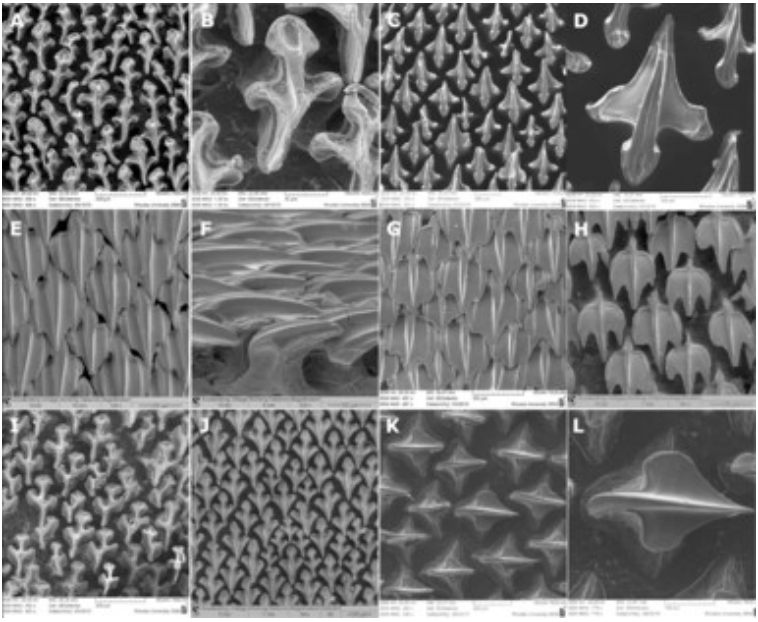

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Figure 6.5—A collage of dermal denticles in the recently discovered extant deep-water shark, Squalus shiraii.

Osteichthyes

One of the biggest changes when looking at the differences between Chondrichthyes and Osteichthyes is the development of bone for the internal skeleton (hence their names). However, there are obviously many other differences between the cartilaginous and bony fishes, and the integument is no exception. The most obvious difference between the two groups is that our bony fishes do not have denticles but instead are covered in **scales**. Both denticles and scales originate in the dermis, but it is there that the similarities end. All denticles, while varying greatly in shape, are composed of the same layers and materials. However, in our bony fishes, there

are a large number of different scale types, the details of which are beyond the scope of our discussion here (for more information, see Elliott, 2011). Some fish have scales that retain elements found in denticles (e.g., gar), while many others do not. One element that the majority of bony fish scales have in common is that central regions of the scales are composed of bone. In Chapters 7–9, bone is going to be discussed heavily as it relates to the skeleton. However, as we discussed above, the presence of bone is not limited to the skeleton. Dermal bone, originating in the integument, is a key evolutionary feature of both extant and extinct Osteichthyes. The size of the scales and the amount of dermal bone present vary incredibly from species to species. The general consensus is that fast-swimming fishes (e.g., tuna) have a large number of small scales, while slower-moving fishes have larger, thicker scales but in smaller numbers (e.g., sturgeon). The theory is that smaller scales still provide some level of mechanical protection against predators but are more conducive to the faster lifestyle—that is, they generate less drag from their shape and size and therefore reduce the energy burden on these fish. Many of the freshwater catfish in the order Siluriformes have even lost scales!

In addition to the structural differences between denticles and scales, their position within the integument is also worth noting. As we mentioned, much of a denticle is exposed to the environment, with only its base being covered by the epidermis. The scales in bony fishes are completely embedded in the integument, completely covered by a thin epidermis. In most species, individual scales overlap each other (Figure 6.6), with a thin layer of epidermis found between the two scales.



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Figure 6.6—The overlapping scales of the common bream, Abramis brama.

This epidermis is full of living cells and tissues and is also the host to a large number of glandular cells. The most common glandular cells are mucus-producing cells known as **goblet cells**. Goblet cells are extremely common not only in the integument but throughout the body. They are also extremely common throughout vertebrates and are the most common cell found in humans. These are different from the glandular cells we observed in lampreys, though the function is the same (the homology of these structures is still unclear).

There are a few additional elements regarding the integument of bony fishes that must be considered before moving on to the tetrapods. The first is that while the hagfishes, lampreys, and cartilaginous fishes are limited to a narrow palette of coloration, the bony fishes have taken advantage of the entire color spectrum. Shades of purple, green, gold, orange, blue, red, and many more can be found throughout the world's water bodies (Figure 6.7). The colors that we see in these fishes are the result of two different

sources: **pigments** and **structural colors**. Structural colors are the result of different structural properties of material, particularly properties that impact how light passes through or is reflected. These could be fibers that scatter light, microscopic structures that reflect different wavelengths, and more. The most common colors that we refer to as structural are blues and anything we might describe as iridescent. Pigmental colors are colors that result from selective absorption of light by molecules. These pigments are often stored in cells called chromatophores, which can be distributed throughout the integument and give rise to all the color patterns we see. As varied as the fish in Figure 6.7 are, there are even more variations of chromatophores.



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Figure 6.7—A variety of Pacific reef fish highlighting a diversity of color patterns.

In our bony fishes, chromatophores mostly occur within the dermis, while the iridescent sheen many fish appear to have is the result of structural coloration within the scales and epidermis.

The last thing we need to discuss regarding the integument of the bony fishes is more functional than structural. With the

hagfishes, lampreys, and cartilaginous fishes, ions and water freely move across the integument. This results in a water/ion balance of a fish's tissue being the same as that in the external environment. However, in many freshwater fishes (and those that may move from fresh to salt water) either the epidermis and dermis act to block the movement of water/ions across the integument or there are ion channels that intentionally move ions. In this manner, the concentration of water and ions in a fish's tissues may be different from that of the external environment. This will become more important when we discuss the tetrapods, which leave the watery environment behind for a life on land.

Tetrapods

The transition from an aquatic environment to a terrestrial one is going to have a drastic impact on many of the systems covered in this book, and the integument is no exception. Again, let us remind ourselves of the ultimate function of the integument, and that is to isolate the body from the external environment. To this point, we have discussed this function in terms of armor (against predators) with some mention of pathogens. As we noted in the last few sentences of the discussion on Osteichthyes, we should not neglect how the integument can regulate the movement of small molecules between the body and the environment. As we begin our discussion on the amphibians and reptiles, armor and protection are still going to be important, but one of the main drivers of the structure of the integument is going to be reducing water loss. Do these organisms have structures that help prevent the movement of water from the body to the environment? How does it impact their biology?

The other major transition that is going to occur is from organisms that are **ectothermic** (i.e., "outside heat") to **endothermic** (i.e., "inside heat"). Ectothermic animals—including fishes (with few

exceptions), amphibians, and reptiles—do not produce their own body heat and are more susceptible to changes in environmental temperatures. The birds and mammals are well-known endotherms, producing heat from metabolic processes that keep their body temperature above that of the external environment and within a relatively stable range of temperatures. The integument is the surface area of these animals and the site of heat loss. Many structures we will examine in these groups will mostly act to prevent the loss of body heat to the external environment, but in some cases, they may be called upon to dump excess heat. In humans, for example, our **sweat glands** are a large contributor to how we maintain our stable body temperature and provide a path for the loss of heat when we get too warm.

Amphibians

In many ways, the amphibians are still tied to the water. The majority of amphibians need to be near water for reproduction and are susceptible to the loss of water through their skin. They have largely lost the ancestral scales (with the exception of a few caecilians), and it is only in the skull where the vestiges of dermal bone can be found. Although the amphibians have not waterproofed their integument as well as the reptiles and mammals, that should not be taken to mean that they lack adaptations suitable to their environment.

What the amphibians can be said to be lacking in waterproofing their integument, they make up for in glands. The epidermis of amphibians varies greatly in thickness (it may be as large as the dermis in some species). Within the epidermis, there is a large variety of glands that can be found in extremely high densities. The majority of these are **mucus glands**. The mucus in amphibians helps trap and retain some water while also providing a physical barrier against the threat of pathogens. As amphibians have relatively permeable skin, it would seem that they are especially

vulnerable to viruses and bacteria. However, many amphibians also have Leydig cells found within their epidermis. It is crucial to note that these are not the same Leydig cells in the reproductive systems of most vertebrates (see Chapter 17). They are similar in structure but vastly different in their function. In the epidermis of amphibians, Leydig cells are considered to assist in mucus production as well as produce secretions that deter the invasion of pathogens.

The other major type of gland found in the skin of amphibians is the **poison gland**. Poison glands are as variable as the amphibians themselves, not only in what they secrete, but also in where those materials come from and where they are found on the body. Some of these poison glands may be large, discrete structures embedded in the epidermis (e.g., parotoid glands of toads), spread across particular regions (e.g., the highly toxic dorsal skin of poison dart frogs), or distributed across the entirety of the body (e.g., the eastern newt of North America). The dangerous bufotoxin found in toads worldwide is produced by the parotoid glands (Figure 6.8), while the famous poison dart frogs acquire their poison by storing the noxious chemicals found in the ants and termites they consume. In fact, in captively maintained populations, the toxicity essentially vanishes, as they have been fed a diet of harmless crickets and springtails.



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Figure 6.8—Left: A cane toad (Rhinella marina) with the parotoid gland highlighted by the white circle. Right: Poison dart frogs (Dendrobates azureus) with the characteristic brightly colored skin.

In addition to the multitude of glands in the integument, many amphibians also respire through their skin. As Chapter 14 on the respiratory system will show in more detail, respiration is fundamentally about moving oxygen and carbon dioxide across membranes, going from the environment to the blood and vice versa. Many amphibians will undertake gas exchange through their skin, and there are some species that will only exchange these gases through the skin, a process known as **cutaneous respiration**. The hellbender, North America's largest salamander, lives in cold, fast-moving streams and is almost completely reliant on cutaneous respiration for its oxygen needs. The lungless salamanders (Plethodontidae), an extensive group of salamanders also found throughout North America (with a few members in Europe and the Koreas), are also completely reliant on cutaneous respiration, with no functional gills or lungs. The adaptations for cutaneous respiration are quite similar to those that we will see later in this book for gills and lungs, increasing the amount of gas exchange that can occur. The first adaptation is that we find a notable increase in the number of capillary beds positioned close to the surface of the skin. The second is that there is often an "excess" of skin, which can be found in folds along the length of the body. The function here is relatively straightforward. The excess skin and capillary beds increase the surface area available for the exchange of gases. With more potential surface area, more gas exchange can take place, enough that these species can get all their oxygen needs through their skin alone. It should be noted, however, that these animals either are relatively small (i.e., higher surface-area-to-volume relationship; e.g., the plethodontids) or live in aquatic environments with an increased amount of dissolved oxygen (e.g., cold and fast-moving streams, like the hellbender).

Nonavian Reptiles

When we get to the reptiles, we see a striking difference from what

we just observed in the amphibians. The reptiles are fully divorced from the aquatic environment, and while many have moved back into the water, as a group they are no longer dependent on the aquatic environment. Waterproofing the body will be of paramount importance for these animals, and therefore many of the adaptations discussed are related to the prevention of water loss. And while several of these adaptations will become highly modified for other functions, many have their origins in the prevention of water loss. There are also some striking features of reptiles that originate in the integument but are subsumed into other systems. The best example of this is the turtle shell. The shell of turtles is one of the best-known structures in vertebrates, a combination of bony plates and scales. The bony underpinning of the shell begins as dermal bone, originating in the skin before fusing with the ribs and vertebrae. The scutes, or scaly parts of the shell overlying the bone, are primarily composed of keratinized scales that we will cover in more detail momentarily. While the bone may grow throughout the turtle's life, the scutes must be shed like any other reptile scale.

The most obvious feature of the integument in reptiles is the return of scales. It is imperative to state that scales in reptiles are not the same as the scales in fishes. Reptile scales are derived from the epidermis as opposed to the dermally derived scales we saw in the fishes. In addition to the developmental differences, there are structural differences in the scales as well. Most scales are composed of two different types of keratin, alpha and beta. Beta-keratin is the most common type in reptiles and birds, making up many of the tougher scales, while alpha-keratin is more commonly found in mammals, making up your hair and fingernails. You can think of beta-keratin as being tougher and less pliable, while the alpha-keratin is much more flexible. The scales are part of the great thickening of the epidermis. The much thicker epidermis in reptiles is heavily **cornified** (filled with keratin), which plays a key role in the prevention of water loss, infection, and injury, acting as a physical barrier. As we recall, keratin is not a living tissue—instead, it is the product of keratinocytes. This means that occasionally it must

be replaced as the keratin becomes damaged or simply no longer "fits" as the individual reptile grows larger. In reptiles, we call the process of replacing the keratin layers **ecdysis** or molting. For this process, let us focus on the lizards and snakes (Squamates). Snakes are famous for "shedding their skin" all at once, but what they are doing is shedding the external layers of older keratin. If you examine the shed skin of a snake, you will see not only the scales but the keratin bridges that form between them. If the head region of the skin has survived the process, you may even see the clear eye scale that is a distinguishing feature of snakes, sometimes known as the spectacle.

In a few groups of lizards and the crocodylians, a special type of scale and dermal bone combination known as the **osteoderm** can be found. Osteoderms are superficially similar to fish scales. They have thickened elements of dermal bone but in addition to that are covered superficially by more layers of keratin (Figure 6.9).



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Figure 6.9—Left: A live Gila monster (Heloderma suspectum). Right: The skull of the Gila monster showing the osteoderms in the skull.

It was originally hypothesized that much like the osteoderm-like scales in fish, these function primarily as armor against potential predation. However, recent work suggests that osteoderms may also play a role in the biomechanics of feeding and

movement in reptiles. In the crocodylians, some muscle tendons attach to osteoderms, enhancing their lateral undulations during swimming, while in skinks, the osteoderms in the head help distribute the high forces and stresses involved in biting. What is also fascinating about osteoderms is how they interact with each other. In many lizards, the osteoderms act similarly to fish scales, overlapping each other but otherwise functioning as independent units. For other lizards, like some geckos and the large Komodo dragon, individual osteoderms may fuse together into much larger bony plates. The remnants of the individual osteoderms can still be observed, but now they are structured as a single cohesive unit.

There are many unique structures within reptiles that we do not have the time to discuss here and are covered in more detail elsewhere (e.g., touch receptors near the jaws of alligators, infrared receptors in snakes). The last aspect of the nonavian reptiles we must discuss are the glands found in the skin. While their skin is not nearly as glandular as that of the amphibians, reptiles nevertheless have their own glands embedded throughout the integument. Both of the examples we will discuss here impact how reptiles are adapted to their environment and spread all over the Earth. The first are salt glands. **Salt glands** are found in reptiles that have reinvaded the marine environment (e.g., sea turtles and marine iguanas). The function of the salt glands can be elucidated by their name. Upon reinvading the marine environment, reptiles have to deal with not just the potential loss of water but also the additional salts that come from ingesting marine food and water. As we discussed earlier, the skin is much less penetrable in reptiles, and so they must expel these excess salts differently. The salt glands are not distributed across the body, as we might expect, but instead are concentrated in the head, near the eyes and nares. The second group of glands we can broadly classify as **pheromone glands**, glands that produce secretions involved in attracting and locating potential mates. Much like the pheromones themselves, these glands are specific to species in terms of their composition, structure, and location on the body. Ovary-bearing (see Chapter 1) red-sided garter snakes have

lipid-producing pheromone glands scattered along their dorsal surface, while certain iguanas have only a few pores on the underside of their femoral region.

Aves

Before diving into all the structures in the bird integument, it is important to remind ourselves that birds are a lineage within the reptiles. Despite all the amazing structures that make birds unique, the origin of those structures is rooted in their evolutionary history. Birds do retain epidermal scales, particularly around the legs and feet, although there are some questions about the underlying genetics that obscure this homology. There is also a physiological innovation that is noted in birds that will impact the structure and function of the integument greatly. This innovation is endothermy, the use of metabolic heat production to maintain a stable internal body temperature. For almost all of the birds, this means the maintenance of an internal body temperature that is greater than the ambient temperature of the environment. Water loss was a major concern for our amphibians and reptiles, and now for our birds (and mammals later on), heat loss is going to be an additional concern. Much like we saw with our lungless amphibians, the surface area of the integument will impact the movement of heat. In this section, and again when we discuss the mammals, we will see how these groups mitigate the loss of heat using the integument and its derivatives. Additionally, birds are famous for their ability to fly. Flight affects many aspects of a bird's body, and the integument is no different. While the thickness of the epidermis and dermis is not notable compared to other classes, it should be noted that these layers are only loosely attached to the underlying hypodermis and muscle. The looser connection provides flexibility for the skin to move freely during flight.

Box 6.1—The Contested Origin of Feathers

If you want to pick a fight with a large number of paleontologists, or evolutionary biologists, or ornithologists, tell them you completely understand how and why feathers evolved. Soft tissues are notoriously difficult to fossilize, and so examples of feathers in the fossil record are sparse outside of the classic *Archaeopteryx*, a small dinosaur with clear feathers. However, there is a growing consensus that many dinosaurs had at least some feathers on their body, including *Tyrannosaurus rex*. If that is the case, then what are these feathers for? Given the size of something like a *Tyrannosaurus*, flight is unlikely. Two competing theories have enough support that there are groups of scientists who support each. The first is that feathers provided insulation and allowed dinosaurs to better thermoregulate and control movement (Prehistoric Planet—Why Do Velociraptors Have Feathers? [YouTube video]). The second is that brightly colored feathers (or any feathers) can be used to signal to other individuals (example from Prehistoric Planet: Courtship Behaviors to a Potential Mate [YouTube video]). What is the evidence for each hypothesis? What seems the best supported? It is worth discussing with your classmates the merits of each hypothesis and seeing what the most up-to-date research says.

The most recognizable aspects of birds are the feathers. **Feathers** are truly amazing structures, accomplishing a wide variety of functions while being fundamentally structured like any other derivative of the integument. Feathers are responsible for powered

flight, waterproofing, insulation against heat loss, and signaling to others (intraspecifically and interspecifically). Feathers are derived from the epidermis and are primarily composed of keratin. However, the type of keratin is different from that of reptile scales. Recent genetic work has found that alligators briefly make the same type of keratin found in bird feathers (an argument in favor of their shared ancestry), but then that gene is suppressed later in an alligator's development. Beyond this, the origin of feathers is still being hotly contested, with fossils, development, and molecular evidence being weighed to this day. Even the function of the first feathers is debated (Box 6.1). Throughout their fossil history, feathers have varied greatly, as the dinosaurs and other contemporaries employed them through time. In modern birds, we can broadly classify feathers into two categories: vaned and downy feathers.

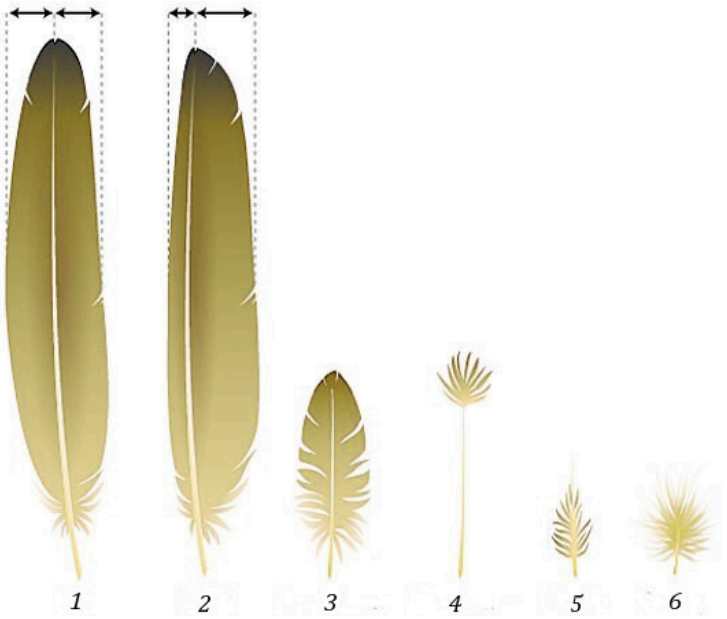

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Figure 6.10—Different types of feathers. (1) Symmetrical, countered flight feather (tail); (2) Asymmetrical, contoured flight feather (wing); (3) Semiplume; (4) Filoplume; (5) Bristle; and (6) Downy.

Vaned feathers have a central vane and shaft with barbs and filaments. The vane/shaft may be central to the feather, but asymmetrical feathers are quite common. These are the feathers that we most often associate with birds. These are the most external layer of feathers, the flight feathers, and may be brightly colored. These feathers can also be shed as they are damaged over time or as necessity dictates. The differences in these feathers can be pretty remarkable. The wood warblers, a group of migratory songbirds in the Western Hemisphere, are well known for such a change. In the spring, as the breeding season approaches, many sperm-producing individuals will become brightly colored with fantastical patterns and colors in an effort to attract mates. At the conclusion of the breeding season, prior to the onset of their southward migration, they will shed these brightly colored feathers. You would (and many do!) struggle to identify these birds in the fall, as they do not resemble their ostentatious past selves at all. The new feathers, much drabber in color, are fresher for the long migration back to their wintering grounds. The other type of feather is the downy feather. Downy feathers are seen in nestlings and are found close to the skin in adult birds. Downy feathers are much smaller with no vane and create a layer of insulation to prevent the loss of heat. Bird down is an incredibly effective insulator. How effective? We currently use bird down in comforters, pillows, and jackets! Within each category, both downy and vaned feathers can be differentiated into many other types. There are even different ways in which feathers interact with others. Some types of vaned feathers have small barbs on them that will hook together with adjacent feathers. An extreme example of this can be found in the penguins. In penguins, famous for inhabiting some of the coldest environments on Earth, the feathers are locked so tightly together they effectively act as a wetsuit. This creates an additional layer of insulation that helps the penguins retain that body heat.

Much like other reptiles, birds have several different types of glands that can be found throughout their integument. Many of the shorebirds and open-ocean birds have salt glands similar to the

other reptiles discussed earlier. These salt glands open onto the dorsal surface of the bill, allowing the excess salt to be ejected. In addition to the salt glands, birds also possess **uropygial glands**, also known as oil glands, which are crucial to the function of feathers. Uropygial glands are located near the base of the tail, producing a secretion that birds spread all over the feathers. This is the behavior we know as preening. The reason they are also known as oil glands is that these secretions are primarily lipids and act to waterproof the feathers. This is extremely important for wading birds, particularly in cold climates, as wetting the feathers would drastically reduce the bird's ability to retain body heat. Wet feathers may also stick or cling, making them ineffective if the bird needed to fly.

Mammals

As we conclude our tour of the vertebrate classes, we reach the mammals. In many ways, mammals are like many of the other vertebrate classes. There is a thick cornified epidermis with an even thicker underlying dermis. There are derivatives in the integument that are important. However, there are many facets of mammalian integument that are unique. With one very notable exception, mammals do not have scales of either epidermal or dermal origin. Mammals do not use osteoderms (or any dermal bone) for armoring, with the exception of the armadillos. Mammals have a myriad of glands embedded throughout their integument, several of which are completely new to the scene. Additionally, many mammals store copious amounts of fat in the hypodermis (humans included). This can serve two purposes. For mammals that slow down their activities in winter, this subcutaneous fat contains the energy reserves they need to survive until warmer weather returns and food is bountiful again. The second purpose involves insulation. Mammals are also endothermic animals, using heat produced from their metabolic processes to elevate their body temperature. Mammals living in colder environments have the same struggle as

the birds: How do you prevent the loss of that heat? One good mechanism is to use a layer of body fat as insulation. This body fat is often poorly vascularized, preventing blood that has been warmed at the core of the body from losing that heat as it travels closer to the surface of the skin. Marine mammals, including whales, dolphins, seals, sea lions, and walruses, use a specialized type of subcutaneous fat that we know as **blubber**. To be clear, blubber is not the same structure as the body fat you or I have. Blubber is a specialized type of body fat that is incredibly efficient at controlling body heat. There are two key areas of difference between our body fat and blubber. The first is that blubber is extremely dense, and the second is that blubber is highly vascularized. While the increased number of capillaries may seem counterproductive to the retention of body heat, they actually allow for the animal to have effectively better control of heat retention or loss. If the body temperature begins to drop, blood flow can be directed away from those capillaries, and the incredibly dense blubber is a more than effective insulator. However, there may be times when the whale is moving through warmer waters or the walrus is sunning on the beach, and too much heat can be an issue. These capillaries can be opened and blood allowed to move to the surface, allowing heat to escape. Many mammals have similar mechanisms for the dispersion of excess body heat. Elephant ears are full of capillaries, and when an individual is getting too warm, they will flood those ears with blood. These elephants can be observed flapping their ears back and forth, cooling the blood before it returns to the core of the body.

The most recognizable feature of the mammalian integument is **hair**. Much like feathers to birds, hair is a trait that even at an early age we are taught is a distinguishing trait (**synapomorphy**) for the mammals. Fundamentally, hair is quite similar across the mammals and in many ways is similarly structured to other integumentary derivatives. Hair is composed primarily of keratin. Hair can be broken into two major regions, the shaft and the root. The root of a hair is where the keratin-producing cells (and other supportive cells) are still alive. The root is embedded

in the dermis. The shaft of the hair contains only dead cells and keratin, found primarily in the epidermis and exposed to the outside environment. Hair itself has three regions internally as well, at the center a medulla, a cortex, and an external cuticle. Hair is also supported by several different cells, tissues, and glands. Collectively, we refer to the hair and its supporting structures as the follicle. Beyond this, hair is extremely variable. As we saw with vaned versus downy feathers in birds, there can be different layers of hair. In most mammals, we refer to the outer layer of hair as the guard hair and the underneath layer as down hair or underfur. The function of the down hair is similar to downy feathers in birds: It provides a layer of insulation.

Hair has been modified to perform a variety of different functions. The quills of porcupines, an apt deterrent of predators, are modified hairs. Wool from sheep is hair. The whiskers of dogs, cats, and many other mammals, formally known as **vibrissae**, are hairs that serve as incredibly sensitive touch receptors (Figure 6.11).



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Figure 6.11—A young cow seal with the many vibrissae present around its nose and mouth.

Most hair on the body has at least some sensory reception; with nerves wrapped around the base of the hair root, any deflection of the hair results in a signal being sent to the brain. The majestic mane of the lion serves as a signal, warning other lions of their prowess and physical threat. Zebras and tigers have stripes on their skin that are also reflected in their hair. For the tiger, this helps hide the predator in tall grass, disrupting their shape and outline as they approach potential prey. The color patterns in zebras were also thought to obscure their outline; however, recently it was determined that the zebra's stripes actually disorient biting flies, making it difficult for them to land. Many mammals use hair for thermoregulation, much as the birds use feathers. The hair traps air close to the surface of the skin, providing an effective layer of insulation that prevents the loss of body heat. These mammals often have thick coats of fur that are composed of multiple layers and different types of hair. Additionally, there are smooth muscles embedded in the dermis known as **arrector pili muscles**. The arrector pili muscles pull on the base of the hair, causing it to straighten upward. When you get goose bumps, you are seeing these muscles in action! Even within the mammals that use hair to thermoregulate (which is most mammals), there are a few exceptional examples. The polar bear is a very interesting case. From our perspective, polar bears are completely white, broken only by black eyes and a small black nose. However, polar bear fur is not white at all but is instead almost perfectly clear. These clear hairs act much like fiber optic cables, trapping light and carrying it down the length of the hair. When that light reaches the skin, it is absorbed as heat. This system is so effective that it is not heat loss that polar bears must be concerned with but actually becoming too warm! Polar bears will roll around in the snow to dump the excess heat from their bodies. All mammals have hair at some point in their life history, though many are greatly reduced. The cetaceans (whales and dolphins) are almost entirely hairless. Hippopotamuses, rhinos, and pinnipeds have reduced amounts of hair as well.

In addition to hair, many mammals also possess a dramatic,

obvious derivative of the integument. Broadly we refer to them as **antlers** or **horns**, and while they may serve a similar purpose, they are different structurally. For the most part, antlers and horns function during the mating season, as testes-bearing individuals will battle one another over access to mates and territory. However, such weaponry on the head also makes for an effective deterrent against predation. Water buffalo and rhinos will use their horns against any perceived threat, and while bighorn sheep battling with each other is quite impressive, wolves and mountain lions would not want to be on the receiving end of such a collision. Both antlers and horns begin as outcroppings of cartilage and bone on the skull. This is different from **tusks**, another popular mammalian weapon, which are actually enlarged teeth (teeth are covered in Chapter 8—The Skull). Antlers are only found within the family Cervidae, which we can think of as "true" deer, moose, reindeer, and others. Antlers begin in the dermis as cartilage, which quickly becomes ossified. During their growth phase, antlers are covered in velvet, a thin layer of highly vascularized integument that helps feed the developing antler. Once the antler is fully grown, the velvet begins to die off, and the underlying bone is exposed. Before weathering and damage take their toll, it is possible to see tracks from the blood vessels on the antler itself. Once the mating season has concluded, antlers are shed, the dermal bone at the base having been removed. The following season, the antlers will regrow. White-tailed deer, a North American cervid, will grow larger antlers in each following season, reaching a maximum size well after sexual maturity. Horns begin in a similar manner (the genetics are different, but that is beyond our scope). Dermal bone grows outward from a base rooted to the skull. There are a few key differences beyond this point. In the majority of species, horns are not shed during an individual's lifetime; rather, they continuously grow until death. Horns also have some component of keratin as part of their structure. Rhino horns have small compartments filled with keratin throughout. The horns of bighorn sheep are covered by a relatively thick layer of keratin on top of the bone. As the bone continues to grow, so does the keratin.

Within the skin, mammals have an array of glands. We are going to focus on two of the major groups of glands: sweat and oil glands. Oil glands, also referred to as **sebaceous glands**, are most commonly associated with hair follicles. Rooted in the dermis, oil glands secrete their products directly onto the root of the hair. The secretion, known as sebum, helps lubricate the hair to pass through the epidermis while also assisting in the waterproofing of both the hair and the pore through which the hair emerges. **Sweat glands** can be subdivided into two further categories, eccrine and apocrine sweat glands. This distinction will become important later in this section, as we discuss mammary glands, a unique mammalian feature. Sweat glands are embedded within the dermis of mammals, with small ducts that lead to the surface of the epidermis, where a small pore allows their secretions to spread on the surface of the skin. The number of sweat glands and their locations vary quite a bit from species to species. Humans have sweat glands all over the body, in fairly high numbers. Domesticated dogs are well known for having many fewer sweat glands, and most of them are located on the pads of their paws. The function of sweat glands is obviously to produce sweat. Sweat, a mostly water-based secretion, has several functional roles. The primary function of sweat is to help get rid of excess body heat through evaporation. Sweat on the surface of the skin absorbs heat, and as it evaporates, it pulls that heat from the body. It also carries nitrogenous wastes that can be left on the skin surface and molecules that act to lower the pH. These lower-pH secretions are referred to in humans as the "acid mantle" and provide an extra level of protection against potentially harmful bacteria and viruses. Most sweat glands that function as we just discussed belong to the category of eccrine sweat glands. Apocrine sweat glands are slightly different. In addition to the water-based secretions, apocrine glands add relatively high numbers of lipids and proteins to the mix. This results in secretions that are thicker than those in the eccrine glands and can be highly specialized depending on location and ultimate function. Some musk glands are apocrine glands, creating the odors that mammals use to mark

territories, advertise mating status, and more. The ceruminous glands of humans are apocrine glands, producing what we know as earwax to trap dirt and other materials that may enter the external ear canal.

Mammary glands, another hallmark of the mammals, are specialized apocrine glands. They produce what we commonly refer to as "milk" for the purposes of feeding offspring after birth. The milk, full of proteins and lipids and other nutrients, is crucial to the health and growth of young. The content of the milk is quite variable from species to species. Whale milk can be almost 50% fat, one of the highest concentrations in mammals. In the monotremes, our echidnas and platypus, each individual mammary gland opens to a pore on the skin, on the ventral surface of the mother. The mother essentially "sweats" milk, which the young then lap up. Other mammals have nipples or teats, individual openings through which multiple mammary glands empty their products. The marsupials concentrate these within the pouch. The placental or eutherian mammals have multiple openings (6–10) on the belly/ventral surface, with the exception of the primates, who often only have two openings in the chest region.

6.5 Humans—Anatomy and Applications

Humans, unsurprisingly, have all the traits of the integument that other mammals possess. We have a keratinized epidermis and a larger dermal layer. We have oil glands, sweat glands, mammary glands, and hair. There are sensory receptors wrapped around the hair that provide sensations when the hair is brushed or moved. Our epidermis and dermis have sensory receptors throughout, providing feedback on touch, pressure, temperature, and pain. In this section, we will focus on structures that are unique to humans, those that are of particular importance to human biology and health, or

common diseases that are the result of damage/issues with the integument.

The epidermis in humans is similar to that of other mammals in terms of both its structure and its constituents. However, some of us develop an extra layer to our epidermis. Known as the stratum lucidum, this extra layer shows up in areas of higher physical stress. The bottoms of your feet, your heel and toes, may seem to have tougher skin than other parts of your body. If you use your hands for a lot of manual labor, you may develop "calluses." This is the effect of the stratum lucidum. It helps prevent the underlying living tissues of both the epidermis and dermis from becoming damaged. In other areas, the epidermis may be thinner. If you were asked to identify an object without looking at it, you may pick the object up and run your fingertips over the object. Your fingertips have higher concentrations of touch receptors and a thinner epidermis, providing excellent feedback on the texture and composition of the object.

Box 6.2—Tattoos

Tattoos are an ancient and ubiquitous part of human culture. Injecting pigments or ink into the skin with symbols and patterns is as old as humanity itself. But have you ever stopped to wonder how tattoos work or why they don't disappear over time? The answer lies in the structure of the skin itself. The epidermis is full of cells, and those cells spend a lot of time dividing and creating new cells. We know from this chapter that there is a lot of turnover in those cells. So how do tattoos survive this? The short answer is they don't. When an artist injects the ink into the skin, they are pushing through the epidermis and placing the ink into

the layers of the dermis. The dermis, being primarily connective tissue (and few cells), does not have the same rate of turnover as the epidermis. The ink is then much more slowly broken down over time. This keeps your tattoo looking new for many years!

As we mentioned before, the sweat and oil glands open to the surface via pores in the epidermis. While the glands themselves are embedded deeper within the dermis, their openings in the epidermis serve as a potential point of entry for unwanted pathogens and other materials. Dirt and other materials may enter a pore and prevent the sweat/oil from being released, effectively clogging the pore. The sweat/oil begins to build up, and bacteria will enter as well. The buildup of sebum and dirt results in what we commonly refer to as a blackhead. They are not themselves dangerous, but if bacteria can enter, infection may occur. White blood cells rush to the scene, working to destroy the pathogens before they cause any damage. However, we might notice the response as the area around the infection becomes inflamed and there is buildup of dead cellular material and pus. This is known by many colloquial names—a pimple or zit, for example. Acne refers to the chronic infection of the skin in this regard. Repeated damage to these areas of the skin can result in painful scarring.

Healthy, normal epidermal tissue is constantly replacing itself. As the outer layers are damaged, new layers of keratin from dying cells are pushed upward to replace them. The rate at which these cells proliferate and produce keratin is tightly controlled. In many people suffering from the condition known as psoriasis, however, the cells of the stratum basale and germinativum grow and reproduce much faster than they should. A result of systemic inflammation, they push themselves to the surface, resulting in patches of skin that are dry and itchy and can be quite painful.

Thought to be an immune disorder, there is no cure for psoriasis, but instead the symptoms are treated in an attempt to provide relief.

The lower levels of the epidermis are also where we find the cells that give color to the skin. The most common and medically relevant of these pigment-producing cells are the **melanocytes**. Melanocytes produce melanin, a dark pigment. **Melanin** absorbs solar radiation and is particularly important for absorbing UV radiation, which is known to damage the DNA within cells. In response to increased exposure to UV radiation, melanocytes will increase in number and in their production of melanin. However, there are two elements of this that are noteworthy for human health. The first is that too much UV exposure at once does not allow enough time for the melanocytes to adapt. The large volume of UV radiation is absorbed by other cells in the skin, resulting in damage to those cells, which you may notice as a sunburn. This is an immediate impact of excessive UV radiation exposure. Over longer periods of time, chronic exposure to excessive UV radiation damages the DNA of the melanocytes themselves, with one effect being that they begin to proliferate uncontrollably. This is a type of skin cancer that we know as melanoma. Melanoma is the most dangerous of skin cancers, as it will metastasize (spread) to other parts of the body, where it may ultimately cause death (Figure 6.12).



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Figure 6.12—Melanoma on a person.

6.6 Summary

The integument is the barrier between an organism and its environment. Any interaction between an organism and its environment (including other organisms in that environment) involves the integument in some manner. It is important to take into account not only the evolutionary history of an organism when considering how its integument may be structured and function but also the environment it currently lives in and what it may encounter in that environment.

Application Questions

1. In humans, burns are assigned degrees (first, second, third). How does burn degree relate to the structure of the integument? Additionally, many people suffering from a third-degree burn initially report no pain at the injury site. Why is this?
2. You have found a dead common garter snake (*Thamnophis sirtalis*) on a hiking trail. The individual is a striking blue color, but in life these snakes are a dark green. What might be the cause of this blue color in the dead individual?
3. Find some human anatomy textbooks and look up their information on melanoma. The majority of the images will show classic melanomas from people of Caucasian ancestry. What challenges do people with darker-toned skin face when it comes to recognizing a potentially life-threatening melanoma?

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7. Bone and Cartilage

BILL RYERSON

Focus Questions—to Guide Your Reading of This Chapter

1. What do the prefixes and suffixes of cell names tell us about the connective tissues?
2. Cells are important in every system, but it seems that everything outside of the cells in bones and cartilage is stressed in this chapter. Why might that be?
3. How do the different types of bone and cartilage and their structural properties impact where we find them in the skeleton?

7.1 Introduction

For the next several chapters, we will take a deep dive into the skeleton. Like the previous chapter on the integument, we will go through a generalized introduction to the different elements of the skeleton, where they originate developmentally, and how their variation across vertebrates can tell us about the evolutionary

history and functional importance of those structures. The skeleton is such a broad system, with so many different moving pieces (pun very much intended), that it would make sense to talk about the components of the skeleton first, how they function, and where they come from before diving into the particular regions.

All vertebrates possess an **endoskeleton**—that is, a skeleton that is internal to most of the body (endo- from the Greek *endon*, meaning "within"). This is somewhat of a vertebrate-defining characteristic, as few other animals possess a rigid internal skeleton. Arthropods, the largest group of animals on the planet (insects, spiders, lobsters, etc.), possess an exoskeleton. There may be skeletal elements that are on the external surface of a vertebrate (see the osteoderms in Chapter 6), but the major pieces of the skeleton are internal to the body. The skeleton may be highly reduced to only a few elements, or it may have hundreds of individual elements. It can be made of bone, as it is in humans, or it can be predominantly cartilage, as in our cartilaginous fishes. There are even some gray areas, where cartilage can look and act like bone, as we see in some of our larger sharks. We will discuss the details of cartilage and bone, how they develop, and their functional roles later in this chapter.

7.2 What Is the Skeleton?

As we just discussed, the skeleton is a primarily internal structure of tough tissues. To really think about the skeleton, we must consider what it does in vertebrates to reflect on how structural variability links with function. We must also consider some of the lesser-known functions of the skeleton, as they may help us understand some of the constraints or limits to the structural variability we see in the skeleton. Skeleton functions include the following:

1. Provide support for the body

2. Protection of internal organs (think about the brain or heart)
3. Anchors for muscles to provide movement (muscles create movement, but the bones actually move)
4. Mineral/nutrient storage
5. Formation of new blood cells—white and red
6. Vitamin synthesis

When we consider the skeleton, we will focus primarily on the first three functions, as they will help us understand the evolution of the skeleton as well as how it impacts the evolutionary history of the vertebrates. However, do not neglect the last three functions, as they do impact the structures of the skeleton and play a vital role in human health.

Despite the fact that the skeleton itself can be thought of as a single system, almost all anatomy textbooks and references (and anatomists themselves!) divide the skeleton itself into a few regions. Some of this is functional in nature, some developmental, and some evolutionary. We will see that the skeleton as well as individual bones and joints will be categorized according to similar patterns, and once we make those patterns obvious, the distinctions will hopefully fall into place.

The first skeletal dichotomy is the **axial skeleton** versus the **appendicular skeleton** (Figure 7.1).

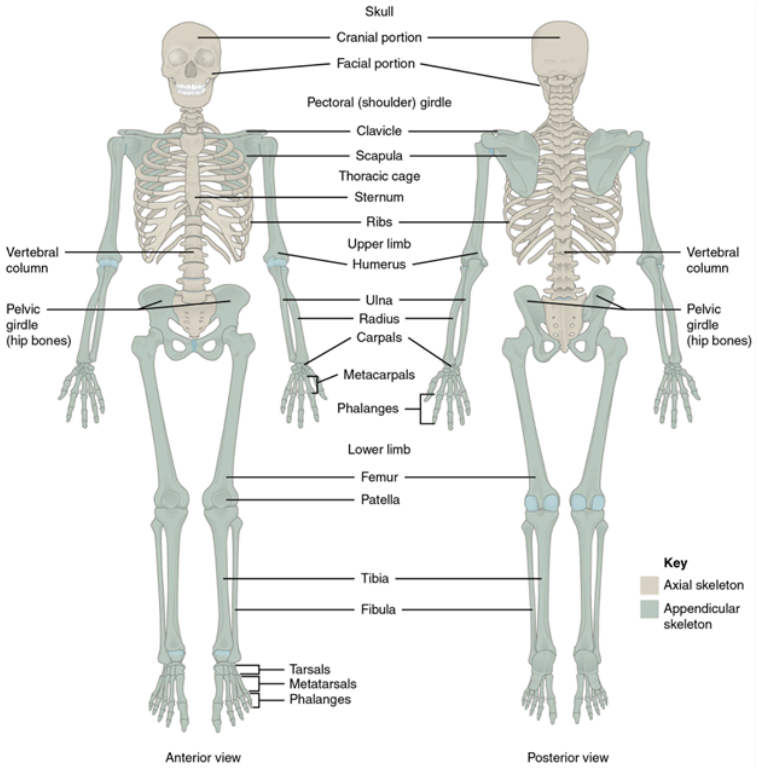

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Figure 7.1—Axial and appendicular skeleton. The axial skeleton supports the head, neck, back, and chest and thus forms the vertical axis of the body. It consists of the skull, vertebral column (including the sacrum and coccyx), and thoracic cage, formed by the ribs and sternum. The appendicular skeleton is made up of all bones of the upper and lower limbs.

Take the human body and make a vertical cut right down the midline (gross), from head to feet. All the bones you would cut through, with two notable exceptions, are part of the axial skeleton. The exceptions are the pelvis (part of the appendicular skeleton) and the ribs, which while you did not cut through them, are part of the axial skeleton. Evolutionarily, these are the "original skeleton." The bones associated with the appendicular skeleton are all the other

bones of the body. Their name hopefully gives them away, as they are the bones that either are part of the appendages or anchor the appendages to the axial skeleton.

The second dichotomy is the **cranial** versus the **postcranial skeleton**. Hopefully this one is even more straightforward than the previous. The "cranial" skeleton is the skull and its associated structures, like the hyoid. This is both a developmental and evolutionary distinction. As we will see in Chapter 8, the skull has a long and storied history, and so many will focus on the skull, leaving the rest of the skeleton on its own as postcranial (see Chapters 9 and 10).

The third dichotomy is dependent on the developmental formation of the bones. The two types are **endochondral** and **intramembranous bones**. These refer to the origin of those bones during development. The process of forming bones in the skeleton is known as **ossification**. Future hint: Wherever you see "oss-," it is referring to bone or something analogous to bone. It is not always bone, as many large sharks and other cartilaginous fish will ossify parts of their jaws, skulls, and vertebrae, which are different from true bone. Later in this chapter, we will distinguish the differences between cartilage and bone. When an embryo is developing, not all bones form in the same way. Some start off as cartilage molds, which are gradually replaced by bone. These are the endochondral bones (endo = within; chondra = cartilage). Other bones will form within developing sheets of connective tissue. These are the intramembranous bones. Before we talk about how these bones will arise, let's take a big step back and introduce the players that will guide these changes.

Bone and **cartilage** are alike in that they are both connective tissues. This means that they share several characteristics and that the differences among these characteristics will help us define the different tissues. Like any tissue type, there are cells. We can define the different bone and cartilage cells by their functions in the body and assign them names based on these functions. We will also define them as "immature" and "mature"

depending on where they are in the life cycle of a cell. There are many types of connective tissues, but for this section we are going to be focusing on just bone and cartilage (and maybe one/two more). We can look at the names given to these cells and break them apart into their prefix and suffix. The prefix will tell us what tissue type that cell belongs to: osteo- for bone and chondro- for cartilage. The suffix will tell us the function and maturity of the cell: -blast for immature cells and -cyte for mature cells. So an **osteoblast** is an immature bone cell. A **chondrocyte** is a mature cartilage cell. Immature cells, for both bone and cartilage, are usually what we refer to as "builders." Builders will create the material that ultimately composes bone and/or cartilage. Mature cells are often referred to as "monitors." These mature cells will remain embedded within the tissue, checking for damage or changes in the chemical environment. They can send their own signals to indicate that the tissue needs to be repaired. We have **osteoblasts**, **osteocytes**, **chondroblasts**, and **chondrocytes**. There is one more cell type to be introduced for now. Bone has a third type of cell, the **osteoclast**. *Clast* has a Greek origin, meaning "to break." Osteoclasts are cells that will break down bone for reasons that we will discuss later. Bone is a very dynamic tissue, always being broken down and replaced depending on activity, age, nutrition, and general health.

While the cells are important, they do not help us define what bone and cartilage are and how they function. All connective tissues have an additional component known as the **extracellular matrix** (ECM). The ECM is just as important as (if not more so than) the cells. The ECM is built from two major elements: **fibers** and a **ground substance**. The fibers are typically one of three types: collagen, elastin, and/or reticular fibers. **Collagen** is the most common protein in the body, found in muscle, bone, cartilage, tendons, ligaments, and more. Bone will have collagen, but some cartilages may have both collagen and elastin. The type of fiber and the amount will determine the flexibility and strength of a material. Collagen is the least flexible but the strongest. **Elastin** is the most flexible but the easiest to damage. The ground substance is just as

important for determining the function of a tissue as the fibers are. There is much more variation here than in the fibers, and so the best approach is to examine each as we delve into the individual tissues.

7.3 Bone and Cartilage Components

There are many players in the building of a skeleton, but for the purposes of brevity, we will discuss only a few major different types: bone, hyaline cartilage, elastic cartilage, and fibrocartilage. Most of this discussion will focus on hyaline cartilage and bone, as they are the most abundant tissues found in the skeleton. They are key components of the adult skeleton and also play a major role in the development of the skeleton.

When considering the skeleton, bone is the first tissue that comes to mind. Bone is one of the strongest biological materials known. In compression, it can withstand over 20,000 psi. The majority of vertebrate groups use bone as the foundation of their skeleton.

Bone has four major cell types: osteoblasts, osteocytes, osteogenic cells, and osteoclasts (Figure 7.2). They are responsible for building, maintaining, and breaking down bone. The major fiber in bone is **collagen**. The ground substance in bone has two major components: an **organic matrix** and an **inorganic matrix**. The organic matrix, known as **osteoid**, helps connect the cells embedded in bone, the collagen fibers, and the inorganic matrix. The inorganic elements of the matrix are known as **hydroxyapatite**. The bulk of the hydroxyapatite is mineral salts, particularly those of calcium and phosphate. The mineral salts compose almost two-thirds of the total mass of bone. It is important to consider the calcium and phosphate together with the collagen fibers in understanding the functional strengths and limits of bone. The hydroxyapatite is extremely strong but extremely brittle. The collagen woven through the ground substance provides bone with some flexibility as well

as increasing bone's strength in tension. Without the collagen and the hydroxyapatite, the function of bone is greatly impaired. This is especially important in the context of human biology and medicine. As we age, the rate at which tissues are replaced slows down. The collagen in our bones loses its limited flexibility, and the bones become more brittle, increasing the likelihood that they will fracture. Additionally, changes in calcium availability and replacement begin to impact the strength of the bone. A lack of calcium in the diet, along with hormonal changes due to aging, can result in the loss of bone density in a condition we refer to as osteoporosis. Osteoporotic bones are much more likely to fracture when subjected to even minor impacts or forces.

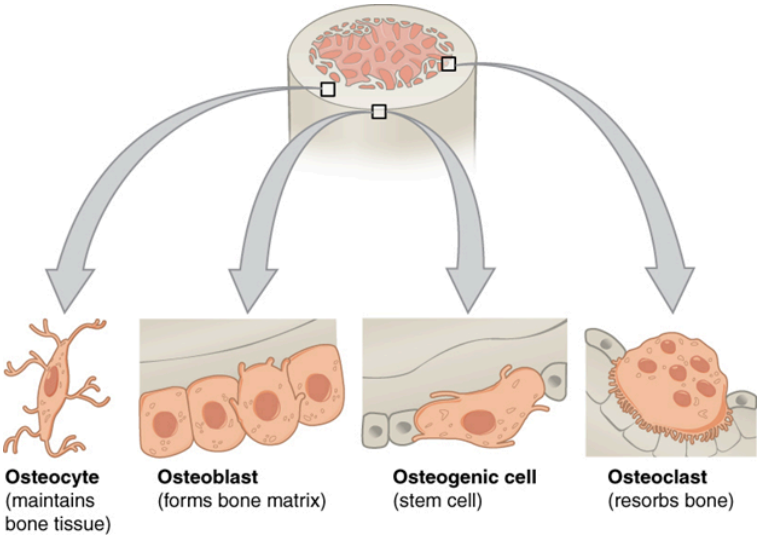

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Figure 7.2—The major cell types of bone cells.

Hyaline cartilage is the most common cartilage in the body. Much like bone, it is a connective tissue, so we can identify the key cells and extracellular matrix that will define hyaline cartilage and how it functions (Figure 7.3).

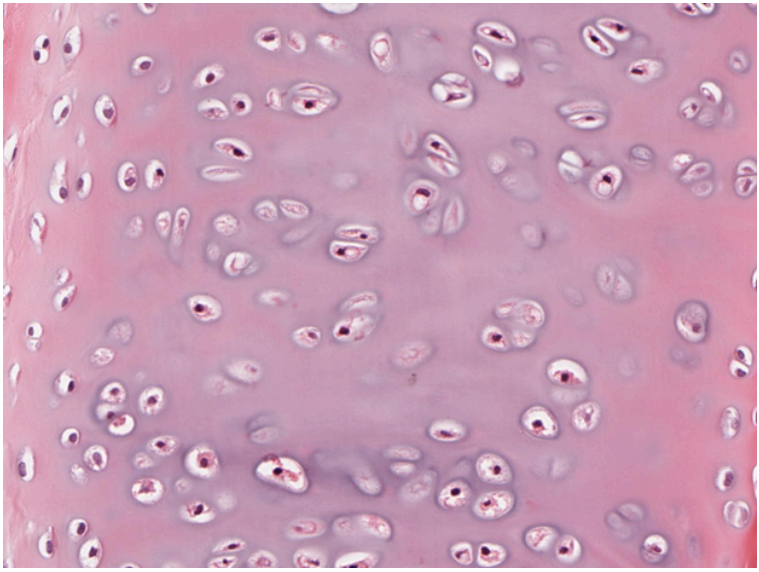

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Figure 7.3—Hyaline cartilage of the trachea. Note the small chondrocytes surrounded by the purple/pink staining ECM.

All of the cartilages have two major types of cells, the **chondroblasts** and **chondrocytes**. Chondro- always refers to cartilage. The -blasts and -cytes work similarly to how they did in bone. Chondroblasts are immature cells that build cartilage. Chondrocytes are embedded within the extracellular matrix, monitoring the mature cartilage for damage. There are **chondroclasts**, cells that may break down and resorb cartilage, but they are rare. In fact, it's only been within the last 10 years that we have started to pin down what chondroclasts are and where they originate. The extracellular matrix for hyaline cartilage is full of collagen fibers, with some elastin. This gives hyaline a little more flexibility than bone. The ground substance is also more flexible than bone. It is composed of three major types of molecules: glycosaminoglycans, proteoglycans, and glycoproteins. These sugar-protein combinations absorb water when placed in the

extracellular matrix and help bind the collagen to the rest of the matrix. This results in a material that is tough (but not as strong as bone) but also fairly flexible. As a result of these properties, you can find hyaline cartilage in many places that require both toughness and flexibility. The ends of many bones have a layer of hyaline cartilage, particularly those involved in joints, which we refer to as articular cartilage. The costal cartilages, which connect the ribs to the sternum in many animals, are also hyaline cartilage. There is hyaline cartilage in nonskeletal locations as well; the cartilage that keeps the trachea open is hyaline. Later in this chapter, when we discuss the development of the skeleton, we will learn that many bones originate as hyaline cartilage before they are replaced with true bone. In humans, we can find remnants of this system in our growth plates, also known as epiphyseal plates, where a bit of hyaline cartilage remains and is the center for bone growth.

Elastic cartilage and **fibrocartilage** (Figure 7.4) play more reduced roles in the body than hyaline cartilage or bone. Elastic cartilage is not involved in the skeleton, found predominantly in the external ears and the tip of the nose in some mammals. It has the same general cell types as hyaline. The difference is in the fibers. Elastic cartilage is almost entirely elastic fibers, composed of a protein known as elastin (repetitive, isn't it?). **Elastin** is the most flexible of all the fiber types, able to be deformed and stretched without damage. However, it is not the toughest material, and it is easily damaged. Fibrocartilage is more common, found in several places: the intervertebral discs between individual vertebrae, menisci in the knees of many vertebrates, and symphyses (the pubic symphysis in humans, for example). All three of these structures sit between bones. In the case of the intervertebral discs, the fibrocartilage surrounds fluid that allows for additional cushioning. Menisci sit between the tibia and femur of most tetrapods and also provide cushioning to the knee joint. Symphyses are fibrocartilage connections between bones. The pubic symphysis provides a small amount of flexibility between the left and right sides of the pelvis.

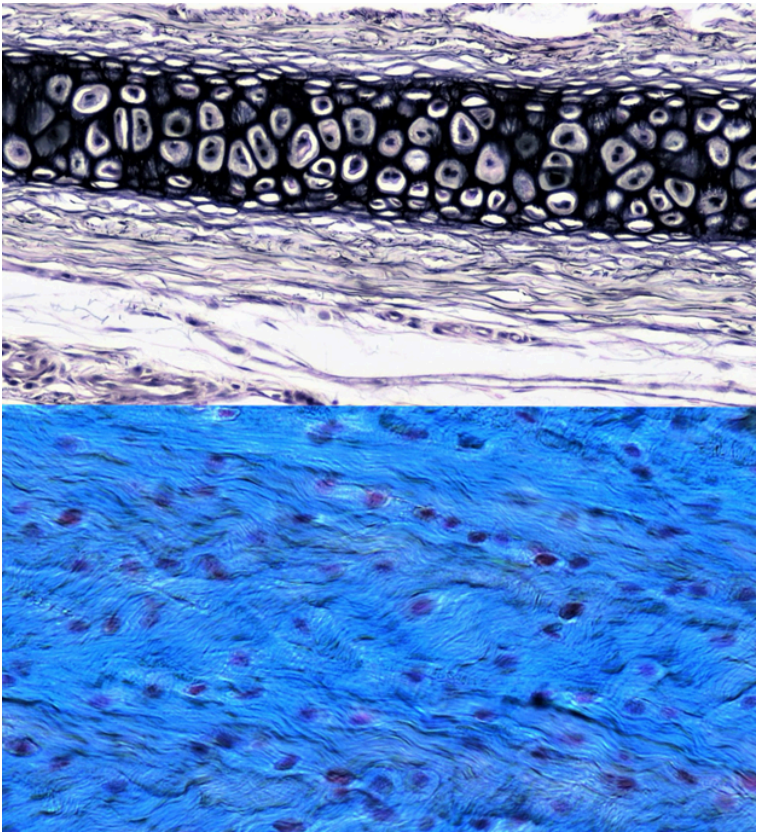

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Figure 7.4—Top: Elastic cartilage. Bottom: Fibrocartilage.

7.4 Bone Structure

Now that we have the cellular components of the skeleton, we can look to individual bones for additional information on how the skeleton is constructed and how it functions. Individual bones can be broken down into several different regions. None of the bones are completely solid and instead may have channels or even large

cavities within them. Using the human femur as an example (Figure 7.5), we can see the regions of a bone.

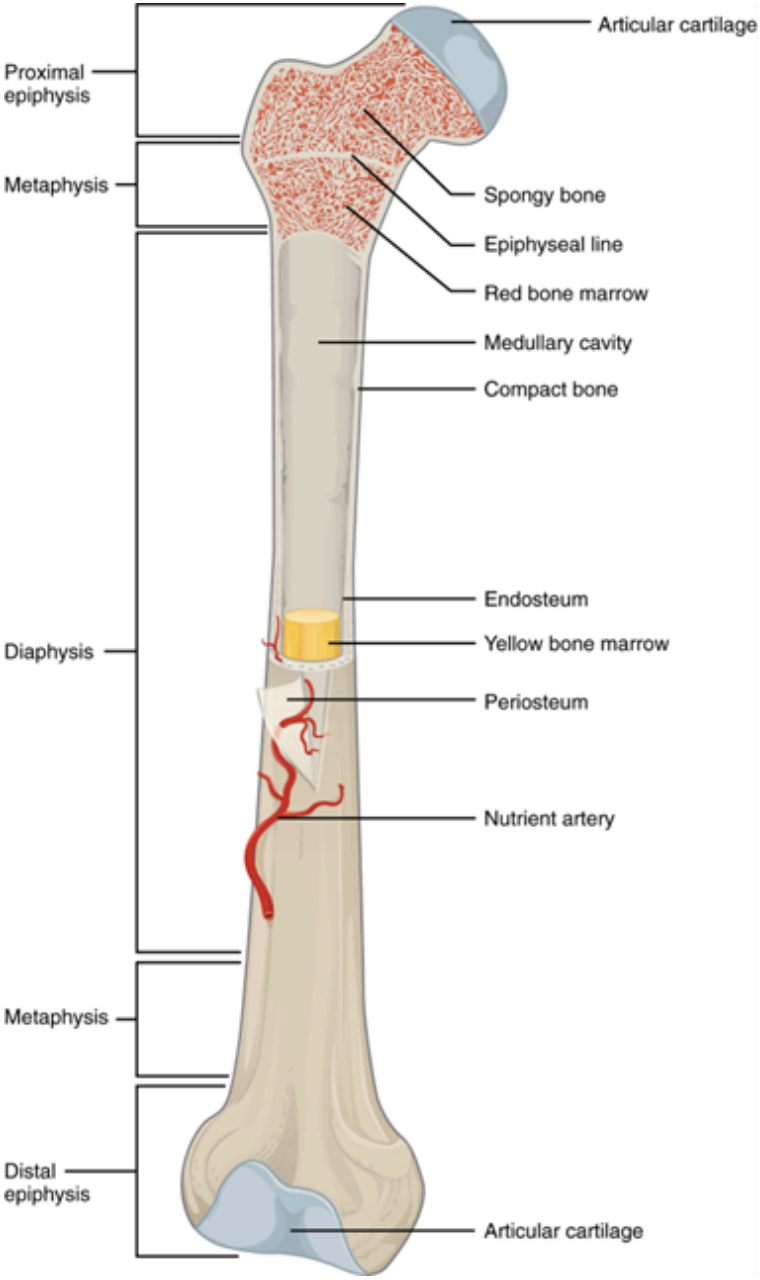
Articular cartilage
Proximal epiphysis
Metaphysis
Spongy bone
Epiphyseal line
Red bone marrow
Medullary cavity
Compact bone
Endosteum
Diaphysis
Yellow bone marrow
Periosteum
Nutrient artery
Metaphysis
Distal epiphysis
Articular cartilage

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Figure 7.5—A human femur with major structures labeled.

The largest section of this bone, sometimes referred to as the shaft of the femur, is known as the **diaphysis**. The diaphysis has a large cavity at its center, often referred to as the marrow cavity. This is where red or yellow bone marrow can be stored, red for the formation of blood cells and yellow for the storage of fats and nutrients. At each end of the bone are the **epiphyses**. The epiphyses are important for bone development as well as bone growth. Within the epiphyses we find the previously mentioned **epiphyseal plates**, or growth plates as we know them in people. Before sexual maturation, the epiphyseal plates are primarily hyaline cartilage and responsible for the growth of bone in length. After maturity, the growth plates fuse and become entirely bone. There are exceptions to this, as many animals can continue to grow in size beyond sexual maturity. Beyond the growth plates, the epiphyses are another place to identify two types of bone that are found throughout the body. When viewed from its external surfaces, bone appears to be quite solid. This is actually a specific type of bone, known as **compact bone**. It is, in fact, quite solid but not continuous throughout the entire bone. In the diaphysis of the long bone, compact bone is found on the **periosteal** (external) and **endosteal** (internal) surfaces. In the epiphyses, it may only appear on the periosteal surface. The internal element is composed of a porous material full of channels and bone struts. This is **trabecular bone** (also referred to as spongy or cancellous bone). Trabecular bone provides the maximum amount of strength with the minimal amount of material. Trabecular bone is heavily regulated and constantly remodeled. The patterns of trabecular bone can be used to understand the forces encountered during movement in an area. The lines of the trabecular bone may match the lines of force.

7.5 Bone Development and Growth

There are two major patterns of bone development: endochondral and intramembranous. The development of the skeleton is a very well-understood process but a little beyond the scope of our work here. Instead, we can briefly discuss the two processes, where they occur, and who the major players are. Intramembranous ossification (making bone) is typically associated with flat bones like the skull and sternum (Figure 7.6).

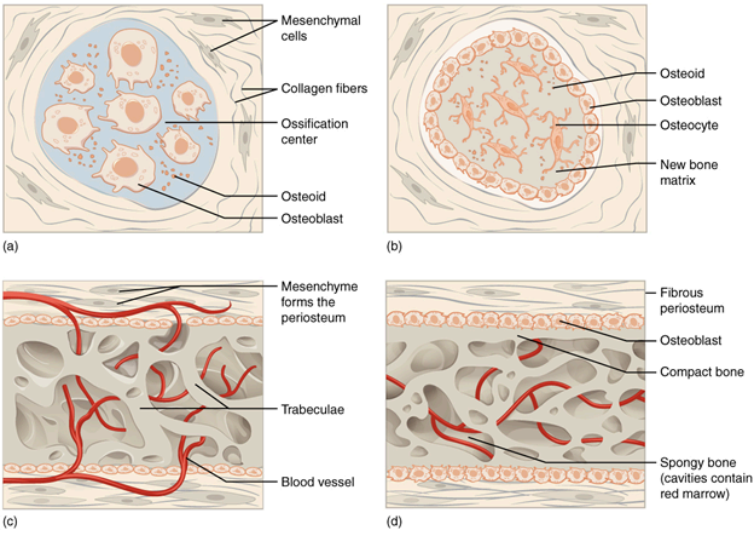

Figure 7.6—The process of intramembranous ossification. Stages occur in order from A to D.

Take a look at a human skull, and find the parietal bone, as it is formed this way. Early in development, mesenchymal cells find their way in between sheets of connective tissue (Figure 7.6A). Responding to environmental and genetic signals, they become osteoblasts and begin to secrete the osteoid we discussed earlier (Figure 7.6B). With many of these cells working, some get trapped in

the bone as it is being laid down. These trapped osteoblasts mature into osteocytes and begin the work on making sure the bone is properly set up. As more and more bone is placed, osteoclasts move in to create openings for nerves and blood vessels (Figure 7.6C). The process continues until the bone is almost fully formed (Figure 7.6D). This pattern of development is primarily used for these flat bones; intricate shapes (like those of the femur/humerus) are not conducive to this type of development. For these bones, the process of endochondral ossification is more common. *Endochondral* roughly translates to "within cartilage," and this is how the process unfolds. You can see the overall process in Figure 7.7. The long bones, like the humerus and femur, begin as cartilage molds (there's that pesky hyaline again; Figure 7.7A). Slowly, that cartilage is replaced by bone.

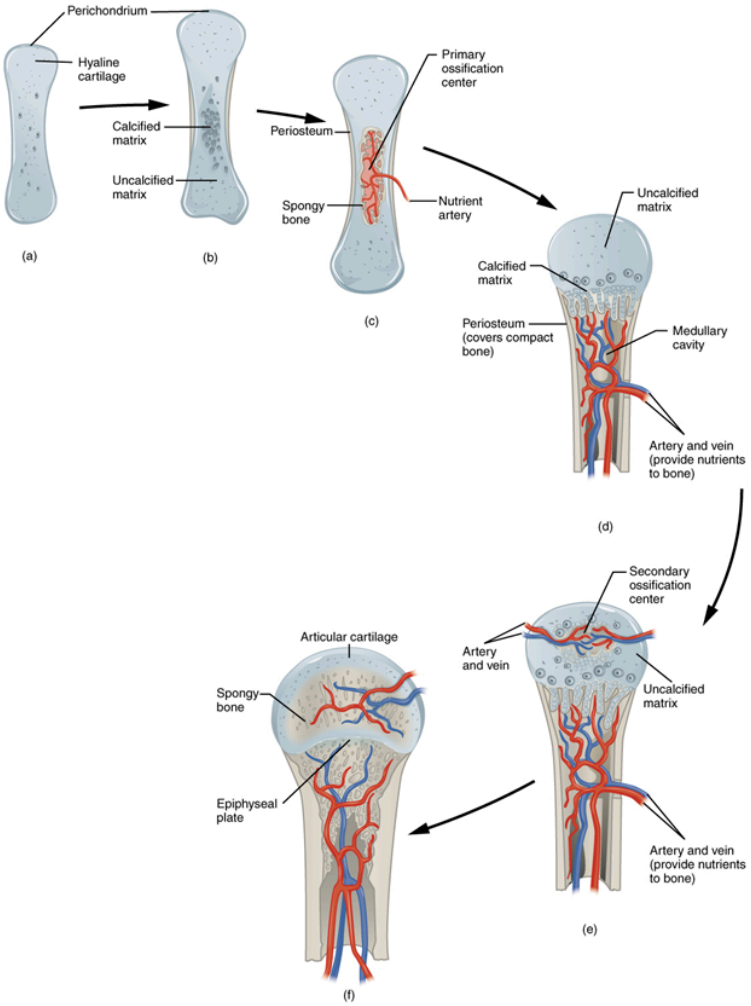

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Figure 7.7—The process of endochondral ossification in a human long bone.

The hyaline cartilage is calcified (Figure 7.7B), and as the chondroblasts and chondrocytes die, they are replaced by osteoblasts. Note the differences in location of the primary and

secondary ossification centers as well as their fates (Figures 7.7C and 7.7E). The primary ossification center will be the diaphysis, with the marrow cavity as its center (Figure 7.7D). The secondary ossification centers will become the epiphyses. As these areas grow, they converge toward one another. However, they don't quite meet. Before birth, these centers stop growing toward one another, leaving a small area of cartilage between them (Figure 7.7F). If this sounds familiar, that is because we recently discussed these areas. These are the epiphyseal growth plates!

Now that the bones have formed, it is easy to think that they will remain the same throughout life. In fact, this couldn't be further from the truth. As an individual animal grows, the bones must grow with them. We can consider bone growth in two different processes: interstitial growth (growth in length) and appositional growth (growth in width). **Interstitial growth** occurs at the epiphyseal growth plates. At the center of those growth plates, hyaline cartilage remained. The chondroblasts and chondrocytes are still active, building new cartilage. As they do so, they push the epiphysis and diaphysis farther from each other, making the bone incrementally longer. As the cartilage moves farther from the center, it starts to become ossified. Calcium and phosphorus salts become embedded in the ECM, and the chondroblasts and chondrocytes die. When they do, this makes space for osteoblasts and osteocytes to move in and convert the ossified cartilage into new bone. Simultaneously, the osteoclasts will be removing excess bone as it moves farther from the epiphyseal growth plate. **Appositional growth** is much simpler. On the periosteal (external) surface of the bone, osteoblasts are adding new bone, while osteoclasts work on the endosteal surface to remove bone. This makes the bone wider, and as long as the osteoblasts are laying down at a higher rate than the osteoclasts can remove it, the bone will also get relatively thicker. This is particularly important for the large weight-bearing bones, like the femur and tibia.

After sexual maturity, many (but not all) vertebrates stop growing in length/height. The epiphyseal plates fully ossify into

bone (in humans, this is in response to the spike in sex hormones), "fusing" the epiphyses and diaphyses together. However, that does not mean that the skeleton is done modifying for the rest of an animal's life. Bone is an extremely dynamic tissue, constantly being turned over, replaced, and changed in response to the needs of an organism. We call this process **remodeling**, much in the way you might remodel your bedroom or a kitchen. It is easiest to consider remodeling in the context of humans. Earlier, we described the spongy bone, or trabeculae, as allowing for maximal stress while minimizing material costs and weight. The trabeculae are laid down along lines of stress, dependent on how the bone is being used. Try this exercise: Find a partner, and have them jog in place. Notice how vertical the femur (their large thigh bone) remains while they are jogging. Now have them squat as low as they can. See how the femur moves into a more horizontal position. For these two activities, the body weight of the person doing the movement travels to the ground along different "lines." If they were a marathon runner or weight lifter, you can imagine that the stresses of their activities would result in bones that look very different. These would be reflected in their trabeculae as well as the thickness of the compact/cortical bone. However, if the marathon runner gives up their sport, for whatever reason, over time the bones will remodel to their new activity pattern. This is a matter of concern for many older adults who struggle to maintain active lifestyles. A lack of activity or becoming more sedentary will often result in a net loss of bone, as the bone that was necessary for movements or stresses is no longer needed and thus removed. It can be restored, but care must be taken, as the process of rebuilding bone slows down significantly as we age.

7.6 Further Reading

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8. The Skull

KASSANDRA FORD AND BILL RYERSON



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Figure 8.1—The skull of the domestic dog, Canis lupus familiaris.

Focus Questions—to Guide Your Reading of This Chapter

1. How is feeding important to the shape and structure of the skull?
2. How does the transition from water to land drastically impact the workings and structure of the skull?
3. What are the three major functions that skulls must undertake, and what trade-offs must be made to accomplish all these functions?

8.1 Introduction

Skulls are ubiquitous in science and popular culture. If you asked someone to draw a skeleton, most would start by drawing a skull, and likely a human skull (Figure 8.2).

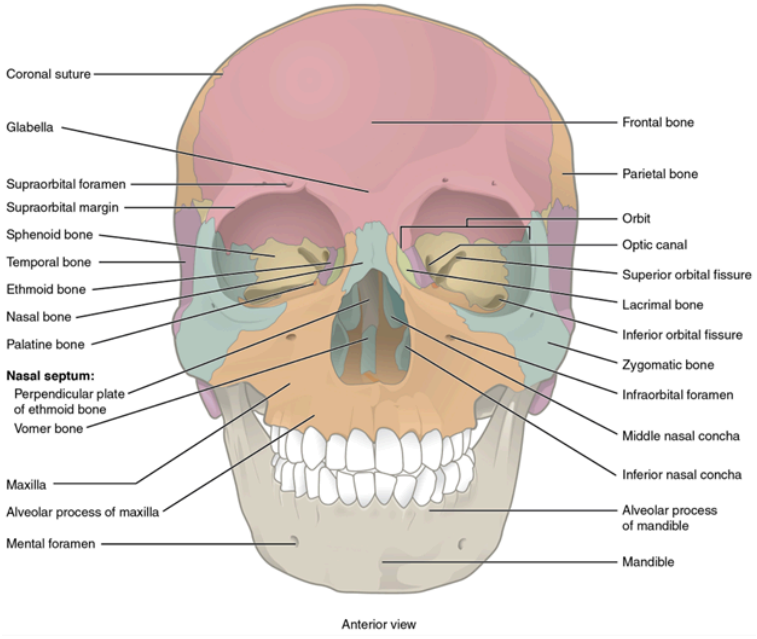

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Figure 8.2—The skull of an adult human, with major bones and structures labeled.

It is the single most recognizable piece of the skeleton. Beyond the draw of its shape, style, and macabre symbolism, skulls are undeniably important in the biology and evolution of vertebrates. Skulls are unique; they are one of the only places in a body where multiple systems converge, competing for space, and additional behavioral elements add complexity. The skull houses the brain, the major player of the central nervous system. The cranial nerves all travel through the skull, requiring holes (foramen/fissures) or canals to pass through. Four of the five major senses are also housed in the skull: smell, taste, vision, and hearing. Intake for the respiratory system occurs in the skull, whether it's air for the lungs or water passing through the gills. Most importantly (according to one of the authors of this chapter), the skull plays

an outsized role in feeding behavior. Teeth, jaws, braincase, orbits, arches, and fenestrae will all be important as we examine the evolution and function of the vertebrate skull. Skulls with weapons, extremely kinetic (movable) skulls, skulls with multiple jaws, skulls with too many teeth, skulls with no teeth, and even more amazing examples will dot this chapter. All these elements, however, cannot overlap perfectly. Instead, we will observe how the different senses, brain evolution, and feeding modalities compete with one another for space and how different species solve this competition.

8.2 General Structure of the Skull

Despite the incredible variety observed in skull structures, shapes, sizes, and functions, there are several key elements that unite skulls together. The purpose of this section is to introduce you to some terminology and structures that are common throughout vertebrates so that as we start to discuss function and evolution, the key components will be familiar to you. There are a few different ways to refer to the structure of the skull depending on your perspective: functionally, developmentally, and evolutionary. There will be some overlap in the regions and areas of a skull, each with its own defining characteristics. Let's take a look at Figure 8.3, a lateral view of a human skull.

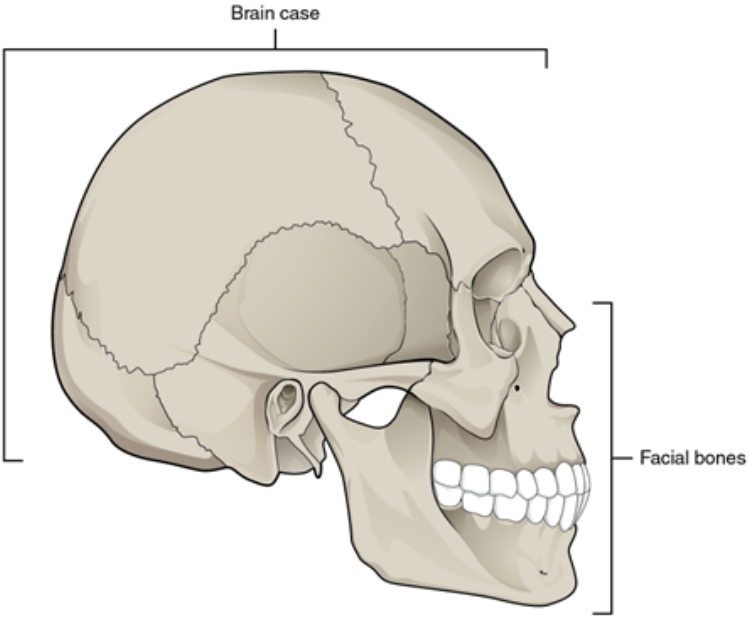

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Figure 8.3—A lateral view of the human skull.

You can see that the skull is separated into two different regions, the **braincase** and **facial bones**. The braincase (one word in most examples) is composed of the bones that encase the brain, simple enough. In humans, the remaining bones are considered the facial bones. There is some variation here between humans and other vertebrates; not all bones in many animals will belong to either a braincase or facial bones. It will help us to define some other regions and elements, particularly as we march our way through evolutionary history.

Neurocranium/Chondrocranium

The most basic type of skull found in vertebrates is known as the neurocranium. The **neurocranium** contains only the elements that enclose the brain, except for the roof. Imagine an empty cardboard box—this is essentially what the neurocranium is. There is no space for eyes, ears, or anything beyond the brain itself. Hagfishes, those deep-sea denizens, have a neurocranium with the addition of a thin roof made of cartilage over the brain. However, very quickly in history, the skull develops additional structures that increase in complexity and require further defining. As the brain and skull grow in size, modifications to the skull include not only additional bones but also landmarks for three special senses: vision, olfaction (smelling), and hearing. In the skull, we call these accommodations "capsules," and they can be remembered as the three O's (optic, olfactory, otic). The addition of a robust roof on the skull and the three capsules is referred to as the **chondrocranium**. This is the type of skull we see in our sharks (Figure 8.4).

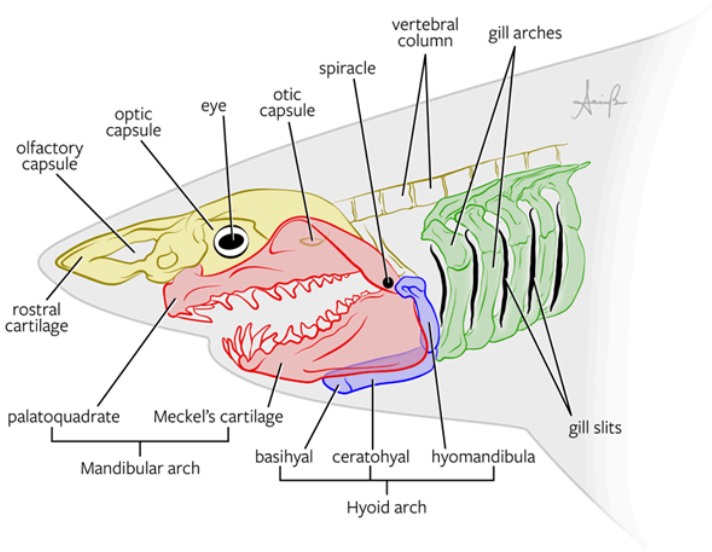

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Figure 8.4—The skull of a shark, lateral view. The chondrocranium is yellow.

Notice that the jaws are not attached to the skull in the shark as they are in the human. When the jaws do attach to the rest of the skull, this is an important evolutionary transition. For now, the jaws are not "part" of the chondrocranium.

Splanchnocranium

The **splanchnocranium** includes several arches and their derivatives, most notably the jaws. These arches include the **mandibular arch**, **hyoid arch**, and **branchial/gill arches** (Figure 8.4). These anterior arches are thought to have evolved to support respiration (moving water across the gills to obtain oxygen) but have since evolved for feeding purposes. All the arches contain either

cartilage or bone (that previously replaced cartilage) and sit in and around the pharynx. The mandibular arch, or the "jaws," contains the palatoquadrate and the lower mandibular bones (either Meckel's cartilage in chondrichthyans or the early-developed dentary system in bony fishes). Posteriorly sits the hyoid arch, which includes a series of bones that support the jaws, such as the basihyal, ceratohyal, and the hyomandibula. The hyomandibula typically connects the otic capsule to the palatoquadrate, suspending the jaws. In chondrichthyans, a structure known as the spiracle is found in between the mandibular and hyoid arches. The spiracle is a small opening from the mouth/pharynx to the external environment. Interestingly, its function is not very well known! Finally, the branchial (or gill) arches are the bony components that surround the gill slits and support respiratory functions. The majority of bony fishes and chondrichthyans have five branchial arches (with five corresponding gill pouches), but some chondrichthyans have evolved additional arches. Each branchial arch contains five bones, all of which form a "series" across the five arches: pharyngobranchials, epibranchials, ceratobranchials, hypobranchials, and basibranchials (Figure 8.4).

Dermatocranium

Key in protecting the cranium is the **dermatocranium**, made up of the surface bones that cover the chondrocranium, the splanchnocranium, and the important internal components of the head (Figure 8.5).

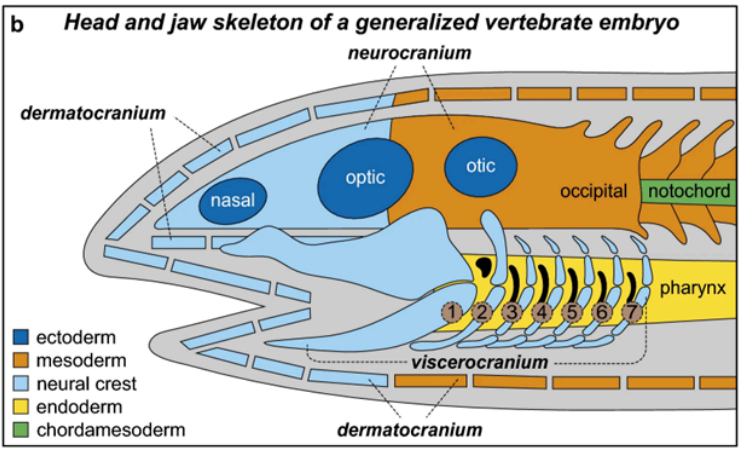

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Figure 8.5—A generalized developing embryo, illustrating the developmental origin of the dermatocranium and where it develops in relation to the other elements of the skull. Here the term viscerocranium refers to the seven arches.

In some groups of vertebrates (e.g., the bowfin fish *Amia*), the dermatocranium sits on top of the cartilaginous neurocranium, while in most groups, that cartilage is replaced by the dermal bones. This includes bones like the maxilla, premaxilla, dentary, hyomandibula, and opercular series, among many others. There are generally considered seven "series" of bones: facial, orbital, temporal, vault, opercular, palatal, and lower jaw (Table 8.1).

Table 8.1—The bones of the skull and the series to which each belongs

			Braincase		
Facial	Orbital	Temporal	Vault	Opercular	Palatal
Premaxilla	*Lacrimal*	*Intertemporal*	*Frontal*	*Preopercle*	*Vomer*
Maxilla	*Prefrontal*	*Supratemporal*	*Parietal*	*Opercle*	*Palatine*
Nasals	*Postfrontal*	*Tabular*	*Postparietal*	*Subopercle*	*Ectopterygoid*
	Jugal	*Squamosal*		*Interopercle*	*Pterygoid*
		Quadratojugal			*Paraspenoid*

Often vertebrates will have teeth on several bony components of their dermatocranium, including on the dentary and premaxilla and often on the palatal bones or parasphenoid. We will cover the diversity of dentition and its placement later in the chapter.

As you are reading all these names and seeing all the figures, it is easy to lose track of all the bones and their names. Instead, focus on the bigger patterns of size, shape, and fusion.

8.3 Development

The development of the vertebrate skull can be thought of in two different forms: the cells and their origins (e.g., neural crest cells) but also the processes by which the individual bones are formed. To understand the different origins and cells involved in the development of the skull, remember that there are three major

components or parts to the vertebrate skull: the chondrocranium, splanchnocranium, and dermatocranium. The chondrocranium, as the name suggests, is formed by cartilage for at least some duration. This requires three different elements that we must consider: neural crest cells, notochord, and mesoderm. The neural tube becomes separate from the ectoderm (which will become epidermal) above it during neurulation, and the cells of the **neural crest** will derive from the neural fold regions near the conjunction of the neural and nonneural ectoderm. Some of the neural crest cells will form the beginning elements of the notochord. The notochord, at its anterior end, grows forward and flattens out, forming the base of the chondrocranium. Mesodermal sclerotomes (blocks of mesoderm-derived cells distinct from neural crest cells but homologous to those that form the vertebrae in the next chapter) come together to form the caudal portion of the braincase, wrapping around the developing nerve cord and forming the foramen magnum. This will eventually become the occipital bones.

The splanchnocranium (including the gill arches, not just the jaws) is also derived from neural crest cells. These neural crest cells are the same as those formed during neurulation, as we just described, but instead these migrate down the body wall during development. In the region of the pharynx, the neural crest cells cease their migration and start laying down the elements of the splanchnocranium.

The development of the dermatocranium can largely be explained by the development of dermal bone in the chapter on the integument (Chapter 6). Briefly, neural crest cells that stay in the vicinity of the integument differentiate into cells we call ectomesenchymal cells. Ecto- and mese- in the names should tell you that these cells can (and do) play a role in the integument and connective tissues in a body. Additionally, dermatomes that are mesodermal in origin will also contribute to the formation of the dermatocranium.

Regarding process, recall that the bones are formed through two different processes: intramembranous ossification and

endochondral ossification (see Section 7.5, Bone Development and Growth). The larger, flatter bones of the skull (parietal, frontal, temporal) are formed via intramembranous ossification, while the bones with more intricate shapes (mandible, squamosal, zygomatic) are formed through endochondral ossification. There is a trickiness to some of this, as elements of the chondrocranium that would be replaced by bone in development actually disappear as the intramembranously formed dermatocranium sinks down beneath the skin.

8.4 Evolution of the Skull

Jawless Fishes

Hagfishes (***Myxinoidea***) lack true jaws and vertebrae; they do have a chondrocranium and a simplified, cartilaginous versions of branchial arches called visceral arches. Within their mouth opening, they have keratinous tooth plates that allow them to scrape and "bite" their prey without the complex jaw system of other craniates.

Lampreys (***Petromyzontioidea***) also lack true jaws but do have a simplified version of vertebrae protecting a notochord (Figure 8.6).

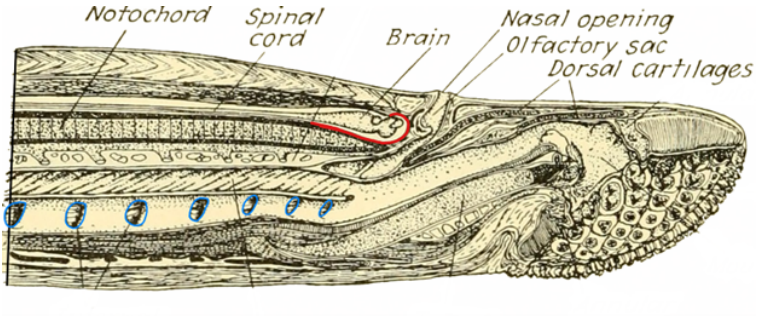

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Figure 8.6—A longitudinal section of a lamprey, with its anterior end to the right. The chondrocranium has been highlighted in red and seven of the eight visceral arches highlighted in blue.

They do not have a dermal component of their cranial skeleton, instead just having a chondrocranium and eight visceral arches that, together, form a branchial basket. They have a characteristic jaw opening filled with keratinous teeth that allows them to latch onto their prey, scrape a wound, and suck the blood of the organism.

†Ostracoderms are an extinct group of jawless vertebrates with incredible armor; they are most known for dermal shields on their flattened heads (see Figure 3.17 for some example ostracoderms). They were thought to be bottom-dwellers; therefore, this dorsoventral shield provided protection from potential predators above and around them. Because they lacked jaws, feeding was likely based on suction feeding rather than biting or crushing. Rapidly opening/expanding the mouth and oral cavity (together known as the **buccal cavity**) creates pressure changes that pull water and food into the mouth.

Gnathostomes

As discussed in Chapter 3, most vertebrates, except for the cyclostomes (hagfishes and lampreys), are part of the group Gnathostoma (Greek: "gnathos" = jaw; "stoma" = mouth), indicating the important evolutionary adaptation of jaws. We will cover jaws in more detail in Section 8.5, but for now it is important to make the distinction between jawless and jawed vertebrates, the latter of which contain almost all vertebrate diversity. Let's dive into that diversity now!

Fishes

Fossil Group of Fishes

Placoderms were a group of jawed, armored fishes that were present from the Silurian to the Devonian. They had thick dermal armor and a joint between their head and thorax, allowing for a decent amount of head and neck movement. One of the most significant finds in recent years indicates that dermal jaw bones (historically thought to have evolved in bony fishes) were present in placoderms and therefore much earlier in evolutionary time than originally thought. It also means that the next group we'll discuss in this section (chondrichthyans) likely lost those dermal bones and retained cartilage instead of bone. It's important to note that researchers are still teasing apart the phylogenetic relationships of these ancient lineages, meaning we may get additional answers about the evolution of jaws in the future.

Chondrichthyans

Chondrichthyes have almost no bone, as they lost that

characteristic in favor of retaining cartilage. Using evidence from the fossil record, we now think that the switch from bone back to cartilage occurred sometime after the jawless fishes branched off from their common ancestor with the rest of the vertebrates. This puts the cartilaginous fishes closer to the bony fishes than the jawless fishes. Because of this loss of bone, they do not have a dermatocranium (the bony head covering most fishes possess) but instead just have a chondrocranium and splanchnocranium. They have expanded that chondrocranium to cover their brain, forming a simplified version of a braincase (Figure 8.7).

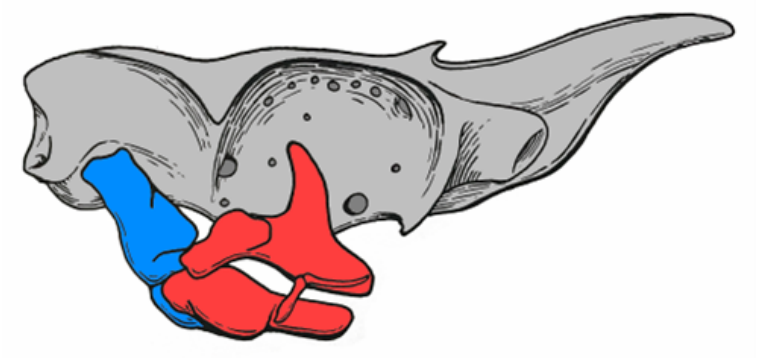

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Figure 8.7—The skull of the spiny dogfish, Squalus acanthias. Anterior side is to the right. Coloring is the same as Figure 8.4, where gray is the chondrocranium, red is the mandibular arch, and blue is the hyoid arch.

Their jaws are formed by the palatoquadrate and Meckel's cartilage (also referred to as the Meckelian element), which are typically suspended from the ceratohyal with a ligament (Figure 8.4). Chondrichthyans typically fall into two different ecological categories: those that capture prey with their jaws and those that use their jaws for crushing prey (holocephalans, including chimeras, along with some skates and rays). They also have five to seven gill openings, with their gills being separated by thin plates of tissue. In some chondrichthyans, the placement of the first gill opening is

somewhat constricted by the ceratohyal and hyomandibula, often leading to it being called a spiracle.

Early Actinopterygians

There is an incredible amount of morphological diversity in Actinopterygii (ray-finned fishes), making it very difficult to describe overall "trends" across the group. Several important adaptations evolved in this lineage, however, leading to increased skull mobility and food-processing abilities.

While chondrichthyans don't have a dermatocranium, most other jawed vertebrates do possess this bony head covering, and it is rather obvious when looking directly at a skull. When looking at the more basal actinopterygians (i.e., *Amia*), nearly the entire skull is covered in dermal bones, leaving very little of the chondrocranium or splanchnocranium visible without further examination (Figure 8.8).

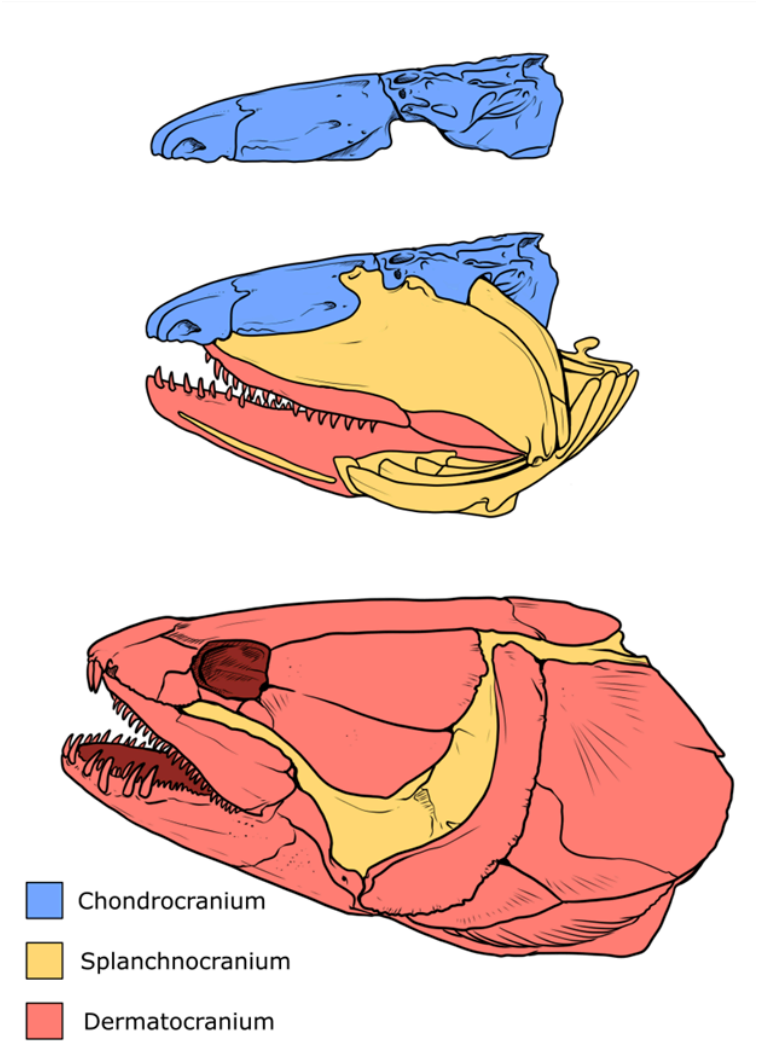

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Figure 8.8—The skull of the bowfin, Amia calva. The chondrocranium is in blue, and the components of the mandibular arch are in yellow. The dermatocranium is red.

There are some gaps in the dermal bone, primarily around the ventral and caudal sides of the skull, allowing for some (but minimal) movement. Movement of bones in the skull (with the exception of the jaws) is known as **cranial kinesis**. This emphasis on the dermatocranium is consistent with many primitive fishes; this offers a large degree of protection but does not do much to dramatically increase mobility. There are several bones in actinopterygians, including the opercular bones and the extrascapulars, that contribute to the overall protection of the chondrocranium and gills.

Actinopterygii is also where we start to see the diversification of morphologies that evolved for food processing. Gill rays, a type of gill raker, are seen for the first time in acanthodians, an extinct group of actinopterygians. These gill rays (eventually becoming gill rakers) were stiff, bony protrusions on the branchial arches. Their position and structure suggest that they were used for filter feeding, sifting the water for food before it passed through the gills.

Actinopterygians and Teleostei

Taking the evolution of cranial kinesis to the maximum is Teleostei, a more derived Actinopterygii group. This group retains the same bones as the more basal Actinopterygii but often reduces the size and restructures the articulations between them (compare Figure 8.8 with Figure 8.9).

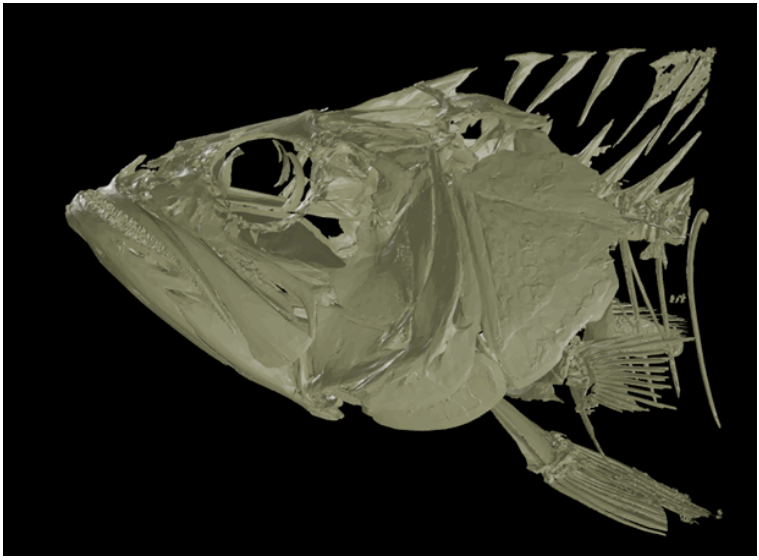

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Figure 8.9—The skull of the largemouth bass, Micropterus salmoides. The image links to the CT scan of this individual.

In teleosts, the premaxilla and maxilla are able to articulate with each other and with the neurocranium in teleosts, allowing for a high degree of rotation and expansive jaw opening. This isn't possible due to the size and robustness of those bones in *Amia*, therefore reducing the overall gape of those early actinopterygian fishes. These adaptations, including slightly reduced dermal bones and differing articulations, lead to increased mobility and therefore the ability to use rapid suction feeding as a method of prey capture (we'll talk more about this soon). It's very likely the ability to suction feed has aided in the rapid diversification of Teleostei, which boasts nearly 30,000 species.

Sarcopterygians

Sister to Actinopterygii (ray-finned fishes) is Sarcopterygii (lobe-finned fishes). See Chapter 10 on the appendicular skeleton for additional information about "ray-finned" versus "lobe-finned." Sarcopterygii contains coelacanths, lungfishes, and all tetrapods (terrestrial vertebrates), although it's important to note that some "terrestrial vertebrates" have evolved to recolonize the oceans permanently (i.e., some members of Cetartiodactyla and Sirenia) or semipermanently (i.e., some members of Carnivora, Rodentia, Monotremata, Afrosoricida, Euliphotyphla, and Didelphimorphia). All sarcopterygians have evolved lungs (in some way), usually aiding in air breathing.

There are several bones in actinopterygians that were lost in sarcopterygians—namely, the opercular bones and the extrascapulars. The loss of "gills" in the sarcopterygian ancestors of the tetrapods meant those bones were no longer necessary to protect that integral section of the skull, leading to dermal bones fusing together. Sarcopterygians are also where we start to see fenestrae, or openings in the outer dermatocranium. As these platelike dermal bones fuse together, openings are necessary for our sensory capsules to transmit information from the organism's surface to its nervous system.

Coelacanths

The first group of sarcopterygians that we'll discuss is the coelacanths (Coelacanthiformes). Coelacanths were thought to have gone extinct approximately 66 million years ago but then were discovered off the coast of South Africa (living!) in 1938. They are considered "living fossils," possibly giving scientists insights into more "primitive" phenotypes from closely related species that went extinct. These fishes have gills, allowing them to breathe underwater, along with a primitive "lung" that remains small and

largely useless after development. Coelacanths, along with all other tetrapods, evolved from an ancestor that had the first functioning lung. Coelacanths are the only remaining sarcopterygian with an intracranial joint, a joint between the anterior and posterior parts of the neurocranium, allowing the neurocranium to bend. Although this characteristic is now only seen in coelacanths, it was present in fossil sarcopterygians, including now-extinct species of lungfishes.

Lungfishes

Lungfishes take their name literally and have true, functioning lungs for air breathing (all other groups after this also have lungs). They begin their lives with gills but become obligate air breathers as their lungs develop. They have autostylic jaw suspension (discussed in Section 8.5 on jaws), allowing them to crush hard prey in a food-processing method called durophagy. They have large tooth plates that are used for crushing prey items.

A Brief Note on Tetrapods

Once the tetrapods begin to move onto land, it is easy to consider the appendicular skeleton as the element of the skeleton that is forced to undergo the most change in the transition from water to land (see Chapter 5 for more!). However, the skull is also going to undergo some extreme changes. Two of these changes we can introduce now, as they will impact all the tetrapods. The first is the evolution of a brand-new structure that we call the tongue. The tongue in all nonmammalian tetrapods has a significant skeletal structure to it, and its evolution creates opportunities for vertebrates to rely on the tongue to play a role in feeding. The second has to do with the environment. Two of the most common types of feeding in fishes, suspension and suction feeding, are not available to the land-dwelling tetrapods (more on this later in this

chapter). Both of those feeding styles rely on moving the relatively denser water (and the prey within it), but moving air is not energetically efficient for tetrapods. Instead, tetrapods will rely heavily on biting (using both jaws to grab and secure the prey) or tongue prehension (using the tongue to grab the prey in the environment). A consequence of this is that those amazing kinetic skulls of actinopterygian fishes will lose a lot of mobility (to create large volumes for suction feeding) and instead become much more robust (a result of different bones fusing together and becoming larger) to handle the larger forces associated with biting and chewing. The increased fusion of the bones in most tetrapods leaves us with often jagged lines known as "sutures." The sutures reflect where the individual bones come together. Sutures can be used to age some vertebrates, as the fusions become harder to identify as individuals get older. Gradually, many individual bones fuse to the point where we can consider them one functional unit. You may find it helpful to refer to the relationships among these groups in Chapter 3.

Amphibians

The first tetrapods to emerge onto land were the amphibians, and our understanding of their skull evolution must begin with their immediate ancestors, many of whom were still predominantly aquatic. One of the most famous fossil finds of the last 100 years is that of *Tiktaalik*, an amphibian ancestor ("fishapod") that still very much lived like a shallow-water fish. *Tiktaalik* is important because its appendicular skeleton shows clearly how the transition from fins to limbs might have occurred. However, that is not the only story that *Tiktaalik* has to tell. The skull of *Tiktaalik*, like that of several other stem tetrapods (e.g., *Ichthyostega*), also shows how all the wondrous elements of the fish skull come together to form that robust skull discussed previously (Figure 8.10).

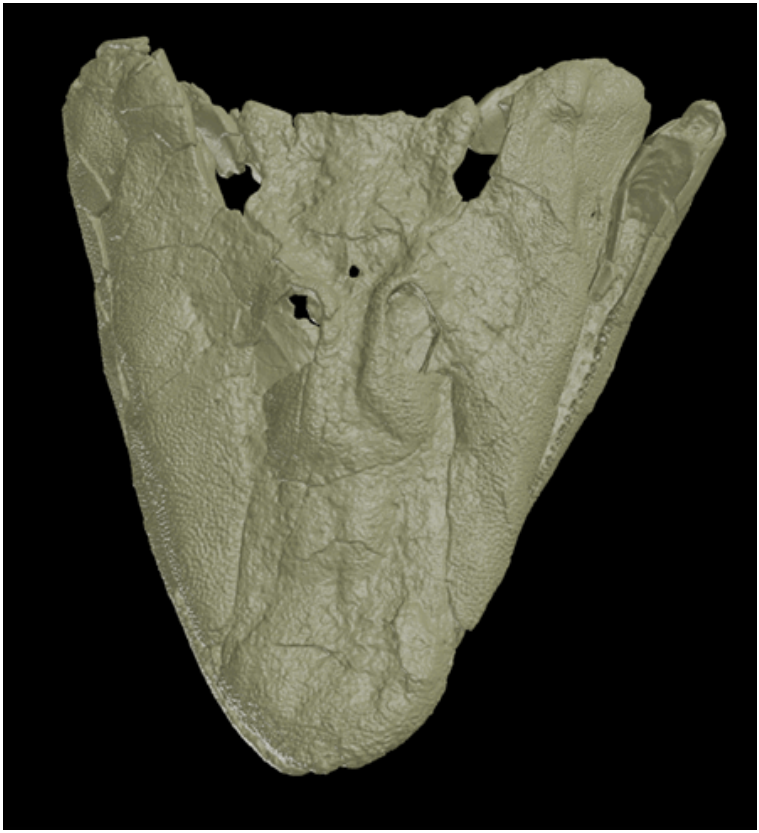

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Figure 8.10—The skull of Tiktaalik. The image links to the CT scan of this fossil.

Notice that the upper jaw is completely fused to the skull along its entire length, from maxilla to quadrate. The lower jaw is quite long and made of several different bones. Teeth on the lower jaw are carried only on the dentary bone. The skull appears to be completely fused together and therefore immobile (also known as **akinetic**), and to our best knowledge this is the case with *Tiktaalik*.

There is one additional bone to note in the amphibians that will play a larger role later on in reptiles, birds, and mammals. The

bone is called the columella, a thin, long bone running from the quadrate to the braincase. This bone carries vibrations from the jaws to the sensory apparatus of the inner ear, with more on this in the sensory chapter (Chapter 20).

Extant amphibians (Lissamphibia) largely retain this robust, immobile skull of their ancestors. From the frogs to the salamanders, no evidence of a kinetic skull can be found. Not all the amphibians resemble the ancestral condition represented by *Tiktaalik*. Many of the amphibians have even further reduced the number of bones of the skull through loss or fusion, making way for larger eyes and jaw muscles (Figure 8.11).

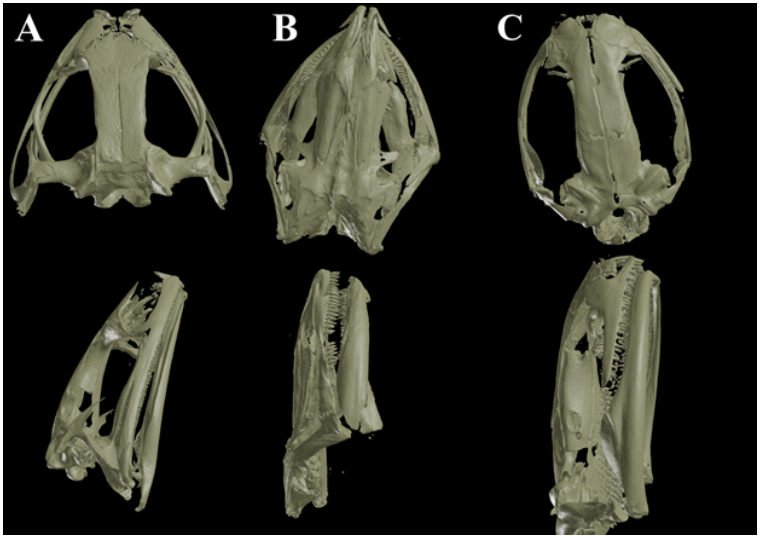

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Figure 8.11—Representative skulls of a few amphibians: (A) *the frog Humerana humeralis*; (B) *the aquatic salamander Necturus beyeri*; (C) *the terrestrial slimy salamander Plethodon glutinosus. Anterior is toward the top of the image and posterior toward the bottom in all images.*

The amphibians have a striking amount of variation in their tongue skeleton. The bones associated with the tongue typically end in the suffix -hyal and are derived from the hyoid arch in fishes.

The tongue skeletons are extremely variable in the amphibians, from the tiny and seemingly irrelevant tongue skeletons found in the mudpuppy *Necturus* to that of the plethodontid salamander *Hydromantes*. *Hydromantes*, found in the mountains of California, have tongues that shoot out twice their body length in the blink of an eye. To support this tongue, the skeletal elements are highly modified to anchor the tongue and muscles. Some of these muscles are so large, they extend all the way down to the hip, and the skeletal elements go almost as far!

Box 8.1—Temporal Roof Evolution—Here's the Weird Stuff That's About to Happen

The dermatocranium of early tetrapods evolved rapidly, and many of the small bones seen in actinoptergyians were lost, resulting in larger, fused bones in the skull. The temporal roof (sometimes called the dermal roof) retained the overall pattern of bones, but the number of connections between them shifted (Figure 8.12). Remember that the opercular series and the extrascapulars were lost in the Sarcopterygii from the Actinopterygii.

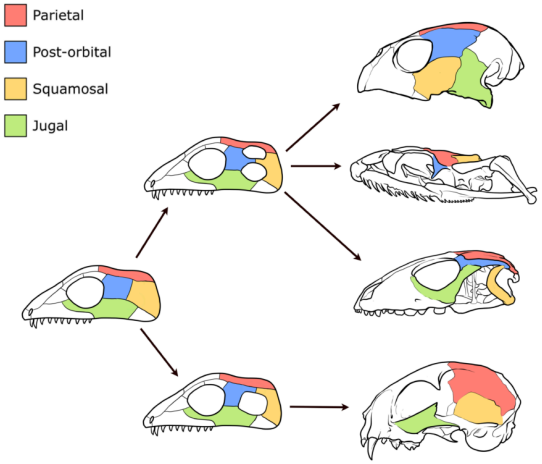

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Figure 8.12—Evolutionary changes resulting in the fusion of certain cranial bones and the temporal roof found in tetrapods. The extant species on the far right are (from top to bottom): Green sea turtle (Chelonia mydas); North American rat snake (Pantherophis alleghaniensis); Caiman lizard (Dracaena guianensis); and domestic cat (Felis catus).

Instead of filling that space with expanded bones, most sarcopterygians shifted bones around, allowing for differences in the size and fusion of the skull bones.

Table 8.2—The bones that compose the temporal roof in tetrapods

Temporal roof of tetrapods				
Lateral tooth-bearing series	Circumorbital series	Cheek series	Median series	Temporal series
Premaxilla	*Septomaxilla*	*Squamosal*	*Nasal*	*Intertemporal*
Maxilla	*Lacrimal*	*Quadratojugal*	*Frontal*	*Supratemporal*
	Prefrontal		*Parietal*	*Tabular*
	Jugal		*Postparietal*	
	Postfrontal			
	Postorbital			

As we stated in the amphibians section, the dermal/temporal roof bones fuse together, becoming extremely resistant to the forces that happen during biting. This robustness comes at a cost. Earlier, we discussed that the robust skulls were limited in movement and that species with very robust skulls were not particularly kinetic. There is, however, a second issue that will arise. In the evolutionary time between the evolution of amphibians and the evolution of the common ancestor that gave rise to both reptiles and mammals, the temporal roof sank deeper below the skin while simultaneously the braincase had to enlarge as the brain changed in size. You might consider this to be a nonissue, as we are talking about bone running into bone. We are forgetting one key element and that is that there is muscle that is trapped between these two layers of bone. In particular, the jaw-closing muscles are the ones that are trapped between these two layers of bone. What's a tetrapod to do? The answer seems to be to create small windows within the temporal roof, giving the jaw-closing muscles enough space to bulge during contraction. We call these windows **temporal fenestrae**, *temporal* referring to the region and *fenestra* being the Latin word for "window."

Amphibians and the common ancestors to reptiles and mammals did not have any temporal fenestrae at all. We call this

condition to be **anapsid**. The "apsid" refers back to the Greek "hapsis," referring to an arch. The names in this case refer to not the windows but the arch of bone that is left behind. *Anapsid* therefore means "no arch." From there, we find that there is a sharp distinction between the lineage that leads to the mammals and the one that leads to the reptiles and birds. Mammals belong to the clade that we refer to as **synapsids**, referring to the fact that they have one arch and one corresponding temporal fenestra. The Reptilia are **diapsids**, with two arches and two temporal fenestrae. When we discuss these lineages, we will rely on a few fossils as examples of what the fenestrae looked like. Note that many changes have occurred since the original evolution of these fenestrae, and many of the modifications are difficult to notice without knowing which lineage they belong to first. Groups like snakes (diapsids), turtles (technically diapsids), and even humans (synapsids) are tough to classify without knowing which group they belong to beforehand.

Reptilia (Diapsids)

Although the amphibian skull was not as altered after the transition to land, as we move onto the reptiles, we find that each major lineage of the reptiles (including the birds) alters the skull in their own way. The functional elements of these changes are varied, and some of them remain a mystery. A few of these (like the turtles) confused taxonomists for years, making their placement on the phylogenetic tree of vertebrates difficult.

To begin, let us approach the lizards and snakes. The common ancestor to all the lizards and snakes is a true diapsid, with both temporal fenestrae clearly visible. In modern lizards and snakes, we refer to the group as modified diapsids, where additional bone loss has made it seem as though there was only ever one temporal fenestra. There is one living example of a true diapsid, the enigmatic tuatara.

The tuatara, while resembling a lizard, is actually the

closest living relative to the lizards and snakes. The two temporal fenestrae are quite clear on this "almost" lizard as the dashed lines in Figure 8.13B.

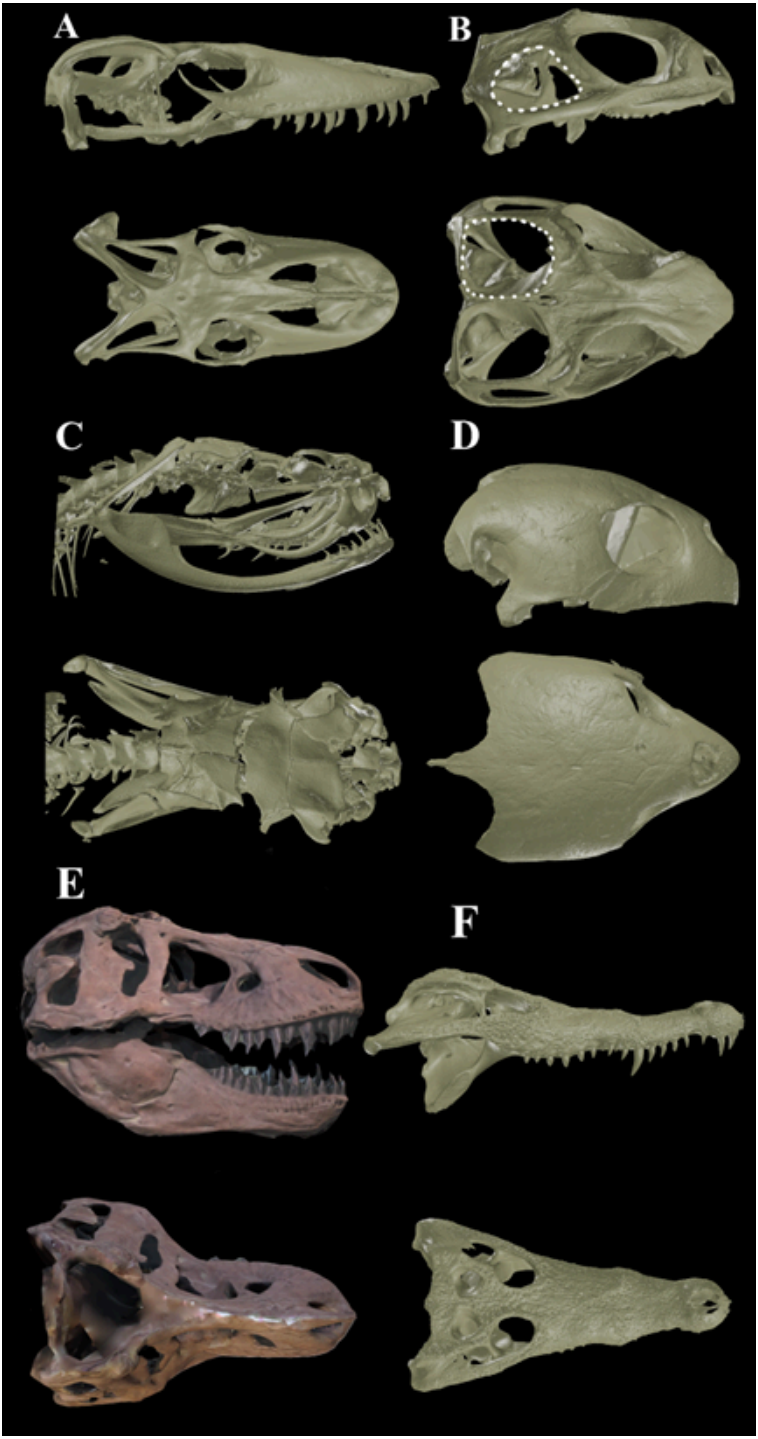
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Figure 8.13—Representative skulls of nonavian reptiles from a lateral and dorsal view, with anterior toward the right and posterior toward the left. Species names link to CT scan data. (A) Savannah monitor, Varanus salvator; (B) Tuatara (with temporal fenestrae highlighted by dashed lines), Sphenodon punctatus; (C) Tropical rattlesnake, Crotalus durissus; (D) Loggerhead sea turtle, Caretta caretta; (E) Tyrannosaurus rex; (F) New Guinea crocodile, Crocodylus novaeguineae.

Beyond the temporal fenestrae, there is an extraordinary amount of variation and diversity in skull shape and structure (Figure 8.13).

One characteristic that reevolves from ancestors with more fused skulls in the lizards is **cranial kinesis**, that ability to move individual bones within the skull. There are different types of cranial kinesis in the reptiles, depending on where the movement occurs within the skull. **Metakinesis** refers to movement of the braincase relative to the rest of the skull. **Mesokinesis** (the most common type among lizards) is movement of the bones of the upper jaw and snout relative to the braincase. Finally, there is **streptostyly**, the movement of the quadrate independently of other bones. Different lizard groups exhibit differing levels of kinesis. Lizards like the Gila monster, with their large robust skulls, exhibit almost no kinesis, while the closely related monitor lizards exhibit varying degrees of mesokinesis. Several lizard groups also possess osteoderms, bony elements in their skulls that act as additional armor (but see the integument in Chapter 6 for more). The snakes are the masters of kinesis of the reptile world, with seemingly every bone in the skull being able to move independently. Snakes exhibit all three types of kinesis, and it seems that their extensive streptostyly is a key element in their ability to create an ultralarge gape and swallow prey many times larger than their head. Many species also have lost several of the bones in the skull, further decreasing their robustness but increasing their cranial kinesis. Compare the typical rattlesnake skull versus the skull of your local

garter snake or water snake and you will see that the rattlesnake skull seems to be a skull missing several bones even when compared to other snakes, further illustrating the diversity of skull shape and structure even within groups.

The turtles are a fascinating group, for their skull as much as their shell. The first thing you may notice about the skulls of turtles is that they lack teeth (more on teeth later). The second thing you may notice is that in comparison to what we have been discussing in the other tetrapods, turtles do not have obvious temporal fenestrae. We refer to turtles as **secondarily anapsid**, having lost the temporal fenestrae during their evolution. The reasons for this remain unclear. It is possible that turtles required the extra armor around the head or that the food being eaten did not require the same level of musculature as in other tetrapods. Turtle skulls are quite robust, with large temporal roof bones and less obvious openings to the inner ear. With the additional robustness, turtle skulls are completely akinetic, lacking the cranial kinesis we previously discussed in the lizards and snakes.

The crocodylians are also characterized by extremely robust skulls. Alligators and crocodiles are flattened dorsoventrally, and this shifts where we consider structures like the temporal fenestrae. When we look at the alligator in comparison to the lizard, you can see how the change in the shape would shift the placement of several bones and other structures. As we saw in the turtles, alligator skulls are akinetic.

Dinosaurs (Diapsids)

Dinosaurs, as a whole group, have some of the most impressive skulls. These large, imposing skulls wow thousands of museum visitors every year. Now that we have a much firmer understanding of the structure and function of skulls, we see that dinosaurs are specialized diapsids, just like the other reptiles. Many of the structural changes we observe in the dinosaurs are by-products of

their size as opposed to different functional demands resulting from dinosaur biology or ecology. In many ways, the smaller dinosaurs more closely resemble the lizards and birds, but we shall focus on one element of the larger dinosaurs that is worth examining. The skull of *Tyrannosaurus rex* (Figure 8.13E) is one of the most recognizable skulls in all the vertebrates. As you take a close look at the skull, you may notice something that jumps out at you. The skull of *Tyrannosaurus*, like many of the large dinosaurs, seems to have more openings/holes than those of other reptiles. We also refer to these openings as fenestrae, just as we did with the temporal fenestrae, and name them for their position in the skull (e.g., anterior fenestrae). The exact function of these fenestrae is unclear. One certain result is that the skulls of these dinosaurs would weigh far less, reducing the energy and muscle costs of lifting that heavy head around. Recently, it has been suggested that these areas may have held concentrations of blood vessels for two possible reasons. The first is that dinosaurs of this size are now thought to have been gigantotherms, their increased body size resulting in elevated body temperatures. These collections of vessels may have been useful to dump excess heat from the body, similar to the large ears of modern-day elephants. The second is that dinosaurs may have been sensitive to infrared radiation, and these areas of vessels may have resulted in hot spots of infrared radiation that served as signals to other dinosaurs nearby.

Birds (Diapsids)

As descendants of the reptiles, birds are also modified diapsids. Birds are extremely variable morphologically, and it should come as no surprise that the skulls of birds are just as variable. There are a few broad patterns that we can observe first and then highlight some of the unique variations. Much like we will see with mammals, there is an increase in the size of the braincase in birds. Like turtles, teeth have been lost, instead replaced with a keratin sheath that

covers the upper and lower jaws. Beyond this point, bird skulls are extremely specialized on a species-level basis (Figure 8.14). This specialization is primarily diet related, but there are many reasons for certain anatomical specialties. Parrot skulls are very kinetic, and recently it has been shown that this kinesis allows them to use their beaks/jaws as a prehensile limb, helping them climb and locomote across branches. The northern gannet is an open-ocean seabird that can dive at high speeds into the water. These birds lack external nares, a prominent feature in all other tetrapods. To breathe, they must open their mouths. Peregrine falcons have small diverticula (dividers) in their nares so that as they dive at 200 miles per hour, the air rushing into their nares does not do any damage to the soft tissues. Owls have extremely large orbits to accommodate their eyes, which are also forward facing for added increased binocular focus. Finally, several woodpeckers have tongues (and tongue skeletons) that are long enough to actually wrap around the top of their head. Changes in bird skull and bill shape can occur quickly in response to changes in the environment and food availability. In fact, it was the beak of a group of birds known as Darwin's finches that was used as strong evidence for how changes to the anatomy of organisms could occur.

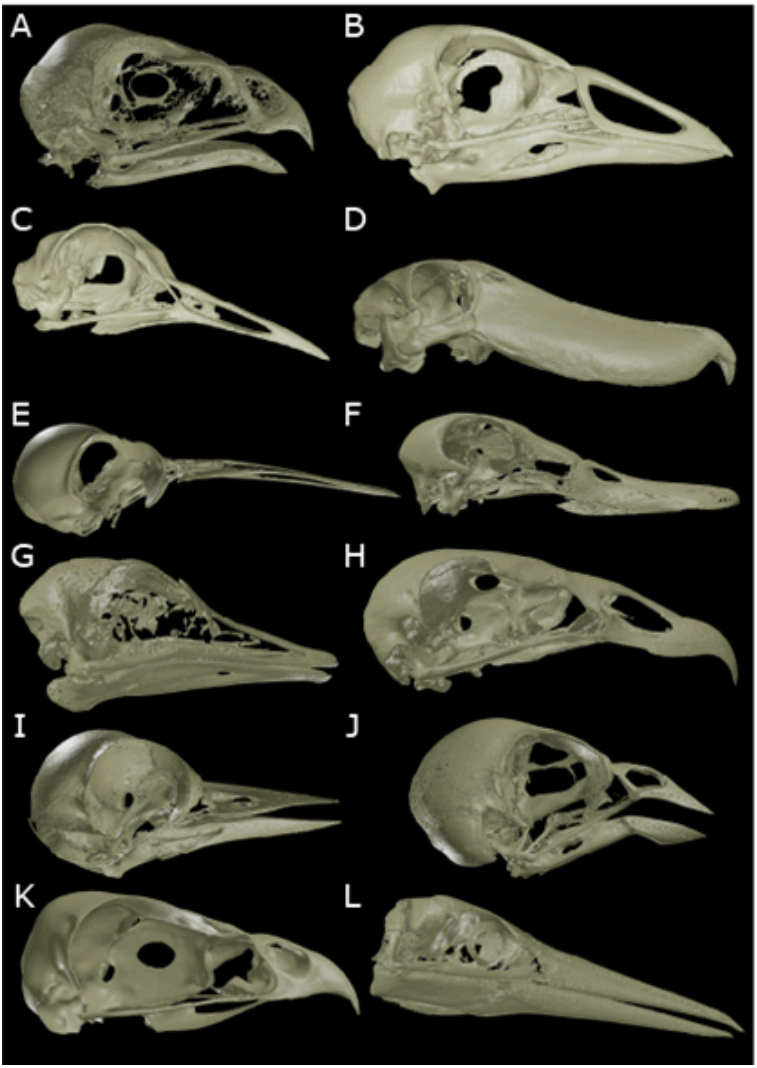

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Figure 8.14—Representative skulls of select bird species with anterior being toward the right. Species names link to CT scans. (A) *Eurasian sparrowhawk, Accipiter nisus*; (B) *Tasmanian nativehen, Gallinula mortierii*; (C) *Kagu, Rhynochetos jubatus*; (D) *Shoebill, Balaeniceps rex*; (E) *Bee hummingbird, Mellisuga minima*; (F) *Green-winged teal, Anas crecca*; (G) *Ostrich, Struthio camelus*; (H) *Lesser yellow-headed vulture, Cathartes burrovianus*; (I) *Yellow-bellied sapsucker, Sphyrapicus varius*; (J) *American tree sparrow, Spizelloides arborea*; (K) *Red-tailed hawk, Buteo jamaicensis*; (L) *Northern*

gannet, *Morus bassanus*.

Synapsids

At the split between the ancestral reptiles and ancestral mammals, we used the temporal fenestrae as a structure that could help us distinguish between the two groups, with the diapsids having two and the synapsids having one. This is an important distinction, as the earliest synapsids resembled reptiles in many ways. The best example of this is the dinosaur-looking (but not a dinosaur!) pelycosaur *Dimetrodon*, famous for its large sail back. In many ways, *Dimetrodon* does closely resemble our image of a dinosaur: sprawled posture, scaly skin, and large blocky head. However, a look at the skull of *Dimetrodon* confirms it to be a synapsid, a distant ancestor of modern mammals (Figure 8.15).

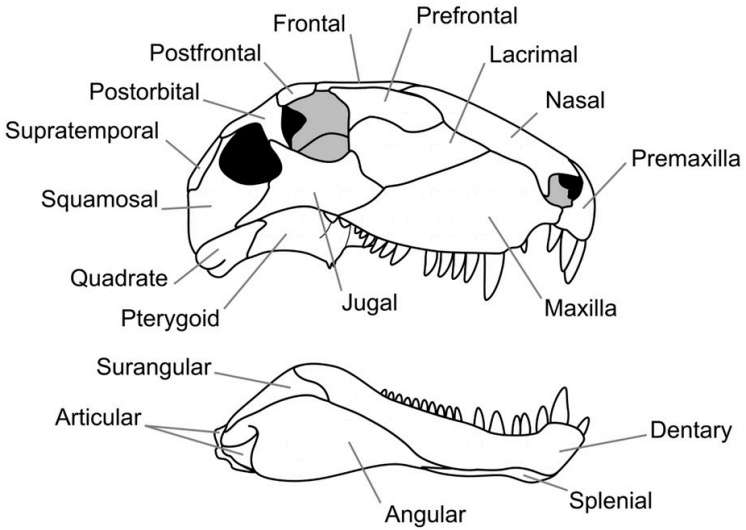

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Figure 8.15—A lateral view of the skull of Dimetrodon, a stem synapsid. The entirely black opening behind the orbit is the one temporal fenestra.

In many ways, we can use *Dimetrodon* as an example of a generalized synapsid, just as we used the tuatara as the example diapsid. While this section has focused on the evolution of the temporal fenestrae as one of the key characteristics that changed greatly during the tetrapod evolution, we will find that this is not the case as we discuss the mammals. At the time of *Dimetrodon*, there is not a lot beyond the temporal fenestrae to distinguish the synapsids from the diapsids.

As we move forward through evolutionary time, a few changes will occur that will additionally distinguish the basal synapsids from the reptiles. One of the most obvious changes is going to be a change in the shape and size of the braincase (Figure 8.16). As the size of the braincase increases, it distorts the other structures that we find useful for identification. Look back to Figure 8.2, the human skull. The large braincase distorts the location of the temporal fenestrae as well as all the facial bones relative to the basal synapsids. One of the earliest changes to appear is the change in the jaw joint. In all our vertebrates to this point, the quadrate (or structure homologous to the quadrate) articulated with the articular (convenient) to form the jaw joint. However, by the time we reach modern mammals, the articulation of the jaw joint is between the maxilla and the mandible. The transition from the basal jaw joint to extant mammals has several cascading impacts, including the evolution of the middle ear bones. The bones of both the upper and lower jaw that were once important for feeding were freed from this function and were co-opted for another very important function. We will explore this transition in more detail in Section 8.5 on the jaws (see Jaw Articulation and Middle Ear Bones), but know that all of this will occur in a series of stages between the basal synapsid *Dimetrodon* and more derived mammals such as the American black bear *Ursus americanus*. The other change that is going to occur in the evolution of more derived mammals from more basal mammals is more subtle but also has big implications. In mammals, a **secondary or hard palate** evolves between the nasal cavity and the mouth (Figure 8.17)—touch your tongue to the roof

of your mouth, and you've found yours. The hard palate is derived from the aptly named **palatine bones**. The palatine bones each have a process that connects to the other, creating an empty channel lying above the mouth. The processes originally did not connect and acted to reinforce the upper jaw when chewing tougher prey. That toughness remains, with the added benefit of separating the airways from the mouth, allowing for breathing while feeding.

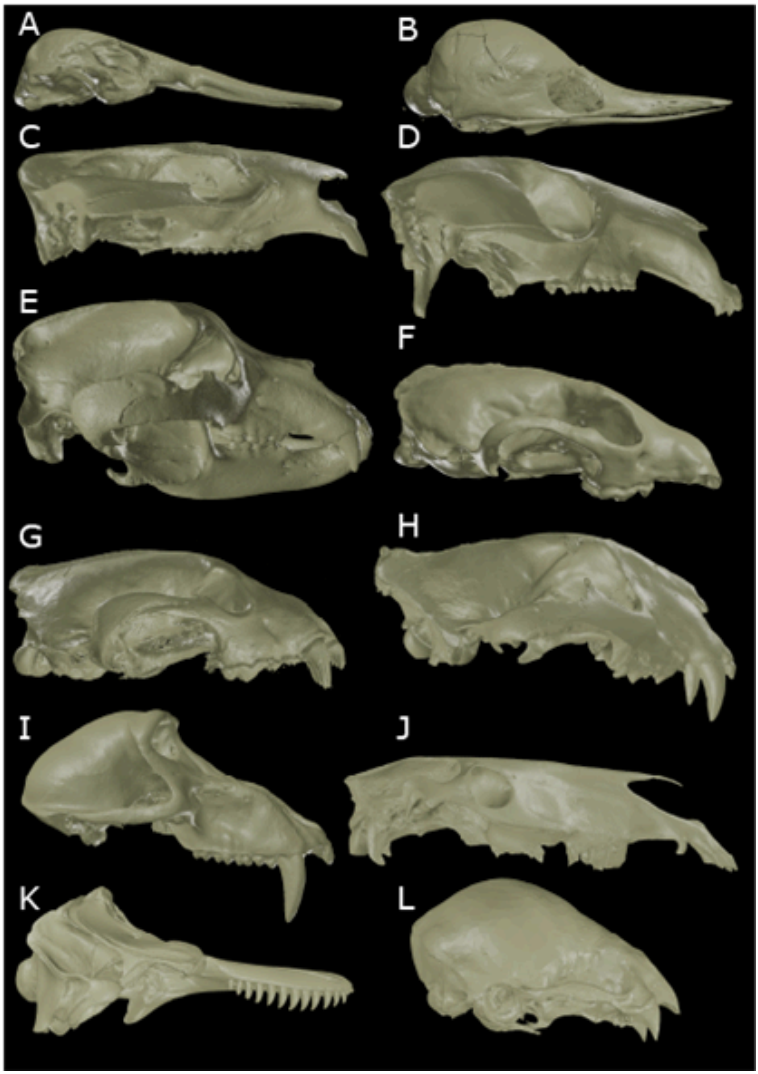

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Figure 8.16—Representative skulls of living mammals. 3D *models are linked in the species name.* (A) *Platypus,* Ornithorhynchus anatinus; (B) *Short-beaked echidna,* Tachyglossus aculeatus; (C) *Common wombat,* Vombatus ursinus; (D) *Black wallaroo,* Osphranter bernardus; (E) *Grizzly bear,* Ursus arctos; (F) *Fisher cat,* Pekania pennanti; (G) *Wolverine,* Gulo gulo; (H) *Tiger,* Panthera tigris; (I) *Chacma baboon,* Papio ursinus; (J) *Domestic horse,* Equus caballus; (K) *Orca,* Orcinus orca; (L) *Vampire bat,* Desmodus rotundus.

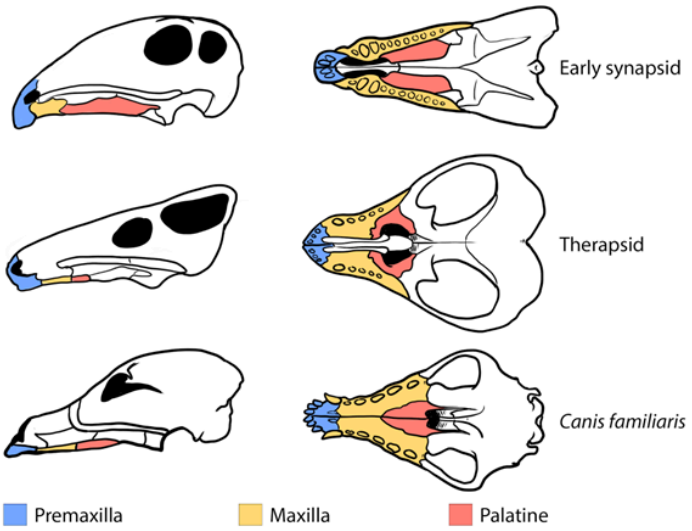

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Figure 8.17—The evolution of the hard palate in mammals. A lateral view is on the left and a ventral view on the right. Note the black openings on the ventral skull images (openings to the nasopharynx).

The monotremes, that earliest diverging group of the mammals, are often considered representatives of the ancestral condition for mammals. However, especially when it comes to the skull, it is important to remember that this group is highly derived in many ways, having had millions of years since they diverged from the rest of the mammals. Both groups of monotremes have lost their teeth, though toothlike structures are present in the juveniles before being lost (Figure 8.16). The separation between the orbit and the temporal fenestrae is gone, making it appear as though there were only one opening.

The marsupials closely resemble the placental mammals in skull features, shape, and size. As we observed in the birds, many of the differences between skulls are highly specific to individual species, so much so that it is possible to identify species without

a complete skull. If presented with an unfamiliar mammal skull, how could you tell if it was a potential marsupial or placental? One distinguishing trait is found on the lower jaw. Near the jaw joint, on the rounded edge of the bottom of the jaw, is the angular process. In placental mammals, the angular process is vertical. Marsupials have an angular process that points medially (toward the midline). This distinctive process makes it much easier to differentiate between the two groups.

The placental mammals, like marsupials, are extremely variable from species to species and even group to group. One unique item that many terrestrial mammals possess is a collection of small, thin bones known as the **nasal turbinates** (or **turbinate bones**). These turbinate bones resemble thin, rolled-up scrolls of paper with lots of little holes punched in them. These bones play a role in the conditioning of air as it enters and leaves the nasal cavities. The thin bones have a large capillary supply within them. As air enters, it is warmed by the blood in capillaries, and its humidity increases before reaching the lungs. This prevents lung damage from inhaling cold, dry air. Conversely, as the air leaves the lungs, some of that heat and humidity can be recaptured. However, we can use different skeletal clues to inform us about what type of mammal we may be looking at. For example, within the order Carnivora, you can use the ratio of two lengths to tell the difference between a few different groups (Figure 8.16). In the family Mustelidae (skunks, weasels, mink, and fisher cats), the length of the skull from the center of the orbit to the back of the braincase is much greater than the length of the tip of the snout to the orbit. In the family Canidae (dogs, wolves, foxes), those two lengths are very similar. Beyond this, however, it is difficult to generalize across broad groups of mammals, as most differences tend to be very species specific, and there are always exceptions within groups. A good example of this is in the Rodentia, which includes mammals like mice, rats, beaver, and the capybara. Groups of rodents have similarly modified skulls that include differences in the infraorbital canal. Other groups have changes involving the teeth that can make them more easily

identified. Some have lost their teeth (anteaters), while others have enlarged teeth that act as tusks from feeding and competition (walrus and elephants). Cetaceans (dolphins, porpoises, and whales) all have highly modified skulls, currently assumed to be the result of accommodating the aquatic lifestyle as well as amplifying their ability to echolocate. One group of whales (the mysticetes or baleen whales) has also lost their teeth, instead using keratinized sheaths (baleen) to filter shrimp and krill from the water.

The teeth of placental mammals are often a distinguishing character for individual species (more later on the importance and structure of teeth). In fact, some fossil mammals are only known from one tooth. Most mammals have teeth that are quite different from one another in certain places in the jaw, known as **morphological heterodonty** (more on this later). Broadly, most mammals have (in order from the front of the jaw to the back) **incisors**, **canines**, **premolars**, and **molars**. The number of each type of tooth, as well as its position, can be used to distinguish individual species of mammals. It is also worth noting that the number and type of teeth may also differ between the upper and lower jaws of a single species. To help us identify different species, we use what is called a **dental formula** to describe this pattern. In humans, for example, our dental formula is 2(I 2/2, C 1/1, P 2/2, M 3/3). The letter indicates the type of tooth (I for incisor), and the numbers indicate both location and number of that tooth type (2/2, two in the upper jaw and two in the lower jaw). The first "2" tells us that this represents one side (left/right), so if you want to count the total number of teeth in the mouth, you must multiply by 2. So now we can compare the human formula to that of a domestic dog: 2 (I_3/I_3, C_1/C_1, P_4/P_4, M_2/M_3). This is different from humans, and also note that there are more molars in a dog's lower jaw than its upper jaw. While this gives us a tool for identifying different species, the hows and whats of this tooth identification are covered in a broader view later, in Section 8.6 on teeth.

Humans

It should come as no surprise that in many ways, the human skull is quite similar to other mammals. Humans have flattened facial bones and a large braincase, as do some closely related primates (Figure 8.2). There is one key difference in the skull of humans, as the shift to bipedalism resulted in changes to the orientation of the skull. The **foramen magnum**, through which the spinal cord leaves the skull, is now shifted ventrally. The orbits also change position, and we end up with two forward-facing eyes, like those seen in owls and cats, allowing for increased depth perception and binocular vision. Human teeth are similar to those of other mammals (more on teeth later). The major bones of the braincase—**frontal**, **parietal**, **temporal**, and **occipital**—are tightly fused together during adulthood. However, they are not completely fused together at the time of birth. The sutures that connect these various bones are still separated quite a bit by bands of connective tissue, leaving "soft spots" on a baby's skull. These soft spots are technically named **fontanels**, and they will close shortly after birth. The current hypothesis is that these soft spots are retained until after birth to provide some level of flexibility during birth. The large braincase is difficult to pass through the birth canal, and the ability to slightly move the skull bones around can ease this process. However, this also means that the brain is not fully protected during these first crucial weeks after birth.

A Brief Note on the Following Section

To this point, we have discussed the generalized function of the skull, its development, and how evolutionary time imposed broad changes on the skull. However, given all we have said about the importance of the skull, it seems that we did emphasize many of these big changes. In this section, we focus on the jaws and how

their evolution sheds light on not only the broader evolution of vertebrates but also how the functional demands of a changing environment forced vertebrates to adapt. We see this most clearly in the jaws of the vertebrates, which we will now explore in detail.

8.5 The Jaws

Hypotheses of Jaw Evolution

The evolutionary transition from jawless to jawed vertebrates was incredibly important to the diversification of vertebrates, but it is also a topic of debate among morphologists and developmental biologists. While the majority of those who study evolution adhere to the theory that jaws (the palatoquadrate, dentary) are modified gill arches, we are not sure about how or why it actually happened.

How?

Several lines of evidence are used to explain the primary theories of jaw evolution, including morphological examinations, developmental studies, and gene expression work. In **gill-arch theory**, scientists view the morphological resemblance between the jaw bones and the visceral/branchial arches as an argument that modifications were made to the anterior arch, resulting in jaws (Figure 8.18). Because the argument relies on bones that develop in series (the branchial arches and the argued derivatives), developmental studies examined sharks and saw similar developmental trends between the jaws and the visceral arches.

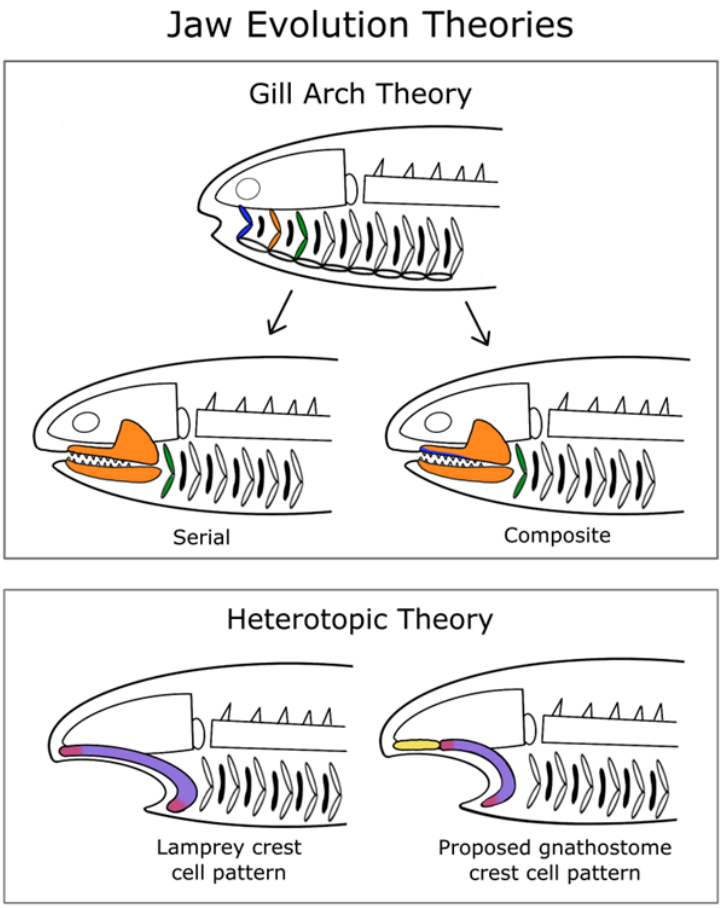

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Figure 8.18—The three leading theories for the evolution of the jaws: serial, composite, and heterotopic. In serial gill-arch theory, the jaws are thought to evolve from the first two branchial arches. Composite gill-arch theory features losses and fusions of branchial arches, resulting in a complex jaw system. The heterotopic theory examined neural crest cells to determine the developmental beginnings of the jaw bones.

While this idea is (fairly) widely accepted, *how* those modifications were made is still up for debate. The main camps

include serial theory, composite theory, and heterotopic theory; each of these is based on developmental and gene expression studies.

Serial theory posits that the mandibular arch was likely modified from either the first or second branchial arch and the hyoid arch from the following arch (some researchers believe the "first" branchial arch was lost, resulting in the second one forming the mandibular arch; Figure 8.18). This is based on the idea of serial homology and the similar development of those arches during the lifespan of an individual.

Composite theory is slightly more complex and also includes data from the fossil record. This theory doesn't follow the "one arch, one mandible" view and instead includes a series of losses and fusions of the arches, resulting in the mandibular and hyoid arches (Figure 8.18). This work is largely based on morphological and developmental comparisons across extinct and extant taxa.

Gene expression work has turned some of these arguments on their heads. These studies, which lead to the **heterotopic theory**, are based on comparisons between larval lampreys and jawed vertebrates (Figure 8.18). There were significant differences in expression of the *Hox* gene in the mandibular and branchial arches across these groups, indicating there may be substantial differences in the developmental origins of these morphologies. This boils down to differences in the neural crest cell patterns across these lineages. Under this theory, the pattern of crest cells responsible for the upper lips in lampreys (Figure 8.18) is not exactly homologous to the pattern seen in the jaws of gnathostomes (Figure 8.18). This work primarily calls into question the genes ultimately responsible for the development of jaws, which may, in turn, change our understanding of how jaws first evolved.

Why Jaws?

Some arguments say that these modifications were to improve

feeding, while others cite improved ventilation. Changing the most anterior arch into a feeding apparatus would help an organism open its mouth and obtain food. Alternatively, the ability to open their mouth could allow aquatic organisms to bring larger amounts of water past the gills, aiding in breathing and acquiring oxygen. Regardless of which purpose came first, the other advantage was likely realized very quickly.

Jaw Suspension and Attachment

While evolving physical jaws was a huge transition for vertebrates, these jaws (and their relevant bones) exist in the context of the entirety of the skull and must move in ways that are (theoretically) efficient for the functions of the organism. This movement is achieved in several ways (through muscles and ligaments), but the bony connections (or articulations) have important impacts on how the jaws are nested in the skull. This idea, called jaw suspension, describes how the jaws are anchored (or not) onto the chondrocranium.

Types of Jaw Suspension

Primitive Autostylic Suspension

In early gnathostomes (e.g., placoderms), the mandibular arch (and especially the palatoquadrate) was anchored to the chondrocranium. This **primitive autostylic suspension** allowed the palatoquadrate to act as a lever, with opening and closing primarily happening via the dentary (Figure 8.19). The hyomandibula was not attached to either the chondrocranium or the palatoquadrate and did not aid in the opening of the jaws. This early style of jaw

suspension allowed for jaw opening and closing, but little processing or "biting" could occur.

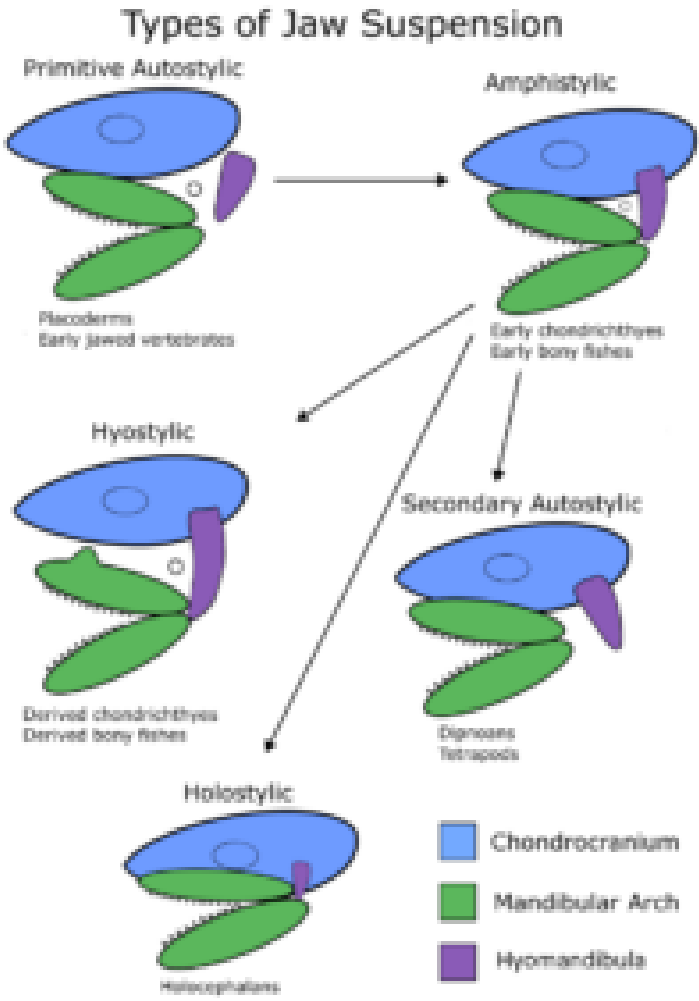

Figure 8.19—Jaw suspension types. (A) Primitive autostylic; (B) Amphistylic; (C) Hyostylic; (D) Secondary autostylic; and (E) Holostylic are shown with example taxa for each type of jaw suspension.

Amphistylic Suspension

From this primitive jaw suspension evolved **amphistylic suspension**, found in early chondrichthyans and primitive bony fishes. In this style of suspension, the palatoquadrate remains attached to the chondrocranium, while the hyomandibula is attached to the otic capsule of the chondrocranium and extends to attach to the posterior tip of the palatoquadrate (Figure 8.19). This produces multiple articulation points in the jaw, greater power, and therefore additional biting and chewing.

Hyostylic Suspension

From amphistylic suspension evolved **hyostylic jaw suspension**, found in derived chondrichthyans and bony fishes. The palatoquadrate is no longer attached to the chondrocranium, and instead the main articulation point is the hyomandibula (Figure 8.19). It remains attached to the otic capsule and the palatoquadrate. While (often) reducing the "power" generated by the jaws, this allows for jaw protrusion and rapid buccal expansion, which generates the negative pressure required for suction feeding. The jaws are able to move forward and downward, and sometimes both, during this movement. In some modern sharks, there is a process on the top of the palatoquadrate, preventing side-to-side motion of the jaws and limiting motion anteriorly and ventrally.

Secondary Autostylic Suspension

Secondary autostylic suspension evolved twice (independently and in different ways) from hyostylic jaw suspension. In holocephalans (e.g., chimeras), the palatoquadrate reattached itself to the chondrocranium, while the hyomandibula remained attached to both the chondrocranium and the palatoquadrate (Figure 8.19). This

leads to a restricted version of the primitive autostylic jaw suspension (sometimes called holostylic jaw suspension), with simple jaw opening and closing and little jaw protrusion.

In Dipnoi and tetrapods, autostylic jaw suspension reevolved, leading to a reattached palatoquadrate and a hyomandibula that is only attached to the otic capsule (Figure 8.19). In this type of jaw suspension, that increased "power" in jaw motion reemerged and the ability to protrude the jaws decreased. In these lineages, toothplates often appeared, aiding in the biting, chewing, and processing of hard prey (durophage).

Jaw Articulation and Middle Ear Bones

Fishes

As discussed in the previous sections, the jaws of the majority of vertebrates (the fishes) include the "upper jaws" (the premaxilla and maxilla) and the "lower jaws" (the mandible consisting of the dentary and the angular-articular fused together). In this group, jaw articulation refers to the connection between the quadrate and angular-articular (Figure 8.20). This articulation point allows for the opening and closing of the jaw by rotating the mandible around this joint. This rotation and the resulting movement of the lower jaws allows for biting, rapid expansion of the mouth, and (primarily) aquatic feeding. Additional work has found another joint in some teleost feeding apparatuses: the **teleost intramandibular joint** (**TIJ**). This secondary joint allows for additional rotation of the bones associated with feeding, allowing for mouth closing while the jaws remain protruded. This possibly allows for additional methods of prey capture. See Section 8.7 on feeding modes for more details about fish jaws.

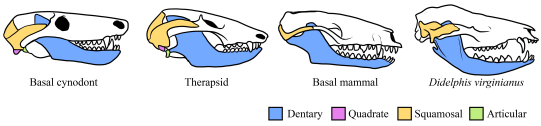

Figure 8.20—Jaw articulation in vertebrates: The connection between the quadrate and the angular-articular allows for the opening and closing of the jaws, but this is different across vertebrates (especially tetrapods).

Amphibians and Reptiles

To set the stage for changes that occur in mammals, it is helpful to remind ourselves of what the common ancestor may have looked like, using the amphibians and reptiles as a useful reference point. The mandible in amphibians and reptiles is made up of several bones, with only the dentary having teeth (Table 8.1). The upper jaw will also contain several bones, including the maxilla, premaxilla, quadrate, and quadratojugal. The jaw articulation joint in our amphibians and reptiles is the same as it was in our fishes, the quadrate and angular-articular bones (Figure 8.20). However, there is one element that we must discuss, as it will influence how we consider the changes that are going to occur in mammals. The bone is known as the **columella**, and at first glance, its very appearance may cause considerable confusion. The columella is a long, thin bone that originates on the braincase. In our frogs, the columella

runs from the braincase to the tympanum, the large circular structure visible in frogs like the American bullfrog. For most reptiles, the tympanum is not so obvious from the surface, instead found deep to an external ear opening. As you have probably surmised, the columella plays a role in sound reception and transduction. However, many reptiles also pick up low-frequency vibrations from the ground through their jaws when they are resting.

Double Articulation in Mammals!

As discussed earlier (see Box 8.1—Temporal Roof Evolution), significant morphological changes happened in the jaws during this evolutionary period, leading to an alternative jaw joint and new **auditory ossicles** (bones that play a role in hearing or the transduction of sound). The exact evolutionary causes of these changes are unclear, but there are multiple factors that together clarify how these changes occurred and what allowed them to do so. The first, seemingly unrelated change to occur has to do with posture. In the evolution of mammals, they changed from a sprawling posture to more of an upright posture, with limbs tucked underneath the body (see Chapter 10—Appendicular Skeleton). This had the effect of raising the body and the head off the ground for longer periods of time. As we discussed in the last section, reptiles and amphibians could transmit sound through their lower jaw to the middle ear. With the head being raised off the ground for longer periods of time, that ability is diminished. But how do you create new ear bones and a new jaw joint without disrupting all the functions?

Thankfully, unlike many other systems, there is a robust fossil record that tells us exactly how this occurred. In the synapsids, well before the rise of the true mammals, there are several fossils with very large dentary bones, so large that they almost make contact with the squamosal bone, part of the braincase

(Figure 8.20). Eventually, the continued increase in the size of the dentary directly results in it making contact with the squamosal bone. This led to the evolution of two distinct articulations of the lower jaw with the skull, and we can see this in stem mammals like *Morganucodon* (Figure 8.21; PBS—Your Inner Fish). These two joints sit right next to each other, with the dentary-squamosal joint being just lateral to the quadrate-articular joint. At the same time, the increasing braincase size and presence of the dentary-squamosal joint push the quadrate into smaller and smaller spaces, drastically reducing its size from what we saw in the reptiles. The new joint between the dentary and squamosal became the primary joint for chewing. This took pressure off the smaller quadrate and articular during feeding. This led to the evolution of new adaptations in these bones. Specifically with this new dentary-squamosal joint, the quadrate and articular bones are free to change. Their feeding function is now redundant; their primary function became hearing. There are many transitional forms, with some stem mammals having middle ear bones that functionally only provide support for hearing but are still firmly anchored to the dentary (now mandible). Over time, the middle ear bones become fully independent of the mandible (Figure 8.21). What once was the columella is now the **stapes**, the quadrate is now the **incus**, and the articular is now the **malleus**. The three bones shift in size and position, growing ever smaller and shifting away from the jaws. By the time we reach the extant mammals, they are completely converted to their role in hearing, and only developmentally can you see their history as the original jaw joint.

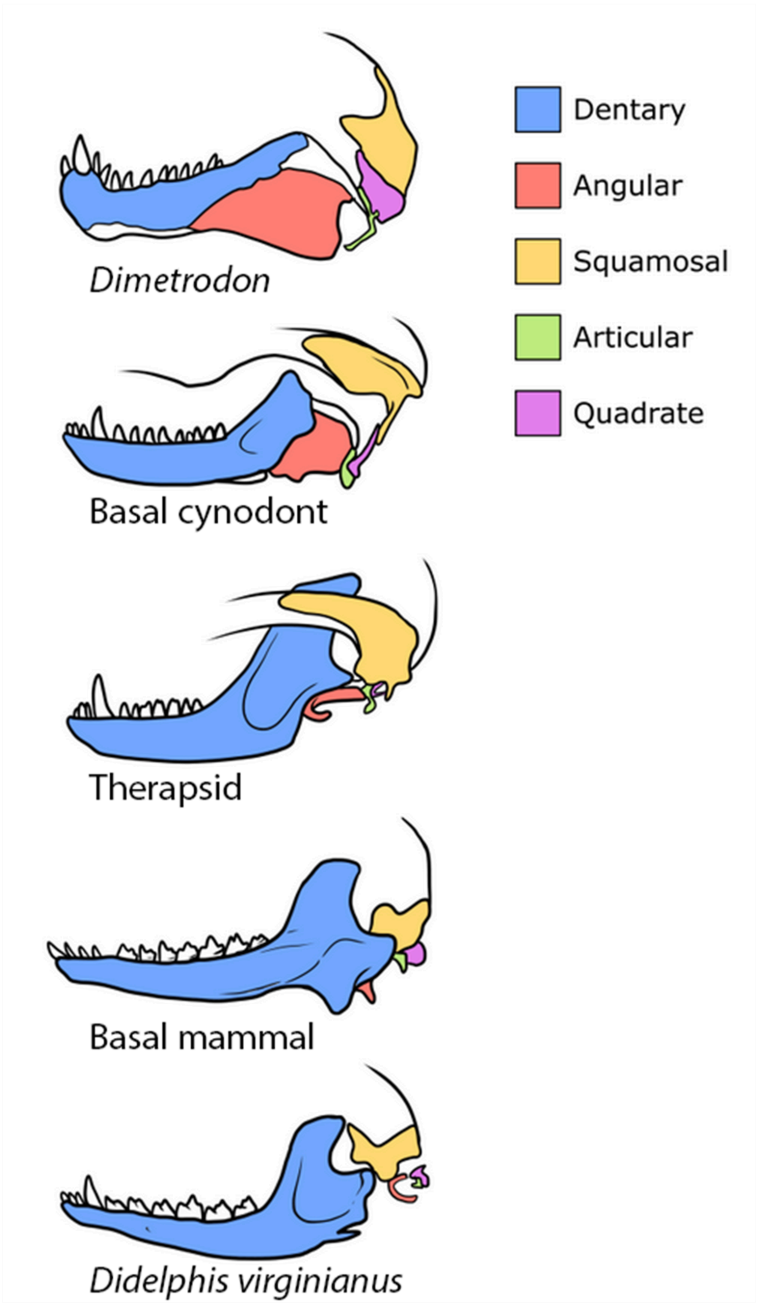
Dentary
Angular
Squamosal
Articular
Quadrate
Dimetrodon
Basal cynodont
Therapsid
Basal mammal
Didelphis virginianus

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Figure 8.21—The evolution of the jaw joint and middle ear bones in mammals.

Secondary and Tertiary Jaws

Pharyngeal Jaws

While the oral jaws (what we've previously discussed) were an incredible adaptation that allowed for better and more efficient prey capture, many species of teleosts took the idea of jaws one step further by evolving a secondary set of jaws, the **pharyngeal jaws**. These jaws typically sit in the throat and are modified gill arches. Originally they were thought to only have evolved once, but newer research estimates at least two evolutions of pharyngeal jaws, with others hypothesizing closer to six instances (Wainwright et al., 2012).

The pharyngeal jaws are considered one of the "key innovations" among vertebrates, drastically improving prey capture and processing and allowing for the rapid diversification of teleosts. The existence of this secondary set of jaws means the oral jaws no longer need dual purposes (both capturing and processing prey prior to digestion) but instead can be the primary mode of prey capture. Prey processing is then accomplished by the pharyngeal jaws, further inside the organism (What's Inside a Moray Eel's Mouth? | Natural History Museum [YouTube captioned video] or [YouTube audio described video]). This duality was thought to have allowed for the independent evolution of each system of jaws, increasing diversification of prey items and speciation. Recent work has put this idea into question: The two systems of jaws appear to be heavily connected and evolving together rather than independently. This likely means there are intense evolutionary pressures for these

jaw systems to remain (somewhat) the same throughout evolutionary history in actinopterygians.

Tongue-Bite-Apparatus

One group of teleosts, Osteoglossomorpha, independently evolved a second set of jaws within the oral cavity, dubbed the **tongue-bite apparatus** (**TBA**). This jaw system consists of patches of teeth on the parasphenoid (on the roof of the mouth) and on the enlarged hyoid and branchial arches. The TBA is incredibly diverse in its size and dentition but, like the pharyngeal jaws, allows for food processing while leaving the oral jaws available for prey capture. Some species have reevolved pharyngeal jaws in addition to retaining the TBA, leaving them with three sets of jaws.

8.6 Teeth

The changes discussed in the previous section (Section 8.5, The Jaws) lead directly to changes in dentition and teeth in terms of location, type, and number of teeth. Cyclostomes (hagfishes and lampreys) have keratinous cones on the mouth and tongue, while the evolution of jaws brought about "true teeth." It's also worth noting that the development of teeth (and the materials they are made of) is a similar process to that of scales. In fact, it is likely that teeth evolved from scales when jaws began to form.

Structure

In general, a tooth has several layers surrounding the pulp cavity, where the blood vessels and nerves are maintained. These layers

include hard enamel covering a bone-like substance called dentine (together forming the “crown” above the gum line). Below the gum line, the pulp cavity is further protected by cement, the periodontal ligaments, and the jawbone itself.

Development

Tooth development originates from two cell sources: epithelium and mesenchyme cells. The epithelium is derived from oral ectoderm, while the mesenchyme is derived from cranial neural crest cells (for more information about these cell types, see Chapter 6—Integument). Initiation of tooth development when specific genes are expressed in sites where the teeth will form lead to the thickening of the oral epithelium. We’ll use Figure 8.22 to show the following process of tooth development.

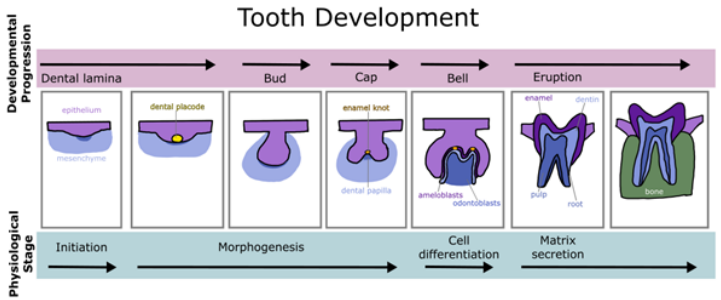

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Figure 8.22—The development of teeth from early embryonic stages, showing the morphological progression and the physiological stages. Based on Thesleff, 2014.

The bud stage immediately afterward is when that epithelial thickening increases and forms the dental lamina, pushing into the dental mesenchyme. This push of the dental lamina creates

the epithelial bud, which in turn forces the mesenchyme cells directly below them to condense. Those mesenchyme cells form the dental papilla. The epithelial bud continues to grow, surrounding the dental papilla. The mesenchyme cells in and around the dental papilla form the dental follicle or sac. The sac starts to increase in size, and the crown begins to develop (producing the tooth!). The shape of the tooth is determined at this stage: whether there will be one or two enamel knots (i.e., the early stages of cusps) resulting in single-cusped teeth or molars, respectively.

Tooth-specific cell types (odontoblasts and ameloblasts) begin to differentiate at this time. The ameloblasts are responsible for producing enamel (the incredibly hard coating around the teeth). The odontoblasts produce dentin, found in the center of the dental papilla. When the tooth is formed (the crown is present), the roots are formed and the tooth erupts.

Types of Teeth

Some organisms have variation in tooth size and shape, while others have incredibly similar tooth features across the mouth. In bony fishes, amphibians, and reptiles, shape tends to remain superficially consistent in the mouth (morphologically homodont), although size can vary. Common tooth shapes in these groups include conical, triangular/bladelike (i.e., great white sharks), peg-like (molariform), or platelike formations of teeth called toothplates (Figure 8.23).

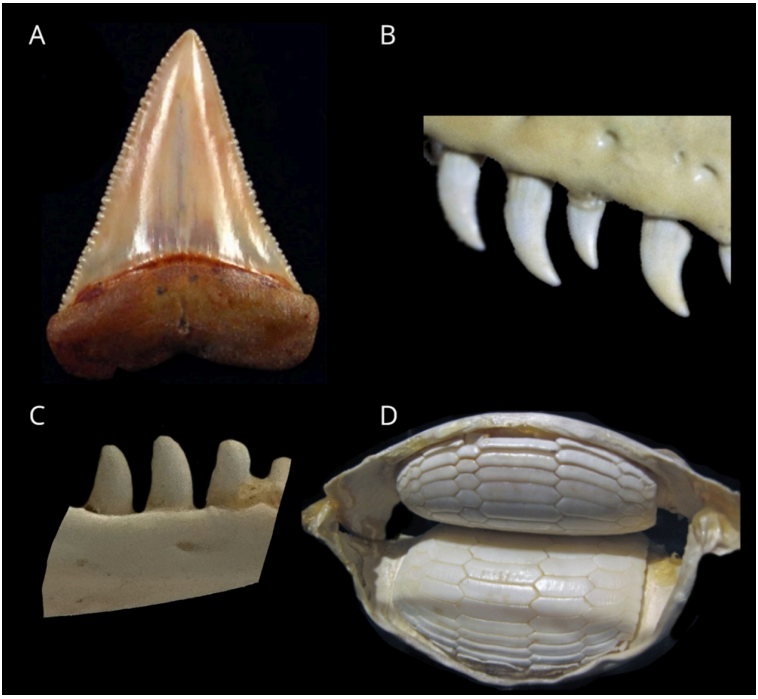

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Figure 8.23—Representative tooth shapes in fishes, amphibians, and reptiles. (A) Triangular tooth of the great white shark, Carcharodon carcarius; (B) Conical teeth of the Komodo dragon, Varanus komodoensis; (C) Peg-like teeth of the Savannah monitor; Varanus exanthematicus; (D) Toothplate of cownose ray, Rhinoptera bonasus.

In mammals, however, tooth size and shape tend to change across the jaws (morphologically heterodont). We can likely picture this by looking inside our own jaws. Placental mammals have matching teeth on either side of the jaws. In primitive mammals, each side (typically) included three incisors found on the rostral part of the jaws, followed by one canine, four premolars, and three molars (Figure 8.24).

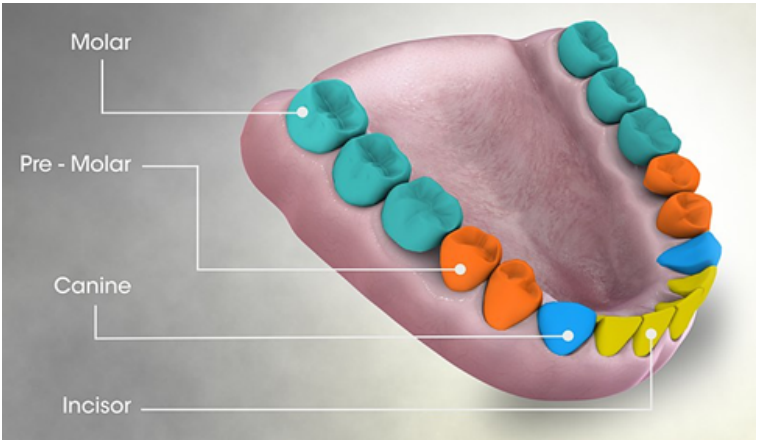

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Figure 8.24—Mammalian tooth types as represented by a human.

This pattern is still found in some mammals, although most mammals (including humans) have lost some of these teeth, altering their pattern. Types of teeth can be documented using a **dental formula**, which describes the teeth found on the upper jaw (numerator) and the lower jaw (denominator) on a given side of the organism. In this formula, incisors are abbreviated "I," canines with "C," premolars with "P," and molars with "M." The common mammal dental formula would be I 3/3, C 1/1, P 4/4, M 3/3. In humans, this shifts to I 2/2, C 1/1, P 2/2, M 3/3.

We would be remiss to ignore some of the odd teeth found in the vertebrate lineage. Enlarged and elongated incisors are often used by mammals for mating purposes, both for physical altercations and for secondary sexual characteristics. Tusks in elephants and Artiodactyla (e.g., wild boar, hippopotamuses) are large teeth, protruding from the skull for combat. Narwhals, also in the Artiodactyla clade, have a singular enlarged incisor, giving them a unique appearance (Figure 8.25).

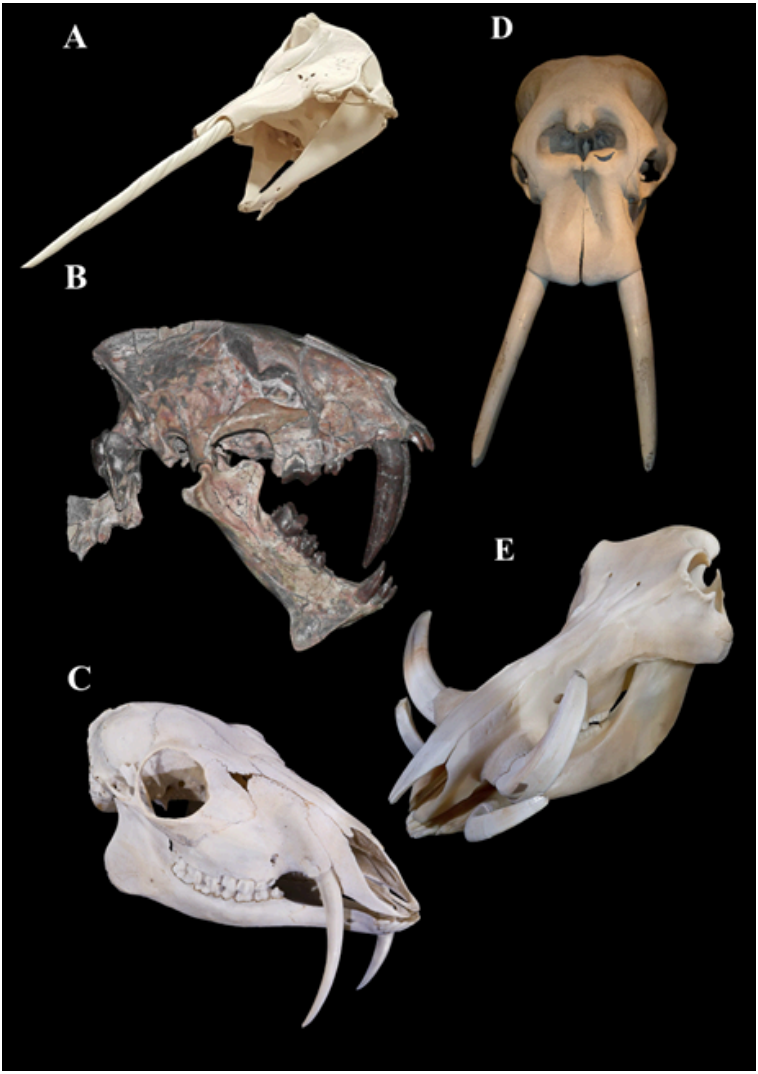

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Figure 8.25—Some examples of modified teeth (tusks) in mammals. (A) *Narwhal*; (B) *Saber-tooth tiger*; (C) *Musk deer*; (D) *Elephant*; (E) *Warthog.*

Substructures

Mammalian teeth often have multiple substructures that allow for multiple functions but also the closing of the jaws during biting (occlusion). You can picture a set of alternating triangles fitting together nicely but often with slightly more complex shapes. We often describe these substructures by the side (toward the tongue or the cheek) of the tooth and by the shape. Structures on the tongue side of the tooth are called **lingual**, while those on the cheek side are **labial**. Over time, cones and cusps evolved to increase the number of shearing surfaces on these teeth. In Carnivora, the third upper premolar and the first lower molar are called **carnassials** and are distinguished by their incredibly large shearing surface (Figures 8.26 and 8.27).

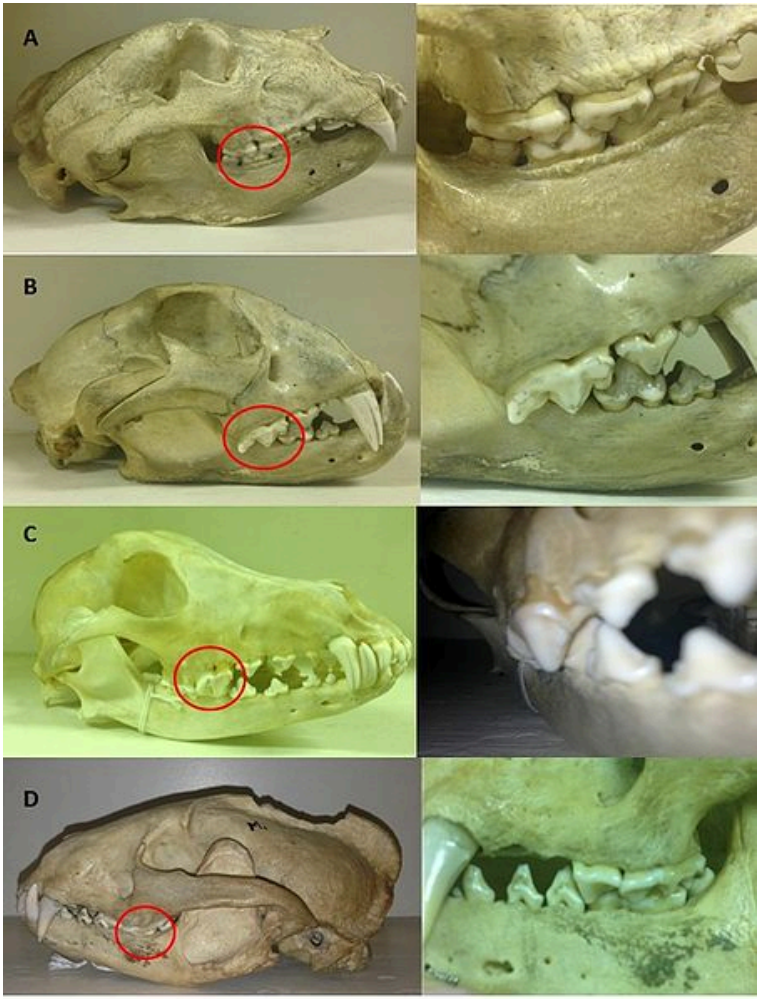

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Figure 8.26—The carnassial teeth of several representative mammals. (A) *European brown bear, Ursus arctos*; (B) *Leopard, Panthera pardus*; (C) *Domestic dog, Canis lupus familiaris*; (D) *European badger, Meles meles.*

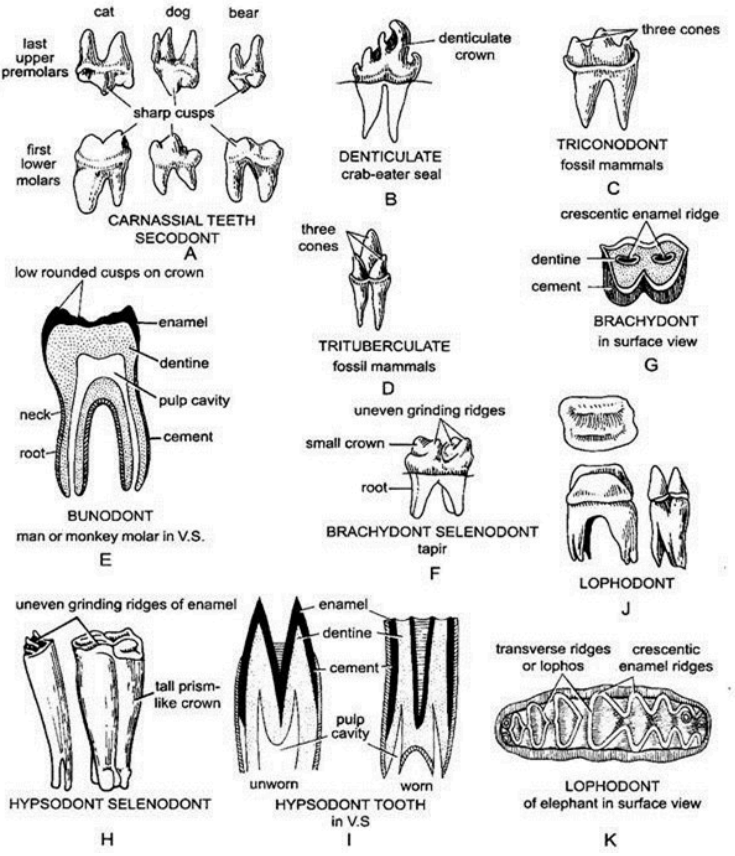

Figure 8.27—Different tooth shapes and styles in mammals.

In herbivores and omnivores, crushing surfaces are emphasized over shearing surfaces. **Bunodont molars** have rounded cusps for crushing and grinding and are typically seen in humans, pigs, and more basal herbivores (Figure 8.27). In contrast, sometimes those cusps fuse together to form ridges in **lophodont molars** in more specialized herbivores (Figure 8.27). Taking it a step further,

those ridges become even taller and longer, eventually forming crescents in **selenodont molars** found in Artiodactyls (Figure 8.27).

Bunodont molars tend to remain fairly low-crowned (brachydont), with their height not extending significantly past the gums (Figure 8.28). Lophodont and selenodont molars are typically high-crowned (hypsodont, with their ridges and crescents extending far beyond the line of the gums; Figure 8.27).

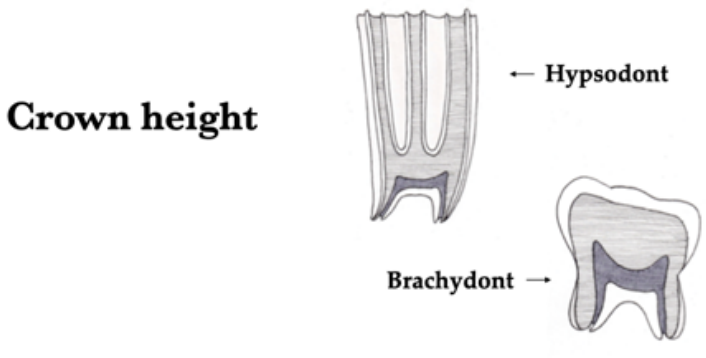

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Figure 8.28—Hypsodont and brachydont teeth.

Tooth Attachments

There are several different types of tooth attachment, primarily distinguished by the degree to which the teeth are "set" in the jawbone. Superficial attachment can be acrodont or pleurodont. **Acrodont teeth** are attached to the top or inside edge of the jaw, and there are limited options for replacement (Figure 8.29A). **Pleurodont teeth** are attached to the outside edge of the jaw (Figure 8.29B). **Thecodont teeth** are set in deep sockets in the jawbone itself and evolved in the ancestors to mammals. They can have one or more roots and are able to withstand greater pressures, and this allows for greater diet disparity (Figures 8.29C and 8.29D).

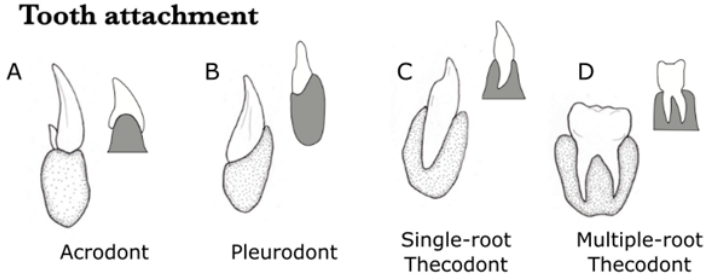

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Figure 8.29—Different styles of tooth attachment to the jaws.

Replacement

The majority of vertebrates (i.e., most fishes) are **polyphyodont**; new teeth can develop as old teeth wear and/or fall out during an organism's life. The new tooth begins to develop prior to the old tooth being lost, leaving little (or no) time when the organism is missing necessary teeth. This is a very complex (and consistent) process. In bony fishes, activation waves of replacement happen from the anterior to posterior part of the jaw. When the new tooth matures, the root is reabsorbed, allowing the old tooth to loosen and fall out. This often leads to the jaw containing a combination of teeth: brand-new teeth that just came in, empty sockets with new teeth erupting soon, and fully formed, mature teeth. The empty sockets do not typically stay empty for long; the activation waves ensure that the majority of teeth are either new or fully mature at any given moment.

Not all vertebrates replace teeth like this; the majority of mammals are **diphyodont** (Greek "diphy" = two), including humans. These organisms have two sets of teeth: milk (deciduous) teeth and permanent teeth. The permanent teeth replace the milk teeth

during the prolonged maturation time. Toothed whales (e.g., dolphins, porpoises, sperm whales) are **monophyodont**, only possessing one set of teeth.

For some organisms, diet plays a crucial role in tooth wear and, therefore, replacement. In most mammals, including humans, incisors are relatively small and typically used for picking up food or making small cuts. In rodents, incisors have been reduced in number but increased in terms of size. Their two pairs of incisors (instead of the usual four) constantly grow because they do not seal their pulp cavity. Rodents typically consume plant materials that wear down teeth quickly. Unless addressed, this problem would result in rodents replacing their incisors constantly. Instead, the wear from eating plants keeps the incisors from overgrowing while removing the need for full tooth replacement. The most anterior part of the tooth is made of enamel, the harder material, which isn't worn down as quickly. The posterior part of the tooth is dentine, which is worn down but also replaced at a faster rate. The resulting teeth have a hard, sharp frontal edge, which is great for cutting.

Function

Different tooth types tend to relate directly to diet. For instance, conical, pointy teeth are much more efficient at piercing prey to prevent its escape, while serrated teeth (like those in some sharks) are better at cutting prey into digestible pieces. Flat-topped, molariform teeth and toothplates are used for crushing hard prey items while reducing stress on the jaws.

Heterodonty Versus Homodonty

An important consideration when thinking about dentition is the

relative size, shape, and position of the teeth within the jaw itself. Position in the tooth row has a direct connection to the amount of pressure or stress a given tooth will be under during a biting motion. If we consider an idealized dentary (Figure 8.30) and place idealized conical teeth on it, we can imagine different stress loads based on the size of those teeth. For instance, in Figure 8.30, all the teeth are exactly the same size from the front of the dentary to the back, making them morphologically homodont. When considering the stress changes across the dentary, however, it is easy to picture the teeth in the back of the jaw experiencing additional pressure or stress during the jaw closing, as they quickly approach the upper palatoquadrate. This means that this jaw example is both morphologically homodont and functionally heterodont. The teeth across the jaw are experiencing different stresses. Alternatively, in the bottom right illustration of Figure 8.30, the size of the teeth changes across the jaw, while the pressure experienced by each tooth remains fairly consistent. This jaw example would be considered morphologically heterodont but functionally homodont.

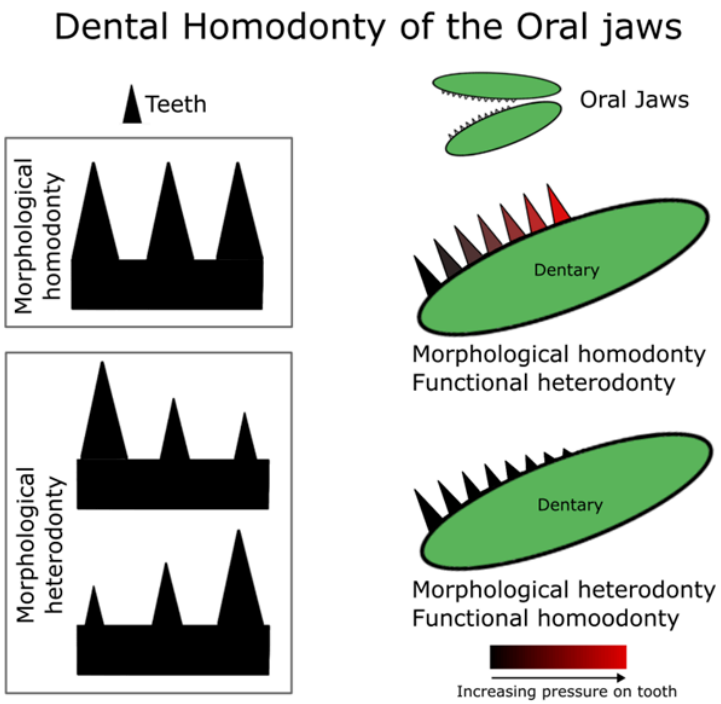

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Figure 8.30—Morphological versus function heterodonty and homodonty. Based on Cohen et al., 2020.

8.7 Feeding Modes

Ram Feeding

With the evolution of jaws and variable dentition came the need to use those jaws for prey capture in aquatic environments (see also Chapter 5). The earliest form of feeding with jaws came from simply opening the mouth using head (epaxial) and body (hypaxial) muscles.

The epaxial muscles lifted the top of the head, while the hypaxial muscles and interhyoideus pulled the dentary down and backward. This motion wasn't incredibly fast but allowed the mouth to open wide. Importantly, the overall volume of the mouth did not increase. The organism (e.g., whale shark) could swim with its mouth open or could open it immediately prior to swimming upon the prey. Many larger aquatic vertebrates use this method of feeding and eat many small prey items during a feeding event. They often have extensive gill rakers to assist with sorting the prey from the water, as water was brought into the mouth at the same time as the prey.

Suction Feeding

We saw a new method of feeding evolve in chondrichthyans and early actinopterygians, but it was improved dramatically in Teleostei. During the evolution of the Teleostei from the more basal actinopterygians, we saw a drastic increase in the number of bones in the dermatocranium, and these bones became incredibly mobile. These changes allowed for significant changes in the overall mobility of the jaw and for complex movements to happen (nearly) simultaneously. In suction feeding, several things happen in very quick succession: The epaxials lift the head up, the hypaxials pull the dentary ventro-caudally, and the levator operculi rotates the gill coverings, further rotating the dentary, premaxilla, and maxilla (Figure 8.31). The rotation of the premaxilla and maxilla causes the upper jaws to protrude (they are practically thrown forward), quickly expanding the volume of the mouth cavity and generating suction (Fish Suction Power: Dynamic Digital Endocast). This suction rapidly brings both water and prey into the mouth for processing (Epibulus insidiator, the slingjaw wrasse). While this increased range of motion is incredibly beneficial to adult organisms, it can lead to challenges in larval and juvenile fishes who aren't able to produce enough suction to adequately feed. Many

larval fishes aren't able to survive due to a lack of suction power (Larval fish failing to suction feed at 1,000 frames per second).

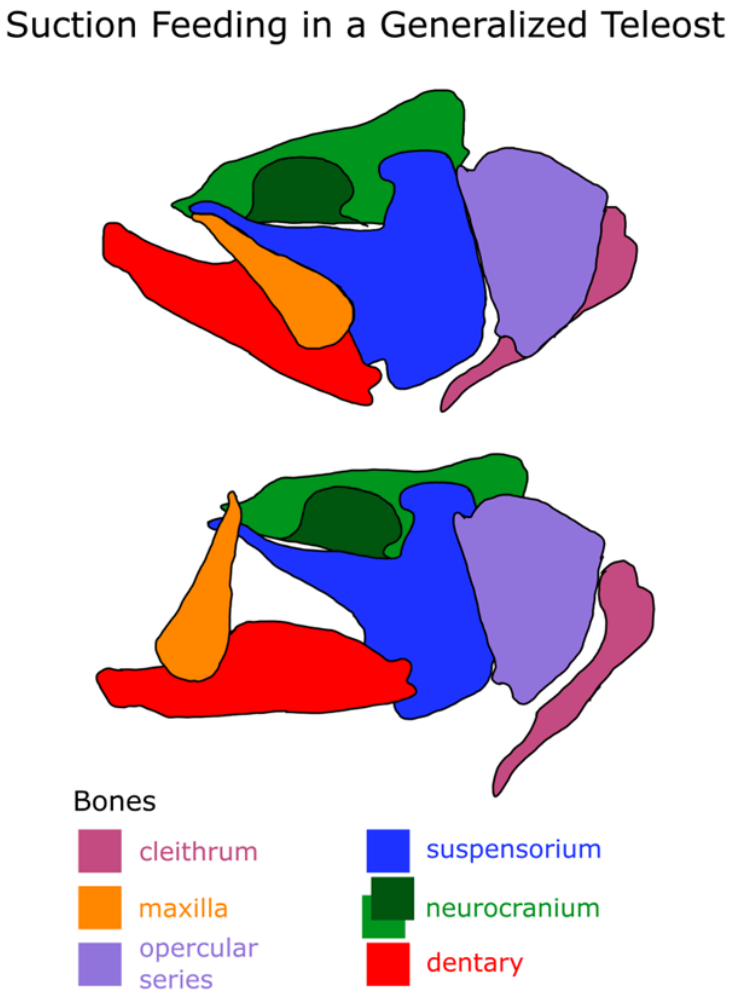

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Figure 8.31—The movements of the skull bones during suction feeding in a largemouth bass, Micropterus salmoides.

Biting/Chewing

Many organisms, including some fishes, rely on biting and/or chewing when processing prey, and this is often visualized by dentition type and pattern in both the oral jaws and the pharyngeal jaws (e.g., conical teeth for piercing versus molariform for crushing, as discussed in Section 8.6, Teeth). Different tooth types likely influence what prey items are processed by an organism, but other changes in the skull were also necessary to allow for true "biting" to occur. Bones tend to be more robust or well-ossified if the organism bites or chews its food, usually brought about by the need for strong muscles and muscle attachments on the head. Each "bite" is produced by torque around the jaw joint, generating force via the teeth onto the prey item. This biting action is true for both terrestrial and aquatic organisms, but they are forced to go about these adaptations in slightly different ways due to the mediums in which they live.

8.8 The Water-to-Land Transition—Tongues and Jaws

One of the key elements in the history of vertebrates was the transition from water to land. This transition impacted so many of the systems in this book, and the jaws certainly exemplify this transition. We will see that not only do the jaws change during the transition, but the lasting impact of that transition will result in continual changes for a considerable length of time.

To understand some of the "why" before the "what," let us revisit the previous section on how vertebrates feed using their jaws. In the aquatic environment, suction feeding is the dominant mode, along with suspension feeding (filter feeding). Both of these modes require food suspended in the environment. When a

largemouth bass sucks in the small minnow, it is not just sucking in the minnow but also ingesting a large volume of water. In fact, you could look at the situation as being reversed: The bass sucks in a large volume of water, and the minnow is carried in with it. Once an animal leaves the aquatic environment, it no longer has the luxury of being able to move the environment and capture the food contained within that environment. Instead, the animal in question must be able to only manipulate the food item itself. This predominantly limits the system to the biting/chewing system described above. In response to this style, you can see the changes to the skull in our early tetrapods: significant fusions of the skull bones and a complete loss of kinesis. This is the result of all the additional forces on the skull.

The second aspect of this transition is what happens once the food is in the mouth. In the water, as long as you can move the water, you can move your food. Transport of food in the mouth and into the esophagus requires moving the water. However, in the terrestrial environment, the anatomical structures are now required to both support and move the food in the mouth and into the esophagus. As we discussed in the section introducing tetrapods (and the gross muscle anatomy in Chapter 12), the skeletal and muscular elements that made up the gills are modified to become the tongue and its skeleton. The highly mobile tongue and its supporting elements play a large role in the ability to move food within the mouth and into the esophagus. Independently, in each group of tetrapods, the tongue has evolved to act not only as food transport but also directly for food capture. Frogs, salamanders, chameleons, woodpeckers, and anteaters all use their tongues to capture their prey.

8.9 Summary

The vertebrate skull is an extremely dynamic structure that has

changed greatly through evolutionary time. As the center for the senses, brain, and feeding, it has been shaped and fine-tuned by natural selection across millions of years. The differences between individual species (and even individuals within a species) are extremely insightful into a species' history, evolution, and ecology.

Application Questions

1. The dinosaurs, despite being diapsids, have more than two fenestrae found throughout their skull. What is the likely functional reason for this change?
2. Why is the trade-off between force and mobility so apparent in skulls?

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9. Postcranial Axial Skeleton

BILL RYERSON

Focus Questions—to Guide Your Reading of This Chapter

1. How does the change in rib function over evolutionary time impact the structure of the ribs (and sternum)?
2. How can the structure of individual vertebrae tell us about a vertebrate's locomotion and general body plan?

9.1 Introduction

The axial skeleton is the anchor of the skeleton. It connects the skull and fins/limbs to the rest of the body (with some exceptions). It plays a vital role in the locomotion of vertebrates, serving as the attachment point for many muscles. The axial skeleton houses the spinal cord, protecting it along its travels down the length of the body. Many of the structures of the axial skeleton appear very early in vertebrate history and change greatly across time.

9.2 General Structure and Function

The **axial skeleton** is composed of the **skull**, **vertebral column**, **ribs**, and **sternum** (Figure 9.1).

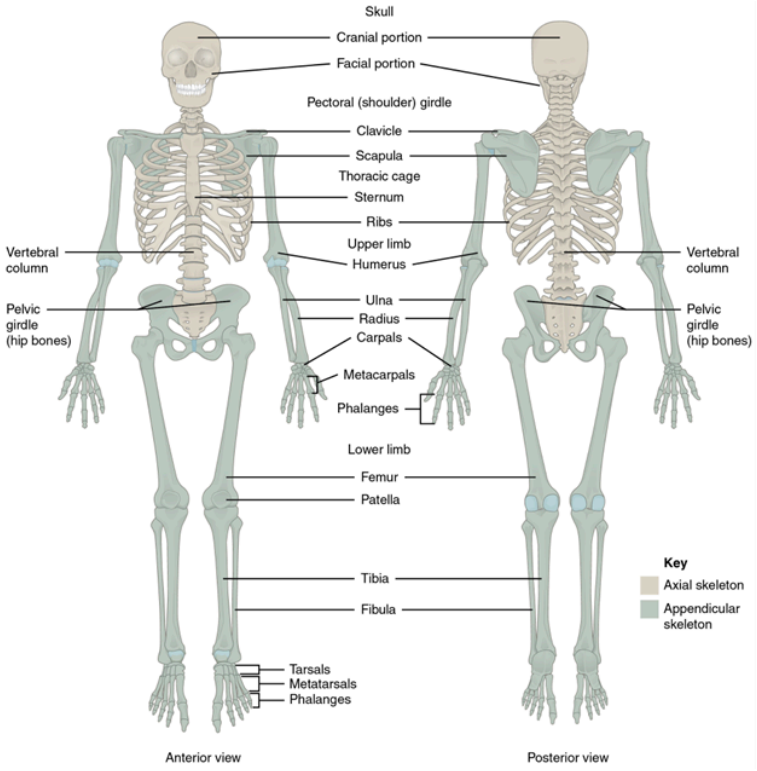

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Figure 9.1—The axial and appendicular skeletons of a human.

The skull has already been discussed in Chapter 8, but we shall cover the remaining elements here (what we will refer to as the **postcranial axial skeleton**). The vertebral column runs from the skull to the end of the tail and attaches to not only the skull but the appendicular skeleton (the fins/limbs and their associated skeletal

elements). It is dorsal in the body, typically covered by a few layers of muscle and the integument. The ribs extend from the vertebral column. In groups that have a sternum, it is a ventral structure and the other connecting point for the ribs.

The vertebral column is a segmented structure composed of individual units we refer to as **vertebrae**. The vertebrae are surprisingly complex structures (Figure 9.2). They provide attachment for epaxial, hypaxial, and other muscles. They have openings for the passages of large blood vessels up and down the body. They also have a single large opening for the passage of the spinal cord. In addition to all these other structures engaging and interacting with the vertebrae, individual vertebrae must also interact and engage with one another. Individual vertebrae moving together at varying degrees drive the side-to-side and dorsal-ventral bending that most vertebrates are capable of (though not all; looking at you, turtles). One of the topics that we will discuss in the evolution of the vertebrae is that of **regionalization**. Regionalization refers to changes in vertebral shape and number that can characterize a particular region of the vertebral column. The number of regions and the characteristics of those regions are variable between the vertebrate groups and are a useful tool for identifying the vertebrate classes broadly when the limbs/fins or skull are not available. The difference between a lizard skeleton and that of a salamander may not be obvious at first, but there are distinct differences in the vertebral column that can be used to narrow down what it is you are looking at. In fact, vertebrae can be so uniquely shaped that it is possible to identify individual species and estimate their size by the presence of a single vertebra (Box 9.1).

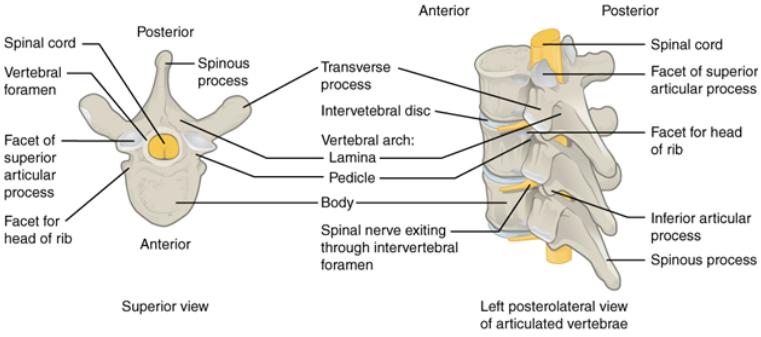

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Figure 9.2—Human thoracic vertebrae, illustrating generalized structure and elements of individual vertebrae.

Box 9.1—The World's Largest Snake...Ever

There are some very large snakes living in the world. The world's heaviest snake, the green anaconda (*Eunectes murinus*), can weigh well over 200 lb. The longest snake in the world, the reticulated python (*Malayopython reticulatus*), can grow to almost 25 ft. in length. Both of these species are dwarfed by the largest snake ever. Known as *Titanoboa*, this relative of the green anaconda and boa constrictors measures in at over 40 ft. in length, and its weight is estimated at over 2,000 lb. How do we know? Not only do careful measurements of fossil vertebrae from Colombia give us information about its close relatives, but we can also use that information to get an estimate of size. All from one vertebra!

Read more: Giant boid snake from the

Palaeocene neotropics reveals hotter past equatorial temperatures.

Between individual vertebrae, **intervertebral discs** are found. Intervertebral discs are small discs of cartilage with a gel-like center, resisting forces that might cause two vertebrae to come into contact with each other. The intervertebral discs also provide a little bit of space for spinal nerves to pass through canals in the vertebrae to reach the spinal cord.

The ribs are flat in cross section, often curved bones that attach to the vertebrae and the sternum. They are highly group specific in terms of number, shape, and function. Primarily, ribs serve two functions: protecting vital organs (e.g., the heart and lungs in mammals) and anchoring for muscle-directed movement. We will see that what is protected by the ribs and which muscles are attached to the ribs will vary greatly across groups. Some muscles may be more heavily involved in locomotion, while others may be almost entirely respiratory.

The sternum is one part of the axial skeleton that is not consistently apparent in all vertebrate groups. It connects the two halves of the ribs together, forming a "cage" in the tetrapods. It is also assumed to play a supportive and protective role in the tetrapods. The sternum seems to help resist the forces of gravity and body weight when a tetrapod is resting on the ground. The additional bone supports the ribs and helps direct the forces away from the vital organs. Many large aquatic species (the whales and sharks) have either no sternum or a reduced one. When they are beached, their sternum and ribs cannot oppose this newfound hazard of supporting their body weight.

9.3 Development

The postcranial axial skeleton is completely derived from mesoderm, although the different elements do not appear at the same stages. The notochord is the first to appear, a solid rod of mesoderm. After the formation of the notochord, blocks of mesenchymal cells congregate along the notochord (on each lateral side), originating from the sclerotome blocks, which will eventually become the individual vertebrae. The mesenchymal cells will differentiate into chondroblasts and begin the process of creating hyaline cartilage "molds" that will be replaced by bone via the process of endochondral ossification, with the exception of our hagfishes, lampreys, and cartilaginous fishes. The development of the ribs will be largely dependent on the species. For most vertebrates, the ribs originally develop as outgrowths of the vertebral cartilage molds. If these outgrowths are to become ribs, the cartilage mold will "break" off from the vertebrae, becoming its own independent unit. If it remains attached, we will come to know these structures as transverse processes (Figure 9.2). The sternum (in those taxa that have one) develops as an independent unit of hyaline cartilage (also mesoderm) along the midline of the embryo. The cartilage begins as two separate pieces that split and fuse depending on the species before ossifying in its adult configuration.

9.4 Evolution of the Ribs and Sternum

The presence, shape, number, and function of the ribs and sternum are highly variable across the vertebrate groups. It is always useful to keep the phylogenetic history in mind when examining the anatomy, but in the case of the ribs and sternum, we must frequently refer to the behavior of the animal and how these

structures must function in the context of behavior. Their structure will also impact the points of articulation with the vertebral column, and so while this section may seem brief compared to that of the vertebral column, you may find yourself referencing it quite a few times.

Hagfishes, Lampreys, and Cartilaginous Fishes

The early diverging vertebrates, from the cyclostomes through the chondrichthyans, do not have any ribs or a sternum to speak of. We might assume that perhaps the foundations of these pieces, like many structures, show up at some point during development and never fully form. However, this does not seem to be the case. The lack of ribs in these groups means that we will also not see the facets (concave attachment sites for a more rounded bone that they articulate with) on the vertebral column that connect the ribs to the vertebral column, making the vertebrae more uniform in appearance down the length of the body than we may see in other groups.

Osteichthyes

In the Actinopterygii (excluding the tetrapods), there is still no evidence of a sternum. However, ribs are present throughout the entire group. Bony fishes have two sets of ribs, aptly named the **dorsal ribs** and the **ventral ribs**. While *dorsal* and *ventral* are helpful in determining their location, to understand their function and evolution, we need to be more specific, especially in the context of other structures in the body wall. If we imagine sectioning a fish in half and rotating it so that we see the cut, the placement of the ribs will be more obvious (Figure 9.3).

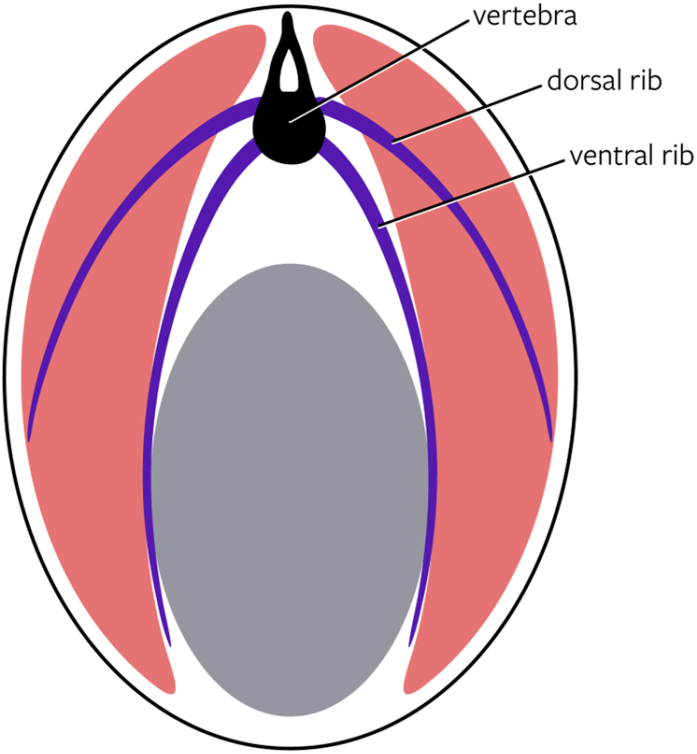

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Figure 9.3—Schematic transverse section of a bony fish. The black is the individual vertebrae, with the purple being the dorsal and ventral ribs. The gray is the coelomic cavity, and the pink are axial muscles.

There are several structures apparent to us in this section. At the most dorsal point, we see one of the vertebrae. Around that vertebra, extending downward laterally, are the axial muscles. The **axial muscles** are named for the placement on the axis of the body (just like the axial skeleton!). Deep to those muscles is the coelomic or body cavity. The **body cavity** is where all the organs are contained. The two sets of ribs help us delineate these different

pieces. The ventral ribs are found between the muscles of the body wall and the body cavity. They serve as an anchor to the body wall muscles as well as protection for the internal organs. The dorsal ribs split the muscles of the body wall (the axial muscles) into two groups: **epaxials** and **hypaxials**. We can think of the epaxials as being above the dorsal ribs and the hypaxials as being below the dorsal ribs. There is much more to these muscles than just this slight difference (see Chapter 12 for more), but the differences between the two groups of muscles can be most easily observed by seeing where they are in relation to the dorsal ribs. Without a sternum, the ribs taper off and end either within the axial muscles (dorsal ribs) or with a loose connective tissue connection to the body wall (ventral ribs).

Tetrapods

The transition to land, as you already know, changes many aspects of the skeleton and other systems. The ribs and sternum are no different in this regard, but unlike the other skeletal systems, it can be a little difficult to directly tie changes we see to the functional ramifications of those changes. As of this writing, the origin of the sternum is unclear. We know its current function in tetrapods: Connect the ribs at the ventral ends, reinforce the rib cage, and serve as one element connecting the pectoral girdle to the axial skeleton. These functional demands will drive the variation we see in later groups. When the sternum first shows up in the close ancestors of the tetrapods is still contested. The classic examples of the transitional fossils, *Tiktaalik* ("fishapod") and *Ichthyostega* (stem tetrapod), both lack a sternum. In fact, most fossils that we use to help us understand the evolution of the tetrapods from the rest of the sarcopterygians also lack evidence of a sternum. However, by the time we reach the modern-day amphibians, the sternum is present.

The ribs will also undergo several changes during the tetrapod evolution. The first is that the tetrapods will lose one set of ribs (the dorsal ribs) and the ventral ribs will be modified. The second is that we will begin to see regionalization of the ribs in some groups of tetrapods, with the eventual reduction and loss of a few of these regions by the time we reach the mammals. The modifications to the ribs are highly dependent on locomotion in these groups, so even within these different groups, the ribs will vary greatly in accordance with how that individual species locomotes and what environment it may be found in.

Amphibians

Across the amphibians, the presence and structure of the ribs and sternum are highly variable. The caecilians—the long, legless, and enigmatic amphibians—lack a sternum completely, with long ribs coming off the many vertebrae. Ribs are found all the way from the first vertebrae to the last few vertebrae before the end of the tail. In the frogs, the sternum is highly reduced, serving to connect the two sides of the pectoral girdle together at the center. Most frog species lack ribs as adults, but there is some developmental research that is suggestive of the ribs either fusing to the vertebral column early in development (while still a tadpole even) or vanishing quickly. The salamanders also, unsurprisingly, have considerable variation in their sterna and ribs. Aquatic salamanders, like *Necturus*, do not have a sternum and have highly reduced ribs. Terrestrial salamanders, like the lungless plethodontid salamanders common to North America, have both a sternum and significant ribs (Figure 9.4).

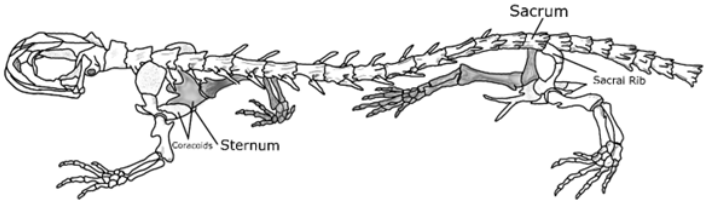

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Figure 9.4—The skeleton of the spotted salamander, Ambystoma maculatum.

As was the case with the frogs, the sternum does not connect to the ribs. Instead, it connects the two opposing sides of the appendicular skeleton and in many salamanders is not fully ossified into bone. However, the ribs of the terrestrial salamanders do begin to specialize in ways that will be repeated in other tetrapods. The first is that the ribs are not the same down the entire length of the body (Figure 9.4). Most terrestrial salamanders have ribs that terminate just prior to the pelvic girdle, where the hind limbs attach to the axial skeleton. Moreover, as we get closer to the pelvic girdle, the ribs that are there continually reduce in size. The same is true for the pectoral girdle, as the ribs closest to the forelimbs are also reduced in size. In addition to the changes in size, the ribs also change in their orientation at different segments of the body. Near the pectoral girdle, the ribs "point" ventrally, forming a quasi-cage around the heart and liver. As we move caudally (toward the tail), the ribs themselves also point caudally. The reason for this difference has not been investigated. We assume that it functions in protection of the organs, while the more caudal vertebrae play a different role, perhaps one linked to locomotion. The final item to note about the ribs in salamanders is that we see the emergence of ribs that connect to the vertebrae in two locations. In the bony fishes, both the dorsal and ventral ribs had one connection to the vertebrae, with one head each. In the salamanders, ribs now have

two heads, which we can refer to as **bicapitate ribs**. Each head articulates with a different vertebra.

Nonavian Reptiles

To better understand the evolution of the ribs and sternum, it is necessary to separate the birds from the other reptiles. Flight will greatly impact how the sternum is structured, and there is considerable variation among the other reptiles to consider before we make our way to flight.

The lizards all possess a sternum, with the exception of the legless families (including snakes). The sternum not only connects the appendicular skeleton to the axial but also now serves as an attachment for the ribs (Figure 9.5).

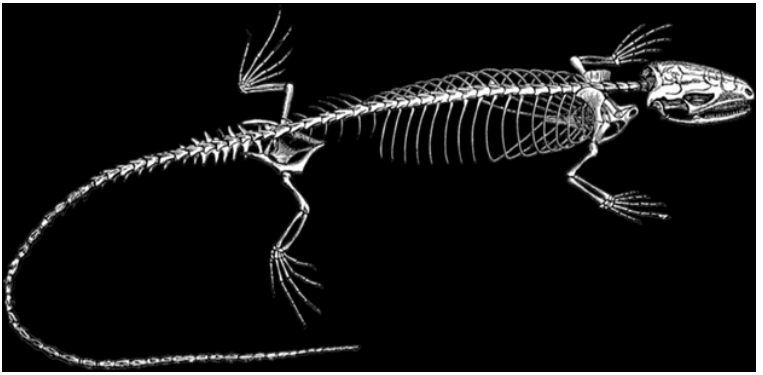

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Figure 9.5—The skeleton of a generalized lizard. Note the changes in ribs throughout the trunk region.

For vertebrates where the ribs are connected to the sternum, we can classify them according to *how* the ribs connect to the sternum. In nearly all cases, there is at least a small branch of hyaline cartilage that runs from the distal end of the rib to the

sternum itself. We will refer to this as the **costal cartilage**. Not every rib has its own costal cartilage. Some ribs will have individual units that merge together to form a single robust costal cartilage that then connects to the sternum. Now we can envision a total of three different categories of ribs, based on their connection to the sternum: one costal cartilage for that rib (true rib), several costal cartilages merging into one (false ribs), or ribs that do not connect to the sternum at all (free/floating ribs). In the lizards with a sternum, we will find examples of all three types of rib. We will also define ribs by their location: **cervical ribs** for those found anterior to where the pectoral girdle meets the axial skeleton and **abdominal ribs** for all the floating ribs posterior to the last **true/false ribs**. The number of ribs that are associated with each region varies considerably. Some lizards do not possess cervical ribs at all, while groups like the monitor lizards may have three or four cervical ribs (Figure 9.6).



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Figure 9.6—The cervical vertebrae of a Savannah monitor (Varanus exanthematicus). Two cervical ribs have been circled in blue.

One of the most interesting changes to occur in the lizards and snakes is not a change in the structure of the ribs themselves but rather one of the functional roles of ribs. To this stage, we have emphasized the role of the ribs in anchoring the axial musculature and introduced their role in protection of the organs. Lizards, having lost the ability to breathe in the same manner that the amphibians have used, must employ different mechanisms for moving air into and out of the lungs (more on this in Chapter 14—Respiratory System). The lizards have employed the ribs for this task. The ribs of lizards can move in several different planes, expanding and contracting the thoracic cavity as needed. Additionally, there is some evidence that rather than moving the ribs as a whole functional unit (as we humans do), some lizards can move individual ribs. Snakes are the undisputed rulers of rib movement. Snakes lack a sternum and the regionalization of the ribs observed in other lizards. Snakes possess ribs along nearly the entire length of the vertebral column, from right behind the head all the way to the cloaca. Ribs in snakes are multifunctional masterpieces. They are integral to protecting the organs, are the only manner by which snakes breathe, and power the many different modes of locomotion that snakes use to get around. However, snakes can also move every rib individually, contributing to their ability not only to move but also to feed. Constricting snakes use their ribs to encircle their prey, generating sufficiently high pressures to kill the prey item. Amazingly, because snakes can move ribs individually, they can constrict their prey with one part of their body and breathe with another!

The turtles, with their robust shells, have moved in seemingly the opposite direction from the snakes and lizards. The ribs and sternum are completely gone in the turtles. What happened to them? Recent fossil and development evidence support the ribs as a key player in the evolution of the shell itself. Several fossil turtles, including *Odontochelys* and *Eunotosaurus*, seem to possess large, flattened ribs (Figure 9.7). These flattened ribs are thought to be the precursors to the bony shell of modern-day turtles, with

developmental data supporting the transition from individual to the large bony shell.

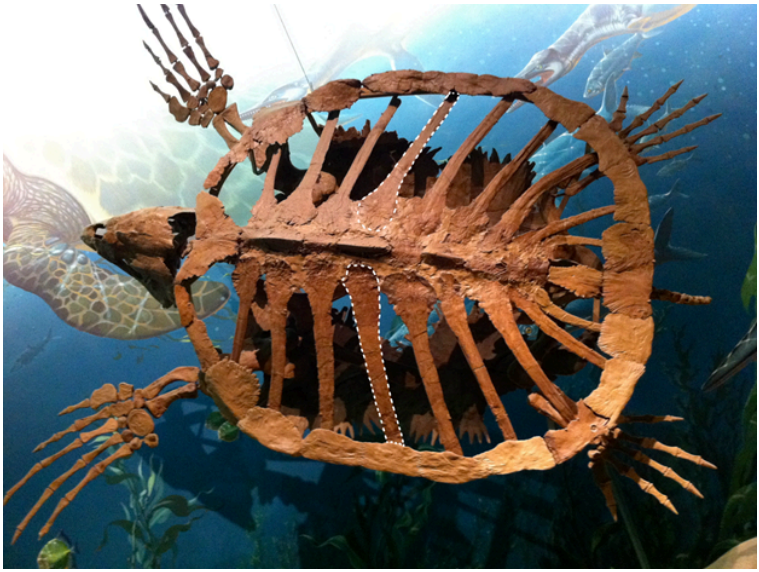

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Figure 9.7—The fossil turtle Eunotosaurus. Two of the ribs are highlighted by white dashed lines.

Leading into the archosaurs, our modern-day alligators, crocodiles, and the extinct dinosaurs, we find a robust sternum with several costal cartilage attachments. The more robust sternum is thought to not only help anchor the larger forelimb bones to the skeleton but also provide additional support for the body when the individual is at rest. Unlike in the lizards, ribs do not move individually in the archosaurs, reducing (but not eliminating) their role in respiration.

Aves

The birds are the next area of considerable variation in the ribs and sternum. The ribs reduce greatly in birds, losing much of their mobility and flexibility in the process. However, the role of the sternum is greatly increased beyond weight bearing and rib attachment. In birds, a key role of the sternum is to serve as an attachment point for the pectoralis muscles. The pectoralis muscles are muscles in the thorax that pull the forelimbs toward the center of the body. For the tetrapod classes we have discussed so far, the pectoral muscles have not been particularly large or of consequence, not impacting the overall structure of the sternum. Birds, being one of the few vertebrate classes capable of powered flight, rely heavily on large pectoral muscles to do so. Large muscles often require large attachment sites, even those that connect via a narrow tendon. In the case of the birds, the large pectoral muscles have had a significant impact on the size and shape of the sternum. The sternum is quite large in most species (Figure 9.8) and can take up more than half the length of the ventral body surface.

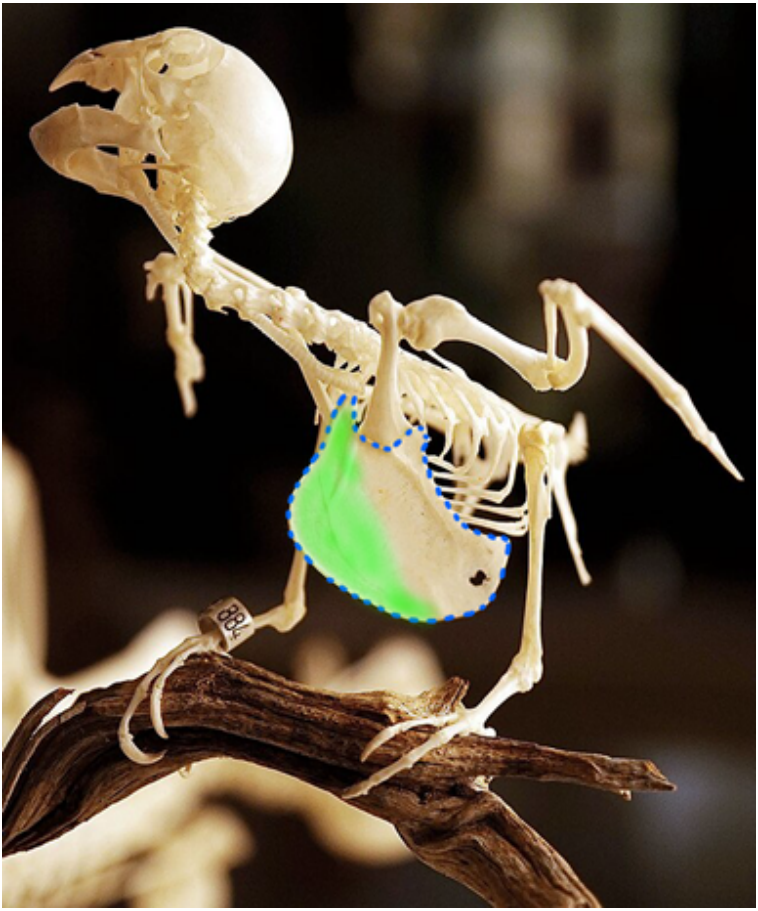

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Figure 9.8—The skeleton of a parrot with its large sternum visible, outlined in blue dashed lines. The prominent keel is highlighted in green.

The size of the sternum is more likely a function of stabilizing the ribs and pectoral girdle during flight rather than anchoring the muscles itself. To anchor the muscles, look at the more striking feature of the bird sternum, the large ridge running right down the midline. This is the **keel** of the sternum and is where

the large pectoral muscles attach. The keel can be as large as the rest of the sternum is wide, particularly in birds like hummingbirds and our domestic fowl (e.g., chickens and turkeys). The ostrich, the largest of the flightless birds, has almost no keel to speak of and a reduced sternum relative to its flighted brethren (Figure 9.9).

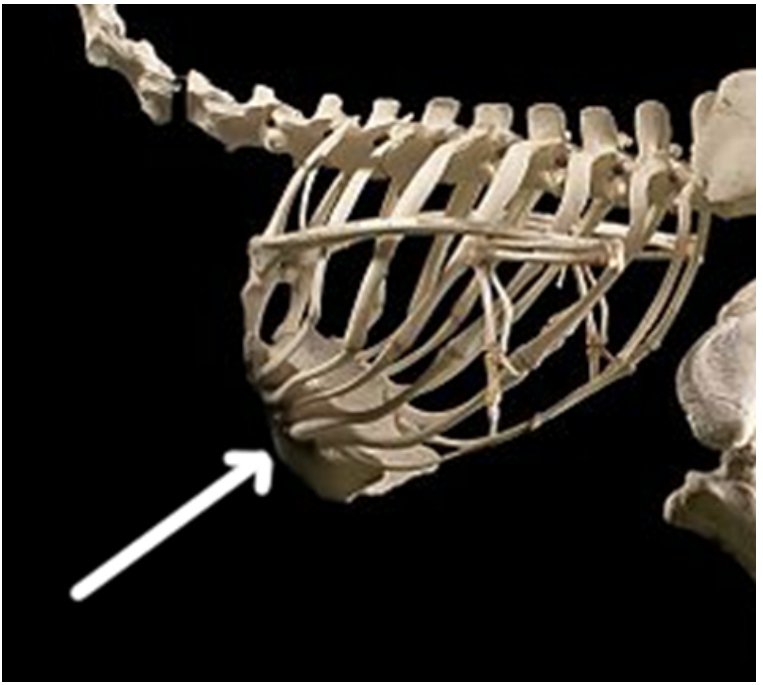

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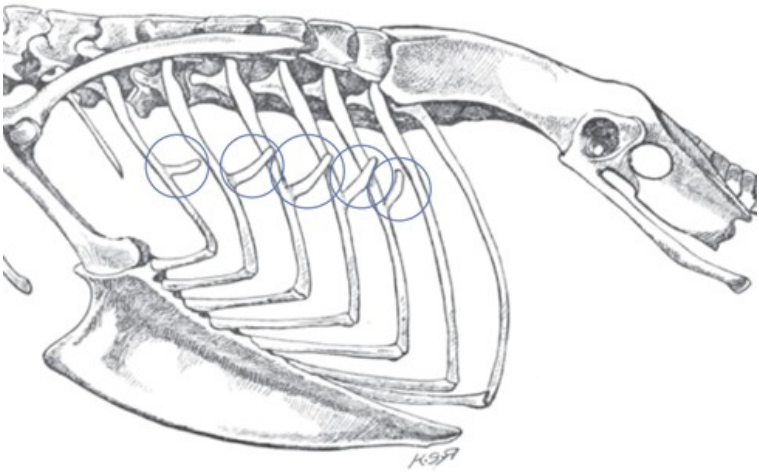
Figure 9.9—The sternum and rib cage of the ostrich (Struthio camelus). Anterior is to the left. The white arrow indicates the sternum.

The ribs of birds are also quite unique. The ribs themselves are a bit broader than in other vertebrates, with very robust connections to the sternum. Bird ribs have **uncinate processes**, hooked caudal extensions on the ribs. The uncinate processes allow the ribs to hook onto their caudal neighbor. In doing so, the whole rib cage is effectively locked together (Figure 9.10).



Thorax of *Aramus* ($\frac{3}{4}$ nat. size).

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Figure 9.10—The rib cage and sternum of a limpkin (Aramus guarauna). The uncinate processes are circled in blue. Anterior is to the left.

Again, this is an adaptation for flight, as the ostrich (Figure 9.9) seemingly lacks these attachments. This also means that the ribs, being less mobile, play a reduced role in respiration, but birds have developed a highly sophisticated respiratory system that does not rely on the same types of movements (see Chapter 14—Respiratory System for more).

Mammals

The ribs and the sternum of mammals are not strikingly different from those of the other tetrapods but are different in a few key ways (Figure 9.11). The sternum in mammals consists of three individual bones: the **manubrium**, the **body**, and the **xiphoid**. The manubrium

is the most anterior/cranial of the three, and it is the attachment point for the clavicle (if there is one) and the first rib. The body is the largest and where the vast majority of the ribs connect. The xiphoid is smaller than the other two components and acts as a connection point for some of the abdominal muscles.

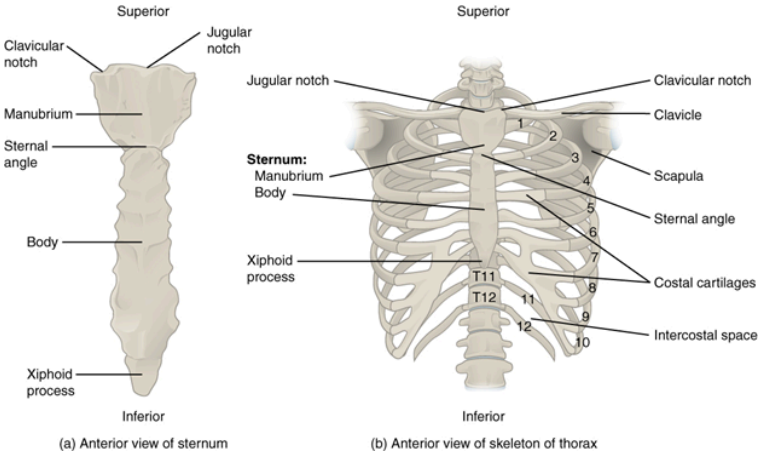

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Figure 9.11—The ribs and sternum of the human skeleton.

Mammalian ribs are limited to only about 13 vertebrae in the body with some variation across species. There are no cervical ribs, and the presence of ribs (and their attachment points to the vertebrae) is a distinguishing character that helps separate the different regions of the mammalian vertebral column (more to come).

Mammals possess all three types of ribs: **true**, **false**, and **floating**. The number of each varies by species. The ribs are mobile in mammals, and while individual muscles (the **intercostals**) have individual units, it appears that no mammals move individual ribs without moving the entire rib cage. The ribs play a role in mammal respiration, but they are not the primary drivers of air movement as we saw in the lizards. The ribs can move to increase the total volume

of a single inspiration (take a very deep breath). At rest, they do not contribute to smaller breaths and are not required to move in order to expel extra volume of air.

9.5 Evolution of the Vertebrae

In the evolution of the ribs and sternum, we largely paid attention to how the bones evolved as entire units. As we begin our exploration of the vertebral column itself, we will need to keep in mind not only the column as a whole unit but also how individual vertebrae may change along the way. It is also important to recall the ancestral condition, as represented by the chordate *Amphioxus* (Figure 9.12).

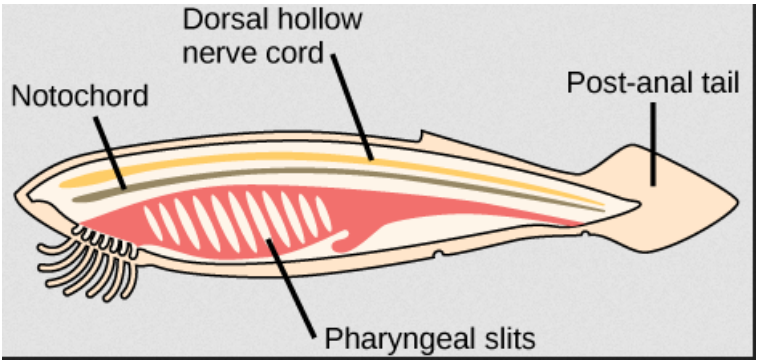

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Figure 9.12—Amphioxus, with its prototypical chordate characteristics.

Two of the classic chordate characteristics will help us begin our journey along the history of the vertebral column: the notochord and the dorsal hollow nerve cord. The notochord is a still, rodlike structure composed of cartilage, a single unit running the full length of the body. It is slightly ventral to the dorsal hollow nerve cord (homologous to the spinal cord). With these two

elements in mind, we can set the stage for the evolution of the vertebral column.

Cyclostomes

It is admittedly a bit odd to begin discussing the vertebrates with two species that previously have not been considered vertebrates because they seem to lack…vertebrae. The presence of vertebrae in the hagfish and lamprey has been contested for almost two hundred years (literally; see Müller, 1834, Ota et al., 2013, and everything in between). Both groups retain the ancestral notochord, and there are no obvious vertebrae present. However, recent developmental work has found "precursors" to the vertebrae that are found in the caudal region of hagfishes and lampreys. To understand these elements, let us visit Figure 9.13. Looking at this image, use the **neural canal** (where the spinal cord passes through) as the center of the vertebrae. Ventral to the neural canal is the **centrum** or body of the vertebrae. Lateral and dorsal to the canal is the **neural arch** with all its associated processes and facets. We can consider the centrum the "ventral element" of the vertebrae and the neural arch the "dorsal element." Why are we making this distinction? Because these different elements are what we observe in the hagfish and the lamprey. In the hagfish, along the caudal section of the notochord, several cartilage-based nodules begin to form, homologous to those ventral elements of the vertebrae. In the lamprey, the opposite is true; the dorsal elements are observed just dorsal to the spinal cord itself. However, as they are not fully formed units and do not extend the length of the body, we do not recognize these as vertebrae.

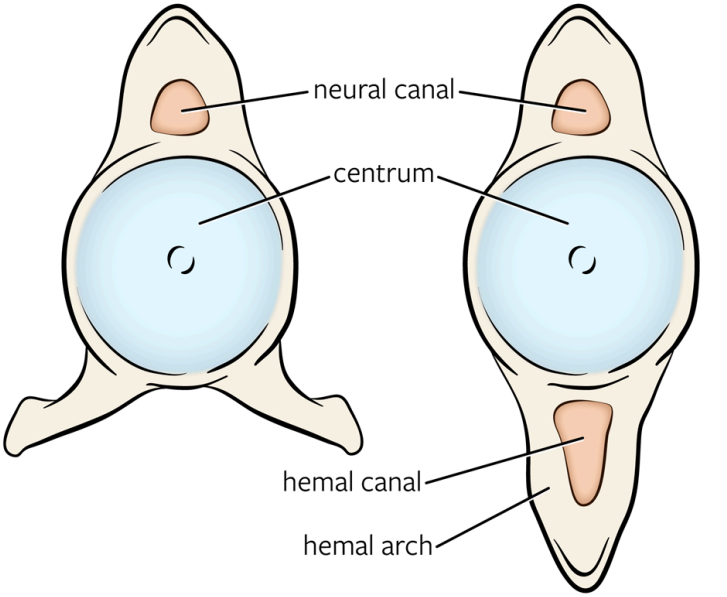

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Figure 9.13—The trunk (left) and tail (right) vertebrae of a generalized shark.

Chondrichthyes

The cartilaginous fishes are the earliest group to exhibit true vertebrae, distinct structures from the notochord. They are also the earliest group in which we see at least some regionalization of the vertebral column. Broadly, we can break the vertebral column of chondrichthyans down into **trunk** and **tail vertebrae** (Figure 9.13). Both vertebrae have large centra and a neural arch through which passes the spinal cord. There are no attachment points for ribs (articulating facets) because as you recall, ribs are not present in these groups. The shape of the centrum may strike you as a little odd in that it seems to indent (be concave) rather than appear flat

like those of humans. This is also a characteristic of many fish, known as **amphicoelous** vertebrae. These spaces are filled with the intervertebral discs, the remnants of the notochord.

The tail vertebrae seem to have two neural arches, but one of those arches is known as the **hemal arch**. The hemal arch serves to protect large vessels that run down the tail to feed those large muscles that drive swimming. The hemal arches essentially prevent the tail from bending in such a way that it "kinks" those blood vessels, pinching them off and blocking blood flow. The trunk vertebrae, being less likely to bend to such a degree, do not have the hemal arches. Instead, you may see small processes coming off the ventral surface of the centrum. These processes still border the large vessels but do not completely enclose them. Where both arches are present, they also have large spines (neural spine and hemal spine) that serve as attachment points for the axial muscles mentioned previously. The pectoral and pelvic girdles (where the limbs and fins attach to the axial skeleton) do not attach to the vertebrae directly, and so there are no processes or facets for those attachments. The large sharks show one interesting adaptation to their vertebral column that distinguishes them from the other cartilaginous fishes. Despite being made of cartilage, the vertebrae show a significant amount of ossification, the deposition of mineral salts (calcium, phosphate) into the cartilage. This is different from bone, which would involve different cells, changes to collagen, infiltration of blood vessels, and a few other changes. However, the mineral salts are similar to bone in that they provide strength to the vertebrae, resisting the forces of the large axial muscles. More and more examples of ossified vertebrae in sharks are being found continuously, even in some species that fall outside of the range of "large sharks."

Osteichthyes

The vertebrae of the bony fishes are very similar to those of the cartilaginous fishes when examined broadly over the entire group. There are trunk vertebrae, and there are tail vertebrae, with the difference between the two types of vertebrae being the same hemal arches as observed previously. In some bony fishes, like the bowfin and the salmon, the dorsal spines can be quite long, much longer than those observed in the sharks and rays. Additionally, the ribs also make an appearance for the first time, and they attach to the vertebrae. The ribs will attach to the vertebrae at locations that we will refer to as **articulating facets** or **parapophyses** (hint: wherever you see -pophyses, think vertebrae). These facets are small, smooth surfaces on the vertebrae that allow ribs to move as needed without grinding against the bone of the vertebrae. The parapophyses, depending on the species, may be found on small processes jutting out from the centrum. If you encounter these vertebrae (and ribs), look for a line that separates the parapophysis from the rib. If it appears to be smooth bone throughout the entire process, you have not found the facet yet. The most striking difference found in some fishes is the retention of the notochord. Lungfishes, sturgeon, and coelacanth all retain distinctive elements of the notochord throughout their vertebral column. In these groups, the intervertebral discs are absent; rather, there is a continuous space running through all the vertebrae through which passes the notochord.

Tetrapods: The Transition from Water to Land

As we will observe in many elements of the skeleton, the transition from water to land imposes changes on the structure and function of many bones. For the vertebral column, there is one large change

that will impact how the vertebrae are structured and function. In the aquatic environment, a fish (or other vertebrate) has its body weight supported by the density of water. Many fishes can control their buoyancy in the water column and are effectively weightless. Upon moving onto the land, an individual must be able to support its own body weight. Envision a salamander and that long torso between the pairs of limbs. How are they not just dragging their bellies on the ground the entire time? The vertebrae and associated muscles must provide the support needed. Some of these changes may seem minor on their own, but as we move into the different groups, they will impact not only other structures but also how the tetrapods move about on the land. One such structure is called the **zygapophyses** (also referred to as **articular processes**). The zygapophyses are predominantly found on the neural arch, often where the neural spine begins (Figure 9.2, articular processes on the human vertebrae). Zygapophyses create points where two vertebrae can link together. Each individual vertebra will have a **prezygapophysis** (on the anterior side) and a **postzygapophysis** (posterior). The prezygapophysis from one vertebra articulates with the postzygapophysis of the vertebrae in front of it. This links the entire vertebral column together. While providing stability to the vertebral column, they limit the overall flexibility. The position of the zygapophyses has been used to better understand movement and how different vertebrate groups employ their vertebral column in locomotion.

In addition to the changes across the entire vertebral column, changes to individual vertebrae will also appear. Beyond the increased regionalization of the vertebral column we will observe, two vertebrae will further differentiate to the point that we will give them specific names (Figure 9.14).

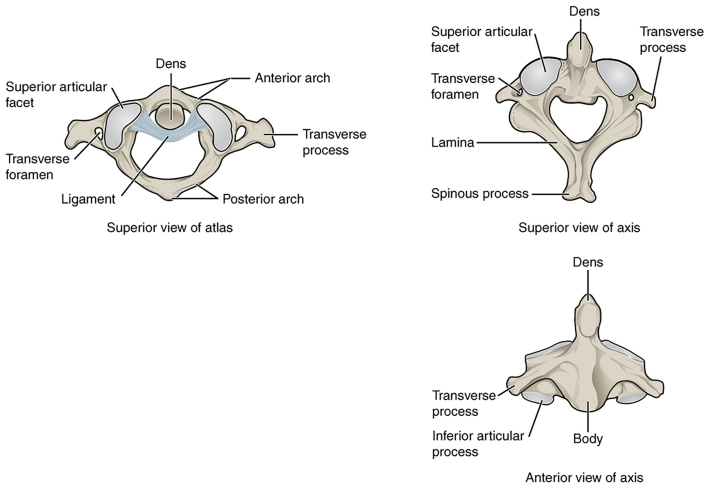

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Figure 9.14—The atlas and axis of the human vertebral column. Note that the figure in the top left shows the dens of the axis as it articulates with the atlas.

The first vertebra, the one that connects the skull to the rest of the vertebral column, is given the name **atlas**. You can remember the atlas by associating it with Atlas, the Greek Titan who held up the sky, as the atlas "holds" the skull. The atlas provides much needed mobility, allowing the skull to move up and down (dorsally and ventrally), a large part of the evolution of the neck (more on this later). In order to pick up food from the ground, movement of the head is needed independent of the rest of the body. The **axis**, the second vertebra right after the atlas, is also highly modified for the evolution of the neck. The axis has a small process known as the dens or odontoid process that articulates with the atlas. This process allows for rotation of the head. Turn your head side to side, as though you were responding "no" to a question. It is the axis that plays a large role in this rotation in addition to other neck vertebrae. This is where the axis gets its name, by

allowing for this rotation. The axis does not appear in every tetrapod group, but those are the exceptions rather than the rule.

Amphibians

With the rise of the amphibians, additional changes to the vertebrae not only distinguish this group as tetrapods but also make them distinct from other tetrapod groups. The first notable change, carried on by other tetrapods, is further regionalization of the vertebral column. In the fishes, we noted the trunk and tail vertebrae, with structural differences between the two corresponding to their function. The amphibians further differentiate regions of the vertebral column: **cervical**, **thoracic/trunk**, **sacral**, and **caudal/tail**. Cervical vertebrae are those we associate with the neck, running from the skull to the pectoral girdle (the site where the appendicular skeleton connects to the axial skeleton). The number of cervical vertebrae varies in our amphibian groups, one or two in the frogs but can be up to five or six in the salamanders. The atlas is the first cervical vertebra, also referred to as C1. There is no axis in the amphibians even if there is a second cervical vertebra in that species. Some species, like the aquatic mudpuppy salamander, may have ribs that attach to the cervical vertebrae at the articular facets. Between the pectoral girdle and pelvic girdle (where the hindlimb skeleton attaches to the axial skeleton) are the trunk or thoracic vertebrae. The naming of this group is more dependent on the source you are referring to as opposed to convention. In mammals and birds, this will always be referred to as thoracic, while in amphibians and reptiles, it may be referred to as trunk or thoracic. The trunk vertebrae are the sites of attachment for the majority of ribs across all species. Where the pelvic girdle meets the axial skeleton, a single vertebra serves as the connection point. This is known as the sacral vertebra or **sacrum** (Figure 9.4). No ribs attach to this vertebra, only the bones

associated with the pelvis (ilium, ischium, and pubis). The sacral vertebra in salamanders is not highly modified and, with the exception of the ribs, appears similar to the trunk and tail vertebrae. In the absence of articular facets from ribs, small lateral projections are found coming off the centrum. These are known as **transverse processes** and in many ways function similarly to ribs. They are attachment points for muscles and other bones. The bones of the pelvis attach to the singular sacral vertebra via these transverse processes in both salamanders and frogs.

The salamanders will also see the change in the shape of the vertebrae, at the centrum. In the fishes, we discussed amphicoelous vertebrae, where the centrum of each vertebra was concave on both sides. Many ancestral tetrapods and some salamanders retain this condition. Other salamanders are **opisthocoelous**: the centrum on the anterior end is convex, and the posterior end is concave. This "tighter" fit to the vertebrae provides stability to the column as the salamander moves about on land.

Frogs possess a unique element to their axial skeleton, shared with no other vertebrate. Caudal to the sacral vertebra, the axial skeleton consists of a single elongated element known as the **urostyle**. The urostyle possesses a neural arch and canal with the spinal cord passing through it. However, unlike the vertebrae, where many individual units function together, the urostyle is one long piece (Figure 9.15).

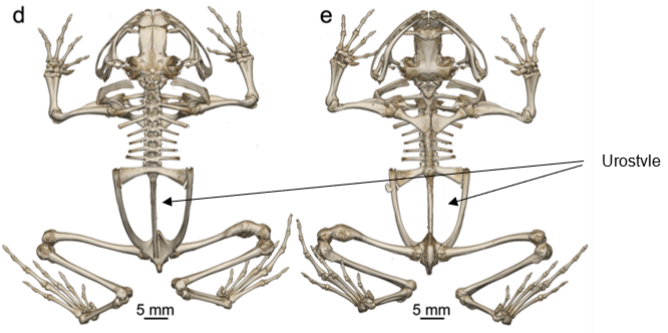

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Figure 9.15—The skeleton of a diamond frog (Rhombophryne nilevine). The left image (d) is dorsal and the right (e) is ventral.

This eliminates any mobility in that region for the frogs. The development and evolution of the urostyle remains unclear. As the tadpole metamorphs into the juvenile frog, the caudal vertebrae shrink and fuse together. This is not completely unusual, as it has also been observed in birds and primates. The frog urostyle also has what is known as a **hypochord**, a developmentally novel structure that enhances the strength and stability of the urostyle.

Nonavian Reptiles

Reptiles have more in common with the salamanders (in terms of their vertebral structure). For the purposes of simplifying our understanding, we can quickly cover the basic plan using the lizards and/or alligators as an example and then move forward to the exceptions (the snakes and turtles). The degree of regionalization is the same with cervical, trunk, sacral, and caudal regions of vertebrae. Ribs do not extend along the entire trunk region, and those without ribs have larger transverse processes than the trunk

vertebrae that do. The numbers of sacral vertebrae differ from those in the amphibians, with the reptiles having two sacral vertebrae to the one of amphibians. Alligator caudal vertebrae have hemal arches running almost the entire length of the tail, as do the majority of large lizards. Smaller lizards lack hemal arches. The degree to which caudal vertebrae differ from one another is variable in lizards, correlating with how they use their tail in the environment. Chameleons with prehensile tails, like the charismatic panther chameleon, have regionalization of the vertebrae within the tail itself, an area of active research.

The other difference between reptiles and amphibians is the shape of the centrum. Remember that in fish both the anterior and posterior centra were concave, what we referred to as amphicoelous. Some amphibians retained that shape, while some of the salamanders evolved vertebrae with the opisthocoelous condition, the anterior centrum being convex and the posterior centrum being concave. The reptiles require us to learn a third condition, **procoelous**. A procoelous vertebra has a concave anterior centrum and convex posterior centrum. Now you should be able to narrow down a lot of skeletons to their vertebrate classes using only a handful of vertebral characters!

Snakes, lacking limbs and therefore girdles (mostly), have lost the regionalization of the vertebral column we discussed in amphibians and other reptiles. Snakes have seemingly "reverted" to a more fishlike pattern, with trunk and caudal vertebrae. There are ribs down the entire length of the body until the cloaca, with articular facets in all the vertebrae along the way. The articular facets for the ribs allow for snake ribs to undertake a large diversity of movements as discussed above.

The turtle vertebral column is also quite the exception. As we discussed with turtle ribs, the development and evolution of the shell have a dramatic impact on the shape and structure of the vertebrae. Turtles have fewer vertebrae than the other reptiles, with the majority of those as cervical vertebrae. The increased number of cervical vertebrae (as we will see in birds) provides increased

flexibility. For the turtles, this means an ability to tuck the head into the shell as well as extend it quite far out (Figure 9.16). Trunk vertebrae are fused to the carapace of the shell, at the location of the neural spine (Figure 9.17).



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Figure 9.16—The extended neck of the Argentine snake-necked turtle (Hydromedusa tectifera).

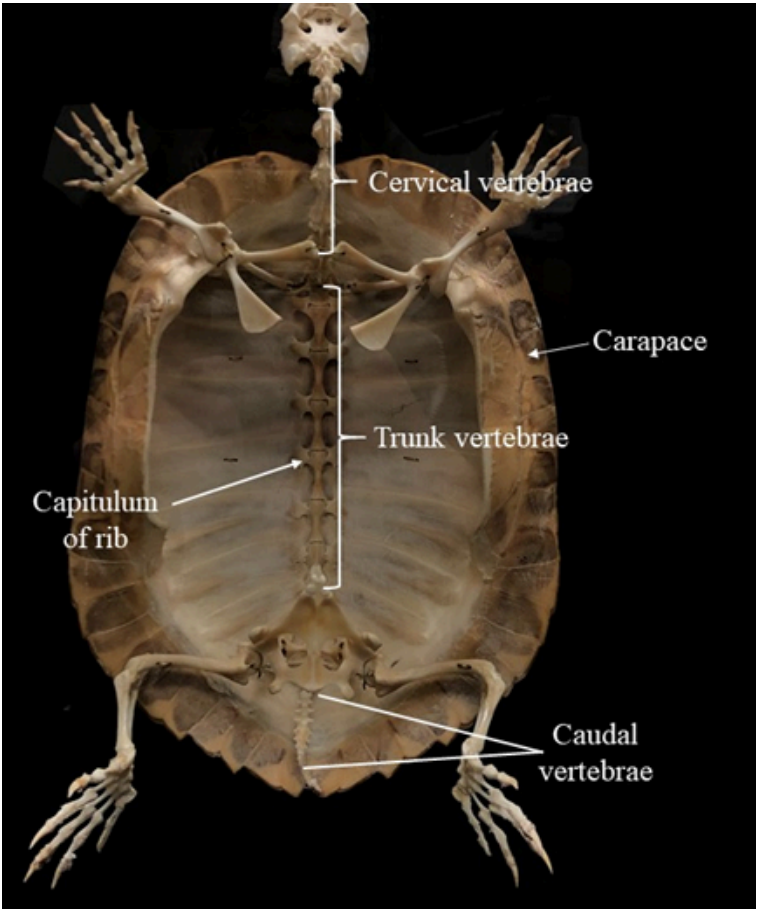

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Figure 9.17—The postcranial axial skeleton of a turtle.

Aves

The vertebral column in the birds stands in stark contrast to all the other tetrapods (Figure 9.18). Increasingly, we find greater variation in the vertebrae of the trunk and tail, allowing for a fine balance

between supporting the body and allowing for increased mobility. The cervical vertebrae lost their ribs but remained few in number and, with the axis and atlas, did not change their structure significantly. Birds have much more variation in the cervical vertebrae than the other vertebrate groups. Bird cervical vertebrae do not have ribs, with some exceptions. The number of cervical vertebrae found in birds is incredibly variable; 10–26 cervical vertebrae have been counted across bird taxa.

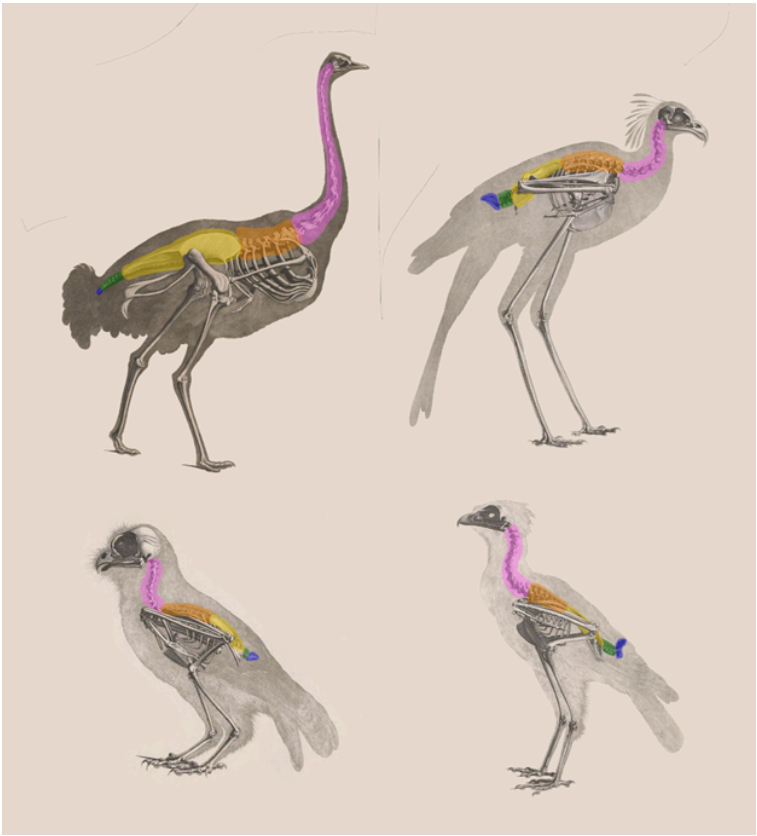

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Figure 9.18—Comparative sample of bird skeletons. The regions of the vertebral column are color coded for homologous regions. The birds from top left moving clockwise are an ostrich, a secretary bird, a tropical screech owl, and a crested serpent-eagle.

The large number of cervical vertebrae gives birds incredible flexibility in neck movements, from elaborate courtship maneuvers to the famous ability of owls to turn their heads almost 270 degrees. The incredible variation within bird cervical vertebrae has been suggested to function more like a fifth limb than a region of the vertebral column and may even have subregions that function as independent units. The centra of the cervical vertebrae are also different. We must introduce **heterocoelous**, a saddle shape to the centra that gives bird necks even more flexibility to maneuver.

For all the variation and flexibility in the cervical vertebrae, the remaining portions of the axial skeleton are fused into distinct units, strengthening the skeleton for the demands of flight. The anterior thoracic vertebrae are fused and articulate with the pectoral girdle into a separate bone called the notarium. The degree and number of fusions are dependent on the group of birds. Interestingly, pterodactyls (not birds but a separate lineage of dinosaurs that evolved flight independently) have converged on a similar arrangement for their anterior thoracic vertebrae and pectoral girdle. This is further support for the role of the fusion of these vertebrae as an adaptation for flight.

The most dramatic alteration of the bird vertebral column occurs further caudally. What appears to be the pelvis of the bird (Figure 9.19) is instead known as the **synsacrum**.

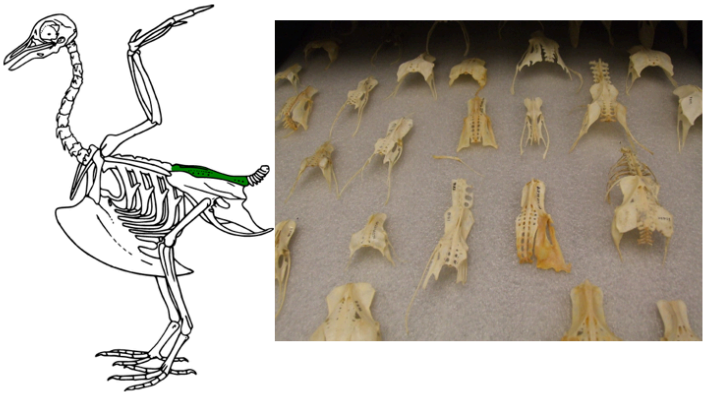

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Figure 9.19—Left: The synsacrum (green) on a bird skeleton. Right: Synsacra from multiple bird species.

The synsacrum is an incredible fusion of the lumbar vertebrae (trunk vertebrae without ribs), sacral vertebrae, a few caudal vertebrae, and the three bones of the pelvis. The synsacrum can be quite thin in some bird species, especially the lighter songbirds. The different elements of the vertebral column and the pelvis are still visible, with demarcations more closely resembling the sutures of the skull bones. Posterior to the synsacrum, there are a few caudal vertebrae, some of which may be fused together into a structure that can be called the **pygostyle**. The pygostyle helps distinguish the skeleton of modern birds from their close extinct ancestors. *Archaeopteryx*, the most famous ancestor to modern birds, does not have a pygostyle. It has been suggested that the fusion of the vertebrae to make up the pygostyle allows for more independent flight control of the tail feathers, increasing maneuverability in flight.

Mammals

The mammalian vertebral column is highly regionalized, with five recognized regions: **cervical**, **thoracic**, **lumbar**, **sacral**, and **caudal vertebrae** (Figure 9.20).

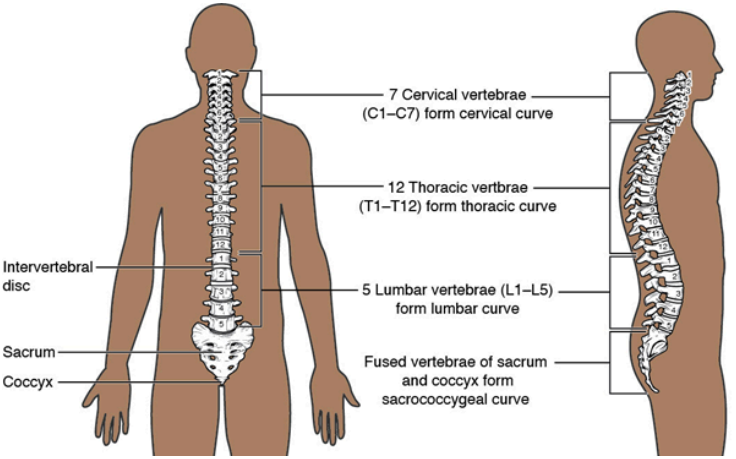

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Figure 9.20—The regions of the human vertebral column.

All vertebrae are individually identified by the region they are found in as well as their order in that region with the exception of the caudal vertebrae. For example, the eighth thoracic vertebra is identified as T8. Many of these regions are similar in structure to those observed in the lizards and alligator. With a few notable exceptions, all mammals have seven cervical vertebrae (Figure 9.21).

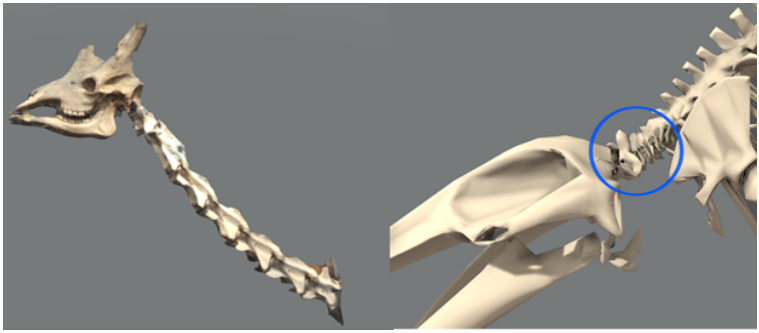

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Figure 9.21—The seven cervical vertebrae of a giraffe (left) and a blue whale (right, circled).

This includes the atlas and axis (C1 and C2, respectively). The thoracic vertebrae are the only vertebrae with ribs and the facets associated with ribs. In mammals, the ribs will attach to two different vertebrae at an articulating facet near the body of two adjacent vertebrae. The lumbar vertebrae are those between the thoracic vertebrae with their ribs and the sacral vertebrae connected to the pelvis. The lumbar vertebrae are characterized by having large transverse processes, attachment sites for many of the axial muscles. The cervical, thoracic, and lumbar vertebrae may also have very large neural spines for muscle attachment, depending entirely on the species. The sacral vertebrae are fused together. Developmentally, they begin as separate vertebrae but are fused together for much of life. There are many openings throughout the sacral vertebrae despite this fusion, allowing for the passage of major blood vessels and nerves. Caudal vertebrae are the most variable across mammals, depending (not surprisingly) on the length of the tail. Even "tailless" mammals such as the primates still retain at least a few caudal vertebrae just posterior to the sacral vertebrae.

One important distinction to make about the mammalian vertebral column involves changes across the vertebrae that correlate directly with changes to the locomotor patterns. To

illustrate, let us go back to the salamander and lizard. To emphasize the changes, we will ignore the roles of the limbs in walking on land but instead focus on how the vertebral column moves while the salamander/lizard is moving. In the majority of those animals, they move by bending their bodies side to side. Earlier, we linked this movement to the orientation of the zygapophyses. The zygapophyses were oriented so that their flat portions were parallel to the substrate. This allows the individual vertebrae to glide by one another in the side-to-side motion. However, you can imagine that when any dorsal-ventral motion is required, the zygapophyses will prevent any motion beyond the most minimal. However, in mammals, there is a notable shift in the position of the zygapophyses, especially pronounced in the lumbar vertebrae. The zygapophyses of these vertebrae are oriented laterally, perpendicular to the substrate. Now we have more freedom to move dorsoventrally, at the cost of lateral bending.

This is exactly what we observe. A horse or cheetah at full gallop will illustrate the principle perfectly; note the bending in the lumbar region (Figure 9.22). The bending of the vertebral column dorsi-ventrally helps contribute to the increased stride length in these very fast mammals. In addition, the vertebrae are loosely connected to each other, providing even more flexibility in their movement (Video: BBC—The Cheetah's Spine).

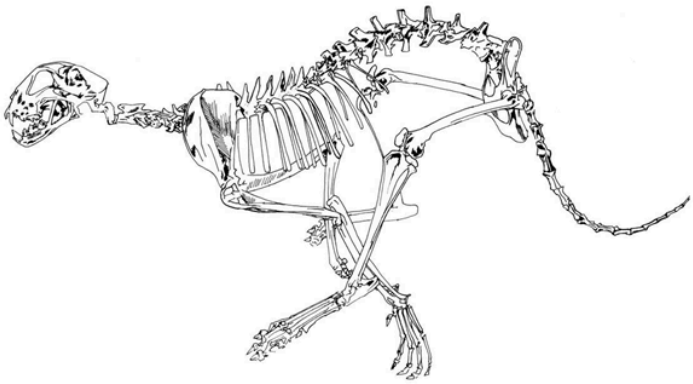

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Figure 9.22—An artistic rendering of a cheetah at full gallop, visualizing the dorsoventral bending of the vertebral column.

Humans

As is the case in other mammals, humans have five regions of their vertebral column, with each region having a specific number of vertebrae: 7 cervical, 12 thoracic, 5 lumbar, 5 sacral (fused), and 3–5 caudal vertebrae. The 3–5 caudal vertebrae are highly reduced and fused together, forming a bone known as the **coccyx**. The vertebral column is curved dorsi-ventrally in humans, with each region curving in opposing directions. The absence of curves or increased curvature in each region is the mark of a pathological condition, named for the region and what is happening to that curve. The vertebral column should not deviate laterally at all; any deviation or curving laterally is identified by the term *scoliosis*.

Of medical importance to humans is the function and dysfunction of the intervertebral discs. Earlier, we introduced the intervertebral discs as remnants of the notochord, a tough cartilage disc filled with a fluid (**nucleus pulposus**). Admittedly, after that brief introduction, we essentially ignored the discs until now. The

discs act as cushions and spacers between individual vertebrae. This is easiest to demonstrate in humans. With our bipedal posture, our body weight is carried through the legs, hips, and the vertebral column itself. When walking or jumping, the physical forces in the movement press vertebrae together. The discs absorb some of that impact and prevent the vertebrae from coming into contact with one another. Between the vertebrae, pairs of spinal nerves (see Chapter 19 for more) enter and leave the spinal cord. Blood vessels also enter and leave in these spaces. If the vertebrae start knocking into one another, there is a chance that the nerves and blood vessels may become damaged. In addition, the discs themselves are not invincible. They may degrade with time or become damaged by considerable impacts or other forces. There are many colloquial terms that describe disc injury, including **slipped disc** (technically incorrect), **bulging disc**, and **herniated disc** (Figure 9.23).

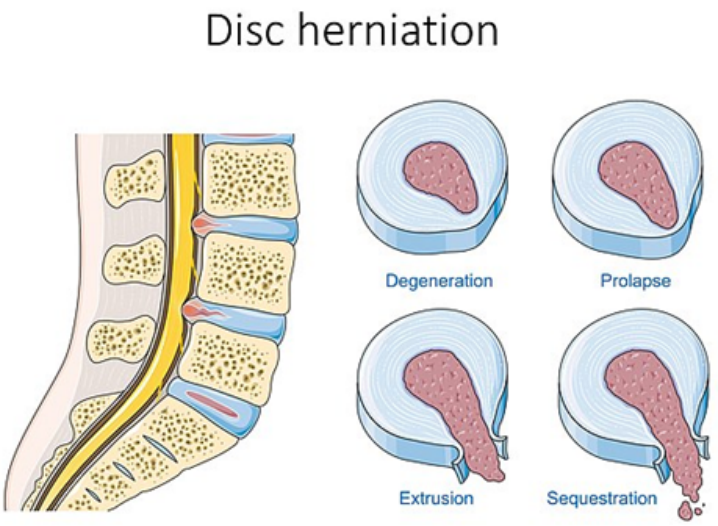

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Figure 9.23—Levels of disc herniation in a human lumbar vertebra.

"Slipped disc" is incorrect because the discs grow as part of the vertebral column, and to slip a disc would necessitate tearing at least part of it. All disc damage can be grouped into the category of herniated disc, with differences in severity. A bulging disc tells us that the outer covering is intact, but the nucleus pulposus is pushing outward as the result of increased pressure. A true herniated disc would have the nucleus pulposus leaking out. In the case of a sequestered disc, pieces of the nucleus pulposus have broken off from the rest.

Application Questions

1. Why do ribs seem to vanish and shrink in many vertebrate groups?
2. How does the orientation of the zygapophyses relate to the locomotion of reptiles and mammals?
3. Do changes in the curvature of the human vertebral column at different points result in different medical conditions?

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10. Appendicular Skeleton

VANESSA K HILLIARD

Focus Questions—to Guide Your Reading of This Chapter

1. Where do the paired appendages come from, starting with fins?
2. What are the component structures of fins?
3. Where the heck did limbs come from?
4. How have limbs diversified across vertebrate taxa?

10.1 Introduction

At this point, you've been introduced to the skeletal tissues (Chapter 7), the skull (Chapter 8), and the axial skeleton (Chapter 9). In this chapter, we'll wrap up the skeletal system by taking a look at the appendicular skeleton, which includes fins and limbs as well as their supporting structures ("girdles"; Figure 10.1). The paired appendages and their girdles are the structures that allow us (and other finned/limbed vertebrates) to propel ourselves and to maneuver in our environments.

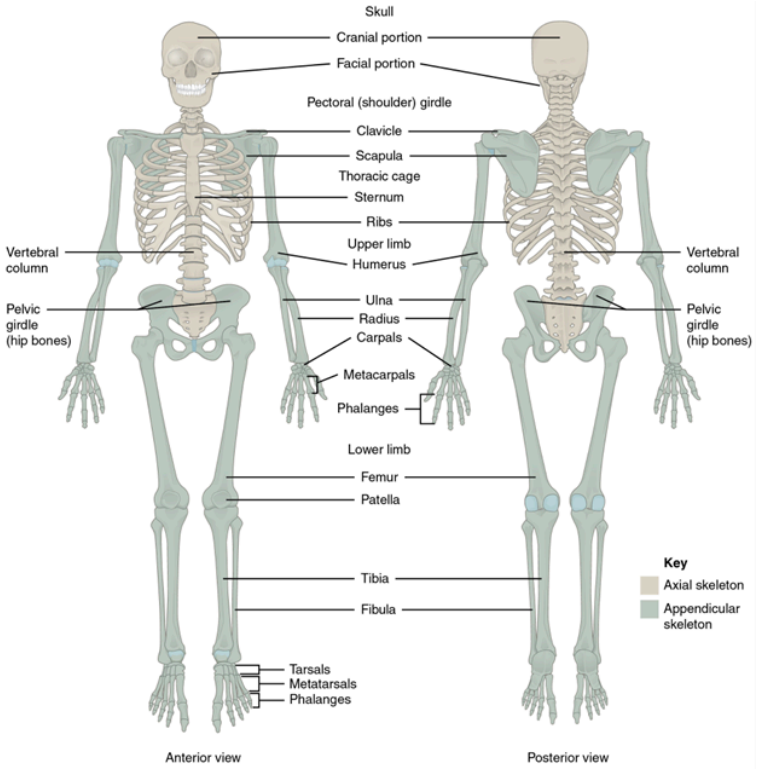

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Figure 10.1—The axial and appendicular skeletons of a human. The limbs and girdles are the structures shown in gray.

We'll begin with a look at where the appendages came from. Then we'll introduce the component structures of the fins, how we get from fins to limbs, and finally limb diversity in vertebrate animals.

10.2 Development

As you know from the vertebrate diversity chapter (Chapter 3), hagfishes and lampreys have no paired appendages. However, later on the evolutionary timeline, we see the emergence of paired appendages in the gnathostomes. But where did these structures come from, and why did they evolve in the first place? Well, if we observe living fishes that lack paired fins, we find that they are pretty lousy swimmers, and by extension, it is likely that those early fishes prior to the evolution of paired fins would have also been pretty unsteady. So fins initially functioned as a stabilizing mechanism during swimming, and this eventually led to the evolution of paired fins and their associated girdles.

Gnathostomes have two pairs of paired fins, pectoral and pelvic, and both are derived from a chain of basal elements. However, there are two long-standing, competing hypotheses that describe the evolution of these fins. These hypotheses are the **gill-arch hypothesis** (distinct from the gill-arch hypothesis introduced in the jaws section of Chapter 8), which posits that the fin elements are derived from gill-arch structures, and the **fin-fold hypothesis**, which posits that the fins are derived from an interruption of two continuous fin folds that ran the length of the body (Figure 10.2).

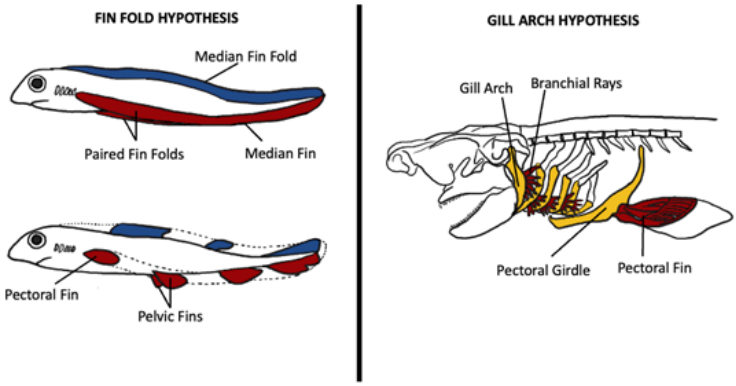

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Figure 10.2—The fin-fold hypothesis (left) suggests the pectoral and pelvic fins evolved from the interruption of paired fin folds that ran the length of the vertebrate body. The gill-arch hypothesis (right) suggests that fin structures are derived from the gill-arch skeleton.

Of these two hypotheses, the fin-fold hypothesis has traditionally had broader support. However, recent work now suggests that both may in fact describe some aspect of fin evolution. Given the conflicting data on the origin of fins from developmental, paleontological, and comparative sources, it is possible that the pectoral appendage in particular may indeed have a dual origin, with the evolution of the pectoral girdle explained by the gill-arch hypothesis and the pectoral appendage explained through the fin-fold hypothesis. Given the far posterior position of the pelvic fin and girdle, there is little dispute that the pelvic structures are likely explained through the fin-fold hypothesis.

10.3 General Structure and Function

If we were to look at the structure of the fin itself, we'd see that distally, the fins are supported by skeletal rays. In chondrichthyans,

these rays are made of cartilage and called **ceratotrichia**. However, in osteichthyans, the distal fin rays are made of dermal bone and called **lepidotrichia**. The fin rays—both ceratotrichia and lepidotrichia—are supported proximally by endochondral condensations called **pterygiophores**. Typically, there's a distal group and a proximal group of pterygiophores. The distal elements, close to the lepidotrichia, are called the **radial pterygiophores**, whereas the proximal elements that support the radials are called the **basal pterygiophores** (Figure 10.3).

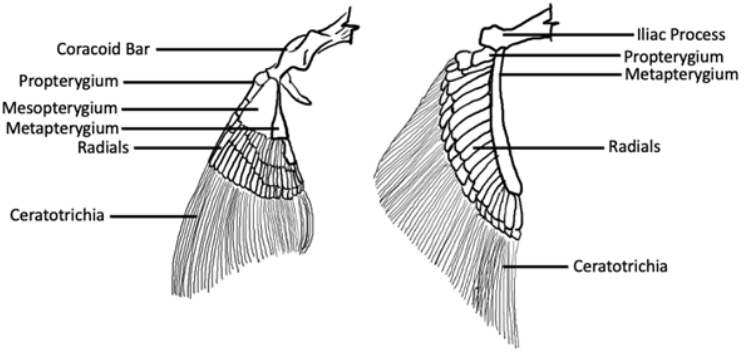

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Figure 10.3—Chondrichthyan pectoral (left) and pelvic fins (right), showing positional relationships of ceratotrichia, radials, and basals. Adapted from Liem et al., 2001.

Ancestrally, in both chondrichthyans and actinopterygians, there were three basal elements: **propterygium**, **mesopterygium**, and **metapterygium** (Figure 10.3). As a result, the fins of these lineages are called **tribasal** (think: *tri* means "three," so "three basals"), and it is thought that as fins became larger through the course of evolution, pterygiophores from each side extended proximally and medially and ultimately coalesced to contribute to the formation of the girdles.

The pelvic girdle is made entirely of endochondral bone,

and ancestrally it does not attach to the vertebral column (Figure 10.4). The pelvic girdle is typically a single element, but it can have three distinct portions or spokes:

1. Dorsally: **iliac portion**
2. Anteroventrally: **pubic portion**
3. Posterioventrally: **ischiac portion**

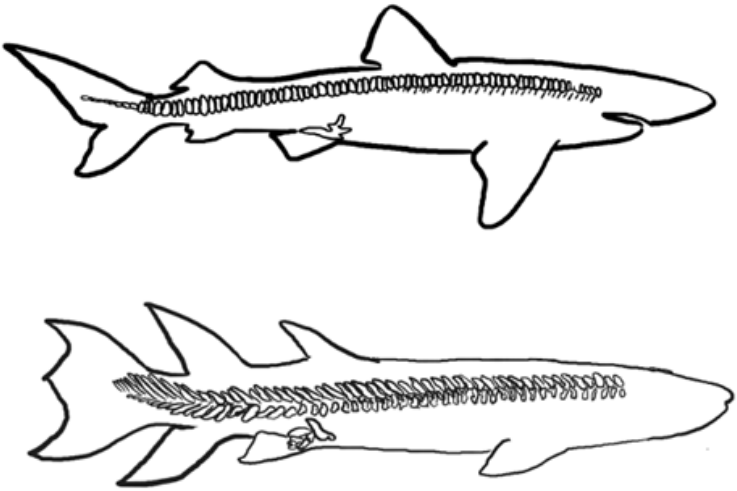

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Figure 10.4—Pelvic girdle in chondrichthyan (top) and osteichthyan fishes (bottom). Notice the position of the pelvic girdle relative to the vertebral column (detached) in both taxa.

The pectoral girdle also incorporates several overlying dermal bones from the posterior cranial region (except in chondrichthyans, due to the loss of dermal bone in this group). These bones function to attach the pectoral appendages to the back of the head, meaning that, functionally, the shoulders and the head are anchored to one another. The endochondral portion

of the pectoral girdle is sometimes a single element called the **scapulocoracoid**, but it may also be formed from discrete scapular and coracoid condensations in some taxa. The dermal portion of the pectoral girdle has several components (Figure 10.5):

1. The aforementioned scapulocoracoid articulates with the largest of these, called the **cleithrum**.
2. Ventrally, there's a small **clavicle**, and in the sarcopterygians, there is sometimes a midline **interclavicle**; these structures unite the girdles from left and right sides.
3. Dorsally, there are **supracleithra** and **postcleithra**; these articulate with the posttemporal bone that connects to the skull.

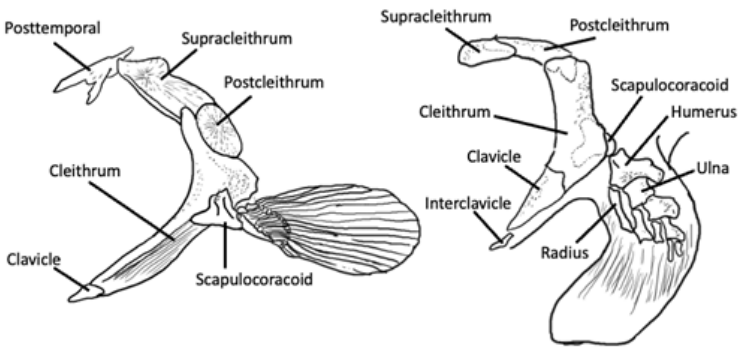

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Figure 10.5—Pectoral girdle of basal osteichthyan (Amia, left) and sarcopterygian (right). Adapted from Liem et al., 2001.

Another major evolutionary transition occurred in the sarcopterygian fin, and that was the shift from a tribasic fin to a monobasic fin, with one basal pterygiophore (*mono*, meaning "one"). Recall that Sarcopterygii includes tetrapods and the other lobe-finned fishes. In the lineage leading to tetrapods, the fin develops along a distinct axis of elements that runs the posterior length

of the fin. As a result, the radial pterygiophores only emerge on the anterior side of this axis. This particular fin configuration is called a **crossopterygian** fin, and if we look at the elements of the crossopterygian fin, we can see clear homologies with the elements of the tetrapod limb (Figure 10.6).

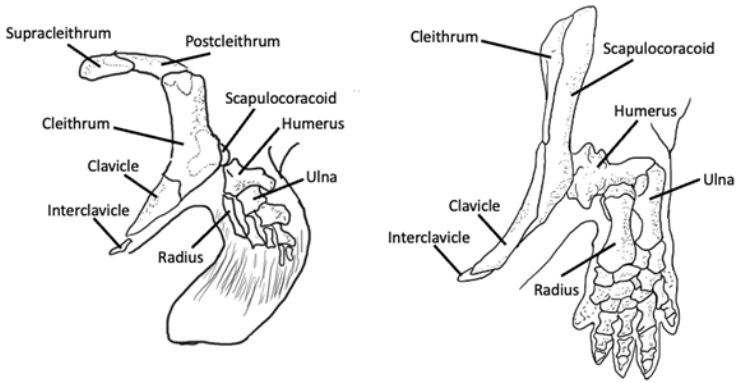

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Figure 10.6—Sarcopterygian appendages: crossopterygian fin (left) and tetrapod limb (right). Notice the single basal pterygiophore and homologous structures in both appendages. Adapted from Liem et al., 2001.

Before delving into the details of the tetrapod limb structure, you should be familiar with the terminology for the sections of the typical tetrapod limb. The standard tetrapod limb has three distinct sections. From proximal to distal, they are as follows:

1. **Stylopodium**: This is the **brachium** (upper arm) of the forelimb and the thigh of the hindlimb.
2. **Zeugopodium**: This is the middle section of the limb and includes the **antebrachium** (radius and ulna) of the forelimb and the **crus** (or "shin"; tibia and fibula) of the hindlimb.
3. **Autopodium**: This is the **manus** (hand) and the **pes** (foot), which are made up of many small bones. These include

a.**carpals** and **tarsals**, the small bones that compose the wrist and ankle, respectively;
b.**metacarpals** and **metatarsals**, which give structure to the palm of the hand and the sole of the foot, respectively; and
c.**phalanges**, which make up the digits (fingers and toes).

In Figure 10.7, the fossil sarcopterygian fish is on the left, and the basal tetrapod is on the right. If you follow along the posterior margin, the axis of pterygiophores is shown with the red line, and you'll see the radials emerging anteriorly. You can also see that in both appendages, there's a single proximal element, followed by two distal elements. However, if we look at the distal area, there's a greater number of distal elements in tetrapods compared to the fossil sarcopterygian. An important point to recognize about the distal elements of the sarcopterygian is that they cannot just be modified fin rays, because the lepidotrichia that make up the fin rays are formed from dermal bone (meaning that they form through the process of intramembranous ossification). However, the digits of tetrapods are endochondral in origin, meaning they are formed through a different ossification process, thus indicating a distinct origin from the lepidotrichia of sarcopterygian fins.

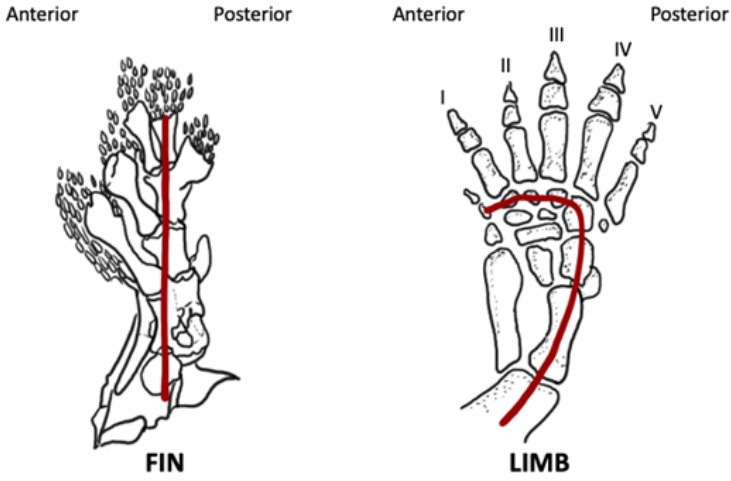

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Figure 10.7—Fossil sarcopterygian fin (left) and tetrapod limb (right). The developmental axis of the fin/limb is indicated by the red line running the length of the posterior side. Adapted from Liem et al., 2001.

As we begin to examine the limbs of tetrapods, you'll notice that the structure of the forelimbs and hindlimbs is very similar, and we typically describe limb structure of both appendages using a series of proximal to distal sections (i.e., stylopod, zeugopod, and autopod). One additional item to be aware of regarding the tetrapod limb, however, is the numbering schemes used to define the phalanges of the autopodium. In fact, there are two numbering schemes you should be aware of:

1. The first number scheme assigns each digit a number, starting from what was ancestrally anterior and moving to posterior. So in humans, the thumb and big toe (**hallux**) are each termed "Digit 1," and the pinky finger and little toe are defined as "Digit 5," with the intermediate fingers/toes numbered in order (i.e., index finger = Digit 2, middle finger = Digit 3, ring finger = Digit

4).

2. The second numbering scheme is what is called the **phalangeal formula**. This formula lists the number of phalanges in each digit in order from Digit 1 to Digit 5; ancestrally, the phalangeal formula was 2-3-4-5-3, meaning that Digit 1 was composed of two phalangeal bones, Digit 2 had three phalangeal bones, Digit 3 had four, and so on.

Recall that the axis of element formation runs along the posterior side of the tetrapod limb (Figure 10.7), and as a result, it was originally thought that this axis extended through the digit that was the longest (ancestrally, Digit 4). This would have meant that the anterior digits were simply extensions of the radial pterygiophores, and only Digit 5 was new, given its posterior position to Digit 4. However, more recent developmental studies have actually shown that the sequence of digit formation turns along the distal edge of the hand or foot (Figure 10.6, notice the curve in the axis); thus all the digits are actually on the opposite side of the axis from the radials and therefore must be novel structures. This pattern also gives us a mechanism for understanding digit reduction: If development along the axis was truncated or terminated early, fewer digits would form!

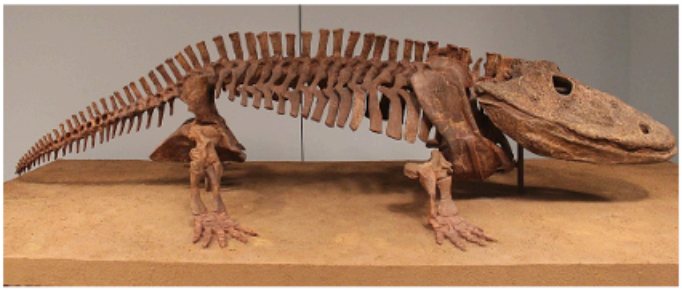
10.4 Evolution

Early Tetrapods

Early tetrapods tended to have big, blocky bones with large processes for muscle attachments. Nonetheless, the earliest limbs were relatively small given the size of the organism (Figure 10.8).



FIG. 28.—*Eogyrinus Attheyi*. Reconstruction of the skeleton about $\frac{1}{12}$ natural size.



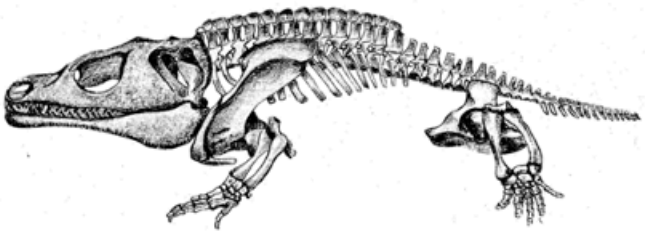


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Figure 10.8—Examples of early tetrapods (Eogyrinus, top; Eryops, center; Cacops, bottom). Notice the relative size of the limbs compared to the body and the blocky bone structure of the appendages, particularly in Eryops and Cacops.

The blockier bones and large sites for muscle attachment help support the body weight of the animal. In the early tetrapods' pectoral girdle, there was a single scapulocoracoid ossification, with the scapula positioned dorsally and coracoid ventrally. The pectoral girdle also had an articular facet for the humeral head, called the **glenoid** (Figure 10.9). However, these animals had reduced dermal

elements in the pectoral girdle compared to fishes. In fact, in tetrapods, only the cleithrum, clavicle, and interclavicle remain.

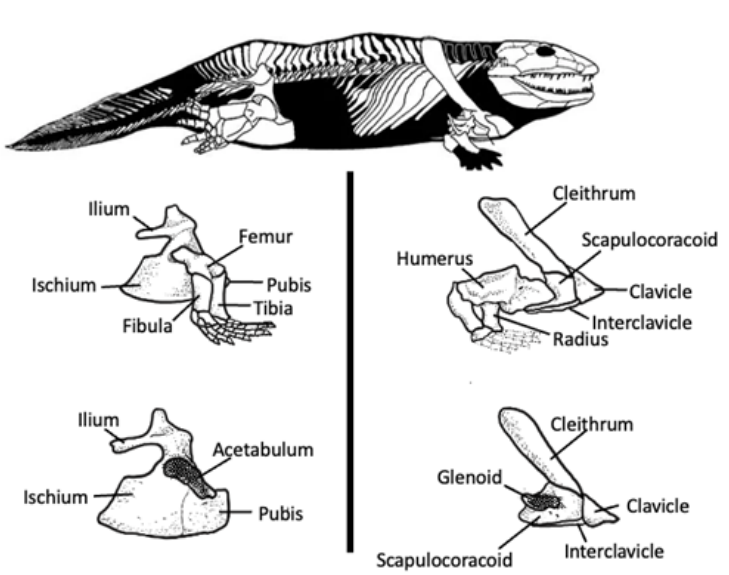

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Figure 10.9—Pectoral and pelvic girdles in early tetrapods (Ichthyostega).

What's more, by this point in the evolutionary timeline, the pectoral girdle was detached from the head (a change that occurred in Devonian tetrapodomorphs, approximately 400 MYA), thereby creating a distinct neck and resulting in a pectoral girdle attached to the body axis only by the presence of a muscular sling (Figure 10.10).

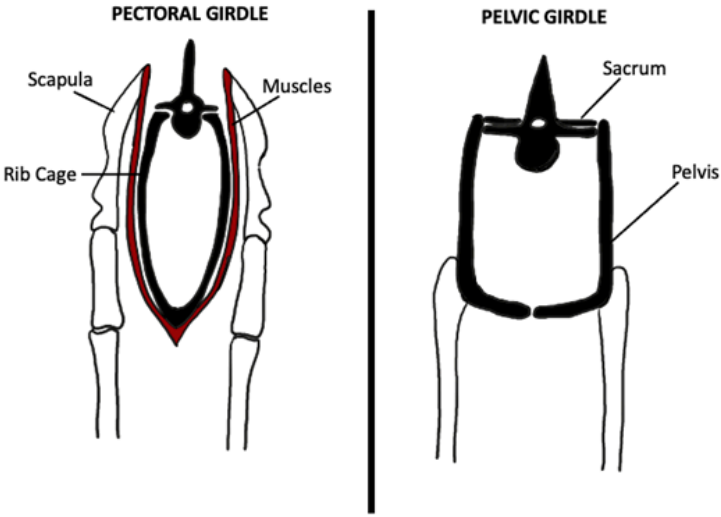

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Figure 10.10—Attachment of the pectoral (left) and pelvic (right) girdles to the tetrapod trunk. Notice that the pectoral girdle is attached only by the presence of a muscular sling. Adapted from Liem et al., 2001.

In contrast to the pectoral girdle, the regions of the pelvic girdle were produced from distinct bones (although these fuse during development to form a fairly unified pelvic structure). The three bones that contribute to the formation of the pelvis are the **ilium** (dorsally), **pubis** (anteriorly), and **ischium** (posteriorly; Figure 10.11). All three of these bones contribute to the formation of the **acetabulum**, better known as the hip socket. Furthermore, the left and right pelves fuse along a midline juncture called the **pelvic** or **pubic symphysis**, thus uniting the left and right pelves (Figure 10.12).

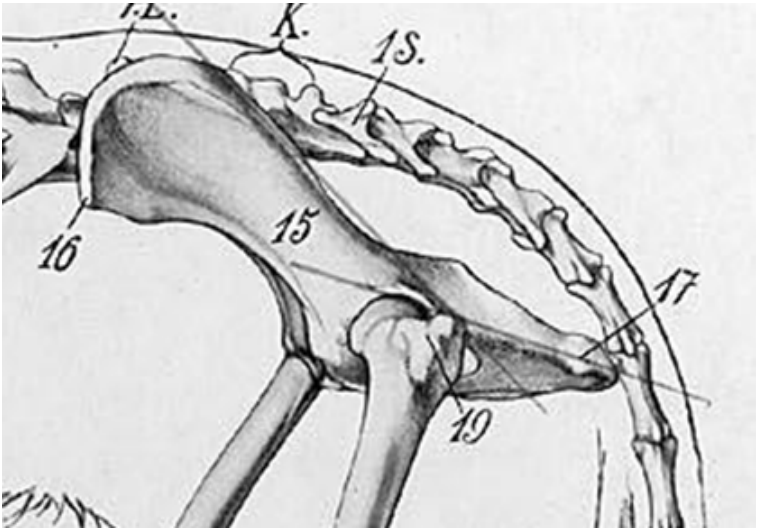

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Figure 10.11—The pelvis of a dog: 15 is the ilium, 17 the ischium, and the region bordered by 19 is the pubis.

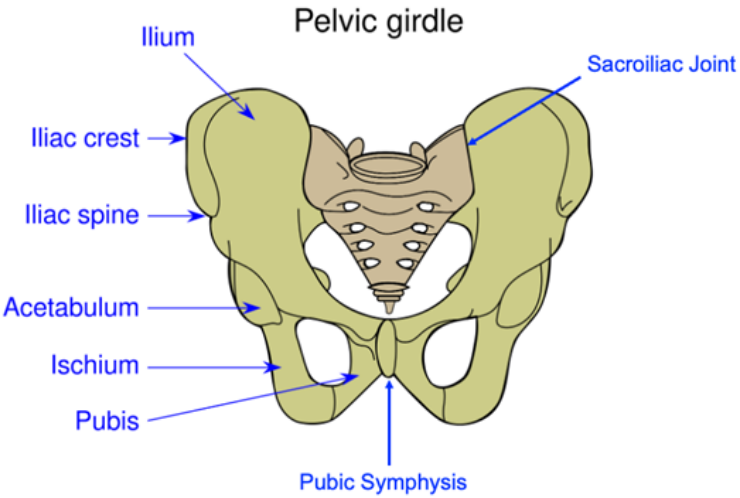

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Figure 10.12—Anatomy of the human pelvis. Notice the pubic symphysis, uniting the left and right pelves.

Remember, as we saw in Chapter 3, based on fossil evidence, these girdle and limb features started to appear in animals with big tail fins. What this tells us is that weight-bearing limbs started to evolve in primarily aquatic animals. So although these structures allowed for locomotion on land, it is likely that they originally helped animals muck around in vegetation-choked shallow-water habitats.

Derived Tetrapods

The blocky structure of the limbs in the first tetrapods kept them low to the ground. A number of later modifications appear and result in limb elongation, elevation of the body off the ground, and modifications of lever mechanics (through the elaborations of

processes on the bones onto which muscles attach). One such early modification occurs in the proximal ulna. Many animals show extensions of the ulna posterior to the elbow joint; this posterior process is called the **olecranon** (Figure 10.13). The lengthening of the olecranon increases the mechanical advantage of the muscles that extend the elbow, which then amplifies the force the limb produces. Recall from Chapter 5 that $F_{in} \times L_{in} = F_{out} \times L_{out}$, and if you increase L_{in} and keep F_{in} the same, you increase F_{out}. Increasing the length of the ulna posterior to the joint in this case increases L_{in} and allows a substantial increase in the force coming out of the system.

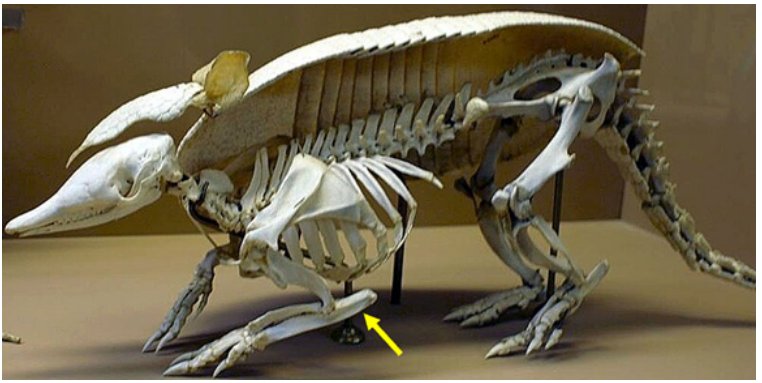

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Figure 10.13—Armadillo forelimb showing huge expansion of the olecranon posterior to the elbow joint (olecranon indicated by yellow arrow).

Amniotes

Amniotes show consolidation of several tarsals into a single structure called the **astragalus** in the ankle (Figure 10.14). The astragalus (also known as the **talus**) serves as a key articulation point between the tibia and fibula of the lower leg and the bones of the foot. This novel structure provides stability to the ankle joint while also allowing a wide range of motions.



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Figure 10.14—Condensation of the tarsals form the astragalus bone (a.k.a. talus; indicated in red) in the tetrapod ankle, shown here in a human.

In addition, amniotes and other derived tetrapods show several changes to the structures of both the pectoral and pelvic girdles:

1. In the pectoral girdle, the tendency is to reduce dermal elements. All derived tetrapods actually lose the cleithrum, and some also lose the clavicle and interclavicle. Furthermore, the scapula and coracoid condense separately in derived tetrapods and amniotes. These changes to the pectoral girdle facilitate increased range of motion in the forelimb.

2. In the pelvic girdle, a foramen develops between the pubis and ischium in many taxa; this foramen expands the margins of the bones, thereby increasing available surface area for muscle attachment. Several lineages also show changes to the pelvic girdle that are related to specific locomotor methods. For example, frogs have a huge anterior projection on the ilium, which functions to increase the surface area for attachment of the muscles that power jumping.

There are also several lineages that show distinctive changes to the structures of the autopodia; these changes are typically related to locomotion, especially swimming and flying. For example, ichthyosaurs form paddles from their forelimbs by radically increasing both the number of digits (called **polydactyly**) and the number of phalanges present in each digit (called **polyphalangy**; Figure 10.15).

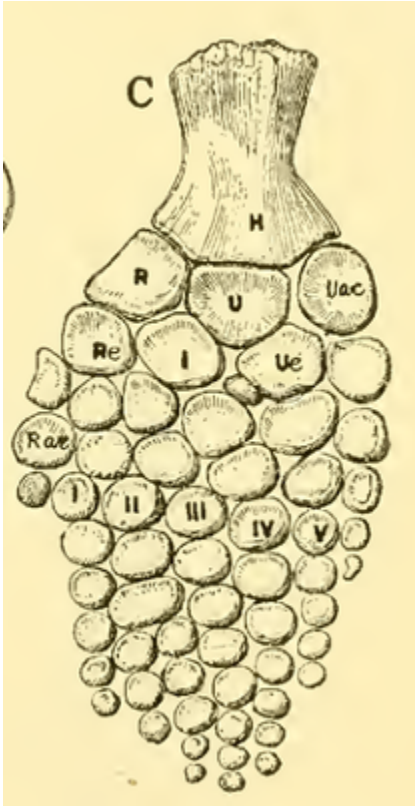

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Figure 10.15—Ichthyosaur paddle. Notice the increase in both the number of digits and the number of phalanges per digit in the autopodium.

Flight also involves a number of substantial modifications to the appendicular skeleton. Three different vertebrate lineages independently evolved flight, and they each evolved a different way to construct a wing (Figure 10.16):

1. Bats elongate all the digits of the hand.
2. Pterosaurs only elongate Digit 4 and shorten proximal bones of

the arm.

3. Birds actually lose two digits on the hand and fuse the carpals and metacarpals into a single structure called the **carpometacarpus** (Figure 10.17).

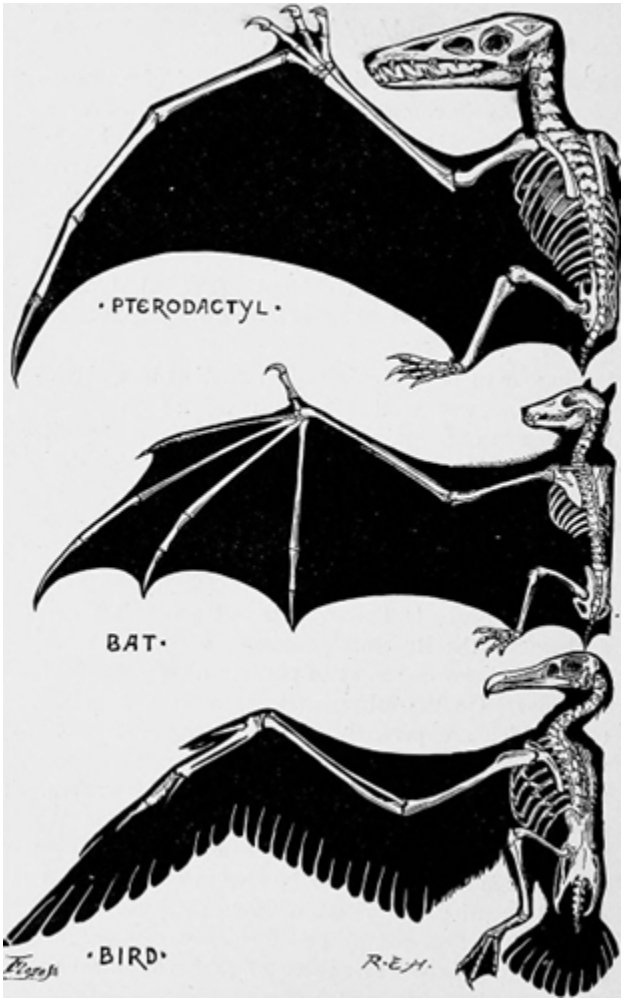

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Figure 10.16—Three different wing configurations in the taxa that evolved powered flight: pterosaurs (top), bats (center), and birds (bottom).

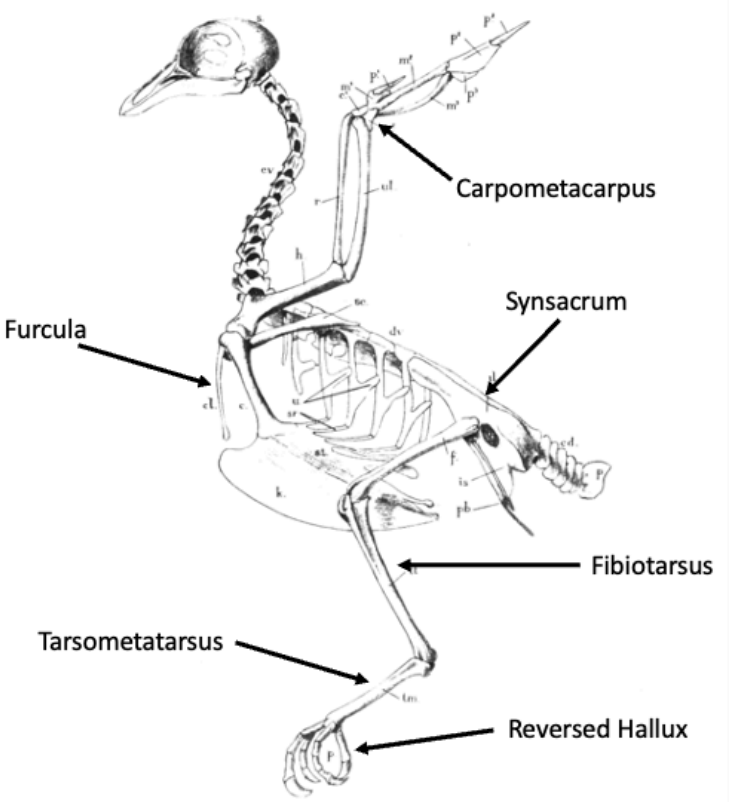

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Figure 10.17—Bird skeletal anatomy. Notice the modifications to both the forelimb and hindlimb structures in this group.

Aves

In addition to the changes to the hand, another forelimb modification unique to birds is the fusion of the left and right clavicles to form a structure called the **furcula**. The downstroke of the wings pulls the clavicular structures apart, but the furcula acts like a spring and recoils when the muscular tension from the wing downstroke is released, thus helping pull the wings back up for the

upstroke and resulting in an energy savings (because less muscular action is needed for the upstroke portion of the wingbeat).

Even though flight is a forelimb-powered activity, appendicular modifications in birds are not just restricted to the forelimb. Indeed, bird hindlimbs also show a number of modifications (Figure 10.17):

1. One of these is the formation of the **synsacrum** in the pelvic region; this structure is partly formed by a posterior rotation of the pubis that helps consolidate the pelvic girdle.
2. Bird hindlimbs are oriented more directly under the body (known as a **parasagittal limb posture**), a posture shared with their cursorial theropod relatives, rather than sprawling out to the side as observed in other reptiles (such as lizards or crocodylians). Bird legs also tend to have a "crouched" position, which functions to reposition the body's center of mass to counterbalance the mass of the large flight muscles.
3. The **fibula** is substantially reduced in birds.
4. The tarsals consolidate onto both the proximal hindlimb element (forming the **fibiotarsus**) and distal element (forming the **tarsometatarsus**).
5. Finally, Digit 1 ("hallux") of the pes of birds rotates posteriorly, thus providing an opposable digit that facilitates grasping and perching.

Mammals

Similar to birds, one major trend in appendicular modifications observed in mammals is that the limbs are brought under the body to produce a more upright posture. And as expected, a number of changes in the limbs and girdles are related to this change in posture. First, there is a further reduction of dermal elements in

the pectoral girdle of mammals, so much so that only the clavicle remains in the therian mammals (Figure 10.18).

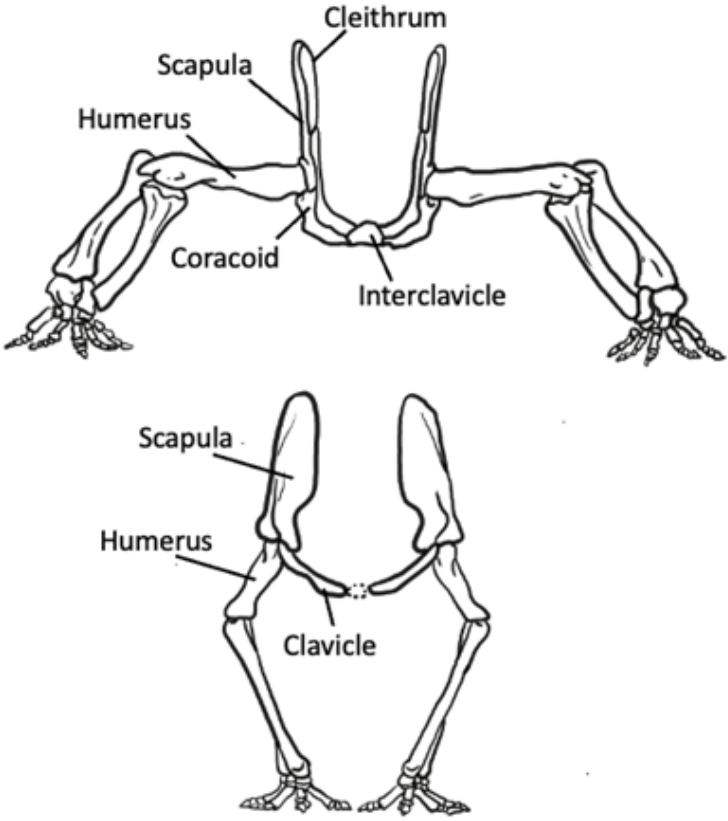

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Figure 10.18—Pectoral girdle of ancestral synapsid (top) versus mammal (bottom). Notice the change in limb posture and accompanying reduction of the ventral girdle elements. Adapted from Liem et al., 2001.

In fact, many mammals reduce or lose the clavicle as well, such that the pectoral girdle is only connected to the axial skeleton via muscles and tendons. Because this upright posture requires

far less ventral musculature, the necessity of skeletal support and structure in that region is less; therefore, it is not surprising that mammals exhibit a reduction in the ventral part of the limb girdle. Ancestrally, nonmammalian synapsids had two coracoid bones in the ventral girdle, but in therian mammals, the anterior coracoid is lost and the posterior coracoid is reduced to a tiny process on the scapula, anterior to the glenoid. As a result of this reduction in the pectoral girdle, the glenoid now faces ventrally rather than laterally, thus directing the humerus downward instead of sideways (Figure 10.18). Furthermore, along with this reduction of the ventral girdle, the dorsal muscles of the shoulder are elaborated. This muscle elaboration is accompanied by a dorsal expansion of the scapula, such that the old cranial border of the scapula actually becomes elevated and forms the **scapular spine** with a supraspinous expansion dorsocranial to it; these changes provide additional surface area for muscle attachment (Figure 10.19). These changes to the pectoral skeleton correspond with changes in the musculature, which exhibits a similar ventral reduction and dorsal elaboration, reflecting the need for increased mechanical support of the shoulder joint as the forelimb swings through longer arcs during locomotion.

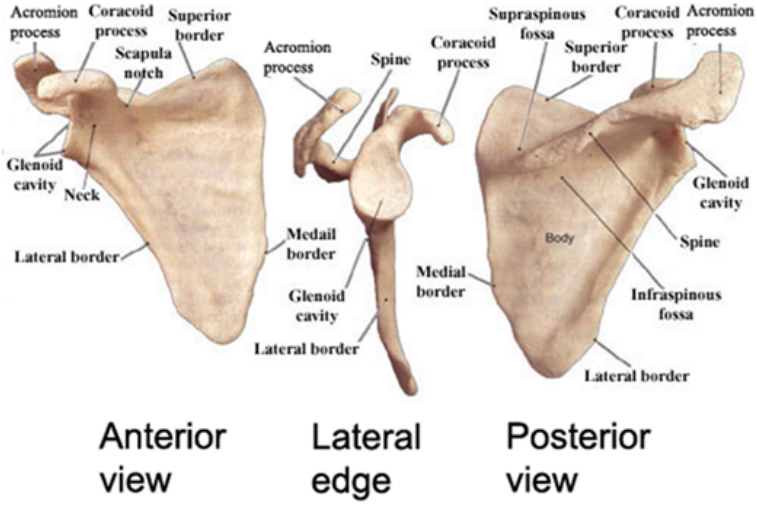

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Figure 10.19—Human scapula anatomy. Notice the small projection anterior to the glenoid (coracoid process) as well as the dorsal expansion of the scapula to form the scapular spine and supraspinous fossa.

There are also a number of changes to the pelvic girdle. The pelves become elaborated dorsally and reduced ventrally in mammals, with a particularly noticeable expansion of the central **obturator foramen**, which was just a small hole that allowed passage for a nerve in the ancestral condition. One major difference in the modifications to the pectoral versus pelvic girdle, though, is the orientation of the joint socket. Recall that in the pectoral girdle, the glenoid becomes oriented downward as ventral elements of the girdle are reduced; however, the acetabulum of the pelvic girdle remains laterally oriented. This means that in order for the femur to be directed downward, the femur's articular head now has to be reoriented to face laterally so it can articulate with the hip (Figure 10.20).

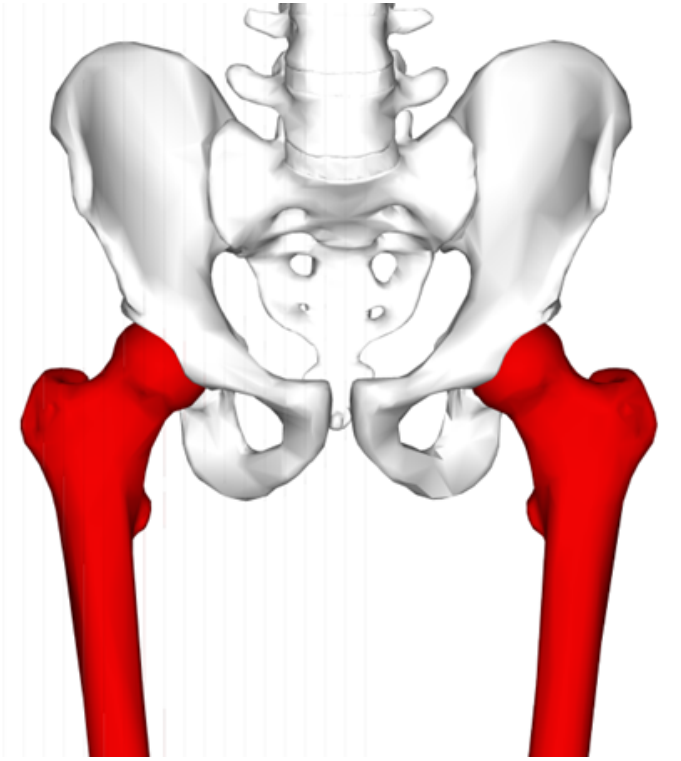

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Figure 10.20—Human femur (red) and pelvis. Notice the lateral orientation of the femoral head to allow articulation with the acetabulum.

In the distal limbs of mammals, the radius and tibia become the primary weight-bearing bones, but the elbow points backward in mammals. This means that for the digits of the hand to point forward, the radius has to rotate about the distal end of the ulna. This rotation is called **pronation**, and you can actually do this with your own arm! The opposite motion is called **supination**. If you grip your forearm and rotate your wrist such that the palm is oriented downward, you can feel your radius pivot and cross over your ulna (pronation). Rotate your palm the other direction so that it faces up

(supination), and you can feel your radius cross back over the ulna so that the two bones now lie parallel to one another (Figure 10.21).

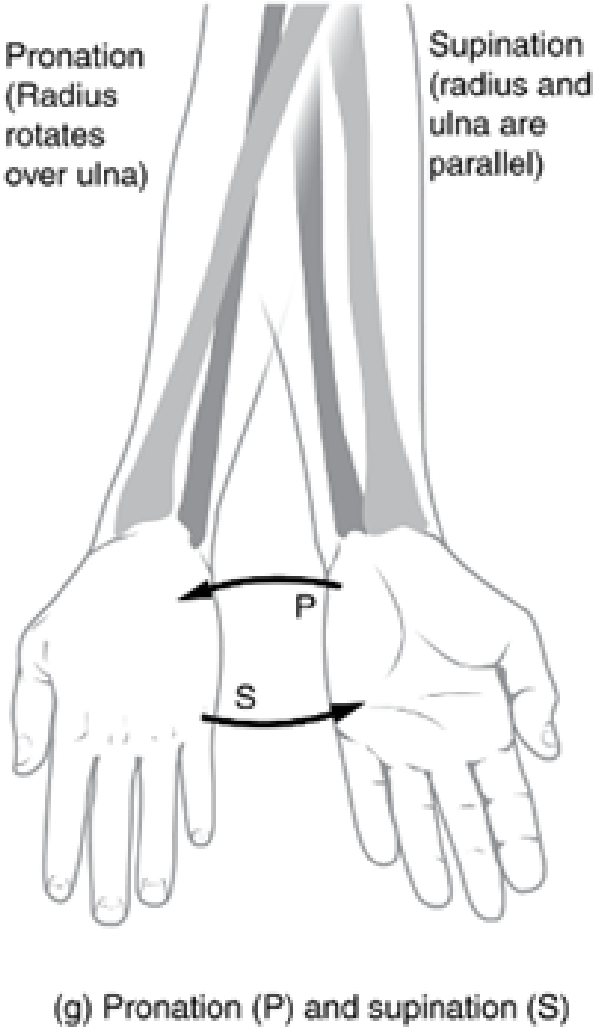

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Figure 10.21—Pronation and supination of the forearm. Notice how the radius pivots to cross over the ulna during pronation.

In contrast, the knee points forward and acts like a simple hinge. One feature of this hinge in mammals is that it often includes an extra, non-weight-bearing bone. This type of bone is called a **sesamoid bone**, and the particular one found in the knee is called the **patella**; you may know it better as the "kneecap." If we look at the knee in lateral view (Figure 10.22), you'll see that what the patella does is increase the moment arm of the muscles that extend the knee. In essence, the patella acts like the olecranon of the elbow to increase mechanical advantage and amplify muscle-force output of the knee joint.

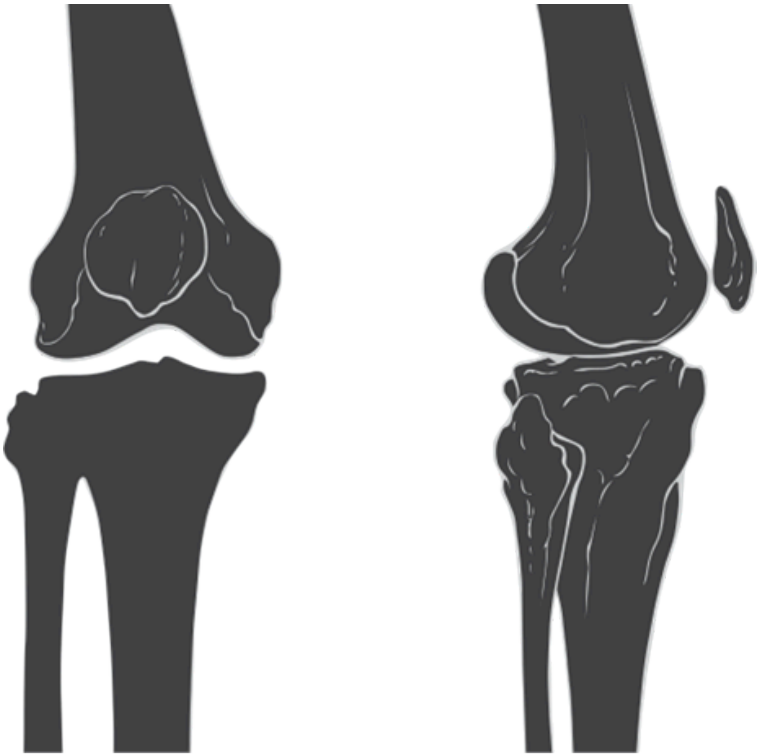

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Figure 10.22—Anterior (left) and lateral (right) illustrations of the human knee. Notice the non-weight-bearing patella bone "floating" in front of the distal femur. This bone is actually embedded in the patellar tendon, a strap of connective tissue that spans the front of the knee joint.

Like reptiles, mammals also have an astragalus in the ankle (called the **talus** in humans), and one of the tarsal bones, the **calcaneus**, develops a posterior process called a **tuber**. The calcaneal tuber functions similarly to the olecranon to increase the mechanical advantage of the muscles that extend the ankle. Interestingly, the calcaneal tuber is not unique to mammals; it is also seen in crocodilian ankles.

Box 10.1—Fins to Limbs to Flippers

The invasion of land by early tetrapods and the evolution of tetrapod limbs from fins have long been considered to be among the landmark events in vertebrate evolution. However, a number of terrestrial vertebrate lineages secondarily returned to aquatic habitats (e.g., whales, mosasaurs, penguins, sea turtles). These secondarily aquatic transitions represent a number of independent returns to the water but are nonetheless characterized by near-universal morphological changes to the appendicular skeleton. Most notably (but not exclusively) for the purposes of this chapter is the evolution of flattened, flipper-like limbs in these taxa. As we know from our study of the relationship between form and function thus far, morphology is often driven—at least in part—by the functional demands of the environment in which an organism lives. But how does one get to a flipper from the typical tubular tetrapod limb?

One likely driver of flipper evolution can be found in differences in limb bone loading between terrestrial and aquatic environments. During terrestrial locomotion, load magnitude is higher due to the lack of

environmental body support on land (i.e., air doesn't help support the body the same way water does). In sprawling taxa, like turtles, there's also a substantial difference in the orientation of limb bone loads during walking compared to swimming, with higher degrees of twisting occurring in the bone on land. Because round cross-sectional shapes resist these twisting loads more effectively than flattened cross-sectional shapes do, these loading regimes likely dictate tubular limb bone shapes on land. However, because twisting loads are reduced during swimming, animals may be released from this functional demand in water, thereby opening the door for the evolution of flattened shapes that confer hydrodynamic advantages.

Phylogenetic history and ancestral swimming style may also contribute to morphological evolution and secondary aquatic transitions. Recent work by **Kiersten Formoso** and colleagues broadly examined secondarily aquatic taxa from both reptile and mammal clades. These different groups would have swum very differently, showing radically different degrees of axial (primarily reptiles) versus appendicular (mammals and birds) propulsion. These clades also exhibit a number of preexisting morphological differences that evolved on land, including differences in posture (sprawling vs. upright) and variations in the structure of the limb elements (consider bird appendicular morphology, described in Section 10.4 of this chapter). As such, it is likely that ancestral terrestrial locomotor methods and morphology influenced swimming style in secondarily aquatic taxa, which by extension could influence aquatic

morphology and flipper evolution. Further work in this area is still needed in order to understand the precise drivers of flipper evolution, but this is an exciting area of current research that requires an integrative approach, bringing together principles of evolution, ecology, development, and biomechanics, among others.

10.5 Summary

Fins and limbs are arguably some of the most fascinating and well-studied structures of the vertebrate body, likely due to their wide variation in structure and relationship to locomotion. The appendages evolved initially as stabilizing structures for aquatic swimming and then diversified to produce structures equipped for locomotion in a range of aquatic, terrestrial, and aerial habitats. Nonetheless, homologies across taxa in the appendicular skeleton point to a common evolutionary origin in early fishes.

Application Questions

1. Once the major limb bones evolve in tetrapods, we see very few instances where those bones are lost.

What functional hypotheses could you consider for this conservation in bone number?

2. There are many instances where limb bones fuse together (wings of a bird, distal limb of a horse). What advantages do these fusions provide for the vertebrates in question?

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11. Muscle Tissue

VANESSA K HILLIARD

Focus Questions—to Guide Your Reading of This Chapter

1. What are the five key properties of muscle tissue? How do these properties facilitate muscle function?
2. Explain the structure of muscle tissue. How does force generation translate from the sarcomere to the whole organ?
3. How is muscle force modulated?

11.1 Introduction

Muscles are the motors of the vertebrate body. But muscles do more than just move us about—they also work in conjunction with the skeletal system to provide body support as well as play a role in the movement of material through the body, such as moving blood through the vasculature or producing pressure changes in the chest to drive air in and out of the lungs. What's more, muscles enable vertebrate bodies to accomplish incredible feats: The shaker muscles of rattlesnake tails can maintain contraction frequencies of

up to 100 Hz for hours. During migrations, eels can swim for months with minimal energy expenditure (four to six times less than that of salmon!). And the smooth muscles of the uterine wall can generate sustained intrauterine pressures of 80–100 mmHg during labor!

This chapter will begin with a brief introduction to muscle development and then transition into an examination of the tissue-level structure of muscle. As we discuss the tissue-level structure of muscle, we'll highlight how this structure facilitates muscle function (namely, contraction).

11.2 Muscle Development

Generally speaking, muscle cells develop from mesenchymal cells from the paraxial mesoderm (myotome). While these cells are actively dividing, they are known as myoblasts and are the precursors to mature muscle cells known as **myocytes** (just like we've seen before with the precursors of bone cells called osteoblasts and precursors of cartilage cells called chondroblasts). When cell division stops, the **myoblasts** begin to elongate and differentiate into myocytes.

Depending on the location in the body, the muscle tissue type, and the ultimate function of the muscle that is developing, the myocytes can form and organize in very different ways. For example, in skeletal muscle, the myoblasts line up longitudinally and fuse together. This produces long, single cells with several hundred nuclei positioned along the outer edge of the cell membrane. This structure is called a **syncytium** and can produce individual myocytes as long as 30 cm in some animals! Because of the unique, elongated shape of the skeletal muscle myocytes, they are often called muscle fibers.

11.3 Structure and Function of Muscle

Muscle Tissue Types

Muscle tissue is specialized for contraction. This tissue type is composed primarily of myocytes ("myo" = muscle, "cyt" = cell; so "myocytes" = "muscle cells"). The cells are considered excitable cells, meaning they respond to electrical or chemical stimulation. The cytoplasm of myocytes is filled with bundles of proteins called **myofilaments**, which give muscle cells their distinctive appearance. Muscle cells can take one of two forms:

1. **Striated**: having alternating light and dark bands formed by regions of myofilament overlap; this gives them a "stripey" appearance (think: striations = striped; see Figures 11.1A and 11.1C).
2. **Smooth**: lacking striations; these muscle cells also contain myofilaments, but the myofilaments are arranged as irregular bundles, so they don't overlap to form striations (Figure 11.1B).

In muscle tissue, a small amount of extracellular matrix surrounds each muscle cell and is called **endomysium** or external lamina; the endomysium functions to hold the muscle cells together in the tissue. We'll revisit this later in the chapter when we talk about gross muscle anatomy. Beyond this general distinction in appearance, we can further classify muscle tissue based on function. There are three types of muscle tissue (Figure 11.1):

1. **Smooth muscle**
2. **Cardiac muscle**
3. **Skeletal muscle**

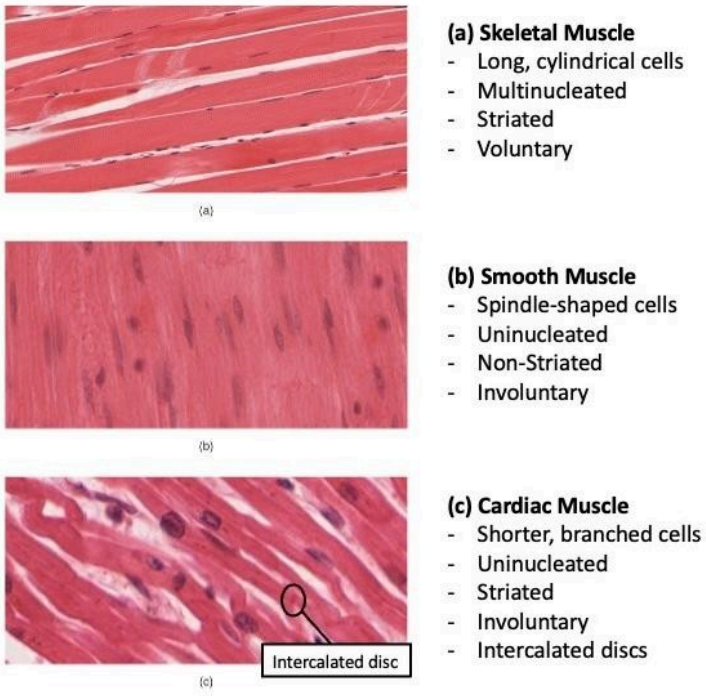

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Figure 11.1—Three types of muscle tissue and their distinguishing features.

Smooth muscle tissue, as its name implies, is composed of smooth muscle cells; contraction of this tissue is involuntary, meaning that we have no conscious control over contraction of this muscle type. We find smooth muscle tissue in the hollow organs of the digestive tract, blood vessels, eyes, skin, and the ducts of some glands. Smooth muscle tissue usually contains specialized junctions ("gap junctions") between cells to facilitate cell-to-cell communication. There are two types of smooth muscle:

1. **Unitary smooth muscle**: This type of smooth muscle undergoes spontaneous, rhythmic contractions that are

generated myogenically (i.e., from within the muscle). This type of smooth muscle is found in the digestive tract, uterus, and urinary ducts.

2. **Multiunit smooth muscle**: This type of smooth muscle is **neurogenic** (i.e., contraction is stimulated by the nervous system). Multiunit contractions allow more refined regulation of control; this type of smooth muscle is typically found in walls of blood vessels, irises of eyes, and sperm ducts.

Cardiac muscle tissue is composed of striated muscle cells called cardiac muscle cells; this type of muscle is found only in the heart. Contraction of cardiac muscle tissue is involuntary (like smooth muscle). Cardiac muscle cells are relatively short and branched; they are also uninucleate (one nucleus per cell). A key characteristic of cardiac muscle tissue is the presence of **intercalated discs**: Within the intercalated discs, we find junctions (i.e., places where neighboring cells connect) that enable rapid cell communication, which helps coordinate heart muscle contraction.

Skeletal muscle is also composed of striated muscle cells, and this muscle tissue type is found mostly attached to skeletal elements. Contraction of skeletal muscle is voluntary and occurs via electrical stimulation from the nervous system. Skeletal muscle is composed of long, thin muscle cells arranged in parallel—these are often called muscle fibers and are formed by myoblasts (immature muscle cells). Because of their incredible length, muscle fibers are **multinucleated**—meaning they have multiple nuclei. This chapter will focus primarily on skeletal muscle.

Even though these three muscle types have their differences, they all perform the same general function, which is to generate a force called **muscle tension**. Muscle tension functions in generating movement as well as maintaining posture, stabilizing joints, generating heat, and regulating the flow of substances through hollow organs. To accomplish the task of generating muscle tension, muscle cells must convert chemical energy in the form of ATP (adenosine triphosphate) into the mechanical energy of muscle

tension. And this task requires that muscles have a set of particular properties, including the following:

1. **Contractility**: ability to contract or the drawing together of the proteins within a muscle cell (rather than necessarily the shortening of the cell)
2. **Excitability**: ability to respond to chemical, mechanical, or electrical stimuli
3. **Conductivity**: ability to transfer electrical change across the entire length of the cell's plasma membrane
4. **Extensibility**: ability to stretch up to three times their resting length without being damaged or rupturing
5. **Elasticity**: ability to return to the cell's original shape after being stretched

As you might imagine, given the tight relationship between form and function, these unique functions of muscle cells come with a bit of unique cellular structure. Muscle cells have the same organelles as other cells, but they also possess some key structural differences (Figure 11.2), which come with their own new set of terms, including the following:

1. **Sarcolemma**: phospholipid bilayer surrounding a muscle cell that houses specialized proteins that assist with cell function; analogous to the plasma membrane
2. **Sarcoplasm**: located within the muscle cell, contains cytosol and organelles, and is analogous to cytoplasm of other cell types
3. **Sarcoplasmic reticulum**: modified smooth endoplasmic reticulum that forms a weblike network around each myofibril and functions in the storage and release of calcium ions—a critical component of muscle contraction
4. **Myofibril**: cylindrical organelle involved in muscle contraction that is composed of bundles of specialized proteins and is the most abundant organelle in skeletal muscle cells

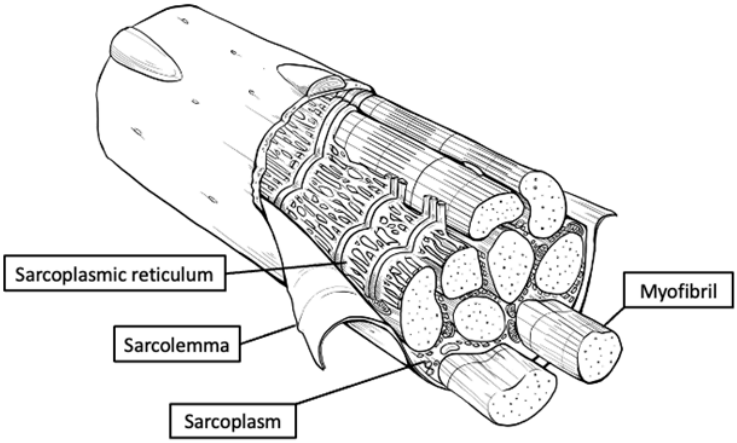

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Figure 11.2—Myocyte anatomy.

Let's unpack the sarcolemma a bit more. Unlike other cells whose plasma membranes are restricted to the exterior surface of a cell, the sarcolemma actually forms inward extensions called **transverse tubules** or **T-tubules** (Figure 11.3). These T-tubules extend deep into the cell and surround each myofibril, creating a tunnel-like network within the cell. Because T-tubules are continuous with the exterior of the cell, they are filled with extracellular fluid. Inside the cell, the T-tubules are flanked on either side by enlarged portions of the sarcoplasmic reticulum called the **terminal cisternae**. Together, the T-tubule and two terminal cisternae are known as a **triad**. The triad is an important structure in the process of muscle contraction.

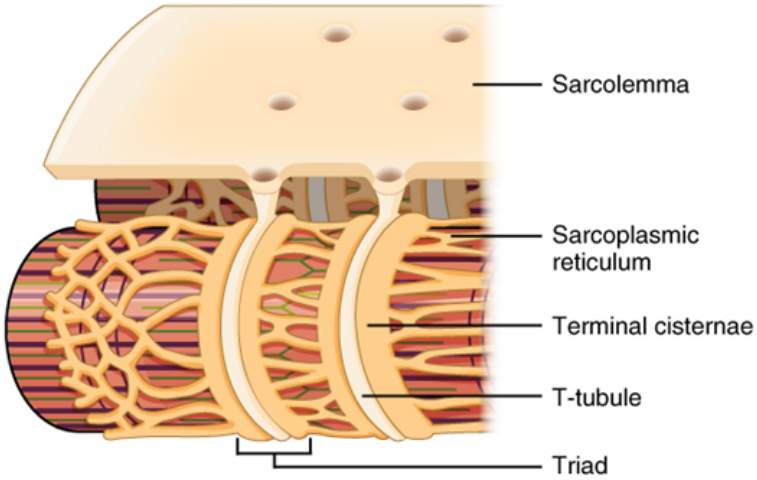

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Figure 11.3—Anatomy of the sarcoplasmic reticulum.

Let's look deeper still and consider the structure of individual myofibrils. A single myofibril is composed of hundreds to thousands of protein bundles called **myofilaments** (Figures 11.4 and 11.5). Myofilaments are made up of one or more of three types of proteins:

1. **Contractile proteins**: produce tension
2. **Regulatory proteins**: control timing of muscle fiber contraction
3. **Structural proteins**: hold myofilaments in place and ensure structural stability of myofibrils and the muscle fiber

Of the myofilaments, there are three types:

1. **Thick** filaments
2. **Thin** filaments
3. **Elastic** filaments

Thick filaments are composed of many molecules of the contractile protein **myosin**. Myosin's structure has two globular heads and two intertwined polypeptide chains that compose the tail. The heads attach to the tail via a flexible neck. Thick filaments are arranged such that the heads, which contain a site for binding to thin filaments, are positioned at the ends of the filament and the tails are in the middle.

Thin filaments are made up of both contractile and regulatory proteins:

1. **Actin:** contractile protein with an active site for binding to myosin head (thick filament); multiple actin subunits string together to form two intertwining strands and form the largest part of the thin filament
2. **Tropomyosin:** long, ropelike regulatory protein that spirals around the actin strands and covers the actin active sites
3. **Troponin:** small, globular regulatory protein that holds tropomyosin in place

Tropomyosin and troponin function in switching on and off (or "regulating") muscle contraction by controlling whether the active site on the actin subunits is available to interact with the myosin heads of the thick filament.

Elastic filaments are composed of a single large structural protein called **titin**. Titin is coil shaped, allowing it to stretch ("extensibility") and recoil ("elasticity"). This filament runs through the core of thick filaments and helps stabilize thick filament structure and resist excessive stretching. Because of its ability to recoil, elastic filaments enable muscle fibers to return to their original lengths after stretching.

Myofibrils are patterned by the arrangement of thick and thin myofilaments; there are regions of the myofibril where only thin or thick filaments are present and regions where the two overlap. You'll recall that one of the characteristics of skeletal muscle fibers is that they are striated—these light and dark bands

("striations") are produced by the repeated overlapping arrangement of thick and thin filaments within discrete units called **sarcomeres**. The anatomy of the sarcomere is as follows (Figures 11.4 and 11.5):

1. **Zone of overlap**: where thick and thin filaments overlap; this is where tension is generated during muscle contraction.
2. **I band**: light regions of a striation; only thin filaments are found here.
3. **A band**: dark regions of a striation; this is the portion of the sarcomere that contains thick filaments. The edges of the A band actually contain both thick and thin filaments, as they overlap here, but the center of the A band is composed only of thick filaments (a region known as the H zone).
4. **M line**: a dark line running down the center of the A band; this consists of structural proteins that function in holding thick filaments in place and serve as an anchoring point for the elastic filaments.
5. **Z disc (sometimes also called the Z line)**: a dark line running down the center of the I band. The Z disc is also composed of structural proteins that function to anchor the thin filaments in place and serve as attachment sites for the elastic filaments. The Z discs attach myofibrils to one another across the diameter of the muscle fiber.

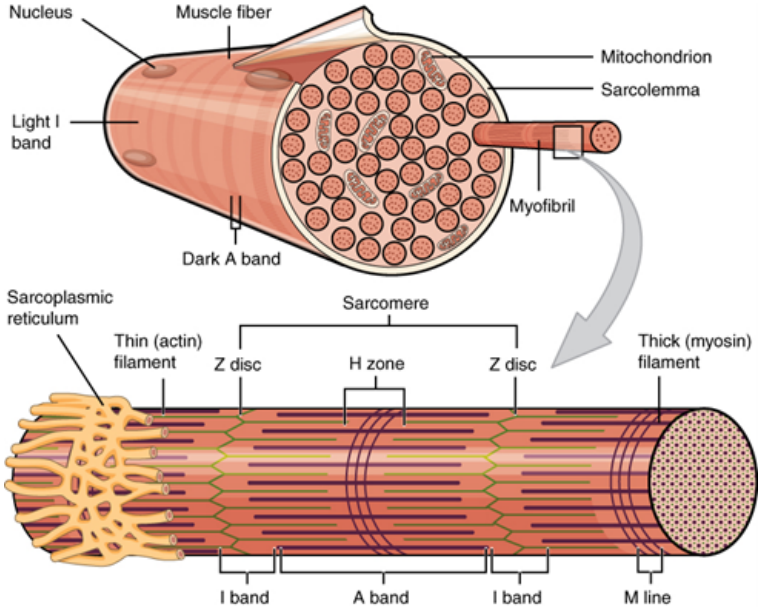

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Figure 11.4—Anatomy and organization of the muscle fiber and myofibrils.

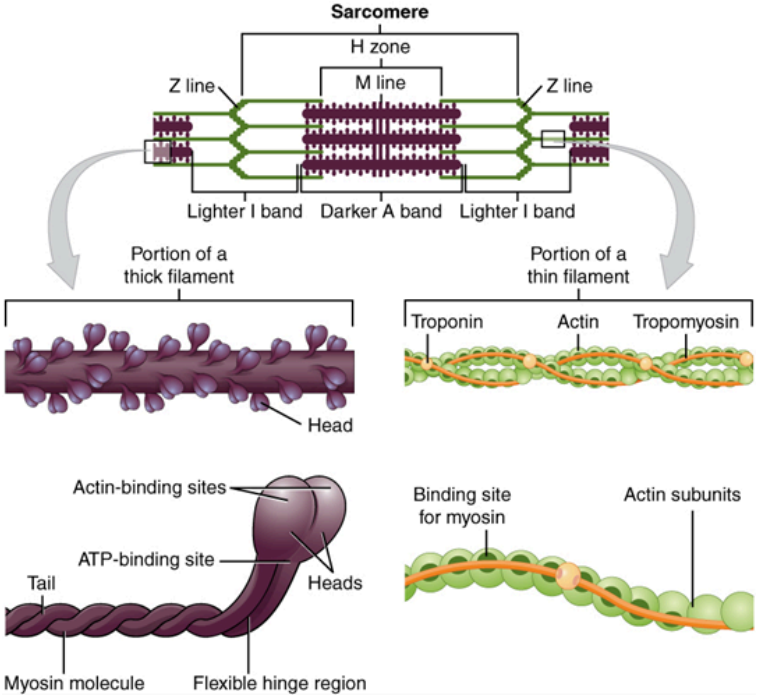

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Figure 11.5—Anatomy and organization of the sarcomere. Note overlap of thick and thin filaments, allowing for interaction between the two proteins and the engagement of the sliding filament mechanism.

The sarcomere is the functional unit of the contraction in skeletal muscle and spans from one Z disc to the next Z disc; therefore, each sarcomere is composed of one A band and two half-I bands. There are many sarcomeres in each myofibril, and the particular arrangement of the sarcomere elements is crucial to allowing skeletal muscle contraction to occur, as we will see in the next section (Muscle Contraction).

Box 11.1—A New Organizational Design in Skeletal Muscle Fibers

The structure of the sarcomere—indeed, of the entire myofibril of skeletal muscle—has been incredibly well conserved across vertebrate taxa. However, recent work by **Eric Parmentier** and **Marc Thiry** has identified a novel sarcomere architecture in the sound-producing muscles of the cusk eel (*Parophidion vassali*; Figure 11.6). Parmentier and Thiry suggest that this peculiar sarcomere structure may be an adaptation that circumvents the trade-off between speed and force often observed in skeletal muscle (i.e., muscle contraction is typically fast or strong but rarely both). In this species, myofilaments have a branched, Y-shaped structure; each of these branches connects to a neighboring myofibril. This branched structure provides a mechanism for increasing the number of crossbridges that can form during muscle contraction, thus amplifying force production. Furthermore, cusk eel sarcomeres have wider Z discs than typically observed in vertebrate muscle. The larger Z disc may contribute to consistent sound features during high-speed contraction.



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Figure 11.6—Cusk eel. Photo credit: NOAA via @DeepSeaImage.

Interestingly, this isn't the only mechanism for sound production in fishes that involves funky musculature. **Verity Cook** and colleagues, at Charité University in Berlin, found that one of the world's smallest fish—*Danionella cerebrum*—can actually produce a drumming sound over 140 dB. These fish accomplish this amazing feat through the use of a specialized muscle that pulls a rib into a small piece of indented cartilage. When the rib is released, it springs back and strikes the swim bladder to create a noise as loud as the bang of a firecracker!

Muscle Contraction

I mentioned that the structure of the sarcomere is critical for the muscle contraction to occur, but why? How does it happen?

The widely accepted model for skeletal muscle contraction is the sliding filament theory. In the sliding filament theory, thin filaments slide past thick filaments and generate tension throughout the sarcomere. During contraction, the I bands and H zone narrow, but the A band does not change. This occurs because the myosin heads of the thick filaments bind to the thin filaments and pull them toward the M line. This brings the Z discs closer together and shortens the sarcomere as a unit. Something important to note here: None of the filaments themselves get shorter! The thin filaments are simply pulled toward the M line. And because the sarcomeres are arranged end-to-end within the myofibril, simultaneous contraction of the sarcomeres results in contraction of the whole muscle fiber (Figure 11.7).

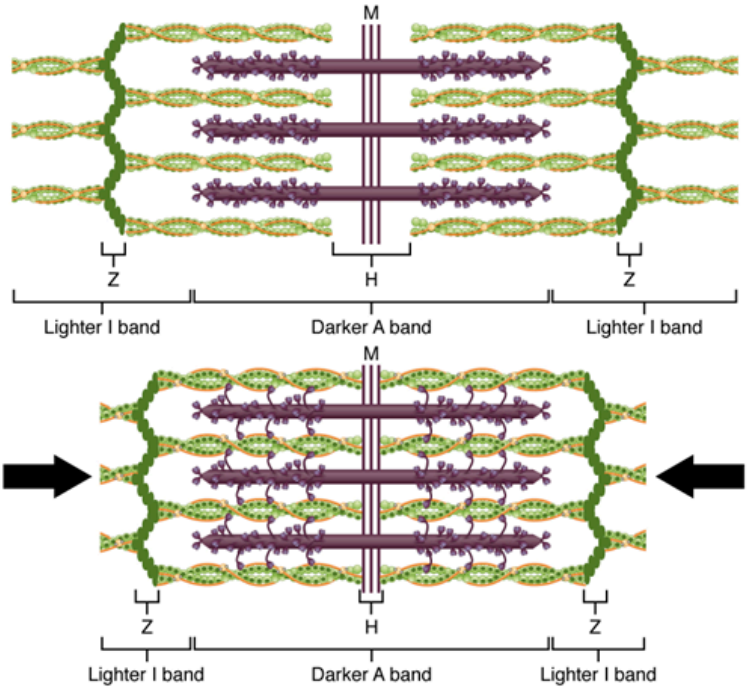

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Figure 11.7—Relative position of the thick and thin filaments during sarcomere contraction. Note that the filaments themselves do not change length; rather, they change their position relative to each other (i.e., the zone of overlap increases in size).

Box 11.2—Winding Filament Theory

Even though the sliding filament theory tends to be the established model for explaining muscle contraction, it fails to account for some aspects of muscle function, including how force is amplified during

stretching and depressed during shortening. **Dr. Kiisa Nishikawa** and colleagues have been working to shed light on some of these less understood aspects of muscle contraction, and their work has led to the development of the **winding filament theory**. This theory focuses on the function of titin, a large elastic protein found in the sarcomeres of skeletal muscle. In the winding filament theory, titin binds to the thin filaments, thus increasing its stiffness. In addition, as the crossbridge cycle is activated and thin filaments are pulled toward the M line (as described in the sliding filament theory), titin is simultaneously wound around the thin filaments. The combination of these two events allows the sarcomere to store potential energy during contraction, which can then be released during shortening. The winding filament theory builds upon the foundation of the sliding filament theory by adding an explanation of the mechanism that allows for force enhancement during muscle stretching and force depression during shortening. It also provides a framework for the development of testable predictions as research of the mechanisms of muscle contraction at the sarcomere level continues.

Now, even though this is an anatomy textbook, it's difficult to understand muscle function (i.e., contraction) without pulling in some physiology. This is because muscle contraction is really dependent on the property of excitability. And even though contraction takes place in the sarcomere, the events leading to contraction begin with electrical changes across the sarcolemma. In muscle cells, the cytosol and extracellular fluid (ECF) are electrically neutral away from the plasma membrane (i.e., there are equal

numbers of positive and negative ions). But at the sarcolemma, we find a thin layer of negative ions in the cytosol and a thin layer of positive ions in the ECF. This separation of positive and negative ions creates an electrical gradient, which represents a source of potential energy. For this reason, this gradient is often called an **electrical potential**.

If we compare the electrical potential of two points—say, one side of a muscle cell versus the other—the difference between the potentials at these two points is called a **voltage**. When we talk about membrane potential, we're referring to this difference in electrical potential across the plasma membrane. For muscle fibers at rest, a typical resting membrane potential is around −85 mV. This resting membrane potential can change as ions cross the sarcolemma.

This brings us to **action potentials**, which are quick, temporary changes in the membrane potential in a single region of the sarcolemma. Action potentials are generated by the opening and closing of channels, which control movement of ions across the membrane.

But why does it matter that these ions cross back and forth and generate action potential? Well, the generation of action potentials is critical for long-distance signaling in the cell. You'll recall from earlier in this chapter that one of the key properties of muscle cells is **conductivity**, or the ability to transfer electrical changes rapidly across the sarcolemma. The action potentials don't just stay in one spot in the cell—they are conducted throughout the sarcolemma, including the T-tubules. And this arrival of an action potential at the T-tubules is what initiates a muscle contraction and enables a muscle fiber to contract as a unit!

Now, we cannot have skeletal muscle contraction without stimulation from the nervous system, so to really understand muscle contraction, we also need a basic understanding of the relationship between the nervous system and the muscular system. The nervous system will be covered in greater detail in other chapters of this book, so the nervous system information we cover

now is really just a generalized introduction to select components of that system for the purposes of understanding muscle function.

All skeletal muscle is **innervated**, meaning that it is connected to a **neuron (motor neuron)**. Each motor neuron communicates with several muscle fibers via a connection called a **synapse**. And the number of muscle fibers innervated by a single motor neuron depends a lot on where we look in the body and whether the structure in question executes gross (i.e., large-scale) movements or precise (i.e., fine-scale) movements. Generally speaking, we see a single motor neuron innervating many fibers in places like the trunk of the body, where gross motion occurs, and few fibers in places like the eyes, hands, and fingers, where precise motion occurs.

The specific term for a synapse between a motor neuron and a muscle fiber is **neuromuscular junction** ("neuro" = nerve, "muscular" = muscle, "junction" = place where two things meet). The neuromuscular junction is where communication between the nerve and a muscle fiber takes place, which is accomplished through transmission of a signal called a **nerve impulse**. The neuromuscular junction is made up of three parts (Figure 11.8):

1. **Axon terminal**: the swollen end of the axon (part of motor neuron). The axon terminal contains synaptic vesicles, which house **neurotransmitters** (i.e., chemicals used to cause change in the cell the neuron communicates with; e.g., **acetylcholine**).
2. **Synaptic cleft**: the space between the axon terminal and the motor end plate.
3. **Motor end plate**: a specialized region of the sarcolemma that houses receptors for neurotransmitters.

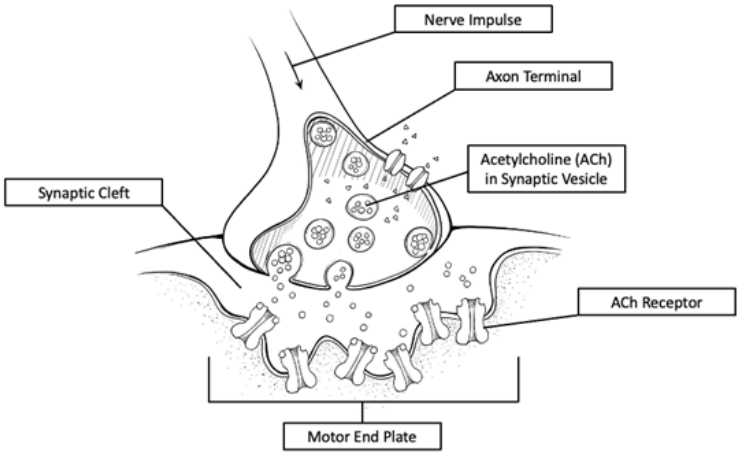

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Figure 11.8—Anatomy of the neuromuscular junction.

OK, we are finally ready to talk about muscle contraction. The process of muscle contraction can be broken down into three parts:

1. Excitation phase
2. Excitation–contraction coupling
3. Contraction phase

During the **excitation phase**, a signal is transmitted from the motor neuron to the sarcolemma of the muscle fiber through the release of **acetylcholine** (**ACh**), a neurotransmitter. This generates a response in the muscle fiber called an **end-plate potential**.

In order to actually produce a muscle contraction, multiple end-plate potentials must be generated. But the ACh released into the synapse is quickly broken down by an enzyme called **acetylcholinesterase** (AChE). So in order to produce the multiple end-plate potentials necessary for contraction to occur, the motor

neuron must continue to generate action potentials and release new ACh molecules.

Upon the generation of the end-plate potential, the second phase of muscle contraction—**excitation–contraction coupling**—is initiated. During this phase, the end-plate potential is propagated across the sarcolemma and down through the T-tubules, which initiates the release of calcium ions from the sarcoplasmic reticulum into the sarcoplasm of the muscle fiber.

The stage is now set to initiate the third phase of generating a muscle contraction—the **contraction phase**. The calcium ions that were released from the sarcoplasmic reticulum bind to troponin, the globular, regulatory protein found in the thin filaments that we discussed earlier in the chapter. This binding of calcium causes the troponin to shift position, which enables tropomyosin—the long, regulatory protein in thin filament—to move and expose the active sites on the actin subunits. Once these active sites are exposed, the myosin heads from the thick filaments can bind to the actin on the thin filaments, and the sliding filament mechanism is engaged. When the sliding filament mechanism is engaged in multiple sarcomeres along the length of the muscle fiber and in multiple muscle fibers in the muscle, whole organ contraction takes place. Now, muscles cannot just contract and stop there—they also have to relax. Muscle relaxation has two components:

1. Release of ACh stops and any ACh left in the synapse is broken down by AChE.
2. Calcium ion concentrations in the sarcoplasm return to resting level and troponin and tropomyosin return to their resting positions, thus blocking the active sites on the thin filament.

One very important thing to note is that muscles cannot lengthen themselves (i.e., muscles can only "pull" on structures, they cannot "push")! For this reason, muscle groups that act about joints are typically organized as antagonist pairs or antagonist groups,

such that the contraction of one muscle or group of muscles moves a body part in one direction, and contraction of the opposing muscle or group of muscles moves the body part in the opposite direction (and thereby relengthens the first set of muscles). So even though muscles cannot actually lengthen themselves, they can be passively relengthened after shortening because they contain parts that behave elastically. In other words, they will return to their original length after being stretched or shortened. These elastic components include connective tissue within the muscle, parallel to the fibers, called **parallel elastic components**, and the tendons that attach muscle to bone, called **series elastic components**. These elastic components help save energy by reducing the force that muscles have to exert to produce opposing motions.

Let's consider an example: My triceps brachii, located on the posterior side of my humerus, extends the elbow when it contracts; the biceps group (which includes biceps brachii, brachialis, and brachioradialis) on the anterior side of my humerus flexes the elbow and relengthens the triceps so it can contract again (Figure 11.9).

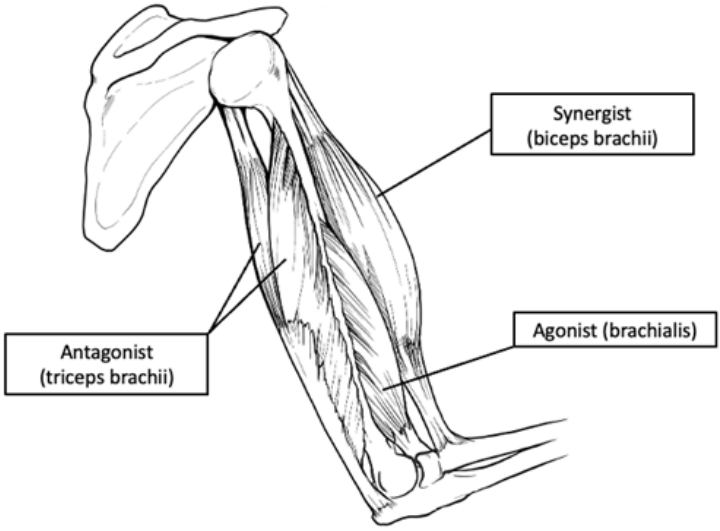

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Figure 11.9—Antagonist muscle groups of the arm.

I described these antagonist pairs as muscle groups, and that is because oftentimes there are multiple muscles in a position to produce a similar motion when they contract across a joint. These muscles that can work together are called **synergists** or **agonists**. This redundancy of muscles crossing joints and producing similar actions allows for more refined control of joint motions; there are also some additional mechanisms for modulating muscle actions and movements, as we'll see shortly.

Muscle Tension

Let's turn our attention to muscle tension for a bit now. When muscle tissue contracts, it produces **tension**. The smallest possible

muscle contraction is called a **muscle twitch**, which is simply the response of a muscle fiber to a single motor neuron action potential. Every muscle twitch produces tension, which translates to some degree of force production and varies from fiber to fiber. The tension produced during a twitch is influenced by several factors, including

1. Timing and frequency of stimulation
2. Resting fiber length
3. Muscle fiber type

Let's look at each of these factors and see how they can influence tension production of muscle fibers. We'll start with timing and frequency of stimulation.

Generally speaking, repeated stimulation of a muscle fiber produces twitches with progressively greater tension. The reason for this is that the sarcoplasmic reticulum pumps don't have enough time to pump all the calcium ions back into the sarcoplasmic reticulum, which results in increasing calcium ion concentrations in the sarcoplasm with each stimulus. This increasing tension is known as wave summation, because the waves of contraction add together.

Depending on the frequency of stimulation, or how quickly the stimulation is occurring, our muscle fiber can wind up in one of two states (Figure 11.10):

1. **Unfused tetanus** (or **incomplete tetanus**): occurs when fiber is stimulated at a frequency of about 50 times per second. This frequency allows partial relaxation between each contraction, so tension increases and decreases slightly with each twitch until a level of maximal tension is reached.
2. **Fused tetanus** (or **complete tetanus**): occurs when a fiber is stimulated at a higher frequency—typically around 80–100 times per second. In this state, the fiber does not have time to relax between contractions, and as a result, tension remains constant at a maximal level.

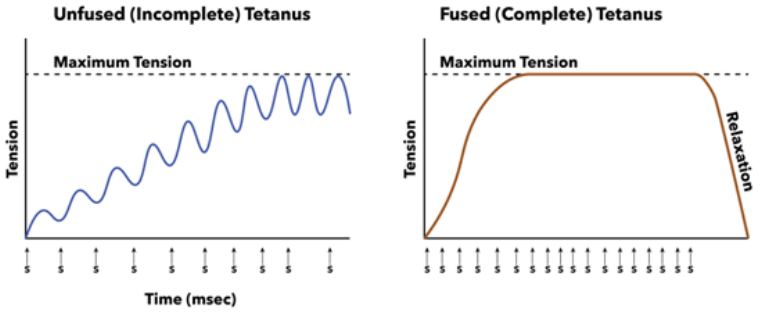

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Figure 11.10—Unfused versus fused tetanus. Note the difference in the frequency of stimulation and how that affects the muscle fiber's ability to relax (or not) between stimuli and the corresponding impact on generating maximum tension.

Our second factor—**resting fiber length**—brings us to the concept of length-tension relationships. The **length-tension relationship** is a principle that states that *the number of crossbridges that can form in a sarcomere depends on the length of the sarcomere prior to contraction*. The optimal length of the sarcomere is the length at which the most crossbridges can form, and the number of crossbridges that can form directly influences the amount of tension the fiber can produce (generally, more crossbridges lead to greater force production and vice versa; Figure 11.11).

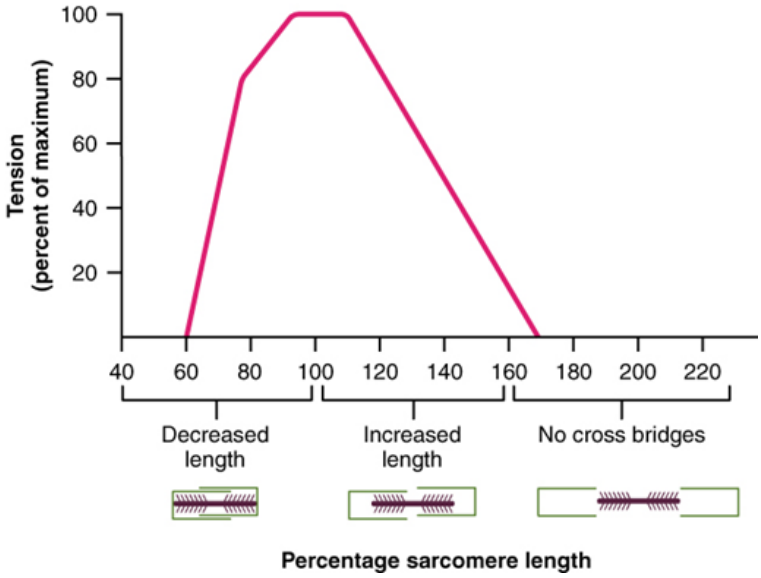

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Figure 11.11—Length-tension relationship. As sarcomere length increases or decreases, the zone of overlap changes size and affects how the thick and thin filaments interact with each other. Because the number of crossbridges is maximized and there is minimal interference of the Z disc at an intermediate length, this is the length of the sarcomere at which tension production peaks.

Box 11.3—Wrist Posture, Sarcomere Length, and Grip Strength

Let's illustrate this concept with a quick experiment. Flex your wrist 90 degrees. Now try to squeeze your pen/pencil/hand dynamometer hard. It's a little tough, right? This is because your flexed wrist position has shortened the muscles of the forearm, causing a great deal of overlap of the thick and thin filaments in the

sarcomeres. Since there is so much overlap, there is not much room for the thick and thin filaments to slide past one another before hitting the Z disc. This results in the formation of very few functional crossbridges, and as a result, the muscle generates less tension.

Let's try again—but hyperextend your wrist this time (90 degrees in the other direction). Now try to squeeze your pen/pencil/dynamometer. Still tough, right? This time, the muscle is stretched, and there isn't enough overlap for the thick and thin filaments to connect properly. Again, this leads to the formation of fewer crossbridges and the production of less tension.

Last, extend your wrist normally and squeeze your pen/pencil/dynamometer. You should notice that your grip strength is strongest in this position. This is because the muscle is at its natural resting length, and the sarcomeres are at optimal length with just the right amount of overlap of the thick and thin filaments; this arrangement maximizes crossbridge formation and increases tension production.

As you can see, the optimal length of the sarcomere is actually an intermediate length. So it's a little bit like a Goldilocks situation: Less tension is produced when the sarcomere is too short and when the sarcomere is too long. But when the sarcomere is "*just* right," the muscle is able to maximize tension—and by extension, force—production.

Recall the elastic filaments that make up part of the myofibril. These filaments enable the myofibril to be stretched, resulting in differing lengths of the myofibril and its sarcomeres.

This, in turn, affects the degree of overlap of the thick and thin filaments in the sarcomere's zone of overlap. Therefore, the size of the zone of overlap is largely dependent upon the position of the muscle prior to contraction.

The third factor that can affect the generation of tension is fiber type.

Muscle Fiber Types

In some muscles, the muscle fibers are supplied by multiple connections from the nerve cells (i.e., they contain multiple motor end plates). These muscles are called **tonic muscles**, and the force of the contraction in this type of muscle fiber is controlled by how quickly the nerve impulses arrive in sequence (**frequency**). Tonic muscles typically contract slowly but are really hard to fatigue. Tonic muscles are uncommon in fishes, birds, and mammals. When observed in mammals, they are typically small and restricted to places requiring fine control, like the muscles that move the eyeball. However, tonic fibers are much more common in amphibians and reptiles and have been shown to contribute to sustained force production by the jaw muscles of some species, enabling them to subdue prey (e.g., horned frogs) or engage in long-lasting courtship behaviors (e.g., alligator lizards).

In contrast, most skeletal muscles have fibers with only a single connection to a nerve cell (i.e., a single motor end plate). These are called **twitch muscles**. In twitch muscles, the nerve impulses spread easily throughout the fiber, so once the threshold impulse level is reached, the fiber will contract in an "all or nothing" fashion and then relax. However, if the fibers are stimulated several times in rapid succession, the forces from each twitch will be summed up to a maximum, which is called **tetanic force**. Twitch muscles tend to be large muscles with several motor units, so forces

can be modulated by varying the number of motor units stimulated as well as the frequency of stimulation.

Twitch fibers differ in structure and function, and as a result of these differences, some fibers twitch more rapidly than others. Whether a muscle fiber is considered a "fast" or "slow" fiber is largely determined by the level of myosin ATPase activity in the cell (this is the enzyme that hydrolyzes ATP to drive the power stroke of contraction).

Muscle fibers with high ATPase activity are called **fast-twitch fibers** because they go through contraction cycles relatively quickly. Fast-twitch fibers tend to be found in body parts that need to move rapidly (e.g., the muscles that move the eyes). In contrast, fibers with low myosin ATPase activity are called **slow-twitch fibers** and tend to be found in muscles that require slow sustained contractions (e.g., postural muscles of the back).

When we consider twitch speed in conjunction with a fiber's primary energy source, we find that skeletal muscle can be categorized into two "type" classes:

1. **Type I fibers**: These are slow-twitch fibers with small diameters, high myoglobin concentration, many mitochondria, and a well-developed blood supply. Myoglobin is an oxygen-binding protein found in vertebrate muscle. The high myoglobin content of Type I fibers makes them red in appearance, so muscles with many Type I fibers are sometimes called **red muscle**. These muscle fibers are also sometimes referred to as **slow-oxidative (SO) fibers** due to their slow contraction times and reliance on oxidative or aerobic metabolism (requires oxygen).
2. **Type II fibers**: These are fast-twitch fibers with large diameters that contract quickly but also fatigue quickly compared to Type I fibers. Type II fibers rely primarily on anaerobic energy production, so they are characterized by having fewer myoglobin and mitochondria compared to Type I fibers. The lack of myoglobin in Type II fibers gives them a

light color, so they are sometimes called **white muscle**. These muscle fibers are also sometimes referred to as **fast-glycolytic (FG) fibers**, because they contract more rapidly and rely on glycolytic or anaerobic metabolism (does not require oxygen).

Up to this point, we've been considering muscle tension at the fiber level, so let's zoom out a bit now and consider tension at the organ level. Recall from earlier that muscle fibers are innervated by motor neurons, and a single motor neuron may innervate multiple fibers. We refer to this motor neuron and all the fibers it innervates as a **motor unit**.

In humans, the average motor unit is made up of one motor neuron and about 150 muscle fibers, but this can vary depending on the part of the body and its function. For example, in areas that require precise movements, like the hand, we find motor units innervating as few as 10 muscle fibers. In contrast, areas of the body with large, powerful muscles, such as in the back, have motor units that innervate upward of 2,000–3,000 fibers. All the muscle fibers in a motor unit are of the same type, though, resulting in slow motor units and fast motor units. When a whole muscle begins a contraction, not all the motor units immediately engage. In fact, the slow motor units typically activate first and are later joined by fast motor units if additional tension is required to complete a task—this is called **recruitment**.

11.4 Summary

In this chapter, we introduced muscles as the motors of the vertebrate body. By looking at muscle development as well as the tissue-level structure of muscle, we were able to gain an understanding of the microscopic organization of muscle as well as how this structure facilitates muscle contraction.

Application Questions

1. In domesticated chickens, the breast meat tends to be "white" meat, whereas the thigh meat tends to be "dark" meat.
    1. Given what you now know about muscle fiber types and their characteristics, why do you suspect this is the case?
    2. What do these differences indicate about muscle function in the breast versus the thigh of these birds?
    3. Do you think this pattern might differ in a migratory game bird, such as ducks? Why or why not?
2. You are at the gym and notice that you have an easier time lifting weights when your arm is in a particular position, compared to other positions. In the context of the length-tension relationship and muscle function, explain why this is the case.

11.5 Further Reading

1. Abbott B. C., and X. M. Aubert. "The force exerted by active striated muscle during and after change of length." *Journal of*

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2. Cook, Verity A., Antonia H. Groneberg, Maximillian Hoffmann, Mykola Kadobianskyi, Johannes Veith, Lisanne Schulze, Jörg Henninger, Ralf Britz, and Benjamin Judkewitz. "Ultrafast sound production mechanism in one of the smallest vertebrates." *Proceedings of the National Academy of Sciences U.S.A.* 121 (2024): e2314017121.
3. Nishikawa, Kiisa C., Jena A. Monroy, Theodore E. Uyeno, Sang Hoon Yeo, Dinesh K. Pai, and Stan L. Lindstedt. "Is titin a 'winding filament'? A new twist on muscle contraction." *Proceedings of the Royal Society B.* 279 (2012): 981–990.
4. Parmentier, Eric, and Marc Thiry. "A new organisational design in skeletal muscle fibres." *Cell and Tissue Research* 393 (2023): 111–117.

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12. Gross Muscle Anatomy

VANESSA K HILLIARD

Focus Questions—to Guide Your Reading of This Chapter

1. Explain the hierarchical structure of muscle tissue.
2. Why are muscles organized into functional groups?
3. Compare and contrast muscle origins versus insertions. How does consideration of origin and insertion inform our understanding of muscle actions?
4. Which muscle groups show highly conserved anatomy across vertebrate taxa? Which muscle groups are highly differentiated? Why might these patterns exist?

12.1 Introduction

In Chapter 11, we explored the microscopic structure of muscle as well as how muscles function at the cellular level to produce tension. In this chapter, we will zoom out and begin considering muscle at the gross anatomical level. We will explore muscle

organization, muscle shapes, and muscle naming conventions. After establishing a foundation of gross anatomical muscle structure and function, we will explore muscle evolution and the diversity of the major muscle groups among vertebrates.

12.2 Hierarchical Anatomy of Muscle

As you know from the previous chapter, skeletal muscle is composed of elongated cells known as muscle fibers or myocytes. But each of these myocytes actually contributes to the formation of a larger organ structure called a muscle, and this muscle has a hierarchical organization pattern.

Each muscle fiber is surrounded by a thin layer of extracellular matrix called **endomysium**. Individual muscle cells and their endomysium are bundled together, and these bundles of fibers form structures called **fascicles**. Fascicles are bound by a layer of connective tissue called **perimysium**. These fascicles and their surrounding perimysium are grouped together into a discrete unit called a **muscle**, which is enclosed by yet another layer of connective tissue called **epimysium**. The fibrous connective tissue of a muscle then forms a **tendon**, which anchors the muscle to the bone and/or the structure that the muscle moves (Figure 12.1).

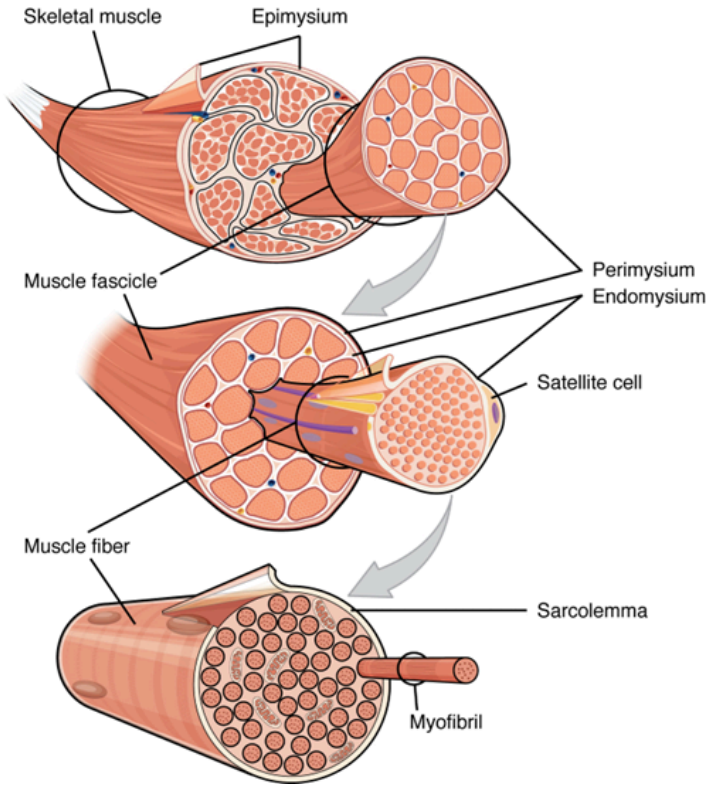

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Figure 12.1—Hierarchical organization of muscle.

This multilayered, bundled arrangement ensures that a muscle pulls as a unit, even if some of the fibers that make up the muscle are weaker than others. As a result of the way muscles are organized, skeletal muscles have distinct attachments to skeletal elements or connective tissue septa. These attachments are called tendons, and they basically consist of an extension of the muscle's connective tissue, as described above, that penetrates the periosteum of the bone. Tendons can take a variety of forms (Figure 12.2):

1. Sometimes tendons are really inconspicuous, so much so that it simply looks as if the muscle grows directly out of bone (e.g., gluteus medius tendon; Figure 12.2A).
2. Other times tendons form prominent cords. The Achilles tendon above our heel is an excellent example of this type of tendon (Figure 12.2B).
3. And still other times tendons form broad sheets called aponeuroses. An example of an aponeurosis can be observed on the abdomen (Figure 12.2C).

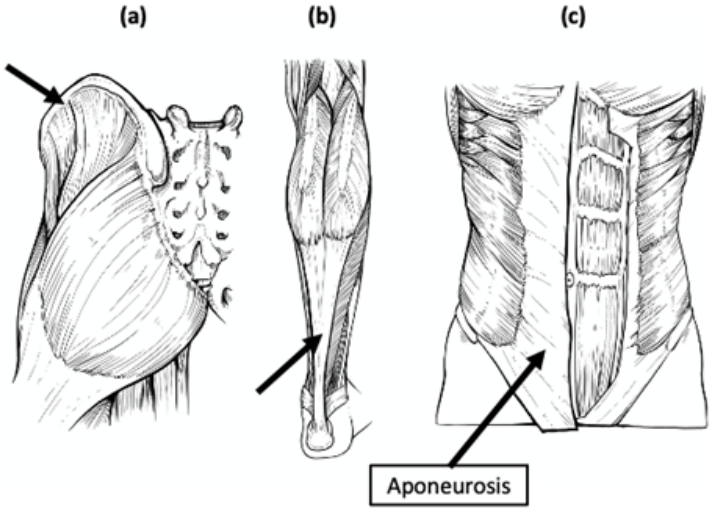

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Figure 12.2—Tendon anatomy.

When we describe the attachments of a muscle, we describe them in reference to two ends (Figure 12.3): There's (1) the end that remains fixed (the **origin**), which is typically more proximal, and (2) the end that moves when the muscle contracts (the **insertion**), typically more distal.

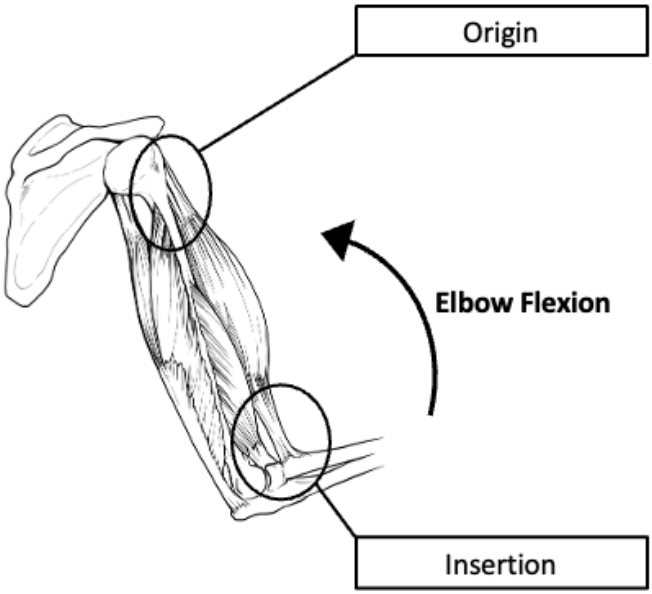

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Figure 12.3—Muscle origin versus insertion during muscle contraction (elbow flexion).

In addition to muscle fibers and connective tissue, we also find blood vessels in muscle, which supply muscle fibers with nutrients and remove metabolic waste. Nerves are also present in muscle, as these are critical structures for coordinating muscle contraction (see **neuromuscular junction**, Figure 11.8).

12.3 Muscle Shapes

Skeletal muscles vary quite a bit in their structures, and this is

related to the pattern of arrangement of the fascicles, which affect the appearance and function of the muscles. We find muscles in the following shapes (Figure 12.4):

1. **Parallel**: evenly spaced fascicles that attach to a tendon of similar width to the muscle. Parallel muscles have a "straplike" appearance (e.g., sartorius muscle of the thigh).
2. **Convergent**: broad and uniformly taper into a single tendon. Convergent muscles tend to be triangular in shape (e.g., pectoralis major of the chest).
3. **Pennate**: fibers and fascicles attach to the tendon at an angle; there are three forms:
    1. **Unipennate**: fascicles angle out on one side only (e.g., flexor pollicis longus of the forearm)
    2. **Bipennate**: fascicles angle out from both sides (e.g., rectus femoris of the thigh)
    3. **Multipennate**: composed of several areas where fascicles angle out from connective tissue separators (e.g., deltoid of the shoulder)
4. **Circular**: encircle a structure. These muscles can be found in areas such as around the eyes and mouth.
5. **Spiral**: characterized by having a twisted appearance; these often wrap around a bone (e.g., supinator muscle of the forearm).
6. **Fusiform**: thick in the middle and taper at either end (e.g., biceps brachii muscle of the upper arm).

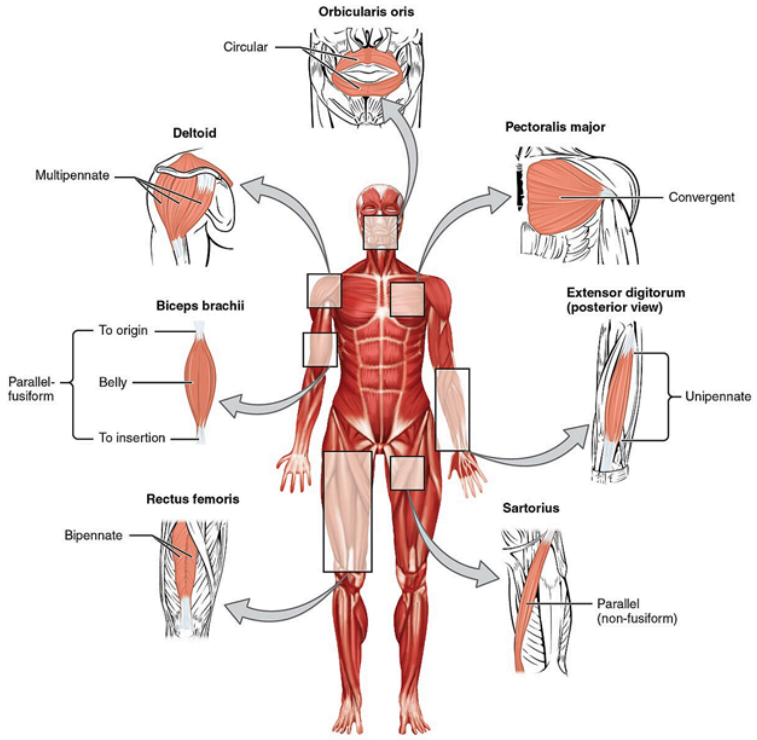

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Figure 12.4—Examples of muscle shapes in the human body.

12.4 Muscle Names

Muscle names can stem from a number of origins, but oftentimes these names are really helpful in informing us about the muscle. Muscle names often include size descriptors such as major (big), minor (small), longus (long), or brevis (short), among others. Similarly, muscle names may also include location information through the use of directional terms such as superior, medial, profundus (deep), capitis (head), and so on. They may also include regional terms, like pectoral or oculi, indicating where in the body

the muscle may be found. In some cases, muscle names indicate the structures to which they attach. For example, the sternocleidomastoid muscle attaches to the sternum ("sterno"), the clavicle ("cleido"), and the mastoid process of the temporal bone ("mastoid"). Muscle names can also give clues about a given muscle's function, such as flexing or extending a limb or elevating or adducting a body part (e.g., flexor pollicis longus flexes the thumb).

12.5 Muscle Evolution and Diversity

Now that we've established a foundational understanding of muscle development, form, and function, we're ready to begin examining muscle evolution and diversity. In an earlier chapter we introduced the distinction between visceral and somatic portions of the body. You'll recall that visceral refers to tissue associated with the gut tube, and somatic refers to everything else. And we can use this same distinction to categorize the muscles of the vertebrate body.

Almost all muscles are derived from mesenchymal mesoderm, but the specific kind of mesoderm differs depending on whether you're dealing with visceral versus somatic muscles. **Visceral muscles**—including those in the walls of the gut tube, blood vessels, and heart—are derived from the splanchnic layer of the lateral plate mesoderm. In contrast, **somatic muscles**—which include the remaining muscles of the body axis, appendages, and head—develop from the somites and somatic layer of the lateral plate mesoderm (see Figure 4.10).

Because the structure of the digestive tract (gut tube) and cardiovascular system (blood vessels and heart) (Chapter 15) will be discussed in detail in later chapters, the rest of this chapter will focus on the six major groups of somatic muscles (and—impressively, four of the six are all located in the head and innervated by cranial nerves). As we introduce these muscle groups below, I will include notes on their innervation (particularly for the

head muscles). But—if the nervous system (Chapter 19) is covered elsewhere in this book, why take the time to highlight the specific cranial nerves associated with the head muscles now? Why is this important? Well, cranial nerve innervation patterns are highly conserved across vertebrates, which means that the specific muscles of vertebrates that are derived from these groups will continue to follow these patterns of innervation!

The six major muscle groups of the vertebrate body (Figure 12.5) are

1. Extrinsic ocular muscles
2. Epibranchial muscles
3. Hypobranchial muscles
4. Branchiomeric muscles
5. Axial muscles
6. Appendicular muscles

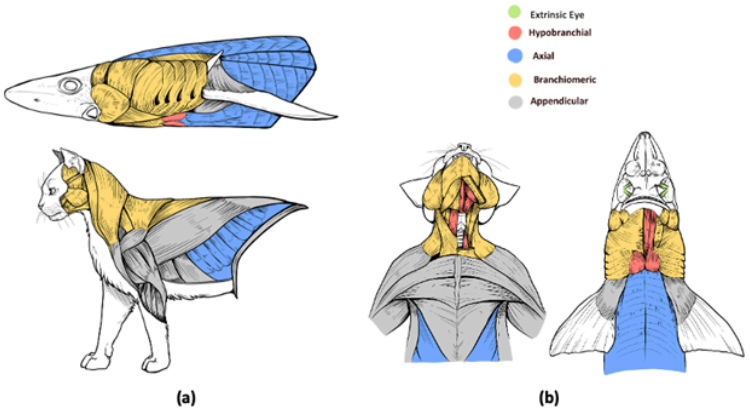

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Figure 12.5—Five major muscle groups of the vertebrate body, shown in the shark and the cat. (a) lateral view; (b) ventral view. Epibranchials not shown.

Extrinsic Ocular Muscles

Let's begin with a look at the **extrinsic ocular muscles**. These muscles are the muscles that move the eyeball (note the term *ocular*, referring to the eye). The extrinsic oculars are derived from the first three **somitomeres** (precursors to vertebrate somites) of the head as well as somitomere 5, and they are supplied by the ventral root nerve of each of the first three head segments. The ventral root nerves for the first three somitomeres are the oculomotor (CN III) for segment 1, trochlear (CN IV) for segment 2, and abducens (CN VI) for segment 3 (these cranial nerves will be covered in greater detail in the chapters on the nervous system and senses). The extrinsic ocular muscles are incredibly conserved throughout the diversification of vertebrates—so the six muscles that move the eye in a shark are essentially the same six muscles that move your own eye (Figure 12.6)!

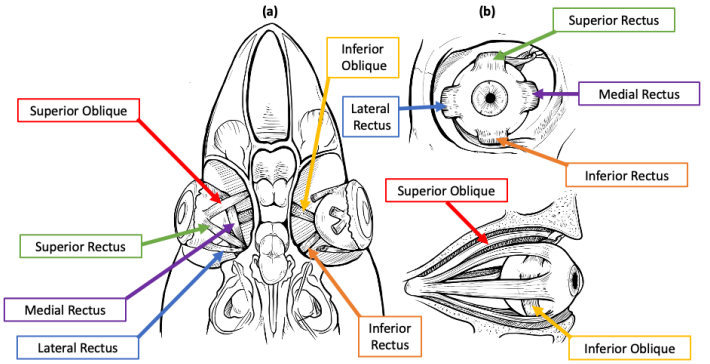

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Figure 12.6—Extrinsic ocular muscles of the (a) shark versus (b) human eye. Note the high degree of anatomical conservation across taxa.

Epibranchial Muscles

The next group of muscles is the epibranchial muscles, which are positioned dorsal to the gill pouches. These muscles are derived from the dorsal parts of the four head somites and are supplied by CN X (vagus nerve). Epibranchial muscles are typically fairly reduced; however, in some fishes and amphibians they contribute to the elevation of the braincase and upper jaw during feeding.

Hypobranchial Muscles

The third group of muscles sits ventral to the gill pouches and are called the **hypobranchial muscles**. The hypobranchials form from myoblasts that migrate ventrally from the somites and are supplied by CN XII (hypoglossial nerve). The hypobranchial muscles are a little more elaborated compared to the epibranchials. These muscles span from the shoulder girdle and sternum to the first and second gill arches and help depress the jaw and open the mouth in fishes. Often, the hypobranchials are classified based on their position relative to the hyoid:

1. **Prehyoid muscles**: muscles anterior to the hyoid arch
2. **Posthyoid muscles**: muscles posterior to the hyoid arch

The hypobranchial muscles become far more complex in tetrapods, because once tetrapods move onto land, their food isn't supported by the environment, and suction feeding is not possible (Table 12.1). As a result, food must be manipulated by muscles within the mouth to facilitate oral processing and transfer to the esophagus to continue through the digestive system (Chapter 13). One of these tetrapod hypobranchial muscles is a newly evolved structure: **the muscular tongue**. The **geniohyoideus** and **genioglossus** also contribute to moving the tongue within the oral

cavity in amphibians. The amniotes further subdivide the hypobranchial muscles, adding **hyoglossus**, **styloglossus**, and **lingualis** to the tongue musculature. In fact, the lingualis contributes the bulk of the tongue structure in mammals. In addition, we also find in mammals the **sternohyoideus**, **sternothyroideus**, **thyrohyoideus**, and **omohyoid** muscles, which contribute to the complex actions of the jaws and throat during swallowing.

Table 12.1—Hypobranchial muscle differentiation across select vertebrate taxa

Muscle group	Shark	Amphibian	Mammal
Prehyoid muscles	Coracomandibular	Genioglossus Geniohyoideus	Genioglossus Lingualis Geniohyoideus Hyoglossus Styloglossus
Posthyoid muscles	Rectus cervicis	Rectus cervicis Omarcuals Pectoriscapularis	Sternohyoideus Sternothyroideus Thyrohyoideus Omohyoid

Branchiomeric Muscles

The fourth group of head muscles are the actual muscles of the gill pouches themselves: the **branchiomeric muscles**. The muscles associated with the first two gill arches are specifically referred to as the **mandibular** muscles and **hyoid** muscles, respectively; the muscles of the remaining gill arches are simply referred to as the branchiomeric muscles. The mandibular muscles develop from somitomere 4 and are supplied by CN V (trigeminal nerve), whereas the hyoid muscles develop from somitomere 6 and are supplied by CN VII (facial nerve). The branchial muscles of the third arch are derived from somitomere 7 and supplied by CN IX

(glossopharyngeal nerve), and the muscles of the remaining gill arches are derived from head somites 1–4 and supplied by CN X (vagus nerve). Unlike some of the more conserved muscle groups we've already been introduced to, the branchiomeric muscles show tremendous diversification across vertebrate taxa (Table 12.2), and much of this diversification is related to changes in feeding and the loss of gills.

Table 12.2—Branchial muscle differentiation across select vertebrate taxa

Muscle group	Shark	Amphibian/ Reptile	Mammal
Mandibular muscles	Adductor mandibulae Levator palatoquadrati Spiracularis Preorbitalis Intermandibularis	Adductor mandibulae Levator pterygoidei Protractor pterygoidei – – Intermandibularis	Temporalis Masseter Pterygoideus Tensor tympani Tensor veli palati – – – Mylohyoideus Digastric (anterior belly)
Hyoid muscles	Levator hyomandibuli Dorsal constrictor Interhyoideus Ventral constrictor	– Depressor mandibuli Branchiohyoideus Interhyoideus Sphincter coli	Stapedius – Stylohyoid Digastric (posterior belly) Platysma Facial muscles
Branchiomeric muscles	Cucullaris Interarcuals Superficial constrictors	Cucullaris Levator arcuum – Dilator laryngis Subarcuals Transversi ventralis Depressors arcuum	Trapezius complex Sternocleidomastoid complex – Intrinsic larynx muscles Some pharyngeal muscles

So let's begin by considering how these muscles work in gilled critters. The muscles that are directly associated with the gills

are known as branchial muscles, and if you think about how gills work, there are two main things they need to do:

1. They need to be able to squeeze and expel water that comes in through the mouth (constrict). The function of constriction is accomplished by the superficial constrictor muscles, which are easily seen in the shark.
2. Then they need to reopen, so they can constrict again. This second function was ancestrally executed by a separate levator muscle for each arch. However, in modern taxa these separate levators have fused together to form a single muscle called the **cucullaris**. The cucullaris runs from the dorsal gill arches to the pectoral girdle, and this position is noteworthy because it shows that the branchiomeric muscles have attachments that reach all the way onto the appendages. In other words, even though these are "head muscles," they are not restricted solely to the head.

And as we examine tetrapod lineages that have lost gills, we find that the cucullaris has migrated and now expands over the limb to form two major muscle groups:

1. the **trapezius group** in the dorsal shoulder and
2. the **sternocleidomastoid group**, ventrally.

The trapezius and sternocleidomastoid groups act to move the arm and head, and even though they span the pectoral girdle, they are in fact still innervated by CN XI (accessory nerve). CN XI is a discrete branch of CN X that innervated the cucullaris, thus revealing clues to the evolutionary history of this muscle group.

The muscles associated with the mandibular and hyoid arches also become specialized for jaw function in the gnathostomes, as these arches are incorporated into the jaw skeleton. Recall from Chapter 8 that one of the major transformations of the tetrapod skull was **fenestration**, which

enabled the expansion of the jaw-closing muscles on the roof of the skull. Ancestrally, the mechanism for jaw closing was dominated by a single, large muscle called the **adductor mandibulae**. However, in mammals, the adductor mandibulae differentiates into several new, discrete muscles with different lines of action (i.e., they pull the jaw in different directions). These derivations include the **temporalis muscle**, which pulls the lower jaw dorsally and posteriorly; the **masseter muscle**, which pulls the lower jaw laterally; and the **pterygoid muscle**, which is deep to the others and pulls the lower jaw medially (Figure 12.7). Because all three of these muscles are associated with the mandibular arch, they're all innervated by the cranial nerve of that arch, which is CN V (trigeminal nerve). Mandibular muscles also contribute to the floor of the mouth by forming the **mylohyoid**, which runs between the mandibles. The **levator palatoquadrati** of the shark differentiates to form the **levator pterygoidei** and the **protractor pterygoidei** in vertebrates with mobile crania (e.g., amphibians, reptiles, and some birds); however, these muscles are lost in animals that lack kinetic skulls (e.g., mammals).

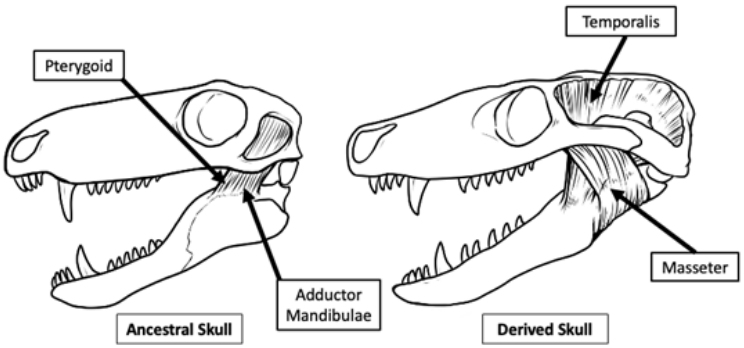

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Figure 12.7—Evolution of the jaw-closing musculature in ancestral (top) versus derived (bottom) synapsids.

Now jaw closing is certainly important, but in order to feed, the jaws have to open too. Ancestrally, jaw depression (which opens the mouth) was accomplished by hypobranchial muscles like the **coracomandibular** and **coracohyoid**. However, as noted above, throughout the course of evolution most of the hypobranchial muscles became involved with control of the tongue in tetrapods, especially mammals. Therefore, mammals needed a different jaw-opening mechanism and indeed evolved a new muscle called the **digastric**. The term *digastric* actually means "two bellies" (*di* = two; *gastric* = stomach) and this name reflects how the muscle is formed from two muscle bundles located between the dentary and mastoid process. The anterior portion of the digastric (**anterior belly**) is derived from the old **intermandibularis** muscle, which was located in the floor of the mouth of animals like sharks. The **posterior belly** is derived from the ancestral **interhyoideus** muscle, which was deep to the intermandibularis in the shark. The intermandibularis is associated with the mandibular arch, so like all the other mandibular arch muscles, it is innervated by the trigeminal nerve (CN V). But the interhyoideus is actually associated with the hyoid arch. And like other hyoid arch muscles, it is innervated by the facial nerve (CN VII). What this means is that the two parts of the digastric muscle are actually innervated by different cranial nerves: The anterior belly is innervated by CN V and the posterior belly by CN VII!

Other muscles of the hyoid arch are retained in mammals in the form of the **stapedius** and the **stylohyoid**. In fact, these are the only two hyoid muscles that retain a connection to the hyoid arch in this group! The remaining hyoid muscles lost their connection to the hyoid arch and instead spread out to become the muscles of the face and the **platysma**, which extends down the front of the neck.

Axial Muscles

The head muscles are considered muscles of the body axis and are therefore technically axial muscles. However, axial muscles are present along the entire length of the vertebrate body, so we must also consider the postcranial axial musculature. Ancestrally, the axial muscles were made up of segmental V-shaped myomeres; these myomeres were separated by myosepta and supplied by spinal nerves. The portions of the myomeres dorsal to the horizontal skeletogenous septum are referred to as **epaxial muscles**, and the portions positioned ventral to the horizontal skeletogenous septum are the **hypaxial muscles**. This myomeric organization is still seen in fishes today, in both the chondrichthyans and the osteichthyans. The muscles attach to the axial skeleton or the notochord, and contractions of axial muscles produce lateral undulations along the length. These undulations exert force on the surrounding water, and the resulting reaction forces produce thrust, resulting in forward swimming of the fish. In fishes, the majority of axial muscle mass is white muscle, which you'll recall from Chapter 11 is composed of fast-glycolytic (FG) fibers (Chapter 11), which contract quickly (but also fatigue rapidly). These rapid-response muscles are excellent for generating propulsion quickly and hence used for escapes.

In tetrapods, however, the major evolutionary trend is toward a decrease in epaxial muscles and increase in hypaxial muscle differentiation (Table 12.3). For example, the hypaxial muscles of the tetrapod's body wall show several distinct lateral layers of muscle. From superficial to deep, these layers form the **external oblique**, **internal oblique**, and **transverse abdominis** muscles, and each of these layers exhibits different fiber angles that bend the trunk in different directions (Figure 12.8). Furthermore, there's also a ventral layer with fibers that run parallel to the body axis: This muscle is called the **rectus abdominis**. Over the course of tetrapod evolution, as animals shift from using sprawling to more upright postures, the hypaxial muscles came to play a reduced role

in trunk stabilization and lateral bending. Instead, these muscles begin to play a greater role in changing the volume of the chest cavity, thus driving the inspiration and expiration of breathing. In fact, the major respiratory muscle of mammals, the diaphragm, is a derivative of the rectus abdominis muscle!

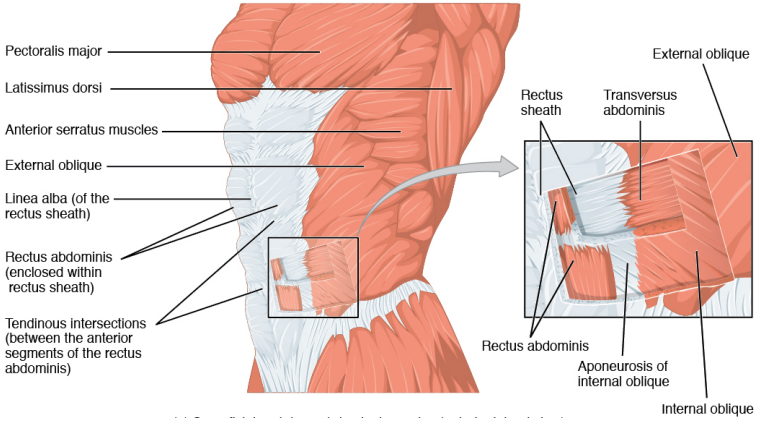

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Figure 12.8—Layers of the body wall, showing fiber orientations of the external oblique, internal oblique, and transverse abdominis muscles.

Table 12.3—Axial muscle differentiation across select vertebrate taxa

Muscle group	Shark	Amphibian	Mammal
Epaxial muscles	Epaxial myomeres	Interspinalis Dorsalis trunci	Interspinalis Intertransversarii Occipitalis Multifidi Spinalis Semispinalis Longissimus dorsi Splenius Iliocostalis
Hypaxial muscles	Hypaxial myomeres	Subvertebralis Levator scapulae Thoraciscapularis External oblique Internal oblique Transversus Rectus abdominis	Longus coli Psoas major Quadratus lumborum Omotransversarius Serratus ventralis Serratus ventralis Rhomboideus Serratus dorsalis Scalenus Rectus thoracis External oblique External intercostals Internal oblique Internal intercostals Transversus abdominis Transversus thoracis Diaphragm Rectus abdominis

Appendicular Muscles

The last major portion of the muscular system includes the **appendicular muscles**. These are the muscles of the limbs and fins and are derived from extensions of myotomes called **limb buds**.

These muscles can also be subdivided into two major groups: **dorsal** and **ventral**. As with the epaxial and hypaxial muscles of the body axis, the appendicular muscles are innervated by segmental spinal nerves.

We've already learned that some of the muscles that act on the forelimb are actually derived from the **branchiomeric muscles** (the trapezius group and the sternocleidomastoid group—see Table 12.2). However, there are also a large number of appendicular muscles that are intrinsic to the appendages themselves. The ancestral organization of the appendicular musculature was very simple, and this can still be observed in sharks and bony fishes. This organization basically consisted of a major dorsal muscle mass called the **extensor** or **abductor** and a ventral muscle mass called the **flexor** or **adductor**. Tetrapods, in contrast, show considerable differentiation of their limb muscles; nonetheless, the basic organization of these muscles can still be traced to the ancestral flexor/extensor groups seen in fishes (Table 12.4).

Many of the differences in the appendicular musculature that we observe in nonfishy vertebrates are related to postural changes in different taxonomic groups. With the shift from sprawling posture (common in amphibians and reptiles) to a more upright posture (common in birds and mammals), the ventral adductors tend to be reduced. In contrast, the dorsal extensors are usually enhanced. Across taxa, there is a general trend toward larger proximal muscles and smaller distal muscles. This pattern is largely related to energetic principles of locomotion. It costs more energy to carry a large mass farther from you than closer to you. Consider walking to class with your backpack—if you carried your bag in your hand, extended away from your body, this would be far more energetically taxing than carrying it slung over your shoulder. So as animals became more upright and longer limbed, there was a distinct energetic advantage to reducing the muscle mass of the distal limbs and shifting those heavy muscles as close to the trunk as possible. But if the muscles are all located close to the trunk, how are the distal limbs moved? Well, the muscles that control the

movement of the distal limb are attached to the distal bones by long, light tendons; this allows the muscles to control the movement of these elements while also keeping the distal limb lightweight.

Table 12.4—Appendicular muscle differentiation across select vertebrate taxa

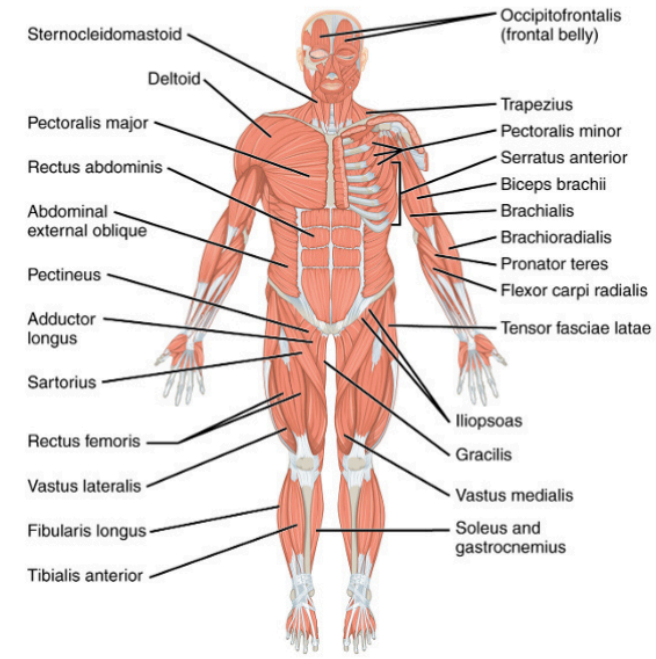
Muscle group	Shark	Reptile	Mammal
Dorsal group	Pectoral extensor Pelvic extensor	Latissimus dorsi Subcoracoscalpularis Deltoid Scapulohumeralis anterior Triceps brachii Antebrachial extensors Subvertebralis Puboischiofemoralis internus Iliotibialis Femorotibialis Ambiens Iliofemoralis Tibialis anterior Extensor digitorum communis Peroneus longus Peroneus brevis Extensor digitorum brevis	Cutaneous trunci Latissimus dorsi Teres major Subscapularis Deltoid complex Teres minor Triceps brachii Tensor fasciae antebrachii Antebrachial extensors Psoas minor Psoas major Iliacus Pectineus Rectus femoris Vasti Sartorius Tensor fascia latae Gluteus minimus Gluteus medius Pyriformis Tibialis anterior Extensor digitorum longus Extensor hallucis longus Peroneus tertius Peroneus longus Peroneus brevis Extensor digitorum brevis

Muscle group	Shark	Reptile	Mammal
Ventral group	Pectoral flexor Pelvic flexor	Pectoralis Supracoracoideus Biceps brachii Brachialis Coracobrachialis Antebrachial flexors Puboischiofemoralis externus Adductor femoris Pubotibialis Caudofemoralis Flexor tibialis externus Flexor tibialis internus II Flexor tibialis internus I Puboischiotibialis Gastrocnemius internus Gastrocnemius externus	Cutaneous trunci Pectoralis complex Supraspinatus Infraspinatus Biceps brachii Brachialis Coracobrachialis Antebrachial flexors Obturator externus Quadratus femoris Adductor femoris brevis Adductor femoris longus Caudofemoralis Dorsal semitendinosus Ventral semitendinosus Biceps femoris Semimembranosus Gracilis Gastrocnemius medialis Flexor hallucis longus Gastrocnemius lateralis Soleus Plantaris

12.6 Human Muscles: A Bipedal Tetrapod

Given our shared evolutionary history as mammalian tetrapods, humans' musculature very closely resembles the musculature of other mammals. This is one reason that animals like cats, rabbits, and minks can be used as dissection stand-ins for human cadavers during anatomy labs—even in strictly human-based anatomy

courses. However, it is worth noting that most mammal species are **quadrupeds** (meaning they move about on all four limbs), whereas humans are **bipedal** tetrapods (walking about on only two limbs). As a result, human bodies do undergo some muscular reconfiguration in order to accommodate bipedal posture and locomotion; these changes are particularly noticeable in the pelvic region and can help explain some common human muscle pathologies (Figure 12.9).


Sternocleidomastoid
Deltoid
Pectoralis major
Rectus abdominis
Abdominal external oblique
Pectineus
Adductor longus
Sartorius
Rectus femoris
Vastus lateralis
Fibularis longus
Tibialis anterior
Occipitofrontalis (frontal belly)
Trapezius
Pectoralis minor
Serratus anterior
Biceps brachii
Brachialis
Brachioradialis
Pronator teres
Flexor carpi radialis
Tensor fasciae latae
Iliopsoas
Gracilis
Vastus medialis
Soleus and gastrocnemius

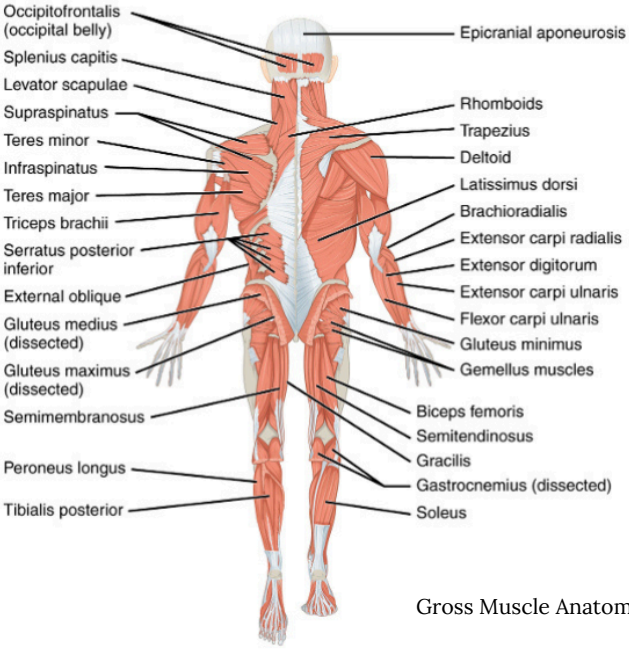

Occipitofrontalis (occipital belly)
Splenius capitis
Levator scapulae
Supraspinatus
Teres minor
Infraspinatus
Teres major
Triceps brachii
Serratus posterior inferior
External oblique
Gluteus medius (dissected)
Gluteus maximus (dissected)
Semimembranosus
Peroneus longus
Tibialis posterior
Epicranial aponeurosis
Rhomboids
Trapezius
Deltoid
Latissimus dorsi
Brachioradialis
Extensor carpi radialis
Extensor digitorum
Extensor carpi ulnaris
Flexor carpi ulnaris
Gluteus minimus
Gemellus muscles
Biceps femoris
Semitendinosus
Gracilis
Gastrocnemius (dissected)
Soleus

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Figure 12.9—Human muscular anatomy.

The **piriformis** muscle is a deep muscle of the gluteal region that functions to laterally rotate the thigh in humans. It originates from the sacrum, passes through the greater sciatic foramen of the pelvis, and inserts on the greater trochanter of the femur. This muscle sits in very close proximity to the sciatic nerve, which also passes through the greater sciatic foramen (Figure 12.10). Hypertrophy or inflammation of the piriformis muscle can cause the muscle to compress the sciatic nerve and result in pain, tingling, or numbness in the gluteal region, in the posterior thigh, and sometimes even down the calf and to the foot. This condition is known as **piriformis syndrome**.

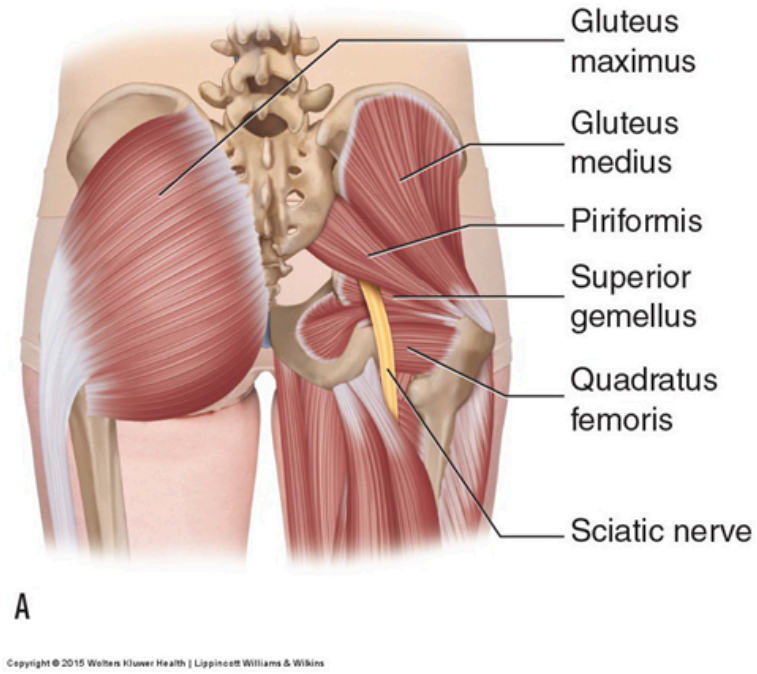

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Figure 12.10—Human pelvic musculature. Notice the relative position of the piriformis muscle and its proximity to the sciatic nerve.

The **iliopsoas** is actually composed of two thigh muscles: **psoas major** and **iliacus** (Figure 12.11). Both of these muscles insert on the lesser trochanter of the femur, but they have different origins. Psoas major originates in the lower back (from the 12th thoracic vertebra to the 5th lumbar vertebra), whereas iliacus originates from the iliac fossa of the pelvis. These muscles function together as a unit to flex the thigh. Psoas major can also laterally flex the vertebral column when the hip joint is held stationary; this is an example of how a muscle's action can change when its origin and insertion are swapped. However, overuse of the iliopsoas through

excessive repetitive flexion can lead to injury or inflammation of the muscle and/or its inserting tendon, leading to **iliopsoas syndrome**. Because the condition is often associated with dancers and high jumpers, it is also sometimes called dancer's hip or jumper's hip. Iliopsoas syndrome can cause lower back pain as well as pain in the hip or groin region, depending on the specific part of the iliopsoas muscle affected.

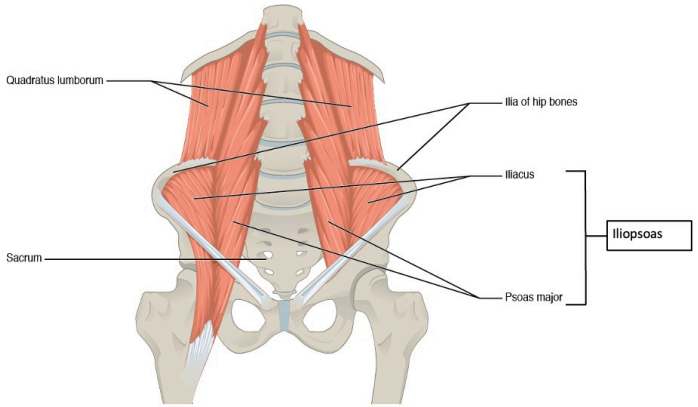

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Figure 12.11—Iliopsoas anatomy. Notice the origin of the psoas muscle on the lumbar vertebrae.

If you have an interest in exercise or physical fitness, you may already be familiar with the **rectus abdominis** muscle. Rectus abdominis is a superficial muscle that runs the length of the ventral abdomen (Figure 12.9). This muscle originates from the xiphoid process of the sternum and the inferior surfaces of ribs 5–7; it inserts on the superior pubis. The rectus abdominis is the muscle that is activated when flexing the trunk anteriorly, such as during crunches or sit-ups. This muscle is characterized by having tendinous insertions along its length, which separate sections of the muscle into smaller "blocks" that are sometimes visible under the

surface of the skin—these are what we commonly refer to as "six-pack abs"!

12.7 Summary

The muscular system really drives the movement of the vertebrate body—both in the sense of moving an animal through its environment and in moving materials through the animal's body. The properties of muscle tissue, along with its structure at both the microanatomic and macroanatomic scales, are intimately tied to muscle function. Furthermore, the muscular system is intimately linked with both the skeletal system, as they work together to give the body structure and movement, and the nervous system, which generates the stimulus for muscle contraction. Vertebrate muscles can be organized into six functional groups, and although some of these groups are highly conserved across vertebrate taxa (e.g., extrinsic eye muscles), others show incredible diversification related to environmental and postural changes associated with tetrapod evolution (e.g., appendicular muscles). Nonetheless, muscle origins can be traced through evolutionary history through a combined examination of muscle development and innervation patterns.

Application Questions

1. How might the abdominal muscles be impacted

during pregnancy? Would a C-section damage the ability of these muscles to function?

2. Back pain is a common condition that millions of people suffer from. What muscles are involved in lower back pain, and how can it be remedied?
3. Many different vertebrates use muscles to generate electrical signals to communicate and incapacitate. How are they able to do this?

12.8 Further Reading

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13. Digestive System

SAMANTHA C. LEIGH AND DONOVAN P. GERMAN

Focus Questions—to Guide Your Reading of This Chapter

1. How do the morphology and organization of digestive organs, such as the stomach and intestines, differ among vertebrates, and how does this impact digestive efficiency? Are all herbivores the same?
2. How do vertebrates adapt their digestive systems to process diverse diets, ranging from plant matter to animal tissue?
3. What are the key structural adaptations in the digestive tracts of vertebrates, and how do they reflect dietary preferences and ecological niches?
4. What are the roles of digestive enzymes and secretions in breaking down macromolecules into absorbable nutrients, and how do they vary across vertebrate species?
5. What insights can comparative digestive anatomy provide into the evolutionary relationships and ecological interactions among vertebrate taxa?

13.1 Introduction

The digestive system is a fundamental physiological system present in all vertebrates, encompassing a series of specialized organs and tissues tasked with the acquisition, processing, and absorption of nutrients essential for growth, maintenance, and energy production. From the smallest fish to the largest mammals, vertebrates exhibit an astonishing diversity of dietary preferences and feeding strategies, each accompanied by anatomical and physiological adaptations within the digestive tract.

At its core, the vertebrate digestive system follows a general blueprint, comprising a sequence of interconnected compartments and tubes, including the **oral cavity**, **pharynx**, **esophagus**, **foregut chamber** (if present), **stomach** (if present), **small intestine**, **large intestine/colon**, **hindgut caecum** (if present), **rectum**, and **anus** (Figure 13.1). Associated accessory organs such as the salivary glands, liver, gallbladder, and pancreas contribute additional digestive secretions and enzymes essential for efficient nutrient processing. The complexity and versatility of the vertebrate digestive system reflect the diverse ecological niches occupied by different species as well as the evolutionary pressures imposed by varying dietary regimes. Carnivorous vertebrates, for example, are equipped with adaptations geared toward the intake of sometimes large amounts of protein and the rapid breakdown and assimilation of proteins and fats (Figure 13.1). These adaptations often include relatively short digestive tracts optimized for swift digestion and absorption of nutrients as well as specialized enzymes and gastric secretions tailored to protein-rich diets. Conversely, herbivorous vertebrates face the challenge of extracting nutrients from plant material, which can be structurally complex, low in protein and lipids, and difficult to digest. To overcome this challenge, herbivores have evolved elaborate digestive strategies, some including physical breakdown of plant cell walls and others including prolonged retention times in the digestive tract, extensive

microbial fermentation chambers, and symbiotic relationships with microbial communities capable of breaking down plant material (e.g., cellulose) that is otherwise indigestible by the animal's own digestive enzymes. These adaptations allow herbivores to extract maximal nutritional value from plant material and thrive on diets rich in fiber and carbohydrates (Figure 13.1).

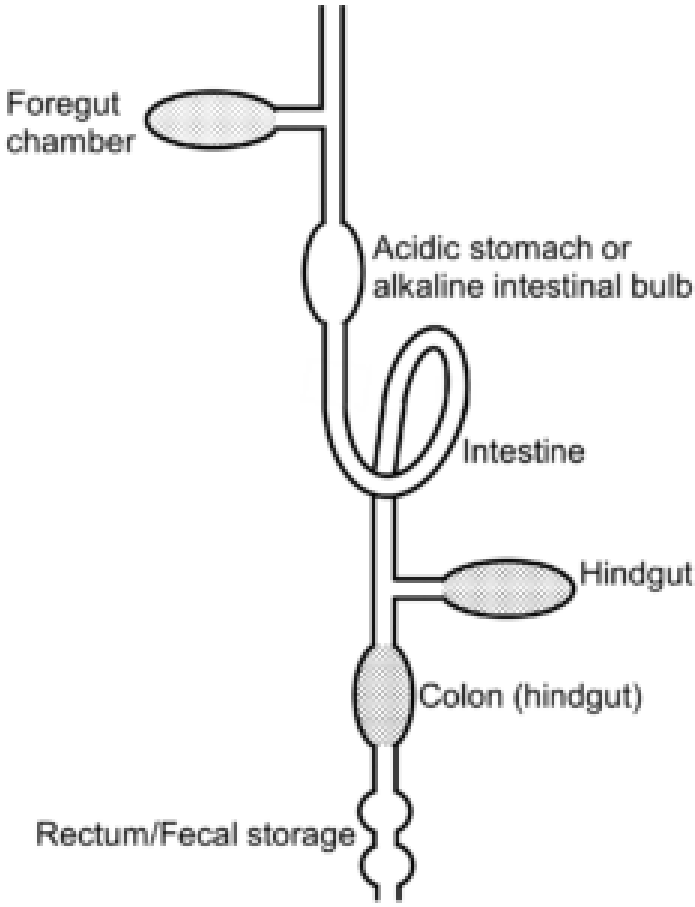

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Figure 13.1—Basic design of a vertebrate gut. Most animals have some form of an intestine but vary as to whether they possess other compartments such as crop, foregut chamber, stomach, or caecum. As a general rule, catalytic enzymatic reactions occur in the intestine, whereas microbial fermentation can occur in the foregut, caecum, and large intestine / colon. Foregut fermentation is known to occur in four major clades of mammals and in at least one avian species (the hoatzin). Hindgut fermentation, in either the caecum or large intestine/colon, occurs in many clades of mammals, birds, reptiles, and fishes. Modified from German and Rose (2021).

Furthermore, the vertebrate digestive system is intimately

intertwined with other physiological systems, including the circulatory, respiratory, and endocrine systems. The circulatory system, for instance, transports absorbed nutrients from the digestive tract to tissues throughout the body, ensuring a constant supply of energy and building blocks for growth and repair. Hormones secreted by the endocrine system regulate various aspects of digestion, including enzyme secretion, gut motility, and appetite control, orchestrating physiological processes to maintain metabolic homeostasis.

Despite the remarkable diversity of vertebrate species and their dietary preferences, certain fundamental principles of digestive physiology remain conserved across taxa. The sequential processing of food through the digestive tract, the enzymatic breakdown of complex nutrients into absorbable molecules, and the selective absorption of essential nutrients while eliminating waste products are universal features of vertebrate digestion. By studying the comparative anatomy and function of the vertebrate digestive system, researchers gain valuable insights into the evolutionary history of organisms, the ecological interactions shaping biodiversity, and the remarkable adaptability of life in its myriad forms.

13.2 Overview of the Vertebrate Digestive System

The **oral cavity** serves as the entry point for food and the initial site of mechanical and chemical digestion. Most extant vertebrates have jaws (with hagfishes and lampreys providing the exceptions), and those jaws can have extensive dentition. **Teeth** are specialized structures composed of enamel, dentin, and cementum that mechanically break down food into smaller, more manageable pieces through the process of mastication. Different types of teeth—such as incisors, canines, premolars, and molars—are adapted for cutting, tearing, and grinding food depending on the

diet of the organism (see Section 8.6). In many fishes, the dentition associated with the oral jaws is limited to grasping prey, as most fish do not chew in the oral cavity. Fishes also possess pharyngeal jaws in their pharynx (see Section 8.5) that can have extensive teeth and grinding plates, where food is masticated.

Many vertebrates have a **tongue** or something similar, which is a muscular organ covered with papillae that assists in the manipulation of food during chewing and helps propel the food bolus (i.e., a semisolid mass of food) toward the pharynx for swallowing. Taste buds located on the surface of the tongue detect various flavors, allowing organisms to assess the palatability of food items. Many fishes have taste buds on their body.

Mammals and some other tetrapod groups have **oral glands** that secrete mucus (and in the case of mammals, **saliva**) into the oral cavity. Saliva is composed primarily of water, electrolytes, mucus, and enzymes such as salivary amylase. Salivary amylase begins the chemical breakdown of starches into maltose, a disaccharide composed of two glucose molecules. Because they live in water, the most speciose vertebrate group (fishes) does not produce saliva.

The **esophagus** is a muscular tube that connects the pharynx to the stomach (if present) and serves as a conduit for the passage of food from the oral cavity to the stomach (Figure 13.1). **Peristaltic contractions**, coordinated waves of muscular contraction and relaxation, propel the food bolus downward through the esophagus toward the alimentary canal. The esophagus contains two specialized sphincter muscles: the **upper esophageal sphincter** (**UES**) at the junction with the pharynx and the **lower esophageal sphincter** (**LES**) at the junction with the stomach. The UES prevents air from entering the esophagus during respiration, while the LES prevents the reflux of stomach contents into the esophagus.

Many bird species have a **crop**, which is a small pouch that precedes the stomach (Figure 13.1). The crop can serve as a space for food storage and releases its contents into the stomach, allowing the animal to ingest a relatively large amount of food and

move to another location quickly to engage more in the digestive process. In some species, like pigeons, the crop can secrete milk-like fluids that can be fed to juveniles instead of food acquired from the environment. Moreover, in one bird species called the hoatzin, the crop has been modified into a foregut fermentation chamber, which facilitates the breakdown of hard-to-digest foods like plants.

The **stomach** is a muscular organ located in the upper abdomen, primarily responsible for the storage, mixing, and initial digestion of food (Figures 13.1 and 13.2). Stomachs have four main tissue layers, from internal to external: mucosa, submucosa, muscularis, and serosa (Figure 13.2). In mammals, the stomach consists of three main regions: the **fundus**, **antrum**, and **pylorus** (Figures 13.2 and 13.3A). **Gastric glands** within the mucosa of the stomach secrete **gastric juice**, a mixture of hydrochloric acid (HCl), **pepsinogen**, mucus, and **intrinsic factor** (a protein that helps with vitamin B_{12} absorption in the intestine). Hydrochloric acid creates an acidic environment (pH 1.5–3.5) in the stomach, which activates pepsinogen to its active form, **pepsin**. Pepsin catalyzes the hydrolysis of peptide bonds in proteins, breaking them down into smaller peptides. The submucosa largely supports the mucosa and connects it to the muscularis. The stomach's muscular walls (muscularis layer) contract rhythmically, mixing food with gastric juices to form a semifluid mixture called **chyme**. The stomach also acts as a temporary reservoir for food storage, regulating the rate at which chyme is released into the small intestine for further digestion and absorption. In birds, reptiles, and some fishes, a gizzard-like stomach can also **triturate** food, reducing particle size by vigorous muscular contractions, causing significant mixing. This process is sometimes aided by the presence of **gastroliths**, ingested sand or gravel against which stomach material is ground. Nearly 8,000 species of fishes, as well as platypus and echidna among the mammals, have lost the gastric stomach as part of their digestive system. They have an esophagus that empties either straight into the intestine or into an **intestinal bulb** (e.g., parrotfishes). These species eat a wide array of foods, including carnivorous diets, and

function without a stomach. Many species of fish have what are called **pyloric caeca** immediately following the stomach, which are blind sacs protruding from the intestine and really are an extension of the intestine in terms of function.

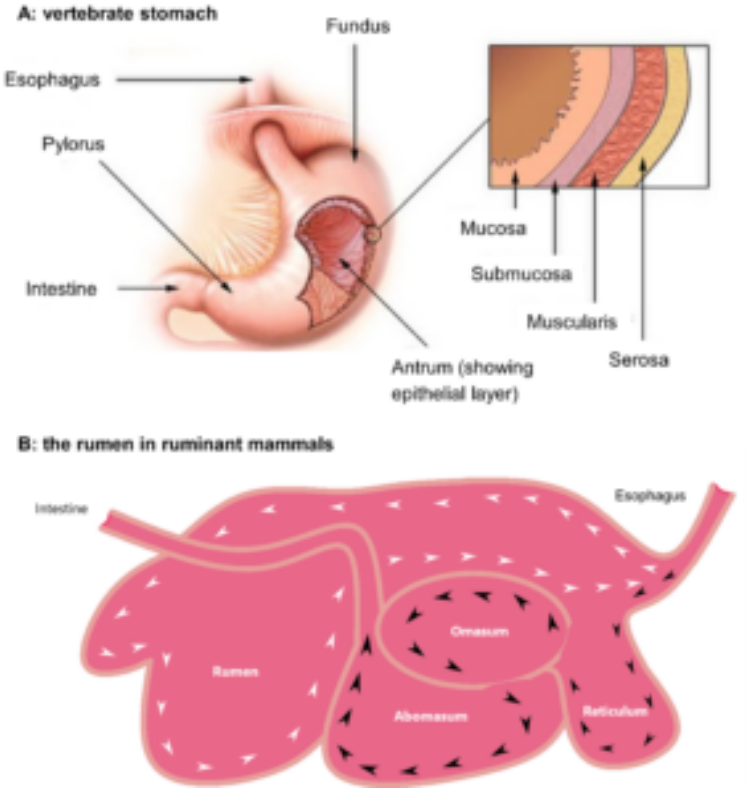

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Figure 13.2—A: Illustration of a mammalian (vertebrate) stomach with the inset showing the tissue layers of mucosa, submucosa, muscularis, and serosa. B: Illustration of the rumen in a ruminant mammal (e.g., a cow). Ingesta enters the rumen via the esophagus. When first ingested, most grass particles are buoyant and follow the white arrows, circulating through the rumen, where contact with microorganisms and their enzymes will digest the grass. As particles become smaller and denser (black arrows), they eventually sink into the reticulorumen (reticulum) and are channeled into the omasum, followed by the abomasum (stomach). Microorganisms also escape the rumen into the omasum and abomasum, where they are digested by the host's enzymes. Microorganisms are the protein sources for ruminants, which by nature consume a low-protein diet.

The **small intestine** is the longest section of the digestive tract and, like the stomach, has the four tissue layers of mucosa, submucosa, muscularis, and serosa (Figures 13.4 and 13.5). In mammals, the small intestine is divided into three segments noted as the **duodenum**, **jejunum**, and **ileum**. However, these terms have specific anatomical meanings in mammals that do not translate directly to other vertebrates; hence, these terms are not universal. The small intestine (or just intestine) plays a critical role in the absorption of nutrients from digested food. Upon entering the proximal intestine (duodenum in mammals), chyme is mixed with bile from the liver and secretions from the pancreas (including buffer and enzymes) and is now called **digesta** (see below for more information on the liver and pancreas). The inner surface of the small intestine is highly specialized for nutrient absorption, characterized by numerous finger-like projections called **villi** (or folds; singular: villus) and **microvilli** (Figures 13.4 and 13.5). Note that the term *villus* is a mammalian term, whereas many other vertebrates have intestinal folds and not true villi. Villi increase the surface area available for absorption, while microvilli, composing the highly convoluted membrane of epithelial cells, further enhance nutrient uptake by increasing the absorptive surface area by orders of magnitude (Figures 13.4 and 13.5). Absorbed nutrients—including amino acids, fatty acids, monosaccharides, vitamins, and minerals—are transported across the epithelial cells of the villi into the bloodstream or lymphatic system for distribution throughout the body (Figure 13.4).

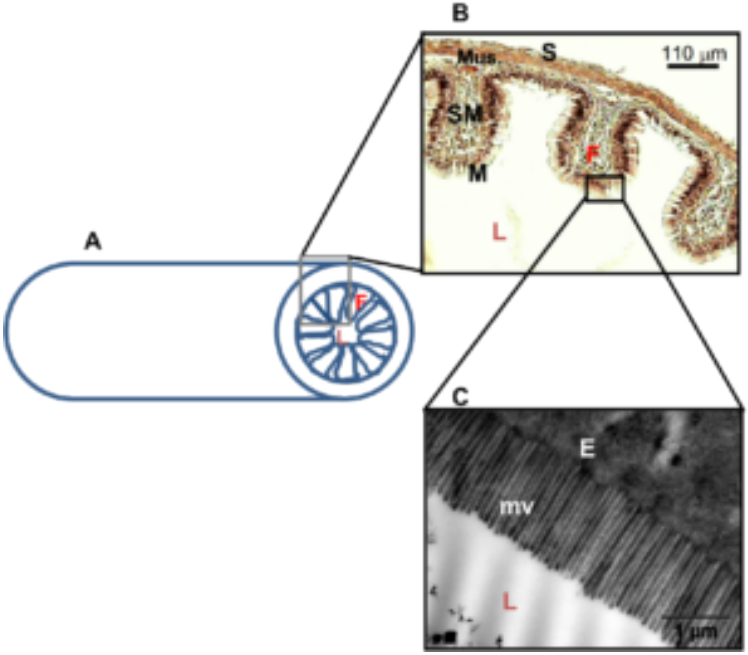

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Figure 13.3—General structure of an intestine. Organism: Pterygoplichthys disjunctivus (armored catfish) A: Illustration of a cross section of an intestine showing the intestinal folds along the mucosal lining of the intestine that increase the surface area. B: Light micrograph of a histological preparation of the midintestine of Pt. disjunctivus showing the intestinal folds. Enterocytes are visible lining the fold. C: Transmission electron micrograph of the microvilli on the surface of epithelial cells of the midintestine of Pt. disjunctivus. The microvilli further increase the surface area of the intestine. E = enterocyte; F = intestinal fold; L = lumen of intestine; M = mucosal layer composing the intestinal epithelium; Mus. = muscularis; mv = microvilli; S = serosa; SM = submucosa. Modified from German and Herrera (2024).

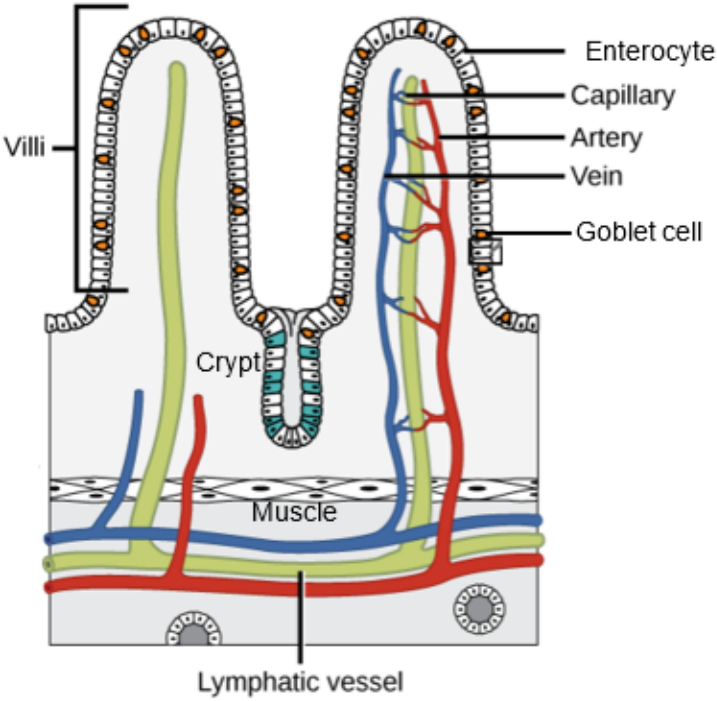

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Figure 13.4—General anatomy of an intestine, showing two villi (folds). Note that each villus is covered in enterocytes (the main absorptive cells of the intestine), and goblet cells, which secrete mucus to protect the epithelial cells. These cells are first synthesized in the crypt and migrate up the villus until they reach the tip. Once they reach the tip, they are typically sloughed off by those cells pushing up from below, and thus, enterocytes only have a life of approximately one week. There are also endocrine cells in the crypt (blue). The same tissue layers shown in Figure 13.2 are also visible here, with the enterocyte cells forming the mucosa, the underlying blood and lymph tissue the submucosa, the muscle cells the muscularis, and the basement membrane the serosa.

The **large intestine** (also called the colon, distal intestine, or hindgut, depending on the animal group) has many functions, including water and electrolyte absorption, formation of feces, and

housing of the gut microbiota (Figures 13.1 and 13.2). As digesta passes through the large intestine, the absorption of water and electrolytes forms more solid waste material. The large intestine also provides a habitat for a diverse microbial community known as the **gut microbiota**, which can reside in the hindgut itself or primarily in the **caecum** (plural: caeca), when present (Figures 13.1 and 13.2). Note that the human appendix is the remnant of what was likely a larger caecum (as in chimpanzees) before humans started cooking their food, essentially predigesting it before consuming it. Symbiotic microorganisms in the hindgut and/or caecum play essential roles in fermentation of indigestible carbohydrates, synthesis of vitamins (e.g., vitamin K, B vitamins) and amino acids (e.g., lysine), and modulation of immune function. Fecal matter is stored in the rectum until it is eliminated through the anus during defecation, a process controlled by the relaxation of the internal anal sphincter and voluntary contraction of the external anal sphincter.

Chondrichthyans and some nonteleost fishes have a **spiral intestine** in the latter part of the intestine. This is a specialized segment of the intestine with regions that are analogous to the small intestine and large intestine of other vertebrates. The morphologies of the spiral intestine come in four main forms: an actual spiral that resembles a spiral staircase, a scroll valve that mimics a rolled scroll of paper with epithelial surface on both sides, funnels oriented anteriorly, and funnels oriented posteriorly. This segment of the intestine is absorptive, with elevated surface area, intestinal enzymatic activities, and nutrient transporters. There is also evidence that the flow of material slows in the spiral intestine, thus allowing for efficient digestion and absorption.

The **pancreas**, **liver**, **gallbladder**, and (when present) **salivary glands** are accessory organs that contribute essential digestive secretions and enzymes to the digestive process. We have already discussed salivary glands, so now we will focus on the other accessory organs. You can also find more information about the

pancreas in Chapter 21 and about the specific enzymes in Section 13.4.

The pancreas, located behind the stomach in those animals with a discernible pancreas, secretes **pancreatic enzymes**—amylase, lipase, and several proteases such as trypsin and chymotrypsin—into the proximal small intestine to aid in the digestion of carbohydrates, lipids, and proteins. The pancreas also secretes bicarbonate ions to neutralize acidic chyme entering the intestine from the stomach, creating a more favorable environment for enzyme activity. Most fish species lack a true pancreas and instead have acinar and islet cells distributed among liver tissue and along the intestine.

The liver, the largest internal organ in vertebrates, contains cells called hepatocytes that secrete **bile**, a complex fluid containing bile salts, cholesterol, and bilirubin. Bile is stored and concentrated in the **gallbladder** before being released into the proximal intestine to emulsify lipids for digestion and absorption. Just like they lack other organs, many fish species lack a gallbladder and simply secrete bile straight from the liver into the intestine. Bile salts tend to be reabsorbed in the large intestine (hindgut), where their status as conjugates can act as a feedback to the liver on the physiological state of the animal.

By coordinating the functions of these specialized organs and structures, the vertebrate digestive system efficiently breaks down ingested food into absorbable nutrients while facilitating the elimination of waste products, ensuring the organism's survival and well-being.

13.3 Structural Adaptations for Digestion

The vertebrate digestive system exhibits remarkable structural adaptations tailored to the dietary preferences and ecological niches of different species. These adaptations include modifications

in organ size, shape, and functionality, as well as the presence of specialized structures optimized for specific digestive processes.

Carnivorous vertebrates tend to share many similarities in their general digestive tract structure. For instance, domestic cat (*Felis catus*), gray wolf (*Canis lupus*), monitor lizards, and carnivorous fish (e.g., tuna) all have digestive systems optimized for processing animal-based diets rich in protein and fats. Their digestive tracts typically exhibit relatively short lengths compared to herbivores, reflecting the carnivores' processing of relatively digestible animal tissues. The stomach of carnivorous vertebrates tends to be highly acidic, with a low-pH environment maintained by the secretion of hydrochloric acid (HCl) from gastric glands. This acidic environment aids in the denaturation of proteins and the activation of pepsinogen to pepsin, facilitating the breakdown of proteins into peptides. Carnivorous vertebrates tend to be efficient at metabolically processing low-carbohydrate diets. Many also possess well-developed teeth (e.g., canines) for gripping and tearing flesh, and mammals can also have sharp molars for shearing and crushing bones. Some carnivorous fishes have specialized pharyngeal tooth plates for crushing and grinding animal material, including pulverizing molluscan shells. These specialized dentitions allow carnivores to efficiently process meat and bone tissues, extracting essential nutrients while discarding indigestible materials.

Herbivorous vertebrates, such as horse (*Equus caballus*), cattle (*Bos taurus*), the hoatzin (*Ophisthocomus hoazin*), green iguana (*Iguana iguana*), and sea chub fish (*Kyphosus* sp.), have evolved digestive systems specialized for the consumption and digestion of plant-based diets rich in fiber and complex carbohydrates (Figure 13.1). Their digestive tracts often feature elongated intestines and enlarged microbial fermentation chambers (foregut for the cow and hoatzin, hindgut for the others; Figures 13.1–13.2), facilitating the breakdown of otherwise indigestible plant material (including cellulose in some and mannitol in others). Foregut fermenters, including the ruminants like cows and deer

and the hoatzin (an avian herbivore), possess a foregut fermentation chamber. In the ruminants, the foregut is divided into a **reticulorumen**, **rumen**, **omasum**, and **abomasum** (Figure 13.2). The reticulorumen and rumen are the main locations of microbial symbionts that digest plant structural polysaccharides and other complex carbohydrates under anaerobic conditions, producing short chain fatty acids (SCFAs, like acetate, propionate, butyrate), which can be absorbed and utilized as energy sources by the host animal. Only the abomasum is a true "stomach" that produces hydrochloric acid and pepsinogen, digesting the proteins produced by the microbes inhabiting the reticulorumen and rumen. Thus, it is a misnomer to state that ruminants have a "four-chambered stomach"; they only have one stomach (abomasum), and the remainder of the foregut is specialized for fermentation. The hoatzin independently evolved an enlarged **crop** that is a foregut fermentation chamber where microbes digest the fibrous compounds of plant leaves, producing SCFAs for the host. The crop of the hoatzin is so large that the bird has reduced flight muscles and cannot fly for long distances. Other mammalian groups (some kangaroos, sloths) have foregut fermentation chambers. Hindgut fermenters have large caeca and or distal intestines (**colon**) where microbial fermentation occurs (Figure 13.1). These fermentation chambers allow hindgut fermenters to extract additional nutrients from plant material that they could not obtain with their own digestive enzymes. There are no known foregut fermenting fishes, so those that do rely on microbial fermentation are hindgut fermenters.

It is noteworthy that not all vertebrate herbivores have symbionts to aid in the digestion of plant material. The giant panda (*Ailuropoda melanoleuca*) provides a perfect example of an animal that consumes fibrous plant material (bamboo) but does not digest much of the fiber with the aid of microbial symbionts. Instead, giant pandas are considered macronutrient carnivores that target protein from the ingested plant material. The low protein content of the panda diet requires that they eat copious amounts of bamboo to

meet their nutritional needs, but they pass this material relatively quickly through an elongated intestine, where protein and soluble carbohydrates are efficiently digested. Many herbivorous fishes, including grass carp, minnows, and armored catfishes, also take this approach of high intake of low-protein food, efficient digestion of protein and soluble carbohydrates, and little reliance on microbes to ferment plant material in their guts.

Omnivorous vertebrates, such as humans (*Homo sapiens*) and brown bear (*Ursus arctos*), and numerous bird, reptile, and fish species, consume a diverse diet consisting of both plant and animal matter. Their digestive systems exhibit a combination of adaptations suited for processing a wide range of food types and tend to be more of intermediate length (Figures 13.1 and 13.5). The human digestive system, for example, features an acidic stomach environment optimized for the digestion of both proteins (e.g., meat) and carbohydrates (e.g., plant-based foods). The small intestine, with its extensive surface area provided by villi and microvilli (Figures 13.3 and 13.4), efficiently absorbs nutrients from diverse food sources, including amino acids, fatty acids, sugars, and vitamins. Some omnivores still enlist the aid of microbial symbionts to aid in the digestion of plant material, whereas others do not. However, any of the features that are relatively extreme in carnivores or herbivores are not as pronounced in omnivores (Figure 13.5).

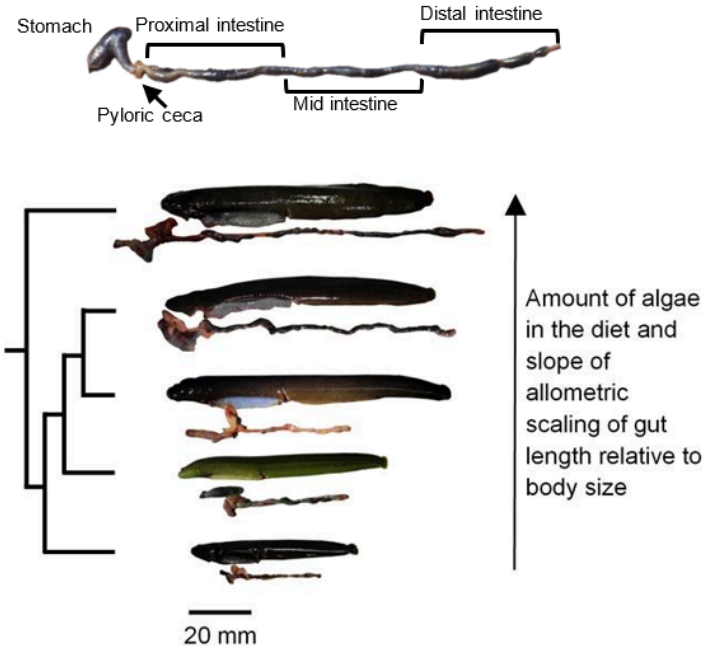

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Figure 13.5—Top: The digestive system of the fish Cebidichthys violaceus (family Stichaeidae) showing the stomach, pyloric caeca, and intestine. Bottom: Trimmed phylogeny and photos of fishes (with their unraveled guts beneath them) in the family Stichaeidae, showing a gradient in the amount of algal material in the diet and gut length relative to body size. Increasing the amount of refractory material in the diet leads to higher intake, leading to more rapid transit of digesta through the gut, requiring a longer gut to maintain digestive efficiency (German and Herrera, 2024; Leigh et al., 2021). From top to bottom: Cebidichthys violaceus (herbivore), Xiphister mucosus (herbivore), X. atropurpureus (omnivore), Phytichthys chirus (omnivore), Anoplarchus purpurescens (carnivore).

Some vertebrates have evolved specialized feeding habits and digestive adaptations to exploit unique ecological niches. For example, **frugivores**, such as fruit bats and some primates, have adaptations for consuming fruits, including specialized dentition for

biting and crushing fruits as well as an elongated digestive tract optimized for processing plant material and seeds. Some frugivorous and **nectivorous** (nectar-drinking) bats and birds achieve blood glucose levels that would pose physiological challenges in other vertebrates, yet they can tolerate these high solute concentrations in their blood. **Insectivores**, such as anteaters and some birds, have adaptations for capturing and consuming insects, including long, sticky tongues for capturing prey and a relatively short digestive tract optimized for processing high-protein insect diets. **Piscivores**, such as dolphins, penguins, and some fish species, have adaptations for capturing and consuming fish, including sharp teeth and streamlined bodies for efficient swimming as well as expandable stomachs capable of rapid digestion of fish proteins and fats. Carnivorous marine mammals have relatively long intestines relative to their body sizes in comparison to terrestrial mammalian carnivores, and this is perhaps to aid in water absorption, since these animals do not drink seawater and must obtain their water from ingested food.

Overall, the structural adaptations observed in the vertebrate digestive system reflect the diverse dietary preferences and ecological strategies employed by different species. These adaptations highlight the remarkable versatility and adaptability of vertebrates in exploiting various food resources and habitats throughout the natural world.

Box 13.1—Make a Model of a Digestive System

Are all guts the same? Take a moment to do online searches of three different vertebrates (preferably not from the same phylogenetic group—for instance, don't pick a cow and a deer) and find images of their digestive tracts. What segments of the system do they have? Does

each have a stomach? What about a caecum in their hindgut? Why is there variation among their guts? If you have time, try drawing or building a model digestive system. What did you include in your model and why? What do you think your model gut would digest well?

13.4 Digestive Enzymes and Secretions

Digestive enzymes serve as catalysts in the digestive process, facilitating the breakdown of complex dietary components into simpler forms that can be absorbed and utilized by the body. Each enzyme is specialized to target specific substrates, initiating biochemical reactions that result in the hydrolysis of bonds within macromolecules. An overview of enzymes can be found in Figure 13.6.

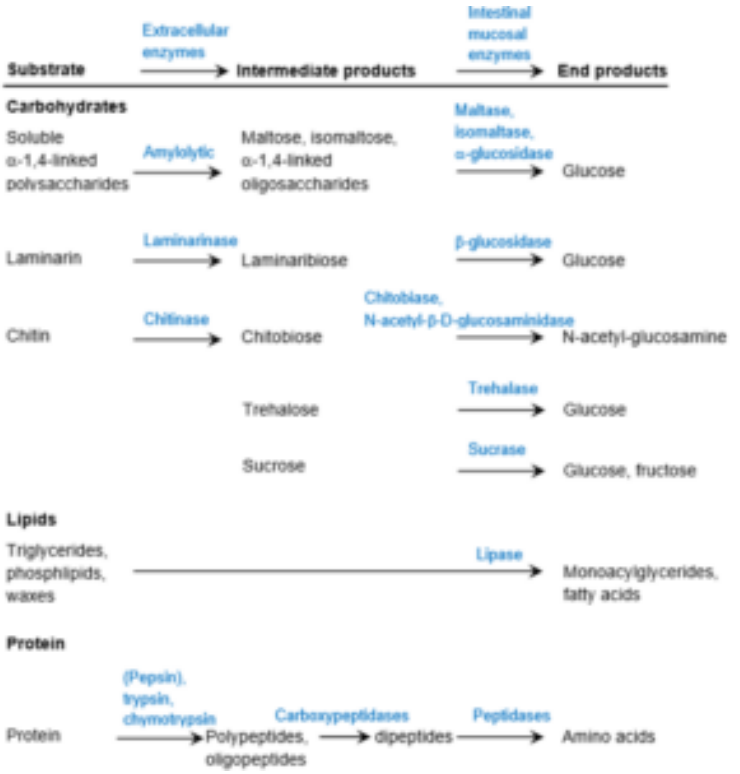

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Figure 13.6—Principal nutrients in food and the endogenous digestive enzymes vertebrates produce to hydrolyze them. Digestion requires multiple steps, beginning with hydrolysis in the lumen by extracellular enzymes and followed by membrane digestion by intestinal mucosal enzymes. For protein, the enzyme pepsin is listed in parentheses because agastric species (i.e., those that lack an acidic stomach) lack this enzyme.

Amylase (pancreatic, and salivary in the case of mammals) initiates the digestion of carbohydrates. This enzyme primarily targets starches and glycogen, which are complex polysaccharides composed of glucose units linked by alpha-1,4 glycosidic bonds (covalent bonds linking sugar molecules to other molecules). Amylase hydrolyzes these bonds, breaking down the

polysaccharides into smaller oligosaccharides, maltose, and dextrins. The hydrolysis reaction involves the addition of water molecules to the glycosidic bonds, resulting in the cleavage of the bonds and the release of maltose molecules. Maltose itself is then hydrolyzed by maltase (◈-glucosidase) into individual glucose molecules that are absorbed by sodium-dependent glucose transporters (SGLT) embedded in the microvilli of epithelial cells. In some vertebrates, chitin can also be digested by an acidic chitinase followed by N-acetyl-◈-D-glucosaminidase, producing monomers of N-acetyl-glucosamine (Figure 13.6). Vertebrates lack the requisite enzymes to digest cellulose and heteropolymers like hemicellulose and thus require the aid of microbial symbionts to digest those compounds (if that is part of the digestive strategy of the animal, again pointing to the panda as an example of an animal that doesn't rely on symbionts to digest plant fiber).

Pepsinogen, produced by oxynticopeptic cells (chief cells in mammals) in the gastric glands of the stomach, is the inactive precursor of pepsin, a proteolytic enzyme involved in protein digestion. Pepsinogen is secreted into the stomach lumen, where it undergoes proteolytic cleavage by hydrochloric acid (HCl) to form pepsin. The acidic environment of the stomach (pH 1.5–3.5) activates pepsinogen by inducing a conformational change that exposes the active site of the enzyme. Once activated, pepsin catalyzes the hydrolysis of peptide bonds within proteins, specifically targeting peptide bonds adjacent to aromatic amino acids such as phenylalanine and tyrosine. Pepsin cleaves proteins into smaller peptides and amino acids through an endopeptidase mechanism, breaking bonds within the interior of protein molecules. This initial protein digestion in the stomach prepares dietary proteins for further enzymatic hydrolysis in the small intestine, facilitating the absorption of amino acids and peptides. Given that the gastric stomach evolved approximately 450 million years ago, this combination of acid hydrolysis, combined with an endopeptidase like pepsin, has been an effective tool with which vertebrates digest protein in the stomach.

As briefly mentioned above, the pancreas secretes a variety of digestive enzymes into the proximal intestine (duodenum in mammals), the first segment of the small intestine, in response to hormonal signals triggered by the presence of food. These enzymes play crucial roles in the digestion of carbohydrates, proteins, and lipids.

Amylase: Pancreatic amylase digests carbohydrates. It hydrolyzes the alpha-1,4 glycosidic bonds present in starches and glycogen, breaking them down into maltose, maltotriose, and oligosaccharides. Amylase exhibits optimal activity at a slightly alkaline pH, allowing for efficient carbohydrate digestion. Amylase activities have been shown to be higher in those animals consuming more starches such as in mammals (including humans), birds, and fishes, and many of these abilities are linked to an increased number of copies of the amylase gene in the genomes of those animals. Note that pancreatic amylase (*amy2*), which is in all known vertebrate animals, is a different gene and protein from salivary amylase (*amy1*) in mammals.

Lipase: Lipase (pancreatic in mammals, and carboxyl ester lipase in other vertebrates) is essential for the digestion of dietary fats (triacylglycerides). Lipase catalyzes the hydrolysis of ester bonds present in the glycerol backbone of triacylglyceride molecules, releasing free fatty acids and monoacylglycerides. This process results in the formation of micelles, small aggregates of lipids, bile salts, and other components, which enhance the absorption of lipids by the intestinal epithelium.

Proteases: Pancreatic proteases, including trypsin, chymotrypsin, and carboxypeptidase, are responsible for protein digestion. Trypsin hydrolyzes peptide bonds adjacent to positively charged amino acids such as lysine and arginine, while chymotrypsin targets bonds adjacent to aromatic amino acids like phenylalanine and tyrosine. Carboxypeptidase cleaves peptide bonds at the carboxyl end of peptides and proteins. These proteases collectively break down dietary proteins into smaller peptides and amino acids, facilitating their absorption in the small intestine.

Pancreatic duct cells and proximal intestinal cells produce **sodium bicarbonate buffer** to raise the pH of acid chyme as it enters the proximal intestine from the stomach. This is not necessary in agastric vertebrates like carps, wrasses, echidna, and platypus.

With the exception of lipases, which already release the appropriate monomeric units of fatty acids and glycerol, the other polymer-degrading digestive enzymes (e.g., amylase, trypsin) produce oligomers and dimers that need to be hydrolyzed further to generate the monomers that are absorbed across the epithelial membrane. Disaccharidases like maltase and N-acetyl-β-D-glucosaminidase, as well as aminopeptidases, are embedded in the mucosal membrane in the microvilli (Figure 13.3) and perform the final hydrolytic step before monomer absorption. Thus, polymer degradation is a complex process that involves an enzymatic cascade to deconstruct complex molecules down to absorbable monomers. Vertebrates invest approximately 25% of their daily energy budgets to digestive tract function, and thus, this is an important aspect of their daily lives.

Bile, produced by hepatocytes in the liver and stored and concentrated in the gallbladder, aids in lipid digestion and absorption. Bile contains bile salts (derivatives of cholic acid), which act as emulsifiers to break down large lipid droplets into smaller droplets known as micelles. This process, called emulsification, increases the surface area of lipid particles, making them more accessible to pancreatic lipase for enzymatic hydrolysis. Bile salts have hydrophobic and hydrophilic regions, allowing them to associate with both water and lipids, thereby stabilizing the emulsified lipid droplets. The formation of micelles facilitates the hydrolysis of triglycerides by pancreatic lipase, leading to the release of fatty acids and monoglycerides that can be absorbed by the intestinal epithelium.

13.5 Digestive System Evolution and Diversity

All animals have the inner tube of life running down the center of their bodies. The need to acquire nutrients, energy, vitamins, and minerals from ingested foods is obvious in heterotrophic organisms. Digestive tract size and diversity of compartments are apparent as one begins to examine guts in different animals. From the ruminant mammals, to stomachless fishes, to flying vertebrates, gut morphology shows wide variation. So too does function, with more or less reliance on microbial symbionts, and wide variation in digestive enzyme activities and transport rates, depending on diet. In this section, we will focus on some of the extreme examples—namely, the smaller guts of flying vertebrates (including birds and bats) and the enigmatic loss of the gastric stomach in over 8,000 species of fishes as well as in the platypus and echidna—and we will finish with the genetics underlying digestive enzyme activity variation in relation to diet. All told, the evolution of trophic and locomotor variation has led to a large diversity in the themes of different gut types (Figures 13.1 and 13.2), each with different consequences for the animals. The diversity of guts is a testament to the fact that there are multiple solutions to solve a single problem.

Flying Vertebrates Have Smaller Digestive Tracts

Imagine you are a sparrow, you've just encountered a large number of seeds on the ground, and you quickly gather as many as you can. You see a shadow above you, and you know that it is a potential predator that is now in pursuit. If you don't quickly leave the area and evade the predator, your life will be cut short. You take off and rapidly fly toward tree cover. Flight, as a mode of locomotion, is expensive; in order to fly, the animal has to be lightweight. Yet the sparrow consumes seeds that are rich in structural polysaccharides

and lipids and likely require a somewhat larger gut with which to digest the seeds, as seen in small nonvolant (nonflying) mammals with granivorous diets. So how does a sparrow, or any volant (flying) bird or mammal, achieve a smaller gut so that they are not weighed down yet still efficiently digest their food? Indeed, the adaptation of the gut of volant vertebrates is an interesting case study in how a digestive system can evolve to fit the necessary function.

First of all, flying vertebrates, as a rule, have smaller digestive systems with lower surface areas than nonvolant vertebrates (Figure 13.7), and this difference is more pronounced the smaller the animal gets. By having smaller guts, their alimentary canals don't weigh as much and thus allow them to be lighter than a comparably sized nonvolant vertebrate. Second, birds have a crop, which is a chamber that precedes the stomach. This allows birds to ingest food items and temporarily store them in the crop before digestion begins. This serves as a way to allow for ingestion and then quick movement to another location before digestion begins, which would serve something small, like a sparrow, well, since it can acquire resources and then retreat to safety before digesting it. Moreover, because many birds regurgitate food items for their young, the crop provides a place to put ingesta (ingested food items) without beginning digestion, thus allowing for nondigested food to be delivered to the young.

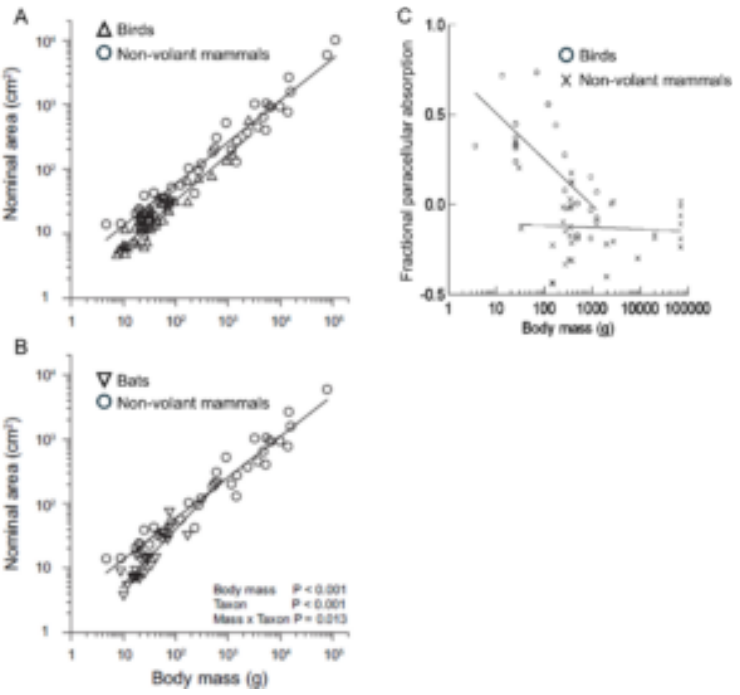

Figure 13.7—(A) Nominal surface area of small intestines of omnivorous birds and nonvolant mammals. Lines were fit to a common slope (0.73), with variable intercepts at line of unity: 1.14 for birds and 1.79 for mammals, showing that birds had 36% lower intestinal surface area than nonvolant mammals. (B) Nominal surface area of bat species compared with the nonflying mammals. (C) Fractional absorption of neutral inert water-soluble compounds for birds and nonvolant mammals.

Third, volant vertebrates, with their shorter guts and lower surface area, make up for this reduction by having greater rates of paracellular absorption. Most vertebrates require transepithelial absorption of most nutrients: Transporters on the brush border membrane of epithelial cells use sodium-dependent transport to bring the nutrients (e.g., glucose, amino acids) into epithelial cells before the nutrients are then transported across the basolateral membrane into the blood for distribution in the body (Figure 13.4).

Efficient absorption using this transepithelial route requires high surface area (microvilli) and many transporters in the membrane, both of which are expensive to maintain and lead to more tissue mass. Volant vertebrates have solved that requirement by just allowing more nonspecific absorption between their epithelial cells, as opposed to requiring nutrients to go the transepithelial route. Paracellular absorption is even more important for smaller birds than larger ones (Figure 13.7C), which again points to this strategy being more about having less mass so as to not impede the ability to fly. Of course, nothing is free, and it should be apparent that less control over what is absorbed paracellularly (i.e., it doesn't have to go through a transporter) comes with a potential cost of absorbing toxins that can harm the animal. Hence this would mean that smaller birds and bats would need to actively avoid toxins or consume compounds that might inhibit the toxicity of various compounds to compensate for this vulnerability, and there is evidence that this is the case.

No Stomach? No Problem!

The **gastric stomach** is an important vertebrate innovation (Figures 13.2, 13.3, and 13.6). An acidic foregut pouch with sphincters separating it from the esophagus and intestine evolved approximately 450 million years ago, along with oral jaws. The acidic environment along with an acidophilic protease (pepsin) likely improved protein digestion. Moreover, this pouch provided an area for some food storage, so the rate of material entering the intestine could be controlled. Add to that the fact that the acidic environment is an important "gate" determining what microbes reach the intestine, and the stomach may be one of the most important aspects of early vertebrate evolution. The fact that greater than 95% of terrestrial vertebrate species and about 75% of all fishes still have a gastric stomach points to its importance. Alternatively, the

fact that this number is not 100% is intriguing. At least 10 times in different groups of fishes, as well as in the platypus and echidna, the stomach has been lost, along with the genes (and proteins) for important transporters, pepsin, and proteins that are key for tight junction formation (**claudins**). Entire orders of fishes (e.g., Cypriniformes, Atherinoformes), as well as the family Labridae (which includes parrotfishes), encompassing thousands of species of fishes, lack a gastric stomach. These fish clades show incredible dietary diversity, ranging from detritivory and herbivory, to carnivory (including piscivory). Thus, one might assume that it would be more efficient to digest a proteinaceous diet with an acidic gastric compartment, yet these fishes get by just fine. The reasons for stomach loss are not clear, as there is not one consistent argument for why stomach loss would be beneficial. Nonetheless, in each of these events of the stomach disappearing, the same suites of genes disappear from the genome, meaning that the stomach phenotype is very unlikely to "reevolve" in those lineages.

Beyond the fishes, the platypus and echidna also each independently lost their stomachs. Each of these lineages also consumes a lot of animal protein and chitin (insects, insect larvae, worms), the digestion of which would potentially benefit from the stomach. The main thing we can conclude from the loss of the stomach phenotype is that it is not a requirement for the vertebrate digestive process; humans who have had gastric bypass surgery also bear this out. Many stomachless fishes have well-developed pharyngeal teeth to reduce particle size before ingesta reaches the intestine, but this is also not a universal rule. Thus, as we see with various structures of the gut—crop, foregut chamber, stomach, caeca (including the appendix in humans)—the intestine, liver, and pancreas (or at least equivalent pancreatic cell types, if not an entire organ) form the basic requirements of a functional digestive system. Beyond those, different animal groups have solved the issues of digestion of different food types in different ways. One may assume that the proteolytic machinery outside of acid and pepsin, so the pancreatic proteases (trypsin, chymotrypsin, carboxypeptidases), as

well as the intestinal aminopeptidases, would be expanded in stomachless vertebrates, and this is a question that has yet to be thoroughly answered and would benefit from a physiological genomics approach comparing relatively closely related species on either side of the loss of the stomach in a given lineage.

The Genetics Underlying Digestive Enzyme Activities

Digestion is a chemical process. Yes, there are physical aspects relating to mastication or trituration, as well as fluid dynamics in the tubular intestinal environment, but by and large, the dismantling of polymers to absorbable monomers is driven by chemistry, and particularly the activity levels of digestive enzymes. When one starts comparing the digestive enzyme activity levels present in different animals with different diets, the first one to jump out as being variable is that of amylase. Amylase activities are elevated in those animals consuming more starch (i.e., herbivores and omnivores) in comparison to those that do not (e.g., carnivores). This pattern has been observed in several mammal groups, including humans, canids (dogs and wolves), mustelids, and bats. Moreover, it is observed in birds and in fishes. The human and canid examples drew much of the attention following the turn of the millennium and the explosion of high-throughput genetic sequencing techniques. It was revealed that humans from agrarian (agricultural) backgrounds had many more copies (up to 14) of the salivary amylase gene (*amy1*) than hunter-gatherer humans, who had 6. That expanded gene copy number led to more gene expression and thus more amylase protein synthesis and elevated amylolytic activities in those people whose lineages have been eating starchy grains for over 10,000 years. Because we have been feeding dogs grains for almost the same length of time, it shouldn't

be surprising that similar effects were seen in dogs, where they have up to 30 copies of the *amy2* gene in comparison to only 2 in wolves.

The elevated amylase in starch-consuming vertebrates doesn't stop there. Bats consuming more starchy fruits have elevated amylase activities in their guts in comparison to carnivorous bats; birds that consume more grains have greater amylase activities than those that don't. In fishes, it gets a little more interesting. Herbivorous and omnivorous fishes have elevated amylase activities in comparison to carnivores in many different clades, but what are the genetic underpinnings of this? Is it the same as seen in mammals with massive increases in gene copy number? The answer is sometimes yes and sometimes no. Comparative genomics and transcriptomics of amylase genes in prickleback fishes make it clear that those that consume more algae have elevated amylolytic activities, elevated expression of the genes, and variation in *amy2* gene copy number. Those prickleback fishes consuming less algae have one *amy* copy (two diploid copies), whereas those with higher activities have two to three (depending on species; Figure 13.8). In the two *Xiphister* pricklebacks, one omnivore and one herbivore, the two copies are identical. However, in *Cebidichthys violaceus* (the monkeyface prickleback, which is also a herbivore), two copies are identical, but the third copy is different from the other two, and the enzyme has a different isoelectric point and pH optimum, suggesting it operates farther down the intestine with more alkaline conditions. Thus, not only can we see gene copy number variation among species with different diets, but we can also see mutations among the proteins that can cause differences.

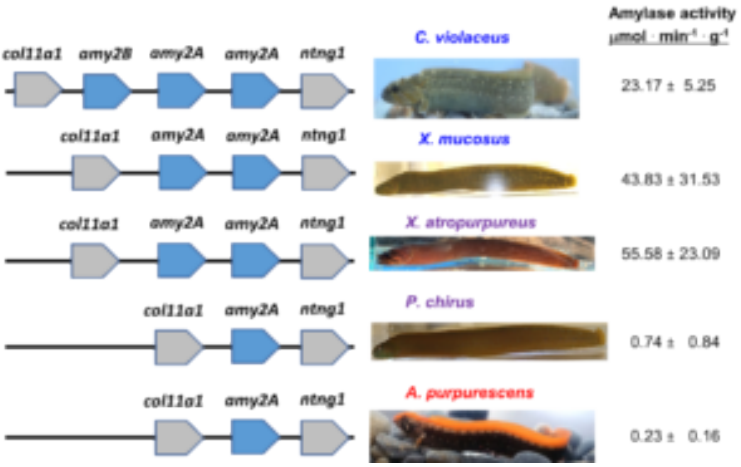

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Figure 13.8—Synteny maps showing the copies of amylase (amy2) genes, their orientation, and distribution on the chromosomes of five prickleback species. C. violaceus and X. *mucosus are herbivorous,* X. *atropurpureus is omnivorous, as is P. chirus, but they consume less algae than* X. *atropurpureus. A. purpurescens is carnivorous. Total gut amylase activities are to the right of the fish pictures (mean ± standard deviation).*

Beyond these observations in amylase, other digestive enzymes are not as well scrutinized, but trypsin may show more copy numbers in some carnivores. This leads to questions about lipase. Shouldn't those also show greater copies in carnivores, which consume more of a lipid-rich diet? The short answer is, it depends on what lipase one is discussing. Mammals have pancreatic lipase, among other lipases, whereas fishes have carboxyl ester lipase as their main lipolytic enzyme. In the prickleback fishes, *C. violaceus* shows the highest lipase activities and has one more copy of the carboxyl ester lipase gene than the prickleback *Anoplarchus purpurescens*. Algae are low in lipids, which suggests that *C. violaceus* has elevated lipase activity to scavenge for scant, and essential, lipids in its algal diet. Much more work is to be done to understand the genetic underpinnings of digestive biochemistry.

Box 13.2—Does Amylase Digest Starch?

Mammals have amylase in their saliva, and you can see it in action with a little chemistry. Did you know that iodine binds to starch, turning a dark-blue color? However, iodine doesn't bind to smaller sugars (like disaccharides or monosaccharides). Wear gloves for this activity. Your instructor can provide you with a 1% starch solution in a buffer. Add a dropperful of starch to a test tube. Then add five drops of iodine, record the color of the solution, and incubate it for 10 minutes. You should see it turn a dark-blue color over time. Now what happens if you add amylase to that starch solution? For safety reasons, your instructor will provide you with an amylase solution, but your own saliva can do the trick! Add a dropperful of starch to a test tube, then add about half a dropper of amylase solution. Shake it to mix it, and hold the test tube in your hand to provide warmth. Let the amylase and starch interact for about 10 minutes. Then just like before, add five drops of iodine to the starch/amylase mixture. Record the color and wait 10 minutes. After those 10 minutes, which was darker? The solution with the amylase and the starch or just the starch? Why is there a difference? Have a class discussion about what is happening. Under controlled conditions, your instructor can show you what their saliva does to starch (*Hint*: It will be like the amylase solution). We just cannot have everyone spitting in vials for sanitary purposes! Measuring the actions of digestive enzymes can be fun with a little chemistry.

13.6 Summary

The vertebrate digestive system is a marvel of evolutionary adaptation, reflecting the diverse dietary preferences and ecological niches occupied by different species. Despite variations in structure and function, fundamental principles of digestion, nutrient absorption, and waste elimination are shared across vertebrate taxa. Understanding the comparative anatomy and function of the vertebrate digestive system provides insights into the evolutionary relationships between organisms and the intricate interplay between form and function in biological systems.

Application Questions

1. You've discovered a fossilized complete theropod dinosaur skeleton. As you examine your discovery, you make note of the fairly wimpy teeth of the specimen, as well as a pile of smooth stones in the ribcage. Given your knowledge of vertebrate evolutionary relationships and the digestive system, what does this suggest about the diet and digestive structures of this species?
2. Describe foregut versus hindgut fermentation. What similarities and differences are present in the digestive systems of animals that do each type of fermentation? What about behavioral differences?

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14. Respiratory System

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Focus Questions—to Guide Your Reading of This Chapter

1. What are the main differences between water and air as respiratory media? How are those differences reflected in the respiratory structures of aquatic and terrestrial animals?
2. Although water and air differ in some key ways, what fundamental anatomical features of respiratory structures are important for effective diffusive gas exchange regardless of respiratory medium?
3. Ways of breathing, or the active ventilation of respiratory organs, are also incredibly diverse across vertebrates. Why do you think that breathing behaviors vary so much, and why does the movement of water or air across a respiratory surface matter for gas exchange?

14.1 Introduction

One of the easiest ways to recognize the importance of your respiratory system is to hold your breath. At first, nothing feels wrong. However, you will quickly feel a tightening of your chest, and your body will begin to fight you. Your chest will start to rise and fall; even as you hold your mouth closed, you will feel a strong need to breathe, until your body finally ignores your mind and breathes in and out several times quickly. In rare cases, people can even lose consciousness at this point, which is why we do not recommend trying it at home. Why do we need to breathe so badly and so often?

Our respiratory systems fulfill a fundamental physiological need: **gas exchange**. At the cellular level, mitochondria perform cellular respiration, converting sugars and other fuels into ATP (raw energy), and this process uses up oxygen (O_2) and produces carbon dioxide (CO_2). As this happens, our cells require fresh oxygen deliveries and a way to remove the carbon dioxide in order to maintain homeostasis. This is true for all animals. The main way that gases move in and out of cells is diffusion. **Diffusion** is a relatively simple process, but it is incredibly important to how animal bodies function. Matter that is in a fluid state is fluid because individual particles are freely moving past each other. This movement is what creates diffusion, such that when different concentrations of particles meet (e.g., a drop of red dye in a cup of undyed water), the concentrations even out over time, simply due to the random movement of particles (Figure 14.1). This is the basic principle behind gas exchange: Expose low-oxygen blood to a high-oxygen source, and oxygen will diffuse into the blood (and CO_2 will diffuse out).

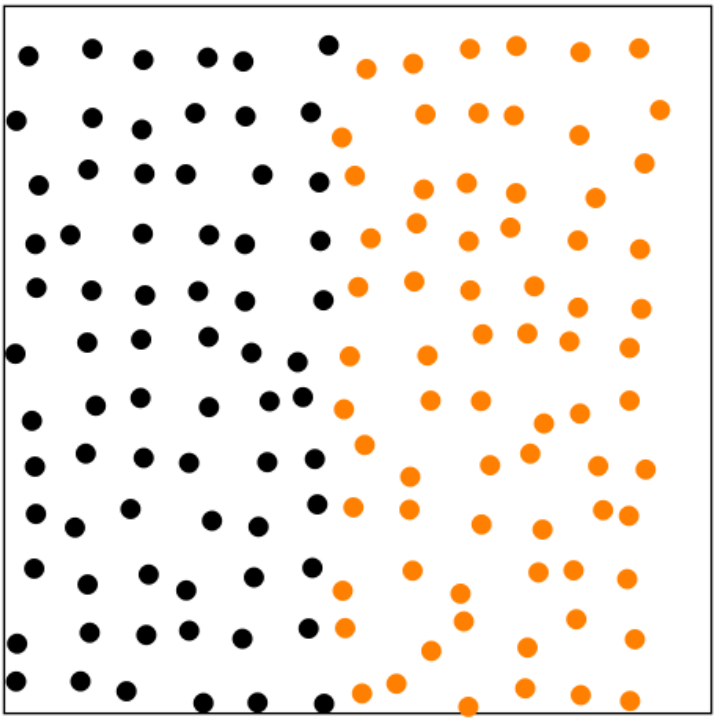

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Figure 14.1—An animation illustrating the basic principle of diffusion. At the beginning, the black dots are on the left and the orange dots are on the right. The dots begin to move randomly and are interspersed by the end.

For a simple, very small animal (e.g., a tardigrade), diffusion from the outside environment alone might be sufficient for gas exchange. Indeed, many small invertebrate animals have no specialized respiratory organs, and oxygen simply diffuses in through their skin. However, this strategy gets harder and harder as animals get bigger. This is a scaling issue, caused by the geometric relationships between **surface area** and volume (Figure 14.2, but also see Chapter 5). As 3D objects get bigger, volume grows faster than surface area, meaning that larger bodies have lower relative

surface areas, making diffusion less efficient. Another problem is that for oxygen to pass through a surface, like skin, that surface must be thin and moist. This is potentially a problem for animals that need defensive structures like scales or want to avoid water loss (the dry skin of desert-living frogs). The way that most animals, including nearly all vertebrates, deal with these problems is to have specialized respiratory organs with large surface areas that do the vast majority of the heavy lifting of gas exchange. The classic respiratory organs seen in vertebrates are **gills** and **lungs**, but **skin** is also an important site of gas exchange for many animals (see Section 14.2, General Structure and Function).

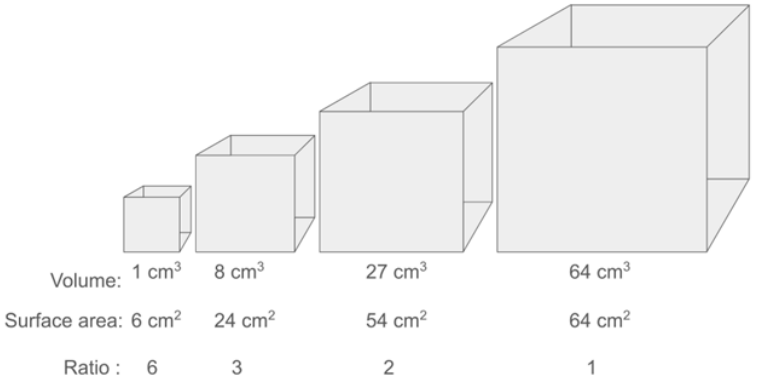

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Figure 14.2—The scaling relationship between surface area and volume, illustrated with a series of cubes of increasing size. Note that the surface-area-to-volume ratio decreases as the cube gets larger.

Such specialized respiratory organs also rely on a vascular system to transport the absorbed oxygen to the brain and muscles and transport carbon dioxide back to the respiratory organ, to be released into the environment. In vertebrates, this is, of course, powered by the heart, which maintains blood flow through the body. The heart partitions freshly oxygenated blood, which is pumped to the body, and low-oxygen blood, which is pumped to the

respiratory organ. This partitioning is a neat trick—by only sending low-oxygen blood to the respiratory membranes, the blood is oxygenated more quickly because of the large difference in concentrations between the blood and the environment. The greater that difference, the faster the change.

Another way that animals increase the efficiency of gas exchange is by controlling and coordinating the flow of environmental oxygen over respiratory organs. Basically, by breathing! There is a reason we do not just hold our mouths open and passively diffuse oxygen down our throats and into our lungs. By moving air into and out of respiratory organs, we can maintain concentration gradients by replacing spent air or water. In the cases of countercurrent and crosscurrent exchange, we can even use flow at the site of diffusion to speed up diffusion even further.

The way that animals exchange **oxygen** and **carbon dioxide** with their environment is dependent on many factors, but perhaps the most fundamental is the respiratory medium: water versus air. Earth's atmosphere is roughly 20.95% oxygen, 78% nitrogen, 1% argon, and just .04% carbon dioxide! That is a lot of oxygen, all produced by Earth's amazing photosynthesizers (plants and algae), and it is available 24/7, 365 days a year. This abundance of atmospheric oxygen is what allows us humans, and other terrestrial animals, to exist. In the water, the dynamics are quite different. Oxygen must be dissolved in water to be accessed by an aquatic animal. Unlike in the atmosphere, oxygen does not make up a significant volume of a lake or stream. Instead, oxygen levels vary throughout the day and part of the year as well as between types of water bodies. And even a bottle of water that has been shaken vigorously to fully saturate it with oxygen has hundreds of times less oxygen than the same volume of air. This means that for many aquatic animals, the primary struggle of gas exchange is consistently getting enough oxygen. For terrestrial animals, oxygen is constantly available, and the more pressing concern is often getting rid of carbon dioxide. In fact, the reason that holding your breath is painful is that your CO_2 levels are too high, not that there

is not enough O_2. Luckily for fish and other aquatic animals, carbon dioxide is highly soluble in water and so readily diffuses out of their gills.

Many different forms of respiratory organs have evolved across vertebrates. The most common respiratory organs today, lungs and gills, are also some of the oldest, having evolved in the ancestors of modern fish hundreds of millions of years ago. Other respiratory strategies are more recent, having evolved in specialized groups. The common themes across the respiratory system are (1) differences in water-based versus air-based respiration; (2) increases in surface area of the respiratory surface; and (3) mechanisms to increase concentration gradients during gas exchange, such as ventilation and coordinated blood flow patterns. Take a breath and dive into the fascinating world of vertebrate respiration.

14.2 General Structure and Function

There are a diverse array of respiratory structures and ventilation behaviors across vertebrates. Later in this chapter we will explore this diversity, but in this section, we will give an overview of the most common respiratory structures and focus on shared properties across vertebrates.

Types of Respiratory Organs

Vertebrate respiratory organs can be grouped into three major categories: **gills**, **air-breathing organs**, and **respiratory skin** (Figure 14.3). Each category faces its own set of physical constraints and demands that influence its **morphology** and mode of **ventilation**.

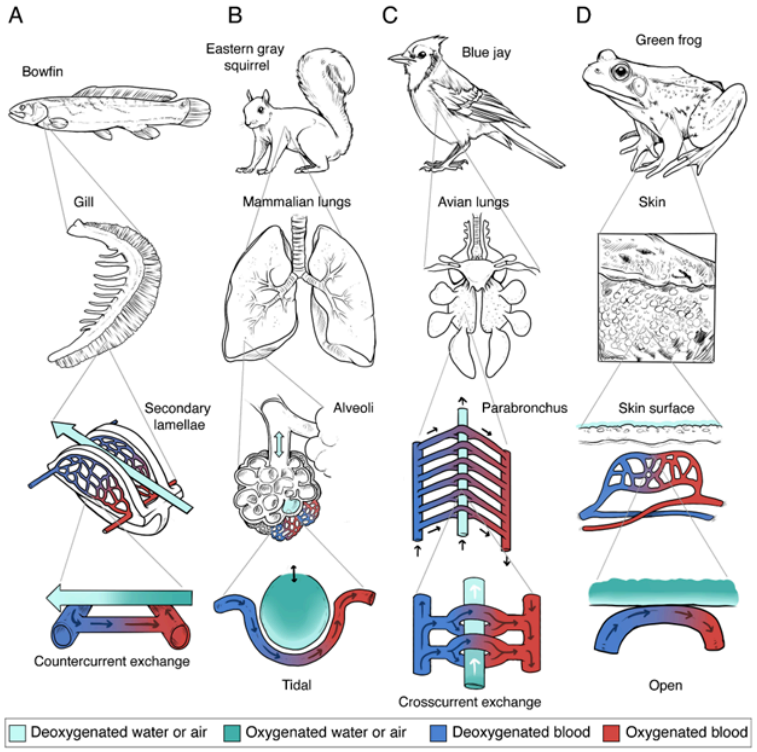

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Figure 14.3—Illustrations of the basic anatomy of four examples of respiratory structures: fish gills (A), mammalian lungs (B), avian lungs (C), and amphibian skin (D). These are shown at different scales: representative organism (top row), gross anatomy of the respiratory structure (second row), microanatomy of the respiratory structure (third row), and the site of diffusion between the capillaries and the respiratory media (fourth row). Arrows indicate the direction of movement of the blood or respiratory media (water or air). Light teal—deoxygenated respiratory fluid. Dark teal—oxygenated respiratory fluid. Navy blue—deoxygenated blood. Red—oxygenated blood.

Gills

With few exceptions, gills are used for gas exchange in water. They are therefore equipped to deal with the high density and

comparatively low oxygen availability of water. Water is much denser and more viscous than air, and so it requires more work to move (i.e., ventilate) water than air. However, ventilation maintains concentration gradients and promotes diffusion and so is quite important. To make matters worse, water contains a fraction of the oxygen contained in air. For example, at 20°C and at sea level, air contains 210 mL of O_2 per L of air, whereas freshwater contains just 6.6 mL of O_2 per L of water. The amount of oxygen dissolved in water depends on the partial pressure of oxygen (pO_2) in the air above the water and on the solubility of oxygen in water, which is fairly low and dependent on temperature and salinity. At high altitude, air pressure decreases, so pO_2 decreases accordingly, and in warmer and saltier water, the solubility of oxygen decreases, creating a lot of variation in how much oxygen is in different water bodies.

The high density of water causes gill ventilation to be energetically expensive. Therefore, gill ventilation must be efficient to offset this energetic cost. In a similar vein, the limited amount of oxygen in water means that gill-breathers often need to maximize their diffusive capacity in order to support their metabolic needs. Gills achieve this by (1) having a large surface area, (2) being ventilated with a unidirectional flow of water, and (3) in many groups, having capillaries arranged to facilitate countercurrent exchange.

Gills have a large surface area because they are covered in small, vascularized folds. The specific design of these folds can vary—for example, the gills of larval actinopterygian fish have thin, filamentous processes, whereas the gills of adult fish have flat plates—but, because these folds are vascularized, they all contribute to a large respiratory surface area. This large surface area is important both because it increases the surface area for diffusion and because it causes flow rate to slow down, increasing the time during which diffusion can take place. Specifically, this is because the velocity of a fluid decreases as it moves from a narrow to a wider region. The combined cross-sectional area of the branchial

arteries is smaller than that of the vascular channels within the gills. Similarly, the cross-sectional area of the pharynx is smaller than the combined cross-sectional area of the passages between the folds of the gills. These mismatches cause the velocities of both their blood and the water in the gills to slow down, increasing time for diffusion of gases.

Unidirectional flow of water over the gills supports an efficient exchange of respiratory gases because it provides a continuous supply of fresh, oxygenated water to the vascular surface where exchange occurs. If gills were more like human lungs and were ventilated bidirectionally—that is, tidally—then half of each ventilation cycle would be spent flushing out water that can no longer be used as a source of oxygen—not an efficient use of energy. Unidirectional flow also enables **countercurrent exchange**, as we will discuss next.

The relative paths of ventilated water and circulated blood in the gills are also critical to their function. For a moment, let us consider what would happen if blood (within the gills) and water (flowing across the gills) were flowing in the same direction. Let us assume that the water starts well oxygenated (10 mg/L), the blood starts out totally anoxic (0 mg/L), and there are equal amounts of water and blood. As the two streams flowed alongside one another, oxygen would diffuse from the water into the bloodstream, and the amount of oxygen in the blood and water would eventually reach equilibrium at some intermediate value (~ 5 mg/L). Unfortunately, in this case, half of oxygen remains in the water! To avoid this, gills use a pattern of flow known as countercurrent exchange (Figure 14.3), which is a clever trick for maximizing diffusion and exchange. During countercurrent exchange, blood flows in the *opposite* direction of water. Therefore, at every point of contact along the path of the blood and the water, the water has a higher concentration of oxygen (and lower concentration of carbon dioxide) than the blood that it is passing, and so the gases keep diffusing the whole time. For example, as a parcel of water loses oxygen to adjacent blood flowing through gill capillaries, the slightly

deoxygenated water flows toward blood that has just entered the gills and contains even less oxygen than the water, enabling further diffusion. In this way, the blood and water never reach equilibrium, and diffusion continues until very little oxygen remains in the water.

Because gills create close contact between capillaries and the surrounding water, heat, water, valuable salts, and nitrogenous wastes also readily diffuse across respiratory gill membranes. In freshwater fishes, water diffuses into the body (toward the higher salt concentration), and in marine fishes, water diffuses out (again, toward the higher salt concentration). As a result, most fishes cannot maintain a body temperature that is warmer than the water they are swimming in, and they require physiological adaptations to maintain internal osmotic balance.

Air-Breathing Organs

Breathing air is, in many ways, the exact opposite of breathing water. Air is oxygen rich, has low **density**, and has low **viscosity**. Carbon dioxide is highly soluble in water and readily diffuses from the gills but is not very soluble in air. Like gills, water readily diffuses across the respiratory membrane of air-breathing organs. While not problematic for aquatic air breathers, water loss across lung membranes during air breathing poses a major challenge for terrestrial air-breathing vertebrates.

The low density of air means that respiratory membranes, and any elaborations that increase its surface area, must be supported to prevent collapse. This is the reason gills are not effective in air, because their filaments rely on water to support them, and on land they clump together. On the other hand, the low density of air makes it much easier to move (ventilate), and its high oxygen content means that gas exchange doesn't need to be incredibly efficient. These two properties make bidirectional **tidal ventilation** and low ventilation rates possible. Air can easily be moved in and out of an air-breathing organ, unlike water, which

has high inertia and viscosity. Additionally, the high oxygen concentration gradient between blood and atmospheric air means that there is plenty of diffusive capacity for most animals' metabolic demands without needing countercurrent exchange. In some taxa, including birds, more complete gas exchange is necessary and is facilitated by adaptations such as unidirectional airflow and **crosscurrent exchange**. Crosscurrent exchange is similar in some ways to countercurrent exchange. In this case, however, the flow of blood is perpendicular to the flow of ventilated air, maintaining concentration gradients more effectively than in typical tidal flow. We see this system in bird lungs (Figure 14.3).

The earliest known vertebrate air-breathing organs evolved in bony fish, allowing these ancestral fishes to store a pocket of air inside their bodies and then continue swimming about. Extant fishes and tetrapods possess lungs and respiratory gas bladders that have evolved from these ancestral air-filled organs. Like lungs, respiratory gas bladders are air-filled sacs that are vascularized and conduct gas exchange with the atmospheric air that is breathed into them. Unlike tetrapod lungs, gas bladders are unpaired, composing a single air bladder. The internal location of lungs and respiratory gas bladders is useful for air-breathing fishes because they can store air for gas exchange. In amphibious and terrestrial vertebrates, this internal location is advantageous because the respiratory membrane remains moist while water loss is reduced. Air within lungs contains a lot of water vapor (evaporating from the moist respiratory surface), but **water loss** is reduced by the low rate of ventilation and low proportion of lung volume that is exchanged with each breath. In addition, air is "conditioned" in the airways before and after entering the lungs, reducing how much moisture leaves the body.

A diverse array of air-breathing organs has evolved in fishes much more recently than the origin of lungs. These organs are subject to the same constraints described above. They must allow the fish to store a pocket of air, the respiratory membranes must

be supported, and low ventilation rates are sufficient, given the high proportion of oxygen in atmospheric air compared to water.

Skin

Aquatic, amphibious, and terrestrial species have evolved adaptations that allow skin to be used as a respiratory organ, most often (but not always) as a supplement to the gills or lungs. In aquatic species, respiratory skin faces similar constraints as gills: The low oxygen availability in water makes it especially important for the respiratory membrane to have a large surface area and maintain a high concentration gradient by vascularizing the surface with low-oxygen blood. In terrestrial species, respiratory skin could just as well be classified as an air-breathing organ and faces the challenges faced by the lungs of terrestrial vertebrates: The respiratory membrane must remain moist but reduce water loss, and CO_2 is not very soluble in air. In addition, skin serves numerous other functions (see Chapter 6), many of which preclude the use of the skin for respiration. Adaptations that enable skin to act as a respiratory organ include having capillary beds positioned close to the surface of the skin and having accessory skin structures (such as vascularized folds or other projections) that increase surface area (Figure 14.4).

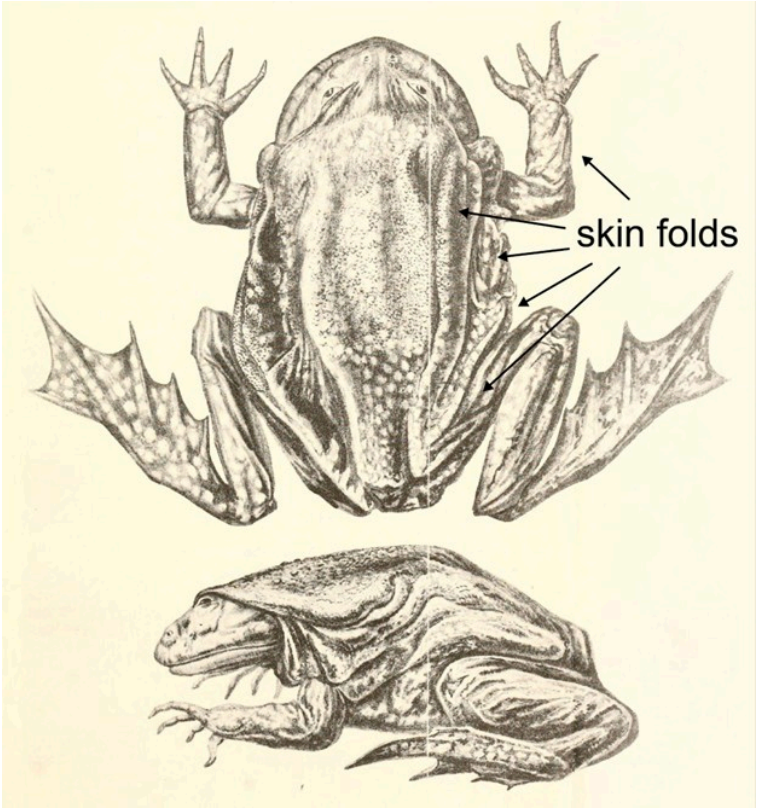

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Figure 14.4—An illustration of the Lake Titicaca frog Telmatobius culeus. Arrows point to the excess skin and skin folds on the surface of the frog.

Mechanisms of Ventilation

The mechanisms for ventilating respiratory structures can be grouped into three major categories: **oropharyngeal** pumping, **aspiration** pumping, and nonpumping **body movement**. Although we have presented three categories of respiratory structures (gills, lungs, and skin) and now three categories of ventilation

mechanisms, they do not match up one-to-one. Oropharyngeal pumping is used to ventilate gills, but it is also used to ventilate air-breathing organs in fish and amphibians. Similarly, body movement can be used to ventilate the surface of the skin, but it is also used for ram ventilation to ventilate the gills of some fish. Aspiration pumping is exclusively a lung-breathing mechanism found within tetrapod vertebrates.

Oropharyngeal Pump

Oropharyngeal pumping uses a combination of expansion and compression of the oral and/or pharyngeal (and neighboring) cavities to produce pressure, driving the flow of fluid (water or air) into and out of the mouth. Variants of this general mechanism are mostly used by fishes and amphibians to ventilate their gills, lungs, gas bladders, and other air-breathing organs. Different forms of oropharyngeal pumping mechanisms have different names based on exactly which cavities are used for ventilation. A **dual pump** is used to ventilate the gills of fishes, and it involves two cavities: the buccal cavity and the parabranchial (in sharks) or opercular (in bony fishes) cavity. Air breathing in fishes and amphibians uses expansion and compression of just a single cavity, the buccal cavity, and so is called a **buccal pump**.

The dual pump produces unidirectional flow over the gills (Figure 14.5A). Described simply, the buccal cavity expands and draws water into the mouth through the open jaws, while the parabranchial/opercular cavity (the name of this cavity differs in sharks and bony fish, respectively) expands and draws water past the gills. Then the buccal cavity compresses and pushes water past the gills, and the parabranchial/opercular cavity compresses and pushes water out of the gill cavities. This cycle repeats to create pulses of unidirectional flow over the gills.

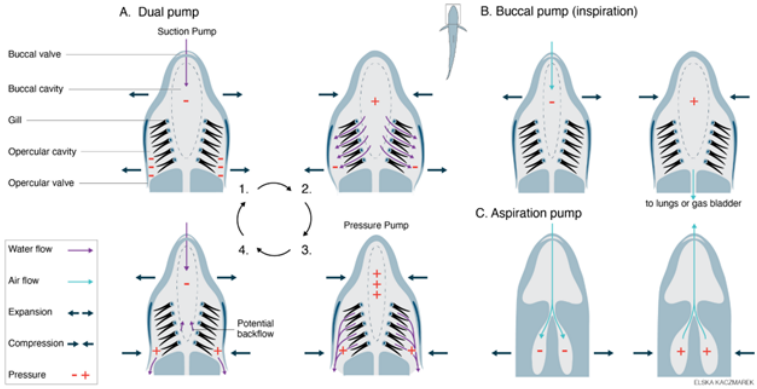

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Figure 14.5—Ventilation mechanisms used for water breathing and air breathing: dual pump (A)*, buccal pump* (B)*, and aspiration pump* (C)*. (A) The dual pump generates unidirectional flow of water over the gills via the cyclical expansion and compression of the buccal cavity and opercular cavity (or parabranchial cavity in sharks). Note that pressure is always lower in the opercular cavity than the buccal cavity. (B) The buccal pump is used to inspire air into the lungs or respiratory gas bladder via the expansion and compression of the buccal cavity. (C) The aspiration is used to inspire and expire air into the lungs of amniotes via expansion and compression of the thorax and/or abdomen. Purple arrows—water flow. Teal arrows—airflow. Dark blue arrows—movement of the animal's head or body. Orange plus and minus symbols—water or air pressure relative to the ambient pressure.*

During air breathing, the buccal pump must move air from the mouth into the air-filled organ (lungs or gas bladder) rather than out via the gill openings, as in the dual pump (Figure 14.5B). This is accomplished by closing the gill openings (i.e., opercular valves) and by opening the glottis to allow air into the lungs or gas bladder. During inspiration, the buccal cavity expands and draws air into the mouth through the open jaws, and then the mouth is compressed after the jaws shut, pushing air into the lungs or gas bladder through the open glottis. Thus, buccal pumping produces inspiration, one-half of the tidal flow that occurs during air breathing.

Expiration of gas (the second half of tidal flow) is not caused by buccal pumping. In fishes, expiration is caused by hydrostatic

pressure on the body wall, elastic recoil of the lung/gas bladder walls, and possibly the contraction of smooth muscle in the lung walls. In contrast, in some amphibians, expiration is caused by contraction of a hypaxial muscle (transverse abdominis) to compress the body wall and lungs. This is called an "expiration pump." When an amphibian is submerged in water, hydrostatic pressure on the body wall also contributes to expiration. (Note that although we have discussed the mechanism of inspiration first and then expiration, in fish and amphibians, expiration of gas typically occurs first, followed by inspiration of new air.)

In air-breathing fishes that do not use lungs or gas bladders (and instead use other diverse air-breathing organs), a buccal pump is typically used to drive airflow, but the pattern of movement may differ from what is described above.

Aspiration Pump

Unlike oropharyngeal pumping, aspiration uses active contraction of muscles in the thorax and abdomen to change lung volume and produce tidal ventilation (Figure 14.5C). During aspiration, the pressure driving airflow is generated by muscles that surround the lungs—the lungs are inside the pump, not on one end of it. Rather than air being forced into the lungs using compression of the mouth, as occurs during buccal pumping, air is sucked or aspirated into the lungs using expansion of the thorax. Muscular compression of the thorax and elastic recoil of the lung walls cause exhalation. The muscles used for aspiration include intercostal muscles, hypaxial muscles, and/or a muscular diaphragm, depending on the taxa.

This may sound familiar because, as we briefly discussed above, contraction of the hypaxial muscles is the mechanism of expiration in some amphibians. The use of axial muscles for expiration (expiration pump) is hypothesized to be an evolutionary intermediate between the use of cranial muscles for inspiration

(buccal pump) and the use of axial muscles for both exhalation and inhalation (aspiration pump). The evolution of the aspiration pump frees the head from the functional demands of producing inspiration, allowing morphological and functional diversification of cranial structures (see Section 14.6, Integration).

Nonpumping Body Movement

This category serves as a catchall for alternative forms of movement-based ventilation aside from oropharyngeal pumping and aspiration. For example, amphibians with external gills contract muscles at the bases of the gills to wave the gills back and forth through the water. The Lake Titicaca frog (*Telmatobius culeus*) has loose folds of skin that it uses for cutaneous respiration in its high-altitude home, and it will perform jerky push-up-like movements to create water flow over its skin and increase gas exchange (Figure 14.4). Many pelagic fish species and some sharks hold their mouths slightly open while swimming, allowing water to flow passively over the gills. This is called **ram ventilation**, and although the drag created by the open mouth reduces swimming efficiency, it saves the energy that would be spent using the dual pump for gill ventilation.

14.3 Development of the Respiratory System

Being equipped to perform respiratory gas exchange is crucial through all stages of life. Not only must newly hatched or newborn animals have at least one functional respiratory organ to survive, but developing embryos must also have an effective mechanism for gas exchange. In this section we will discuss how gills and lungs arise during embryonic development, how embryos themselves perform gas exchange, and how lungs are stretched throughout

development so that they will be healthy and ready for a lifetime of breaths.

Development of Gas-Exchange Organs

Vertebrate gills arise from a mix of **ectoderm** and **endoderm** on the pharyngeal arches. They typically appear during the embryonic stage, becoming fully functional soon after hatching into the water. Animals with aquatic eggs, like fishes and amphibians, typically have an aquatic larval stage and greatly depend on cutaneous gas exchange and their gills to survive to adulthood. In vertebrates with a biphasic life cycle (where the organism metamorphoses between the larval and adult stages), like frogs and salamanders, the gills are usually resorbed at metamorphosis in anticipation of the move onto solid land.

During embryological development, lungs and gas bladders form as endodermal outpocketings from the foregut (Figure 14.6). Lungs are typically (but not always) paired and bud off the ventral side of the foregut, whereas the gas bladder is unpaired and buds off the dorsal side of the foregut. The connective tissues of the respiratory system are derived from mesodermal tissue groups, forming the vasculature, smooth muscle, and cartilaginous elements of the lungs. The hollow connection to the mouth or foregut is called the trachea (in tetrapods) or pneumatic duct (in nontetrapod fishes). The opening to both the trachea and pneumatic duct is called the glottis, which is closed and opened via muscular control.

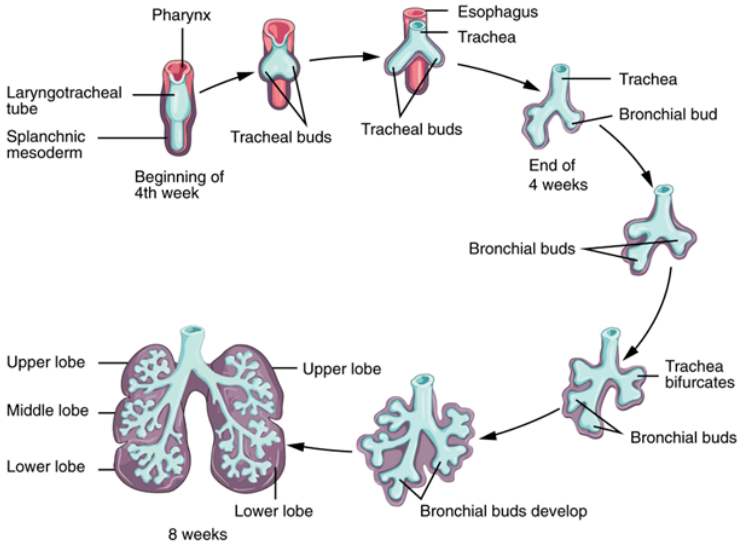

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Figure 14.6—The branching development of the lungs in humans and other mammals. Human lungs develop as small buds on the ventral side of the anterior gut tube, and then successively branch, like a tree, over the next four weeks.

Embryonic Gas Exchange

Animals have gas-exchange requirements for their entire life cycle, not just when they are adults. This creates a tricky situation: How is an animal supposed to acquire oxygen while it is still building (i.e., developing) its lungs or gills?

For many fish and amphibians, the struggle for gas exchange begins when aquatic eggs are fertilized and laid in the water (Figure 14.7). For these tiny developing embryos, oxygen must diffuse through any jelly coatings to reach the developing embryo, where oxygen diffuses either directly through the skin or via gill-like structures (Figure 14.8). Because diffusion is most effective

when there is a large surface-area-to-volume ratio, and because water is relatively oxygen poor, oxygen diffusion is a major limiting factor for these groups. Often embryos must hatch at small sizes and early developmental stages to avoid asphyxiation. Some amphibians have special adaptations for gas exchange in the egg, such as endosymbiotic algae that live in the egg and are passed down across generations. These algae perform photosynthesis, using up carbon dioxide and nitrogenous wastes excreted by the developing embryo, and provide oxygen in return (Figure 14.7). In many embryonic fish and amphibians, long gill-like structures branch out from the embryo to the surface of the egg to facilitate gas exchange. In others, adults stick around after mating to fan the eggs, creating flow, which maintains **concentration gradients** of oxygen, allowing for diffusion to take place.



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Figure 14.7—Salamander eggs from a vernal pool. The faint green color comes from symbiotic algae that photosynthesize, producing oxygen used by the developing embryos. In other groups, oxygen must diffuse through the jelly coatings of the egg mass, limiting egg size.

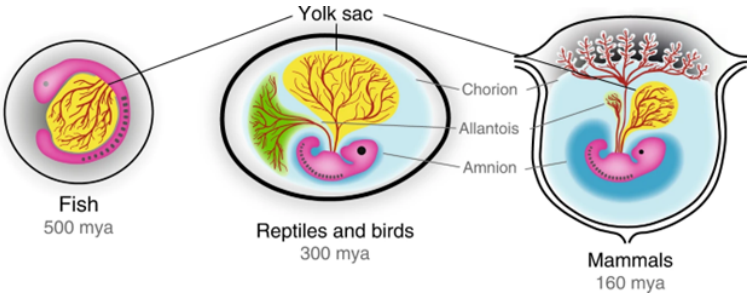

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Figure 14.8—The diversity of egg types in vertebrates. Amphibian eggs are similar to the "Fish" egg shown here, while reptiles, birds, and mammals all have the amniotic condition. In nonmonotreme mammals, the external egg has been lost. The dates shown indicate estimates of when this egg type first evolved.

Animals with hard-shelled, terrestrial eggs, such as nonavian reptiles, birds, and monotreme mammals, take a different approach to embryonic gas exchange. These groups are all part of the Amniota, a group defined by their egg type (Figure 14.8). Like all animals, developing amniote embryos also have gas-exchange requirements, but unlike many fish or amphibian eggs, amniote eggs are terrestrial, meaning they are exposed to the atmosphere. On one hand, this is advantageous for gas exchange, as there is far more oxygen available in the atmosphere than is dissolved in water bodies. On the other hand, however, amniote eggs must resist desiccation while still allowing for oxygen diffusion, a fundamental trade-off in respiratory biology you have seen throughout this chapter. Amniote eggs use a semipermeable shell to deal with this trade-off, which allows gases to diffuse while keeping water inside the egg. Amniote embryos also have two **extraembryonic membranes** (the **allantois** and **chorion**) that facilitate gas exchange between the embryo and the external atmosphere, leading many to call amniotic eggs "air-breathing." The evolutionary origin of the amniotic egg was an important step that permitted this lineage to

spend more of their life cycle away from water and to diversify and radiate in new ways.

In humans and other placental mammals, the **chorion** and **allantois** fuse to form the **placenta**, which facilitates gas exchange from the bloodstream of the mother to the developing fetus. Oxygen is taken in by the mother's lungs and then transported to the placenta, where it diffuses into the fetal bloodstream.

Box 14.1—Intersections Between Respiration, Development, and Conservation Biology

For any living creature to persist in any habitat for very long, their basic physiological needs must be met. For vertebrate animals, this means they need food to sustain themselves, enough water to keep them from drying out and to facilitate waste removal, and finally, oxygen to breathe. For aquatic animals especially, this last requirement can be a big problem. As we have covered throughout this chapter, hypoxia (low levels of oxygen) is a common occurrence for many aquatic habitats and fishes and amphibians that depend on dissolved oxygen. Aquatic adult animals deal with hypoxia in myriad ways, from coming to the surface to breathe air to using complex countercurrent exchange systems in the gills that extract every bit of oxygen dissolved in the water. However, developing fish and amphibian eggs, embryos, and larvae sometimes do not have this luxury. Often, these developmental stages strongly depend on naturally high environmental levels of oxygen. Here, we will discuss what happens when we humans change the environment, impacting the natural

oxygen levels of streams and the animals that call them home.

Along the Pacific coast of the United States, Coho salmon were once an iconic feature of the landscape and the lives of Indigenous peoples of the region. Today, Coho salmon are extirpated across much of their native range, especially in California. While many factors have contributed to this decline, one major contributing factor that remains a "hot-button" issue today is related to respiratory biology during development. Salmon spend much of their adult lives out in the open ocean but return to inland freshwater streams to breed. Breeding adults swim from the ocean upstream to their breeding grounds, where they spawn millions of eggs that develop into fry and then juveniles before eventually returning to the ocean. As salmon develop, they are highly susceptible to low oxygen levels. When water levels in their breeding streams fall, so too do oxygen levels, sometimes leading to mass mortality and failed breeding events. This is particularly damaging for Coho salmon, which are semelparous, meaning they only reproduce once in their lives.

There are several ways that humans impact water levels in streams across the world. Groundwater feeds streams at their sources, and irrigation in California commonly uses groundwater from underground "aquifers." Agriculture is incredibly important to the economy of modern California, but there is a heavy toll that using all that water takes on the natural ecology of the landscape. Aquifers are replenished by rain, but increasingly common and

severe droughts predicted by models of climate change have also contributed to lower groundwater levels. During an especially severe drought from 2011 to 2016, salmon populations were decimated across California, largely because developing salmon did not survive the low-oxygen conditions found in their streams.

Understanding the respiratory physiology of organisms, especially during development, is a critical component of understanding how to conserve the natural world. If organisms' physiological needs cannot be met, they will not persist in nature. When humans alter the natural landscape, it is critical for us to understand how those alterations impact the living creatures around us. In the case of Coho salmon, there are now much stricter regulations in place to protect the streams that still host breeding populations, and there are even places where the salmon are coming back to their ancestral breeding grounds. By understanding the respiratory requirements and then taking action to ensure those needs are met, humans have the power to help endangered organisms persist into the future.

Lung Development

Nearly all tetrapod vertebrates depend on lungs for gas exchange as adults. Lungs are interesting from a developmental perspective because inflation is an important step in lung development. In other words, lung tissue must be stretched in order to properly develop.

What does this mean? Tissue development is a very complicated process, likely beyond the scope of this course. In brief, however, tissue development is controlled by complex cascades of gene expression. This means that one gene might control another gene, which might control another gene or set of genes, and organs develop correctly when the right set of genes is turned on at the right time. In lungs, some of those genes do not turn on unless the tissue is "stretched." Experiments have shown that living lung tissue in a petri dish will not develop correctly unless it is pulled taut.

In amphibians, most lung development happens during the larval stage, such that air breathing starts while the larval frog or salamander is still reliant on gills for gas exchange. This means that the lungs can develop, be stretched out, and become functional gas-exchange organs without needing to be the primary source of oxygen during this process. However, in some cases, lungs are also important gas-exchange organs for larval amphibians, especially in hypoxic conditions. When amphibians metamorphose, they leave the water and lose their gills, and their lungs are fully functional and ready for the terrestrial world.

Amniote vertebrates do not have the luxury of a prolonged larval stage during which to stretch out and develop their lungs. Instead, they must emerge from the egg or womb with fully developed lungs that are ready to go. It is actually a remarkable feat that human babies are able to breathe within seconds of being born, considering that their lungs need to be stretched during development. It turns out that mammal embryos actually fill their lungs with liquid while in the womb, performing "fetal breathing movements" where that liquid is breathed in and out of the lungs. This stretches the lungs, and experiments in sheep and other animals have shown that stopping these fetal movements prevents proper development. This research has been applied to human medicine in efforts to prevent lung birth defects. Other amniotes, such as *Anolis* lizards, are similar to humans and other mammals, in that the lungs are inflated with liquid while in the egg, where the lung fully develops in anticipation of hatching.

14.4 Evolution of the Respiratory System

The evolutionary history of the vertebrate respiratory system is one full of twists and turns (literally in some cases!). While the earliest vertebrate ancestors lacked lungs and probably had primitive gills, this story is not a stepwise journey from fishlike gills to human lungs. Remember, there are no living ancestors of vertebrates, and all living organisms have had an equally long and fascinating evolutionary history (Figures 14.9 and 14.10).

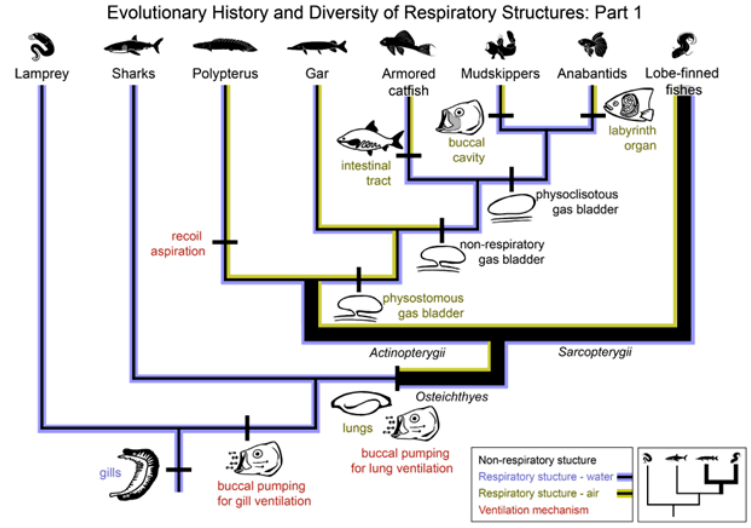

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Figure 14.9—Evolutionary history and diversity of respiratory structures: Part 1. The phylogeny of living vertebrates (including Cyclostomata, Chondrichthyes, and Osteichthyes) is shown, with an emphasis on the diversity of respiratory structures within the Actinopterygii branch of Osteichthyes. Relatively few actinopterygian species can breathe air; however, we overrepresent air-breathing species here in order to highlight the diversity of air-breathing structures present in Actinopterygii. Evolutionary origins or transitions in respiratory structures (purple—structures for water breathing; yellow—structures for air breathing) or ventilatory mechanisms (red) are labeled. Branches are highlighted to indicate the respiratory medium used by each lineage (purple—breathe water; yellow—breathe air; purple and yellow—breathe water and air, "bimodal breathers").

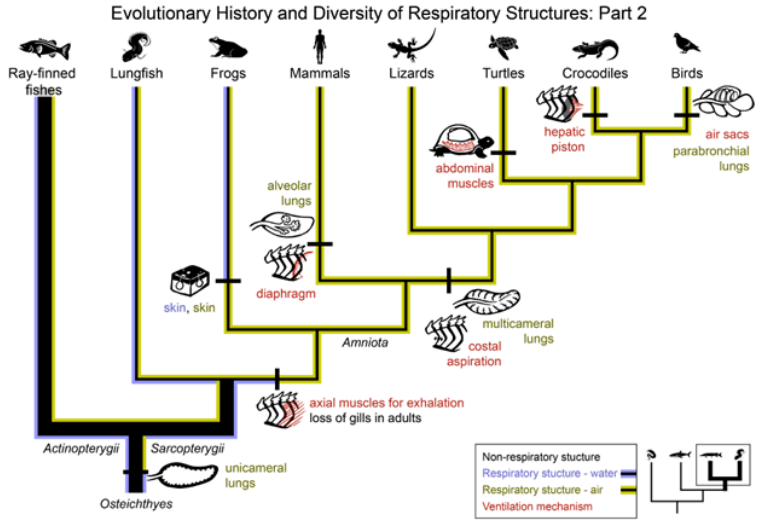

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Figure 14.10—Evolutionary history and diversity of respiratory structures: Part 2. The phylogeny of Osteichthyes (Actinopterygii and Sarcopterygii) is shown, with an emphasis on the diversity of respiratory structures within the Sarcopterygii branch. Evolutionary origins or transitions in respiratory structures (purple—structures for water breathing; yellow—structures for air breathing) or ventilatory mechanisms (red) are labeled. Branches are highlighted to indicate the respiratory medium used by each lineage (purple—breathe water; yellow—breathe air; purple and yellow—breathe water and air, "bimodal breathers").

Jawless Fishes

The earliest common ancestors of modern vertebrates swam in warm seas more than 500 million years ago. These fishlike animals lacked true teeth and had not yet even evolved jaws, though they had mouth openings. However, these animals had **gill pouches** to breathe water just like hagfishes and lampreys today.

The ability to breathe water through gills requires a mechanism for moving water into the mouth and over the gills. An ancient mechanism for creating water flow is the cyclic expansion

and compression (in other words, pumping) of the oropharyngeal and gill chambers. Expansion draws water in through the open mouth, and compression pushes the water out past a set of vascular gills. Water flow *ventilates* the gills, greatly increasing the efficiency of gas exchange, as opposed to simply exposing the gills to the water around a fish. This pumping mechanism is key to understanding the transitions in ventilation strategy across all vertebrates.

Unidirectional flow of water over the gills also enabled the evolution of countercurrent flow. While water is pumped over the gills in one direction, capillaries are arranged so that blood flows in the opposite direction. This design greatly increases the rate and efficiency of gas exchange.

Gnathostomes

The next major transition in the vertebrate tree of life was the evolution of jaws, seen today in all living gnathostomes (up to and including you and me). The first jawed fishes retained the pumping mechanism used for gill ventilation in their jawless relatives but lacked a bony skeleton. Roughly 450 million years ago, these early jawed fish split into two major groups that are still alive today: the cartilaginous fish (sharks and rays) and bony fish (basically everything else).

Chondrichthyes

The cartilaginous fish for the most part retain many respiratory characteristics of early jawed vertebrates, including five gill slits (typically), no air-breathing organs, and an absence of dense, ossified bone.

Osteichthyes

The bony fishes are an extremely diverse group, including typical fishes, like the perches and trout of the world, and bizarre, terrestrial fishes that lack gills, wear shirts, and do their taxes (the Osteichthyes as a formal group includes all descendants of bony fish, and therefore includes amphibians, reptiles, and even mammals). However, their earliest common ancestors were very different from the bony fish around today.

A major development during early bony fish evolution was the origin of air breathing. Some early bony fish, which evolved to use oropharyngeal pumping (specifically the dual pump) for gill ventilation, came to use the buccal pump to breathe air (see Section 14.2, General Structure and Function, for a discussion of oropharyngeal pumping). Exactly what purpose the first air breaths served is not well understood today, though both respiration and buoyancy control have been cited as likely original functions of air breathing. This was probably a very early event in bony fish evolution, because both major lineages of bony fish alive today (ray-finned and lobe-finned fishes) have air-breathing organs (Figures 14.9 and 14.10). Among ray-finned fishes (most typical fishes), some have paired, respiratory lungs and others have unpaired, respiratory gas bladders. Like lungs, respiratory **gas bladders** are vascularized and conduct gas exchange with the atmospheric air that is breathed in. On the other hand, nearly all lobe-finned fishes (lungfishes and tetrapods) have paired, respiratory lungs. The fact that the earliest diverging extant lineage of ray-finned fishes also has paired lungs has led many scientists to suggest that the ancestor of all bony fishes had lungs as well and that lungs evolved into the gas bladders seen in most ray-finned fishes today.

Regardless of the directionality, it is clear that gas bladders (including nonrespiratory gas bladders known as swim bladders) and lungs are **homologous**, meaning they are descended from the same ancestral structure. It is possible lungs evolved first (and unpaired gas bladders evolved from them), lungs evolved multiple

times from unpaired gas bladders, or lungs and gas bladders evolved independently multiple times from some third structure that served as a precursor.

There is an interesting and somewhat mysterious difference worth noting in the ventilation patterns of air breathing between ray-finned and lobe-finned fishes. Both groups ancestrally used a buccal pump, but there are major differences in the way this buccal pump is deployed (Figure 14.11). The ray-finned fishes generally use a "four-stroke" breath type: (1) the oropharynx expands to expire gas from the lungs/gas bladder, (2) compresses to push that gas back out of the mouth, (3) expands again to draw new air into the mouth, and then (4) compresses a final time to push that air into the air-filled organ. The lobe-finned fishes, on the other hand, use a "two-stroke" breath. Lobe-finned fishes like lungfish use a single expansion of the oropharynx to both expel air from the lungs and draw fresh air into the mouth. Following this, two-stroke breathers compress the oropharynx to fill the lungs back up under positive pressure. With few exceptions, lobe-finned fishes (lungfish and tetrapods) use two-stroke air breaths, and ray-finned fishes use four-stroke air breaths. This has led researchers to hypothesize that these breath types are ancestral to each lineage. It is unknown whether a four-stroke or two-stroke breath is ancestral to the other, but it has been suggested they both evolved from simpler expiration and inspiration breaths that were common to both lineages.

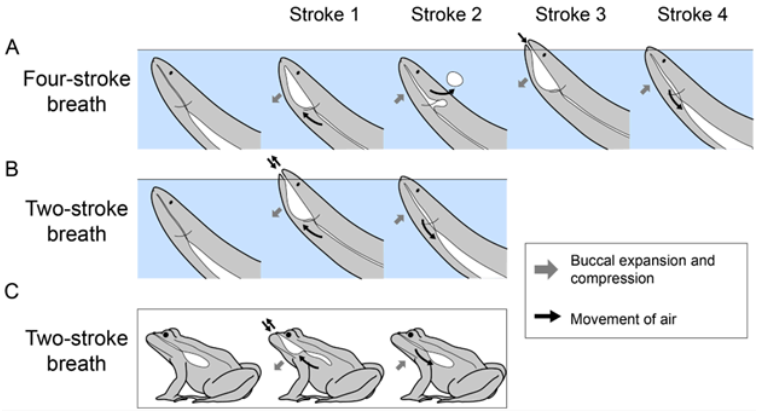

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Figure 14.11—The phases of two-stroke and four-stroke air breathing. Each stroke is an expansion or compression of the buccal cavity. (A) During four-stroke breaths, strokes 1 and 2 produce expiration of air, and strokes 3 and 4 produce inspiration of air. (B) During two-stroke breaths, stroke 1 produces expiration and stroke 2 produces inspiration. Note that A and B are illustrated for a generic fishlike animal, but no single species is known to take both two-stroke and four-stroke air breaths. (C) Two-stroke breaths are also used by amphibians (using the same pattern of movement shown in B). Gray arrows—expansion or compression of the buccal cavity. Black arrows—movement of air.

Actinopterygians

While the gas bladders of only some fishes are respiratory (i.e., vascularized and used for gas exchange), all gas bladders (both respiratory and nonrespiratory) are hydrostatic because the gas inside them provides buoyancy. In fact, for most species, the gas bladder is nonrespiratory and is solely hydrostatic (i.e., functioning to increase and regulate buoyancy). Some of these species (called **physostomous**) retain a connection between the swim bladder and the mouth, while most species (called **physoclistous**) lose that connection and instead fill the bladder with nitrogen gas.

However, even among physoclistous fish, which cannot use their swim bladders for respiration, air breathing has reevolved

many times. This has produced a diversity of air-breathing organs, including elaborately modified gills, respiratory membranes within the mouth, and even respiratory portions of the digestive tract (Figure 14.9). The evolution of various forms of air breathing is a common theme across the fish tree of life.

Sarcopterygians

Lobe-finned fishes, which include lungfishes and tetrapods, are nearly universally lunged. For these groups, lungs are typically the primary site of gas exchange, with some exceptions. The fascinating coelacanths (*Latimeria*) are an amazing group of deep-water lobe-finned fishes that were first described from fossils that are millions of years old and were considered to be a fascinating but extinct group of early sarcopterygians. Amazingly, fresh specimens of these giant deep-sea dwellers were later discovered by South African scientists in the bycatch of local fishermen in 1938, and now much more is known about this mysterious group. Coelacanths lack functional lungs and use their gills for respiration. They do, however, have small fatty organs that scientists believe to be vestigial lungs, similar to how your coccyx is a vestigial tail. The Australian lungfish, *Neoceratodus*, is another lobe-finned fish with a unique respiratory apparatus. Unlike other lungfishes, *Neoceratodus* has a single, unpaired lung and retains a lot of gill functionality. This lungfish appears to mostly use its lung as a buoyancy organ, and only when aquatic oxygen levels drop does it use its lung as a respiratory organ to supplement its gills.

Generally, however, lobe-finned fishes are quite reliant on their lungs to meet their respiratory needs. Perhaps because of this very fact, the most significant evolutionary event in the history of this group was the water-to-land transition where amphibious, tetrapod-like animals like the famous *Tiktaalik* began moving onto land for the very first time. This transition was only possible because these aquatic animals already had lungs and so could

breathe air and acquire oxygen on land (Figure 14.10). These early land-dwellers likely shared many characteristics with modern amphibians and probably maintained an aquatic larval phase reliant on gills for respiration.

Tetrapods

While some of the early lobe-finned fishes went on to become lungfishes, retaining many "fishy" characters, others eventually evolved into the tetrapods of today: amphibians, reptiles (including birds), and mammals. These groups all are distinct in various ways with different respiratory strategies, lung morphologies, and ventilation patterns.

The amphibians evolved from early tetrapods and retain many respiratory traits that are probably similar to those groups. Most amphibians retain an aquatic larval phase, with gills of various shapes and sizes depending on the species. Amphibians also retain the ancestral buccal pump, which they use to ventilate the gills as larvae and then lungs as adults. Some amphibians, however, use muscular contractions in the body wall to actively empty the lungs in conjunction with the buccal pump used to fill the lungs. This is a major transition in ventilatory mechanics foreshadowing the transitions that would occur in other tetrapod groups. Amphibian lungs are generally simple and unicameral, consisting of two open chambers connected to a glottis at the base of the mouth. Amphibian lungs are vascular and somewhat septate, but the degree of complexity greatly differs across species.

Amphibians are the most variable tetrapod group in terms of respiratory strategy, perhaps because they employ their skin as another major source of gas exchange. Many amphibians even use their skin as the *primary* source of gas exchange. Others use gills as a primary source of gas exchange, not only during the larval stage, but all the way through adulthood. There are also actually amphibian species that have evolved to lose their lungs entirely,

using only their skin and the lining of the mouth for gas exchange. Lacking gills, some aquatic amphibians have evolved to increase skin surface area, making gas exchange faster and alleviating some of the physical limitations of diffusion that make skin respiration difficult at large sizes (Figure 14.4). Others have evolved to retain their larval gills into adulthood, a developmental process called "neoteny."

The final extant major tetrapod group are the amniotes, which include reptiles, mammals, and other related but now-extinct groups like the dinosaurs. The amniotes are distinguished by their key evolutionary innovation: the terrestrial, amniotic egg (see Section 14.3, Development of the Respiratory System). Early amniotes were fully terrestrial, laying eggs on land and relying on lungs for essentially all gas exchange. Modern amniotes generally retain these traits, with some key exceptions.

A major feature of amniote respiration is what is called **aspiration breathing**. Aspiration breathing is a set of ventilatory patterns defined by the use of active, muscular movement of the thorax and abdomen to pull air directly into the lungs. This pattern of breathing differs from the ancestral buccal pump because air moves directly into the lungs and does not require head musculature to contribute to ventilatory flow. Most modern reptiles use rib musculature to expand the body wall and pull air into the lungs. Mammals have an alternative breathing system, which uses a muscular diaphragm to expand the pleural cavity, lowering pressure in the lungs and pulling air in.

Amniote lungs vary greatly in shape. The lungs of the first amniotes were probably similar to those of amphibians: paired and unicameral. In the course of amniote evolution, other lung types have arisen, from the elongate, unpaired lung in snakes, to elaborate bird lungs with many chambers that allow for unidirectional airflow, to the branching, tree-like lungs of modern mammals. This explosion of diversity is most often explained by the higher metabolic rates of some amniotes, which require more oxygen and therefore lungs that are more complex and effective at extracting oxygen.

14.5 Diversity of the Respiratory System

The evolutionary history of the vertebrate respiratory system is a tale with many changes and innovations, shaped by changes in the ecology and physiology of ancestral species. This history has produced rich diversity in the **respiratory structures** and **ventilation mechanisms** of vertebrate species alive today (Figures 14.9, 14.10, and 14.12). In this section, we will dive in and explore this diversity in more detail.

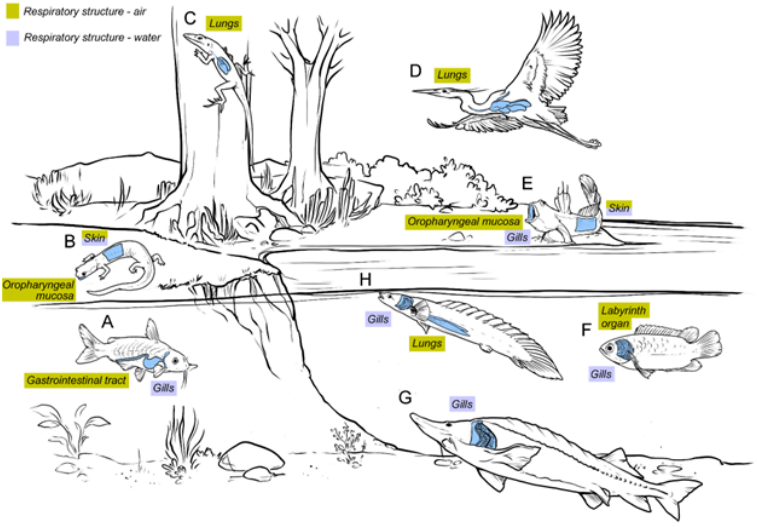

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Figure 14.12—Illustration of vertebrates and their respiratory structures in a variety of habitats. Air-breathing structures are found in fully aquatic, amphibious, and fully terrestrial species, while water-breathing structures are found in aquatic and amphibious species. (A) Armored catfish—gills (water) and gastrointestinal tract (air). (B) Lungless salamander—skin (water and air) and oropharyngeal mucosa (air). (C) Basilisk lizard—lungs (air). (D) Great blue heron—lungs (air). (E) Mudskipper—skin (air and water), oropharyngeal mucosa (air), and gills (water). (F) Climbing gourami—gills (water) and labyrinth organ (air). (G) Sturgeon—gills (water). (H) Bichir—gills (water) and lungs (air).

Fishes

Gills

Gills are paired structures that have capillary-rich membranes with a large surface area and that enable respiratory gas exchange in aquatic environments. The gills of fishes (cyclostomes, elasmobranchs [sharks and rays], and actinopterygians) share many features in common but also differ in notable ways, as described in Figure 14.13. The gills and ventilation mechanism of hagfishes are the most different from the other clades, so we will discuss them last.

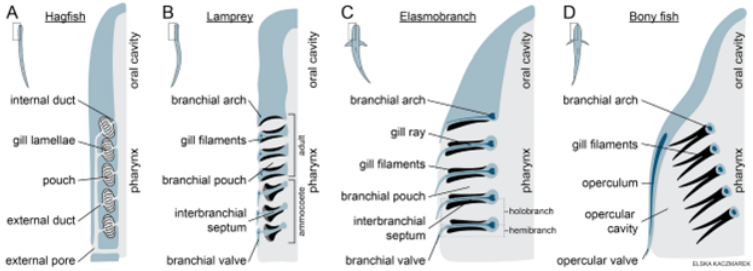

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Figure 14.13—Anatomy of gills in fishes. (A) Hagfish gills are located in branchial pouches. (B) Lamprey gills are arranged in holobranchs supported by branchial arches and interbranchial septa. Larval lamprey (ammocoetes) have branchial valves to maintain unidirectional flow, while adult lamprey lack branchial valves and use tidal flow. (C) Shark gills are arranged in holobranchs supported by branchial arches and interbranchial septa. (D) Bony fish gills are supported by branchial arches, but the gill filaments extend freely without interbranchial septa.

Lamprey, elasmobranchs, and bony fishes have gills arranged as paired holobranchs (Figure 14.13). "**Hemibranch**" refers to one set of **gill filaments**, while **"holobranch"** refers to both sets supported by the same **branchial arch** (also known as a gill arch).

A branchial arch is a paired series of bones that articulate dorsally on or near the head and meet ventrally along the midline. Two sets of gill filaments (**primary lamellae**) extend from the branchial arch, one on the anterior side and one on the posterior side. The space between the hemibranchs on adjacent branchial arches is called a branchial pouch.

In lamprey and elasmobranchs, an interbranchial septum (connective tissue) lies between and anchors the anterior and posterior sets of gill filaments of each holobranch (Figure 14.13). Elasmobranchs have a cartilaginous gill ray within each interbranchial septum, providing structural support. Elasmobranchs and larval lamprey have branchial valves (flaps that extend posteriorly and laterally from each holobranch) that create separate gill openings for water to exit through after passing through the branchial pouches. Importantly, the branchial valves also maintain unidirectional flow of water—they are pushed open when water flows out of the branchial pouches but are pushed closed by the reverse flow of water. Adult lamprey do not have branchial valves, which enables tidal ventilation, crucial for their ability to respire while feeding.

In contrast to lamprey and elasmobranchs, in bony fish, the anterior and posterior sets of gill filaments extend from the branchial arch separately, with no interbranchial septum (Figure 14.13). Instead of water flowing through distinct branchial pouches and then exiting through separate openings (divided by the branchial valves), in bony fish, water flows past each set of gill filaments and then combines and flows out of a single (typically large) opening on each side of the head: the opercular valve. The opercula are paired flat bones that cover the gills and move during ventilation. If you have ever watched a bony fish in an aquarium, you have likely seen its opercula flare out and in with every ventilation cycle. Like the branchial valves of lamprey and elasmobranchs, opercular membranes (flaps of skin extending from each operculum) function as valves that maintain unidirectional flow of water.

The gill filaments of lamprey, elasmobranchs, and bony fish are covered in tiny **secondary lamellae**, platelike ridges that contain respiratory capillary beds. Water flows over the secondary lamellae in the opposite direction as the blood flowing in the capillaries. This countercurrent arrangement maximizes gas exchange, and as a result, fish are able to extract a higher percentage of oxygen from their respiratory fluid than all other vertebrates. This feat is important to their survival because water contains much less oxygen than air.

Gill Ventilation

Countercurrent exchange requires unidirectional flow of ventilated water. Fishes generate unidirectional flow of water using compression and expansion of structures in the head (with the exception of adult lamprey—which use tidal, not unidirectional, flow—and species that use ram ventilation instead of cyclic movements of cranial elements).

Larval lamprey ventilate their gills using their branchial basket and their velum. The branchial basket, also known as the branchial apparatus, consists of the branchial arches and associated structures in the pharynx. The velum is a structure with muscular flap-like folds positioned between the pharynx and the mouth. To pump water out of the pharynx through the gill openings, the velum seals closed (blocking flow back into the mouth) and the branchial basket compresses. To draw water into the branchial basket through the mouth, the velum and branchial basket relax and the branchial basket elastically springs back into its expanded shape. While the velum is always used to generate flow, the branchial basket contracts and expands more when the larval lamprey has greater oxygen demand. In contrast, if the branchial basket is not contributing, then there is little flow laterally over the gills, and flow is instead used for filter feeding.

Postmetamorphosis, adult lamprey generate tidal flow (in

and out of the gill openings) using only contraction and relaxation of the branchial basket. The velum separates ventilatory flow in the pharynx from the mouth. In species that are parasitic feeders as adults, this enables the lamprey to respire while its mouth is attached to the side of a fish as it feeds on its prey.

Elasmobranchs and bony fish produce unidirectional flow by compressing and expanding their buccal chamber and their parabranchial (in elasmobranchs) or opercular (in bony fishes) chambers (Figure 14.5). This mechanism is often called a **dual pump** because it involves two chambers or is called a "two-phase pump" because it has a suction phase and a pressure phase.

The suction phase begins with the chambers compressed and the oral and parabranchial/opercular valves closed. The buccal chamber begins to expand, the oral valve opens, and then the parabranchial/opercular chamber expands. Because the parabranchial/opercular valves remain closed, the expansion of the parabranchial/opercular chamber creates subambient pressure that is lower than that in the buccal chamber. Therefore, water flows through the buccal chamber into the parabranchial/opercular chamber, following the pressure gradient. During the pressure phase, the oral valve closes, the buccal chamber begins to compress, and then the parabranchial/opercular cavity begins to compress. Because the oral valve is closed but the parabranchial/opercular valves are pushed open, there is greater pressure in the buccal cavity, causing water to flow into the parabranchial/opercular chamber and out of the gill openings. The parabranchial/opercular valves limit reversal of flow, but flow reversals have been observed in some species, especially when there is lower respiratory drive.

Elasmobranchs that are benthic will often bury themselves in the sediment and cannot use their mouth for ventilation. Instead, they draw water in through their spiracles, which are small paired openings on the dorsal surface of the head that open into the buccal chamber. Some bottom-dwelling sharks will also use the spiracles when not buried, and in some cases bidirectional flow (i.e., flow in and out of the spiracles rather than out of the branchial valves),

has even been observed, perhaps to avoid disturbing the sediment around them. Exclusive use of spiracles for ventilation has only been observed in benthic species, but spiracles are present in most elasmobranchs, including both benthic and nonbenthic species. Aside from gill ventilation, spiracles may also be used to sample water passing by for chemosensation.

Gills and Gill Ventilation in Hagfishes

The gills of hagfishes are located within branchial pouches, which are posterior to the pharynx and are not supported by branchial arches (Figure 14.11). The branchial pouches have muscular outer walls and gill lamellae (folds) contained within. Like all other fishes, hagfishes have countercurrent gas exchange. Water flows unidirectionally, entering each branchial pouch through its afferent duct and exiting through its efferent duct, while blood flows through the gill lamella in the opposite direction. Gill ventilation is driven by the movement of the velum and peristaltic contractions of the branchial pouch walls. Unlike the velum of lamprey, in hagfishes, the velum is shaped like two curled sheets of paper, and it furls and unfurls to pump water through the head. Water is drawn in through the nostril or mouth, passes through the pharynx, is pumped into the afferent ducts and out of the efferent ducts of the branchial pouches, and then flows out of the external gill openings. Some species have one common opening on each side of the hagfish, and some have separate openings for each branchial pouch.

Lungs and Gas Bladders

Lungs and gas bladders are air-filled sacs within the body, found in lobe-finned and ray-finned fishes (Figure 14.14). Lungs are present in all lobe-finned fishes (lungfishes and tetrapods) as well as in

some ray-finned fishes (specifically polypterid species). All other ray-finned fishes possess gas bladders (either respiratory or nonrespiratory). While nearly all lungs are well vascularized and used for respiratory gas exchange, the gas bladders of only some fishes are vascularized and used for respiration. These respiratory gas bladders connect to the mouth via a pneumatic duct (i.e., are **physostomous**) and are ventilated using air breaths, just like lungs. Nonrespiratory gas bladders are often called "swim bladders" and in most species lack a passage to the mouth (i.e., are **physoclistous**). In most air-breathing fishes, air breathing is used to supplement gill ventilation, either when dissolved oxygen is too low to meet their oxygen demand or when their oxygen demand is elevated (e.g., because of high physical activity) and cannot be met by gill ventilation. In some air-breathing fishes, air breathing is the primary source of oxygen, and the gills are reduced. These species typically live in habitats with prolonged hypoxia, and their reduced gills minimize the loss of oxygen to the water.

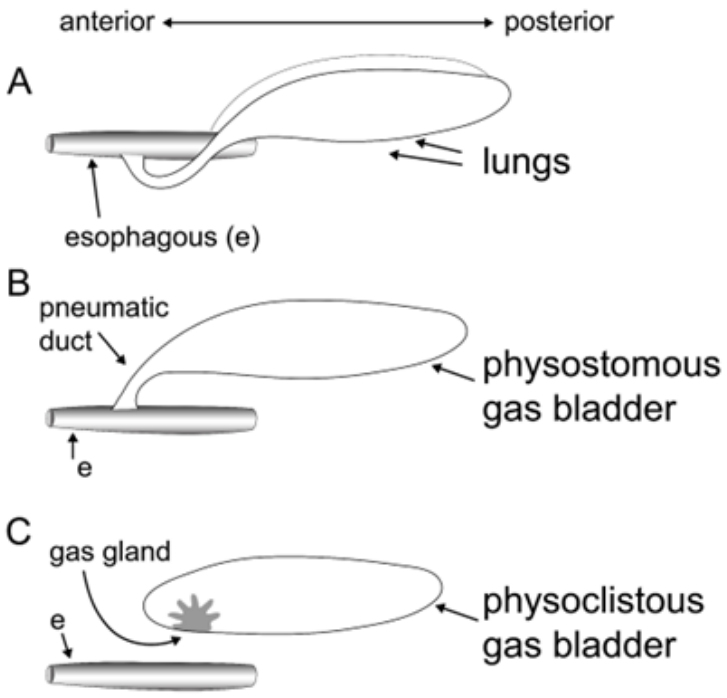

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Figure 14.14—Basic anatomy of air-filled organs. (A) Lungs are paired air-filled organs. In nontetrapod fishes, the lungs connect to the esophagus (as illustrated here) via a pneumatic duct. In tetrapods, the lungs connect to the mouth via the trachea. (B) Physostomous gas bladders are unpaired air-filled organs that connect to the esophagus via a pneumatic duct. Physostomous gas bladders may be respiratory (i.e., vascular) or nonrespiratory. Some have a gas gland for buoyancy control. (C) Physoclistous gas bladders are unpaired air-filled organs that lack a connection to the esophagus. A gas gland is used to control volume and buoyancy via diffusion of gas into or out of the gas bladder.

Air Breathing: Ventilation of Lungs and Gas Bladders

Air-breathing fishes ventilate their lungs and gas bladders using buccal pumping. Unlike the dual pump for gill ventilation, the buccal pump only uses expansion and compression of the buccal chamber

and creates tidal flow of air into and out of the lungs or gas bladder (Figure 14.5). While buccal pumping causes airflow during most of the air breath (including changes to buccal volume and lung / gas bladder inflation), deflation of the lung / gas bladder is driven by hydrostatic pressure on the body wall, as well as possible contributions from smooth muscle fibers in the walls of the lungs or gas bladder.

Air-breathing fishes use one of two air breath types, a **two-stroke** breath or a **four-stroke** breath, both of which utilize buccal pumping but differ in their sequences of movement and airflow (Figure 14.11). The "strokes" refer to the number of expansions and compressions of the buccal chamber, by analogy to two-stroke and four-stroke piston engines (see Section 14.4, Evolution of the Respiratory System).

One clade of ray-finned fishes, Polypteriformes, has evolved a mechanism for aspirating air rather than using buccal pumping. Aspiration occurs when lung ventilation is caused by active movement of the body wall and thereby the lung wall. This is in contrast to buccal pumping, where airflow is caused by active movement of the head. In Polypteriformes, contraction of muscles in the body wall compresses the lungs and causes expiration. It also deforms the interlocking scales of the fish ("scale-jacket"), which surround the body like a suit of armor. When the muscles relax, the scale-jacket recoils, reexpanding the lungs and causing air to be inspired through the open mouth. This is the only nontetrapod fish known to use aspiration. Notably, these fish still move their buccal chamber in the pattern of a typical four-stroke air breath, the breath type expected for these ray-finned fishes.

Accessory Air-Breathing Organs

We have focused most of our attention on air-breathing fishes that utilize **lungs** or respiratory **gas bladders** retained from the ancestral air-breathing species at the base of Osteichthyes. However, the

majority of air-breathing fishes have nonrespiratory gas bladders and instead use a variety of other air-breathing organs (Figure 14.9). In other words, these fishes belong to lineages that ancestrally lost the ability to breathe air into their physoclistous gas bladders and have gone on to reevolve different forms of air breathing. These novel air-breathing organs can be classified as involving adaptations to the gills, the membranes lining the oropharyngeal cavity, the digestive tract, and the skin. Species with elaborated gill structures or highly vascularized buccal membranes take atmospheric air into their mouths and hold it there to enable gas exchange. Many catfish species use their digestive tract for respiration (Figure 14.12). Instead of using buccal pumping to move air into the gas bladder, they pump air into the esophagus. This air moves through the digestive tract, and oxygen diffuses across the highly vascularized epithelia. Last, some species supplement their oxygen uptake using their skin and may move onto land temporarily, exposing their moist skin to the greater concentration of oxygen that is in the atmosphere.

Amphibians

Cutaneous and Oropharyngeal Respiration

The amphibians (frogs, salamanders, and the enigmatic caecilians) are the only major tetrapod group that uses the skin as a major gas-exchange organ. In order to facilitate cutaneous gas exchange, amphibian skin is generally thin, moist, and well vascularized. Many amphibians have evolved intricate, unique skin structures hypothesized to increase surface area and thus respiratory capacity. In extreme cases, some amphibians have either reduced or totally lost lungs, most famously in the salamander family **Plethodontidae**, relying entirely on cutaneous forms of respiration.

Oropharyngeal respiration is a specific form of **cutaneous**

respiration that takes place across the skin lining the mouth and pharynx. In order to perform this respiratory strategy, an animal must pull air into the mouth, but it does not require the active filling of a lung or other internal organ. Many amphibians, both lunged and lungless, perform buccal pulsing, quickly filling the mouth with air and emptying it to exchange gases across the oropharyngeal surfaces.

The trade-offs between **water loss** and **gas exchange** are fundamental to how respiratory anatomy has evolved. Cutaneous respiration is particularly problematic from this perspective, because the entire exterior surface is potentially exposed to drying factors, as opposed to more internal gills or lungs. If a frog was to exchange oxygen across the entire skin organ, that frog would dry out very quickly in any dry environment. Unsurprisingly then, many amphibians are restricted to cool, moist environments. However, those amphibian species that venture into drier, hotter environments have reduced cutaneous respiration, with thicker, drier skin that is less susceptible to water loss, and they rely more strongly on lung respiration.

Gills and Gill Ventilation

While lungs are the dominant respiratory structure across tetrapods, many amphibians retain some form of gills over the course of their development. In the prototypical life cycle of amphibians, an aquatic egg hatches into a larva with gills, which later transforms into a more terrestrial adult that lacks gills. This is true for many frogs, salamanders, and caecilians. However, some neotenic salamanders retain gills into adulthood, perhaps most famously in the axolotl (*Ambystoma mexicanum*), a highly endangered salamander native to central Mexico that is now common in the pet trade.

Amphibian gills generally fall into two groups: internal and external. Internal gills are contained within a chamber of the mouth,

and a series of vascular gill tufts provide gas exchange when water is pumped into the mouth, through the gills, and out an opening or series of openings. External gills are outside the mouth, with large, branching tufts typically sitting directly behind the head. These gills are ventilated not by moving water through the mouth but instead by moving the head and/or moving the external gills through the water.

Frog tadpoles typically have internal gills, while salamanders with gills typically have both internal and external gills. In salamanders, the internal gills have a series of openings on either side of the head, so water pulled into the mouth can be pumped out in every direction. Frog tadpoles have a different system, where the gills empty into a single spiracle, typically on the left side of the body.

Lungs, Buccal Pumping, and Active Expiration

Even with vascular skin, and gills in some cases, lungs are still an important site of gas exchange for many amphibians. This is particularly true for oxygen uptake, as the skin and gills are more effective as CO_2 eliminators. Amphibian lungs are generally unicameral: Each lung consists of a single primary chamber. This chamber is surrounded by a net of connective tissues including blood vessels and a capillary net for gas exchange as well as smooth muscle bands that squeeze the lungs into a honeycomb shape, increasing surface area.

Amphibian lung ventilation typically follows a two-stroke pattern of breathing that is similar to that seen in lungfish (Figure 14.11). Air breathing begins with oropharyngeal expansion, during which the lungs empty and fresh air is pulled into the mouth. Following this, a single compression of the oropharynx refills the lungs, completing a single breath. Unlike lungfish, however, some amphibians use axial musculature to empty the lungs.

Larval Lung Ventilation and Bubble Sucking

People often assume that gills and lungs do not overlap in the life cycle of frogs and that larval tadpoles use gills and then switch over to lungs at adulthood. In reality, however, many larval amphibians breathe air into the lungs at a very early age, in some cases even before feeding begins.

In order to breathe air at such small sizes, tadpoles must contend with the **surface tension** of water. Surface tension is a phenomenon created by extra hydrogen bonds between water molecules at the interface of water and air. For large animals, the surface tension of water is a trivial barrier to reaching the surface, and so whales and other aquatic animals easily breach the surface to breathe in air. However, breaking through the surface tension is a serious feat for smaller animals. Tadpoles deal with this problem by "**bubble sucking**," a form of air breathing where instead of breaking through the surface, the surface is sucked down into the mouth as small bubbles and then bubbles of air are compressed into the lungs. As tadpoles grow larger, they eventually become large enough to breach the surface and so transition from bubble sucking to breach breathing (Figure 14.15).

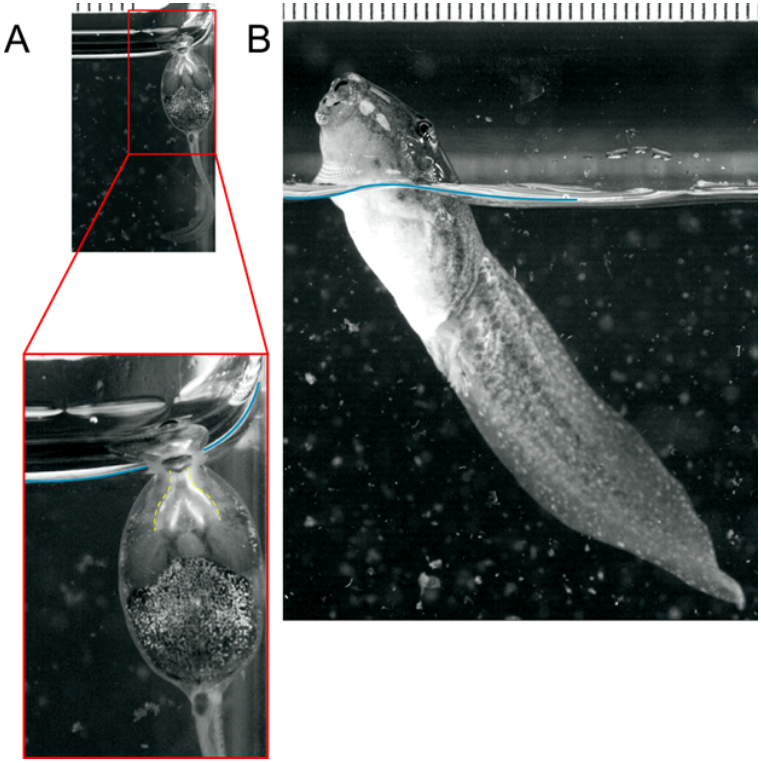

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Figure 14.15—Bubble-sucking and breaching frog tadpoles. (A) *A small Rana tadpole bubble sucks. Notice that the air bubble is sucked into the mouth (outlined in yellow in the inset) from the level of the surface tension (in blue).* (B) *A large Rana tadpole easily breaks through the surface, breaching to breathe air well above the surface. The two photos at the top share the same scale, showing the great increase in size over development. Stills taken from videos by Jackson R. Phillips and Kurt Schwenk.*

Nonavian Reptiles

In addition to the diversity of lung anatomy and structural differences among nonavian reptiles, we will see that there are additional mechanisms for lung ventilation. We will tackle these

structural and functional elements simultaneously, as it will help us better understand the linkage between structure and function if we can visualize the lungs ventilating along with the changes in their structural elements.

One of the largest differences between the lungs of many reptiles and amphibians is the subdivision of the lung into distinct large chambers (lobes, as we would refer to them in mammals). Amphibians generally have **unicameral** lungs—that is, each lung has one large chamber. Reptiles (and other amniotes) generally have **multicameral** lungs, meaning each individual lung may have multiple chambers (Figure 14.16). At a superficial level, the division of one lung into multiple chambers allows for increasing the surface area available for the diffusion of oxygen and carbon dioxide. As reptiles (and other amniotes) evolved into larger sizes and some became more active, the additional surface area helped support the additional energy requirements. Whether air moves from one chamber to another is highly dependent on species and stage of ventilation.

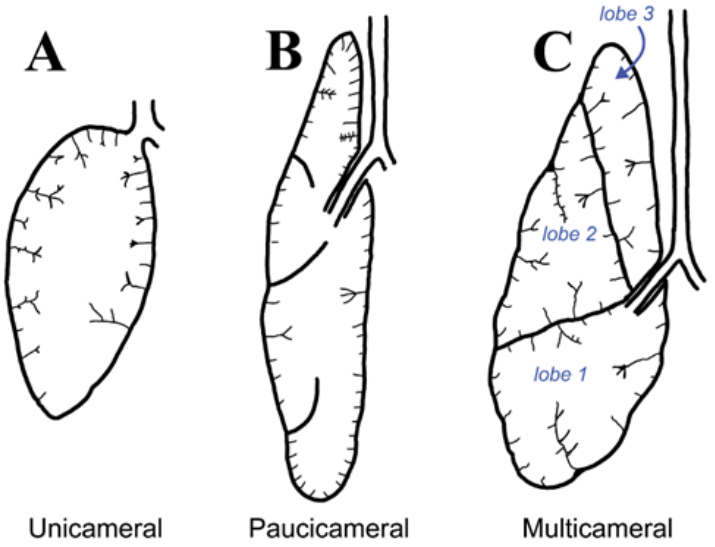

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Figure 14.16—The three different lung types found in reptiles. (A) Unicameral lungs have a single chamber, surrounded by folds that increase surface area and carry vasculature. (B) Paucicameral lungs have larger divisions between different areas of the lung, further increasing surface area and topographical complexity. (C) Multicameral lungs have full divisions between different lung lobes and have high surface area and functional complexity.

The lizards will serve as a convenient starting point for investigating changes in both the structure of the lungs and their ventilation. Partially, this is because lizard lungs are extremely variable across different lineages. Some lizard lungs are unicameral like the amphibians, some are multicameral with distinct chambers, and finally some are **paucicameral** (incomplete separation of lung chambers; Figure 14.16). To further complicate the issue, it is difficult to pull apart trends within lizard groups as to who has which type of lung. The monitor lizards (Varanidae) have complex multicameral lungs, while their neighbors on the phylogenetic tree have both unicameral lungs (tegus, Teiidae) and paucicameral lungs

(iguana, Iguanidae). Even within a group like the monitor lizards, lung complexity is very different, especially if you are comparing a Komodo dragon to a Savannah monitor.

With great variation in complexity comes great variation in behavior. There are multiple mechanisms of ventilation in lizards. The most common of these is known as **costal aspiration**. Costal refers to the ribs, which in this case are the main drivers of ventilation. Remember that the lungs are ventilated via changes in pressure. In the amphibians, positive pressure in the buccal cavity pushes air into the lungs. The lizards are our first example of using negative pressure to draw air into the lungs rather than push it. In costal aspiration, the ribs rotate outward (we are simplifying the motions), increasing the volume of the body cavity. Increasing volume decreases the pressure, and air is pulled into the lungs to balance the pressure. To exhale, the muscles that rotated the ribs can relax, and as the ribs return to their original location, the positive pressure they generate helps push the air back out of the lungs. However, if you have ever seen a lizard run, you know that they bend their bodies quite a bit side to side while they move. That lateral bending also involves active control of the ribs (and obviously the vertebral column), so how do lizards breathe and move at the same time? For many lizards the answer is simple; they do not. Many species will simply hold their breath while they are running, necessitating that they must stop periodically to quite literally catch their breath. However, there are other methods that some lizards can employ. Smaller lizards, like the anoles, can still make use of buccal and gular pumping to push air into the lungs, particularly after quick bouts of intense locomotion. Larger lizards, like the monitor lizards, have increased amounts of smooth muscle associated with the lungs, hypothesized to help move air through the lungs.

We have described a fair bit of variation in the morphology of lizard lungs while neglecting one very large group of lizards: the snakes. This is intentional, as snakes represent a distinct change in body plan that impacts many different body systems. Like many

of the other organ systems in snakes (and snakes themselves), the respiratory tract becomes quite elongated in snakes. There are two key elements, one structural and one functional, that make snakes unique. In the most recently diverging groups of snakes—the Colubridae, Elapidae, and Viperidae, among others—there are technically two lungs, but one becomes highly reduced during development and in many cases disappears. In all these groups, it is the left lung that is highly reduced or has vanished. The earlier diverging snake groups (the boas and pythons, for example) retain two individual lungs. In the elongated lungs, there is also evidence that the entire lung is not used for respiration. The most posterior section of the lung is often referred to as the saccular portion of the lungs, while the more anterior sections are the respiratory portion. How can you tell the difference? The saccular portion lacks blood vessels associated with the lung itself, making it impossible for gas exchange to take place. Additionally, it appears that at least a few species of snakes can breathe using different regions of the lung independently. For example, boa snakes are able to breathe with different parts of the lung during prey constriction to ensure they are able to both squeeze their prey and ventilate their lungs.

Moving to the turtles, some of our base assumptions for how reptiles breathe need to be updated. This is primarily because of the change to the ribs in turtles (see Chapter 9—Postcranial Axial Skeleton). The ribs, becoming fused to and playing a vital role in the structure of the shell, are no longer mobile enough to enable costal aspiration. So how do turtles create the pressure differences needed to move air into and out of the lungs? There are mobile elements of the skeletal system of turtles: The limbs and some of the abdominal muscles can be manipulated. The muscles associated with the limbs and the abdominals function to create the same pressure changes we saw in the lizards. However, the shell limits the mobility of these elements. So how is the pressure created? In turtles, we see that if the skeletal body wall cannot be moved, then the organs themselves must move. By moving the viscera within

the body cavity, resulting pressure changes allow for the lungs to expand and contract.

Turtles are also famous (infamous?) for developing an additional method for breathing. Many turtles spend their winters buried in the mud of ponds and rivers, awaiting the warmth of spring. However, this means that they are unable to breathe with their lungs during their long submerged slumber. While their metabolic rates drop precipitously, they do not completely shut down and still require oxygen. Their alternative method of respiration involves gas exchange through their cloaca. Turtles have a highly vascularized cloaca, with a thin layer of tissue separating the blood vessels from the external environment. This use of cloacal ventilation provides enough oxygen to meet all the turtle's metabolic needs during the winter until it can resume using its lungs in the spring.

The crocodylians, to no surprise, also have a few anatomical innovations for their respiratory system. In many ways, we can point back to what we observed in the lizards (and in particular the monitor lizards) for some of the structures and behaviors present in the crocodylians. Crocodylians have multicameral lungs, with extensive subdivisions and highly complex surfaces for increasing surface area. However, crocodylians possess a muscle that is not found in other vertebrates and is used for ventilation. Running from the pelvis to the liver is a muscle known as the **diaphragmaticus** (Figure 14.17). Convergent to the mammalian diaphragm, the independently evolved diaphragmaticus works in a very similar but subtly different way in the crocodylians. The diaphragmaticus attaches posteriorly to the liver (and other structures). The liver is physically connected to the lungs. When the diaphragmaticus contracts, the liver and lungs are pulled posteriorly. This creates the negative pressure to pull air into the lungs, and when it relaxes, the positive pressure created by the liver returning to its resting position pushes the air back out. We refer to this as the hepatic piston, moving the liver back and forth in such a way to change the pressures.

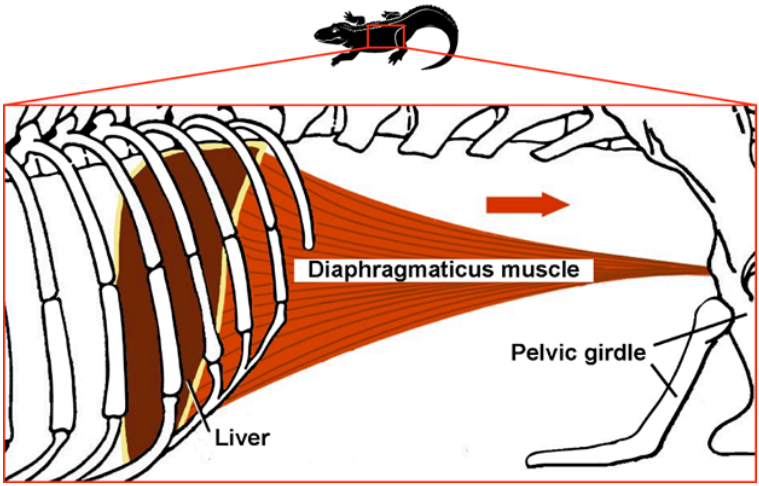

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Figure 14.17—The diaphragmaticus muscle in crocodylians. Contraction of the diaphragmaticus muscle (red arrow) pulls the liver posteriorly and expands the lungs (which are anterior to the liver), which causes inspiration. Relaxation of the diaphragmaticus and contraction of abdominal muscles moves the liver anteriorly and compresses the lungs, causing expiration.

Aves

When considering the respiratory system of the birds, it is helpful to remind ourselves of two important features of bird behavior and physiology that impact the respiratory system. The first is that most birds undertake **powered flight**. Filling the body with air instead of water may help with flight, reducing the density of an individual. The second is that birds are **endothermic**, which comes with an increased metabolic rate. If more energy is needed to maintain a stable internal body temperature, then more oxygen is required to make use of that energy. It should come as no surprise then that the avian respiratory system is quite effective at obtaining oxygen. Importantly, birds have **unidirectional airflow** throughout

their respiratory tract, which promotes efficient gas exchange by maintaining larger concentration gradients, as we discussed earlier in this chapter.

To begin, we should define some new characters. Birds possess all the major structural elements of vertebrate respiratory systems: larynx, trachea, bronchi, and lungs. Birds also possess a **syrinx**, a structure that allows for the production of sound, as well as a series of structures known as **air sacs**. The air sacs are not directly involved in gas exchange; instead, they play a more complex role in the movement of air throughout the respiratory system of birds. The number of air sacs and their position in the respiratory system is quite variable, with new discoveries being made continuously (in fact, as the authors write this, a new paper came out in *Nature* on a type of air sac in soaring birds). Let us use a simplified model to illustrate how the air sacs work in conjunction with the other elements of the respiratory system to visualize how birds generate the unidirectional airflow (Figure 14.18).

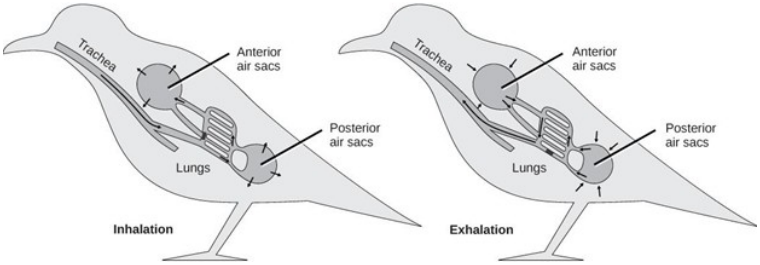

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Figure 14.18—Simplified anatomy of the avian respiratory system. Two cycles of expansion and compression of the air sacs must occur in order to move a parcel of air through the entire respiratory system. The first inhalation draws air through the trachea and bronchi into the posterior air sacs and lungs. The first exhalation causes that air to move from the posterior air sacs into the lungs. The second inhalation draws air from the lungs into the anterior air sacs. Last, the second exhalation expels air from the anterior air sacs out through the bronchi and trachea.

In our model as presented in Figure 14.18, there are two

air sacs: anterior and posterior. The air sacs help separate the functional roles of ventilation and gas exchange. In nonavian reptiles, the lungs have to be stretched open, creating the negative pressure that moves air into the lungs. In birds, the lungs no longer have to be flexible and elastic because the air sacs can be stretched instead. The air sacs are completely avascular, and their entire role is to act as "bellows," expanding and contracting to push air through the lungs. The expansion and contraction of the air sacs are accomplished through movement of the ribs, another example of costal aspiration as we observed in nonavian reptiles. Additionally, many birds can move their sternum a significant amount, enough to also generate pressure changes and the movement of air. In contrast to the air sacs, the lungs themselves are relatively rigid, not expanding or contracting as air is moved through them. This allows the lung tissue to be more specialized for gas exchange without the constraint of needing to be flexible for ventilation. Specifically, the lung tissue between the blood capillaries and the air can be extremely thin because this tissue does not need to stretch. Air moving through the lungs passes first through **parabronchi**, then narrow passageways known as **air capillaries**. The walls of the air capillaries are extremely thin, much thinner than that of the other gas-exchange surfaces in vertebrates. If this was not enough, the orientation of the blood vessels also increases the efficiency of gas exchange. Recall that earlier we discussed crosscurrent exchange, where blood moves at a perpendicular angle to the air, which creates an increased oxygen concentration gradient between the air and the blood (Figure 14.3).

How does this complex system ensure one-way airflow through the whole tract? Let us follow one "air molecule" as it makes its way through the system, using Figure 14.18's simplified tract. Note that it is going to take two full breaths to go from start to finish for this air molecule, and we will have to refer to stages like inhalation 1 and inhalation 2. During inhalation 1, one air molecule enters the nares, passes through the trachea and bronchi, and travels into the posterior air sacs. Not all of the air travels

into the air sacs; some will move directly into the lungs. During exhalation 1, the air molecule is pushed out of the posterior air sacs and travels through the parabronchi in the lungs. During inhalation 2, it moves from the lungs into the anterior air sacs. Finally, it is during exhalation 2 that the air molecule is pushed from the anterior air sacs back into the trachea and out of the body.

The adaptations of bird lungs, including extremely thin blood-gas barriers, crosscurrent exchange, and unidirectional airflow, create an incredibly efficient system of extracting oxygen from the air. It accommodates the intense oxygen requirements of endothermy and flight. In fact, it has allowed birds to take flight to some extremes previously thought impossible. Bar-headed geese have been observed flying over Mt. Everest, over 8 km above sea level. The availability of oxygen at that height was thought to be too low to support any animal, but the bar-headed goose can simply cruise over Mt. Everest thanks to its respiratory system and some physiological tricks.

Box 14.2—Scientist Spotlight: Colleen Farmer and the Evolution of Unidirectional Airflow in Reptilians

Birds have perhaps the most bizarre lungs among all vertebrates. For as long as humans have interacted with birds, we have known that bird lungs are attached to strange sacs that extend around the body, unlike any other group of animals. These structures were first formally described in 1573 by Volcher Coiter, an important figure in the field of comparative anatomy who was one of the first Europeans to study comparative osteology.

Today, we understand that air sacs in bird lungs have several functions, but one dominant function is

facilitating unidirectional airflow through the lungs. The first experiments to demonstrate unidirectional airflow in bird lungs used powdered charcoal to track the passage of air through the bird respiratory system. Exactly how unidirectional breathing was accomplished was hotly debated at the time, with some scientists suggesting that some valve preventing backflow must be present in order to explain the one-way flow of air through the lungs. The modern understanding of bird lung ventilation was established by Dr. E. H. Hazelhoff, who advanced a hydrodynamic theory of airflow in the bird lung, suggesting that a combination of looped passages and air sacs produce unidirectional airflow in birds without needing any valves. After this breakthrough, the following 70 years saw hundreds of papers published on bird lungs and their unidirectional flow. Because the complex air sacs and looped passages are only seen in bird lungs, it was a foregone conclusion that unidirectional airflow was unique to birds.

Unidirectional airflow has many advantages, because it allows fresh, oxygenated air to pass continuously across a vascular surface. This continuous flow allows for crosscurrent gas exchange, increasing the rate of oxygen uptake to very high levels. Unidirectional air breathing and crosscurrent exchange were originally thought to have uniquely evolved in birds to support the high metabolism required for endothermy and powered flight. This paradigm was intuitive and unchallenged for over 100 years, until Dr. Colleen Farmer of the University of Utah totally changed the game.

Dr. Farmer made the first crack in the armor in 2011 when she published a paper describing unidirectional airflow in the lungs of the American alligator (*Alligator mississippiensis*), a crocodylian, in the journal *Science*. Crocodylians are decidedly not a flying species and are not even endotherms, so the presence of unidirectional flow seemed totally out of place. However, crocodylians *are* relatively closely related to birds, with the two together forming the living archosaurs. Dr. Farmer suggested that unidirectional air breathing was an ancestral trait of archosaurs that was likely also shared by extinct members like the dinosaurs and pterosaurs.

The literature has not stopped there, either. Two members of Dr. Farmer's lab, Drs. Emma Schachner and Bob Cieri, went on to describe unidirectional airflow in a series of lizards. They found that multiple unrelated lizard groups, including monitor lizards and iguanas, all have unidirectional airflow in the lungs, pushing the hypothesized origin of unidirectional air breathing all the way back to the origin of reptilian tetrapods. Finding unidirectional airflow in iguanas was especially surprising, given their relatively simple, unicameral lungs. These findings have revolutionized the field and opened up many new hypotheses and possibilities for how, when, and why different types of air breathing have evolved.

So often in science, a good, reasonable hypothesis is slain with a bit of investigation. Fifty years ago, no biologist would have thought that an iguana lizard could possibly have unidirectional airflow in the

lungs. However, as the evidence builds, it would hardly be surprising if any reptile was found to have unidirectional airflow in its lungs today.

Mammals

The mammalian lung is unique in many ways but in some ways requires us to go back to where we began, with the nonavian reptile lungs. Mammals have biphasic, **tidal breathing** (one inhalation, one exhalation), where air enters and leaves through the same pathway. Mammalian lungs are multicameral, with completely separated lobes. The number of lobes varies in mammals and even within an individual. For example, dogs have four lobes in the right lung and two in the left. Humans have three on the right and two on the left (Figure 14.19).

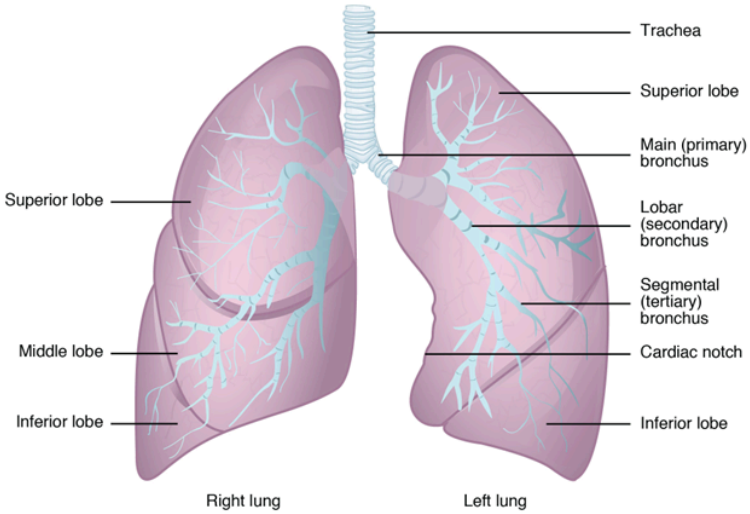

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Figure 14.19—Human lungs with lobes labeled. The right lung has three lobes (superior, middle, and inferior). The left lung has two lobes (superior and inferior) and accommodates the heart, which is located slightly left of midline.

The lungs themselves are surprisingly dense compared to other groups. In the section on birds, we discussed how air travels through progressively narrower passageways (from parabronchi to air capillaries), which increases the amount of surface area available for gas exchange. Mammals will take a different approach with a similar outcome. In mammals, the bronchi divide into **bronchioles**. These bronchioles will continue to divide into smaller and smaller channels for air to pass through. However, unlike in the birds, where the channels become air capillaries, the passages dead-end in mammals. They dead-end in areas we refer to as **alveolar sacs**. The alveolar sacs appear as extremely bumpy balloons, but each bump can be considered an **alveolus** (Figure 14.3). The alveolus is where gas exchange occurs in mammals. Each alveolus is completely wrapped in capillaries, minimizing the distance between the inhaled air and the blood supply. The lung is completely full of alveoli,

making use of every possible space to dramatically increase the surface area available for gas exchange to occur. How many alveoli are there? Estimates in humans range, but recent work has put the number at 480 million alveoli in the average person, with some estimates at almost 800 million. While the movement of air through mammalian lungs may be not as efficient as we observe in the birds, the incredible increase in surface area available for gas exchange allows the mammals to be endothermic and highly active.

While mammalian lungs use bidirectional flow for moving air in and out of the lungs, the manner in which they do so is unique among vertebrates. Mammals have the ability to alter the volume of air coming into/out of the lungs with any particular breath. Your "normal" breathing, the standard in and out that you often don't think about, is what we refer to as *tidal breathing*. It moves in and out of its own accord, like the tides. The ventilation of tidal breathing is largely done by the diaphragm. The diaphragm is a skeletal muscle that sits between the liver/stomach and the lungs. The diaphragm, at rest, is shaped like a hill, with its highest point in the center and the edges much more inferior. The edges are connected to the last few ribs, thoracic vertebrae, and the abdominal body wall. At its center, a central tendon connects the diaphragm to the pericardium, the membrane surrounding the heart. When the diaphragm contracts, it pulls downward/inferiorly, increasing the volume of the thoracic cavity, thereby reducing the pressure and causing the lungs to fill with air (Figure 14.20). In order to exhale, the diaphragm relaxes, and the elasticity of the lungs pulls the diaphragm to its previous position and pushes the air outward under positive pressure.

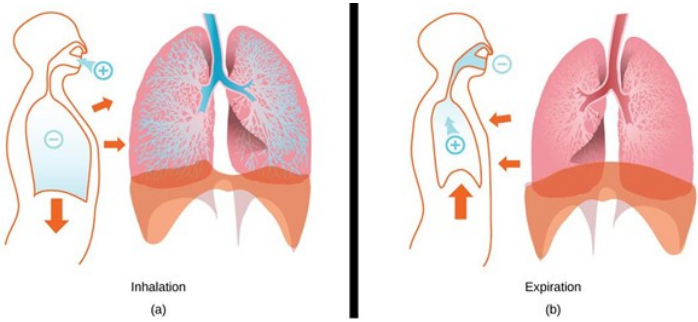

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Figure 14.20—The mechanics of lung ventilation in humans. (A) During inhalation, the diaphragm and intercostal muscles contract, expanding the pleural cavity and the lungs. This draws air into the lungs through the mouth or nose. (B) During exhalation, the diaphragm relaxes, allowing the lungs to elastically reduce in size and causing air to be exhaled.

However, mammals can do more than just tidal breathing. Mammals can consciously take very large breaths, for inhalation as well as exhalation. To accomplish this, they must involve other structures. The volume that would be displaced by tidal breathing is still done by the diaphragm. To increase the volume of the thoracic cavity even further, mammals incorporate costal aspiration as well. The ribs, while less individually mobile than they are in the reptiles, rotate (as an entire unit) outwards and cranially. This further increases the volume of the thoracic cavity, allowing for the intake of even more air.

14.6 Integration

Like everything in the body, the respiratory system does not and cannot exist in isolation. In humans, oxygen brought in by our lungs must go somewhere, and the carbon dioxide expired must come from somewhere. Effective respiratory organs also need input from

our central nervous systems to ensure we keep breathing (even when we are asleep!). The respiratory system is also integrated with other systems from an evolutionary perspective. Evolutionary changes in other organs must accommodate the respiratory system and vice versa. Due to the critical nature of respiratory function, these effects can be quite powerful in shaping the evolutionary history of lineages. The integration of the respiratory system is central to understanding its **evolutionary history** and **functional role** in animals today.

The Path of Oxygen: Connecting the Respiratory and Cardiovascular Systems

Nearly every molecule of oxygen gas used by any Earth-borne, vertebrate animal, was originally formed by some form of natural photosynthesis and released into the atmosphere. That oxygen must then diffuse into the bodies of vertebrates to be used, typically through the skin, gills, or lungs. Dissolved oxygen is then transported by the bloodstream to a cell in need, where it is used in cellular respiration. Vertebrate blood has special oxygen-carrying ferroproteins (iron-proteins), which bind oxygen and carry it around the body. Eventually, most of that oxygen diffuses into mitochondria, which use oxygen to convert macromolecules like glucose into cellular energy (ATP) that powers an animal's metabolism. During that process, the by-product carbon dioxide is created, which then diffuses back into the bloodstream. When dissolved in blood, carbon dioxide converts into carbonic acid, acidifying blood and creating a potential problem (our bodies cannot operate with strongly acidic blood). To deal with this problem, our blood carries carbon dioxide to the lungs, where it diffuses out into the air we exhale. The cardiovascular system enables the respiratory system to function at a basic level, and the two are deeply and fundamentally integrated.

Breathing Control: Nervous Control of Respiration

When you actively think about breathing air, you can easily control how quickly and deeply you breathe. However, during and after exercise or in stressful moments, controlling your breathing can be very difficult. On the other hand, you breathe constantly without being aware of doing so, and you breathe every night while sleeping without any active input. The respiratory center in your brain is located in the brain stem. This brain region is ancient and deeply conserved across vertebrate taxa. The respiratory center controls air breathing, allowing you to breathe without thinking about it, but it also adjusts respiratory rates to environmental signals.

One obvious way in which our nervous systems adjust breathing rates is during exercise. When we exercise, more cellular energy is used in our cells, requiring more oxygen and creating more CO_2, acidifying our blood. Our body and brain notice this change, and one of the first responses is to increase breathing rates, allowing us to expire more CO_2 and inspire more O_2. The same thing happens at high elevation, where there is less oxygen in the air and our brains respond by unconsciously increasing breathing rates. The brain also responds to stress by increasing breathing rates, presumably to prepare for a stressful event, but in many parts of the world today, this response can be maladaptive, as our stressors tend to be less "saber-toothed tiger" and more "homework due on Monday."

In nonhuman animals, similar or identical systems also exist. For many air-breathing fishes and aquatic amphibians, air breathing is facultative (while gill ventilation remains obligatory). This means that many species rarely breathe air normally but significantly increase air-breathing rates when environmental oxygen levels fall or when physiological demand for oxygen increases (such as during a high level of activity). Similarly, hyperoxygenation of the environment lowers rates of air breathing

for many species. While the nervous system orchestrates the actions of the respiratory system, the respiratory system in turn provides the oxygen needed to generate nerve impulses in the first place.

Box 14.3—Integration Breathing Activity: With a Partner, Learn How to Take Basic Vital Signs and Understand How We Can and Cannot Control Our Respiratory Biology

First Thing*: *Baseline Vitals

Two of the most important vital signs are also the easiest to learn how to measure: the pulse and breathing rate. The pulse is a rhythmic measurement associated with the speed of contractions in the beating heart. This beat radiates out as a "pulse" across the body, and so any major artery in the body has a pulse. The two most common places to measure a pulse are the radial artery in your wrist and the carotid artery in your neck.

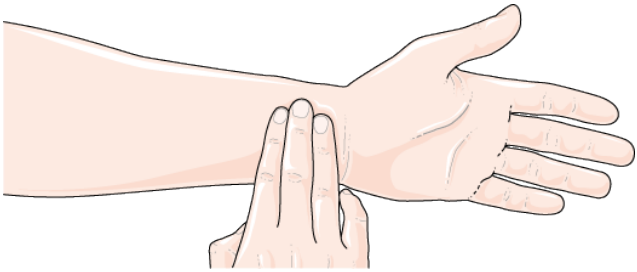

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Figure 14.21—How to take a radial pulse using three fingers to press firmly on the radial artery, which runs over the thumb side of your lower forearm and wrist.

To begin, place your three middle fingers on your own wrist just below the thumb, as shown in Figure 14.21. With a firm pressure, move your fingers around slightly until you can feel the pulse, increasing pressure if necessary. If you are having difficulty, you can try the carotid artery instead, feeling on the side of your neck just next to your esophagus with two fingers. Once you can easily find your own pulse, obtain permission from your partner to try to find their pulse as well. To measure a pulse, set a timer for 30 seconds and then count the number of beats during that time period. To get the number of beats per minute, multiply your number by two. Write that number down in a format like Table 14.1.

Next, practice measuring breaths. This is probably easy to do for yourself, but because we can consciously control our breathing, it is usually best to have someone else measure your breaths. Try watching

your partner's shoulders or back rise and fall to count the number of breaths. Once again, count the number of breaths for 30 seconds and multiply by two to get the number of breaths per minute, and then add the value to the first column of Table 14.1.

Table 14.1—Experimental data of your own breathing

	Baseline	Experiment 1	Experiment 2
Pulse (beats/min)			
Breathing rate (breaths/min)			

Experiment 1: What Can We Control?

You may not be able to consciously contract your heart to beat whenever you want, but you can behaviorally control your breathing in most scenarios. For this experiment, we will see whether we can indirectly control our hearts using our lungs.

For two minutes (or longer), try to actively slow down your breathing. Only go as far as is comfortable, and *do not* hold your breath. Instead, try to make your inhalations and exhalations longer. Use your diaphragm to fill your lungs all the way up, then slowly let all the air go. It can be helpful to consider all the information you have learned about the respiratory system to this point, and think about the flow of air, blood, and oxygen that is allowing you to even perform this little exercise.

Once you have reached a consistent rate of

breathing for at least a minute, have your partner measure your pulse and breathing rates as described above. Enter those values into Table 14.1, then decide whether you would like to return to the activity or take another few minutes to just breathe. Do you feel different after this experiment? In what ways?

Experiment 2: What Can't We Control?

A different form of respiratory control is physiological rather than behavioral. Here we will simulate a high-stress event using physical exercise. If you have a medical condition that makes exercise dangerous, please consider skipping this activity. Otherwise, pick your favorite form of exercise, such as jumping jacks, push-ups, running down a hallway, or going up and down stairs. Do one or more of these activities vigorously for at least two minutes and then have your partner help measure your vitals as quickly as possible. If you can, measure your own pulse while your partner measures your breathing rate and enter them into Table 14.1.

Follow-Up Questions

1. In experiment 1, you actively slowed down your breathing. Did your pulse also change with your breathing? What organ systems are being integrated to form that response?
2. After exercising in experiment 2, what happened to your pulse and breathing rate?
3. Why are these responses important? What is happening at the cellular level during exercise that makes these responses critical?

Connections Between Ventilation and Other Behaviors

Ventilation, or the active process of breathing, is shared among nearly all vertebrates. There are differences, of course, as fish and frog tadpoles ventilate their gills with water, while lizards, lungfish, and lions ventilate their lungs with air. However, what all these animals share is that ventilation happens through the mouth (though many tetrapods use their nostrils as well). Water or air enters the mouth and then proceeds to a respiratory organ of some kind. In fishes, gill ventilation behavior is nearly always produced by some form of oropharyngeal pump. During inhalation, the mouth opens and the interior pharynx expands in volume, drawing water in. During exhalation, the mouth closes and the pharynx compresses, squeezing water out through the gills. For the oropharyngeal pump to work, fish mouths need to be able to perform these actions. Of course, fish mouths also have a lot of other jobs, like eating. Over evolutionary time, fish mouths have evolved many amazing shapes and forms for different feeding strategies, but all these forms are constrained by the need to continuously pump water over the gills.

In terrestrial amphibians, the buccal pump is used for air breathing, and this pump is powered by movements of the hyobranchial apparatus, including the hyoid bone. This set of bones in the throat is also critical for tongue movements. An interesting trend across salamanders is that species with reduced or absent lungs often have strongly divergent feeding modes and hyoid shapes not seen in salamanders with large lungs. It has been hypothesized that the evolutionary loss of lungs in salamanders allowed for the hyoid to evolve in new ways because the buccal pump was no longer needed.

A similar story has been told to describe the situation for amniotes, which made the transition from the buccal pump to aspiration breathing for lung ventilation (see Section 14.4, Evolution

of the Respiratory System). Many reptiles use costal respiration to ventilate the lungs, but these same species also rely on the ribs for flexion of the body wall during locomotion. For many species, the ribs cannot be used for breathing and running at the same time. This is a big potential problem, because running is an energy-intensive activity where carbon dioxide elimination and oxygen uptake are critically important. Many lizards deal with this by optimizing their use of anaerobic metabolic pathways, which is a way to generate cellular ATP without oxygen. Anaerobic metabolism results in lactic acid buildup, and many lizards are extremely tolerant of lactic acid, often resulting in amounts that would be devastating to a typical mammal.

Mammals took a different evolutionary route than lizards and other reptiles. Mammal ancestors likely also relied on costal ventilation but eventually transitioned to a muscular diaphragm, which decoupled locomotion from air breathing. Many mammals, including humans, are particularly good at sustained activity via aerobic metabolism, as they can continuously ventilate the lungs during locomotion, providing sustained oxygen to muscle cells and quickly eliminating carbon dioxide.

Breathing air is a powerful route to bringing oxygen into the body. A necessary side effect of breathing air is that the addition of a large volume of air inside a body results in a change in buoyancy—air is less dense than all solid materials, including animal bodies. In some cases, this is useful, as we see for many fish that use swim bladders to achieve neutral buoyancy and improve swimming efficiency. Indeed, many animals adjust the volume of gas in their lungs or gas bladders to achieve different buoyancy similar to how scuba divers adjust their buoyancy control devices (BCDs) to stay neutrally buoyant. Some fishes have even evolved physoclistous swim bladders with no respiratory role (Figure 14.14). These bladders can be inflated and deflated to maintain neutral buoyancy without trips to the surface using a “gas gland” with a complex series of blood vessels (called a rete mirabile) set up for countercurrent exchange to fill the bladder with nitrogen gas. In physostomous

species, the pneumatic duct (connection between the swim bladder and the mouth) is retained. Some physostomous species control buoyancy using a gas gland (like physoclistous species), some use air breaths (i.e., either release gas through the pneumatic duct or take in additional air from the surface and pump it into the gas bladder), and some may use both. Physostomous air-breathing fish also use air breaths to regulate their buoyancy. In other aquatic animals, sometimes negative buoyancy is useful. Crocodylians are thought to swallow large stones (**gastroliths**, literally "stomach stones") at least in part to allow for easier buoyancy control and to stay at the bottom of water even with the lungs partially full of air.

Box 14.4—The Evolution of Vocalization in Vertebrates

The evolution of air breathing and lungs in vertebrates enabled the subsequent evolution of vocalization in tetrapods. The birds you hear chirping in the morning, the dogs barking down the street, the lecture your professor gave today, and even the text on this page (a representation of spoken words) can all be traced back to the evolution of air breathing. In the evolution of vocal communication, ventilatory airflow that originally served a respiratory role became co-opted for communication. Unlike water, it does not take much energy to vibrate air (i.e., produce sound), and the larynx and syrinx of tetrapods are adapted to produce and modify these vibrations.

In its simplest form, the **glottis** is the opening between the oropharyngeal cavity and the airways (and lungs). In lungfish, the glottis functions as a valve: It is a slit in the floor of the pharynx that is closed and opened

by a sphincter muscle. In tetrapods, the glottis became more robust. Tetrapods evolved three cartilages (a pair of lateral **arytenoid cartilages** and a ring-shaped **cricoid cartilage**) that support the glottis. Muscles control the movement of the arytenoid cartilages, allowing tetrapods to actively open and close their glottises at will. Inferior to the glottis and larynx, reptiles (including birds) and mammals possess additional rings of cartilages around the airway, called a **trachea**.

Larynxes are diverse across tetrapods. Some lineages possess **vocal cords**, which are tissues attached to the arytenoid cartilages that vibrate when they are close to each other and air flows past them. Movement of the arytenoid cartilages modulates the pitch of the vibrations. While frogs are known for the sounds they make with their vocal cords (and vocal sac, as we will discuss next), all other amphibians, as well as most nonavian reptiles, lack vocal cords. In these cordless species, some still vocalize by rapidly expelling air from the lungs. Of course, mammals have vocal cords, and the sounds produced when the cords vibrate are modified or "shaped" by the position and movements of structures in the oropharynx, allowing for the great vocal flexibility observed in many mammals. Some mammals, such as red deer stags, even lower the position of the larynx in order to lengthen the vocal tract and alter the quality of their calls.

The characteristic croaking and chirping of frogs are possible thanks to both their vocal cords and vocal sacs. While all frogs have vocal cords, typically only testes-bearing frogs have resonating vocal sacs and

produce mating calls. The **vocal sac** is an elastic chamber beneath the floor of the buccal cavity. To prepare to call, a frog inspires extra air into the lungs, then contracts muscles in the body wall (with its nares closed) to transfer gas back into the buccal cavity, where it flows into the air sac via slits in the buccal floor. These slits are closed by muscle around it. Now that the air sac is inflated, the frog starts to call: The frog cycles gas between the lungs and the buccal cavity, and as gas flows past the vocal cords, they vibrate. These vibrations are amplified by the inflated, resonating vocal sac.

Birds are renowned for their musical sound production, but they do not vocalize with their larynx. Instead, they have a **syrinx**, a structure located at the inferior end of the trachea, where the trachea splits into two bronchi. The cartilaginous rings in this region are specialized, and drum-like membranes between the rings (in the walls of the syrinx) vibrate as air passes. Sound characteristics are affected by muscles that modulate the tension of the membranes, by the shape of the trachea and oropharyngeal cavity, and by tongue movements. In some birds, the syrinx only spans the inferior end of the trachea, while in others the syrinx only spans the superior ends of the bronchi, and in most birds (including songbirds), the syrinx spans both the trachea and the bronchi. This anatomical complexity allows birds to simultaneously produce different sounds in different regions of the syrinx (e.g., membranes in the left and right bronchi), creating the musical complexity we know and love.

14.7 Human Respiratory System

Our lungs have the same characteristics that are shared by the lungs of other mammals. Our airways branch over and over again, ending in hundreds of millions of **alveoli**. Because of surface tension, these moist spherical structures (~0.2 mm diameter) want to deflate like little balloons. To reduce their inward pull, our lungs are coated in **surfactant**. When we are relaxed, we contract our diaphragm and intercostal muscles to inhale, and we exhale passively by relaxing our muscles and letting our alveoli pull inward. When we are breathing heavily, we recruit our abdominal and intercostal muscles to help with exhalation. The large cumulative surface area of our alveoli supports our high metabolic load, which results from being endothermic and having large, energy-hungry brains. Our gas-exchange capacity is larger than we typically need, allowing us to endure more extreme conditions, such as exercising at high altitudes or surviving disease. In this section, we will focus on the intersection between **respiratory anatomy** and **human health**.

Segments

Our airways bifurcate approximately 23 times ("generations") before becoming alveoli. Arteries travel and branch along with the airways, matching the structure of the respiratory tree (Figure 14.22). This may sound like a trivial fun fact, but it has medical importance. If a portion of the lungs becomes diseased (e.g., becomes cancerous), then that portion can be removed without affecting arterial blood flow to other portions of the lungs. This procedure is called a **segmentectomy** or a **lobectomy**, depending on how large a portion is resected from the lung.

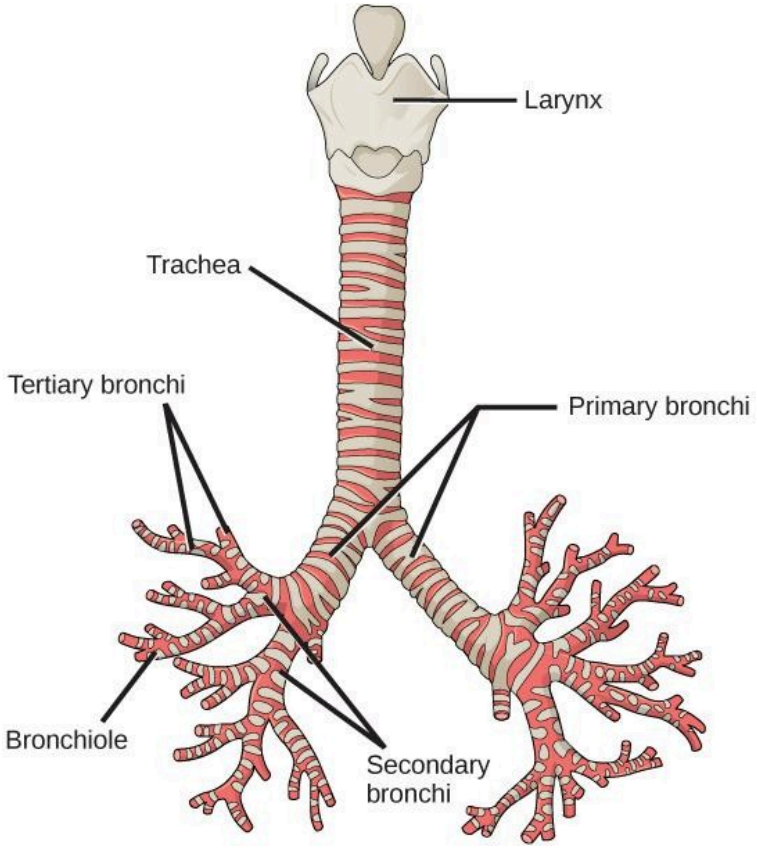

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Figure 14.22—The branching pattern of the airways in the human lungs. On average, human airways have approximately 23 branching generations. The branching of the pulmonary arteries closely matches the branching of the airways. The left primary bronchus is oriented more horizontally than the right primary bronchus because the left bronchus passes over the heart.

Surfactant

At first glance, it may seem like only small organisms, like the tadpoles discussed in Section 14.5, Diversity of the Respiratory

System, meaningfully interact with the forces of surface tension, not large organisms like us. However, surface tension is also an important force in the context of our respiration. Alveoli must remain moist to permit gas exchange, and the surface tension of this thin layer of water adds up to a large inward force. This force is useful because it drives exhalation, along with the elasticity of the lung wall. However, it also makes it more difficult to inhale. Surfactants are molecules that reduce surface tension, and they reduce how much force our muscles must produce when we take a breath.

Unfortunately, fetal surfactant production matures during the last 10 weeks (weeks 30–40) of gestation. Infants born before 34 weeks of gestation often develop **respiratory distress syndrome** (RDS) because there is not enough surfactant in their lungs. In these cases, infants have difficulty breathing because of the extra work to resist surface tension and because their alveoli may collapse. RDS is treated by administering artificial surfactant, providing breathing support, and providing a higher concentration of oxygen. Scientists and clinicians are still refining this treatment, as lung damage can also result from ventilating the lungs at too high a pressure or from providing too high an oxygen concentration.

Intrapleural Space

The left and right lungs each sit within their own cavity within the thorax. Each cavity is bounded by the ribcage, diaphragm, and pericardium (the sac surrounding the heart). These boundaries are lined by a **pleural membrane**, and another pleural membrane envelopes the lung (Figure 14.23). The pleural membranes touch and slide past each other as the lung expands and shrinks with every breath. To minimize friction as the membranes slide, there is a small amount of serous fluid between them that acts as a lubricant (imagine holding a plastic zip-top bag with a little bit of water inside

[and no air] and sliding the walls of the bag past each other). The space between the membranes is called the pleural space, and it is considered a "potential space" because it is extremely small in healthy people. The pleural spaces (left and right) are crucial to our ability to breathe. If an injury causes a tear in one of the pleural membranes, the potential space will become an actual space, and the lung will collapse.

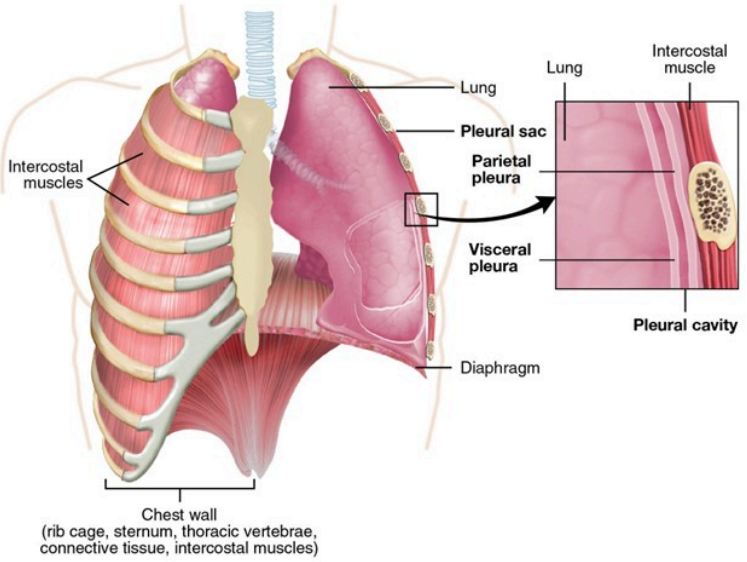

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Figure 14.23—Anatomy of human lungs and pleural cavity. Each lung is located within a separate pleural cavity. The outside of each lung is covered in a visceral pleural membrane (i.e., pleura), and the margins of the pleural cavity (the rib cage, diaphragm, and pericardium) are lined by a parietal pleura. Between the visceral and parietal pleurae is the pleural cavity, which contains a small amount of pleural fluid.

Pneumothorax and **hemothorax** occur when air or blood (respectively) enters the pleural space. These occur when either the chest wall or the wall of the lung is damaged. This can be caused by blunt trauma (e.g., fractured rib, knife wound, gunshot wound), by

lung diseases (e.g., COPD [chronic obstructive pulmonary disease], cystic fibrosis, tuberculosis), by connective tissue disorders (e.g., Marfan's syndrome), or by certain medical procedures. Because each lung is surrounded by a separate pleural space, only the lung on the affected side will collapse.

In certain cases, called **tension pneumothorax**, the damaged pleural membrane acts as a one-way valve. Air enters the pleural space as the person breathes in but cannot leave the pleural space as the person breathes out. This causes air to accumulate in the pleural space, which ultimately puts pressure on the other lung and the heart, pushing them aside within the thoracic cavity and making it more difficult for the heart to pump. This is treated by needle **thoracostomy**—inserting a needle into the pleural space so that air is no longer trapped there.

Box 14.5—Reading Human Chest Radiographs

Imaging the lungs enables physicians to diagnose abnormal, injured, or diseased conditions. Radiography (X-ray imaging) is well suited for visualizing the lungs because X-rays produce contrast when adjacent structures have different densities (or, more accurately, different X-ray absorption coefficients), and the lungs are radio-lucent structures surrounded by radio-opaque structures. X-rays easily pass through the lungs but are absorbed into the soft tissue surrounding them, making it easy to see the margins of the lungs, fluid in the lungs, diseased or scarred lung tissue, or other indications of injury or poor health. In the images provided here, radio-lucent structures (such as the lungs) appear dark, and radio-dense structures (such as bones and muscles) appear bright.

In order to recognize when something is wrong, physicians must be familiar with how healthy lungs appear in chest X-rays. The first image is a chest X-ray of a person with relatively healthy lungs (however, note that they have cardiomegaly [i.e., an enlarged heart], and there are artifacts in the image related to their treatment, specifically four EKG [electrocardiogram] leads and circular artifacts along the midline; Figure 14.24). Before dragging the slider over to look at the second image, try to identify the location of the anatomical features listed on the left and right of the image. When you are ready, look at the second image, where these features are labeled.

(Note: **Hemidiaphragms** are the left and right halves of the diaphragm. **Costodiaphragmatic recesses** are the potential spaces where the costal and diaphragmatic pleura meet and where fluid is likely to pool.)

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https://pressbooks.palni.org/comparativevertebrateandhumananatomy/?p=534#h5p-1

Next let us look at a chest X-ray of a person with a diseased lung (Figure 14.25). Examine the image,

consider the position of the left lung, consider the radio density of the material in the left pleural cavity, and then describe what condition you think this person has. When you are ready, drag the slider over to look at the following image and see the labels.



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Lung Capacities

When a healthy adult breathes in as deeply as they can, their lungs are filled with 6 L of air, on average (**total lung capacity**, TLC). And when a healthy adult exhales as deeply as they can, their lungs contain an average of 1.2 L of air (**residual volume**, RV). Yet the average amount of air that is exhaled and inhaled with each breath at rest is only 450 mL (**tidal volume**, TV). These terms (TLC, RV, TV) and many others have been created to refer to the volume of air in the lungs at different time points during respiration (Figure 14.26). By quantifying typical values for these volumes in healthy populations, clinicians have determined what values are abnormal and indicate impaired respiratory performance.

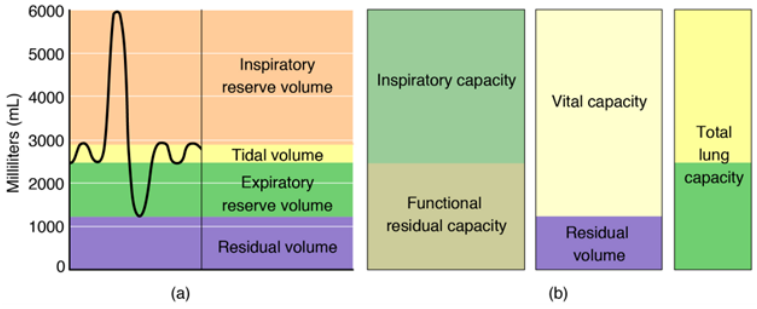

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Figure 14.26—Graph of human lung volumes and capacities. Volume is plotted in milliliters. (A) Respiratory volumes during breathing at rest and during maximal ventilation. (B) Lung capacities that combine to equate to total lung capacity. Tidal volume: the volume of air ventilated during a typical breath at rest. Inspiratory capacity: maximum amount of air that can be inhaled at the end of a normal exhalation at rest. Inspiratory reserve volume: inspiratory capacity minus tidal volume. Expiratory reserve volume: the maximum amount of air that can be exhaled, excluding tidal volume. Residual volume: the volume of air that remains in the lungs at maximal exhalation. Functional residual capacity: the amount of air that remains in the lungs after a typical exhalation at rest. Vital capacity: the maximum amount of air that can be exhaled after taking a maximal inhalation.

One common test that clinicians use to assess respiratory performance is **spirometry**. While breathing through a spirometer, a patient is asked to inhale as deeply as possible and then exhale as forcefully as possible (forced vital capacity, FVC). From this, the volume of air that was forcefully exhaled during the first second of exhalation (FEV1) can be measured. These measurements can help diagnose a variety of diseases, such as COPD or asthma. For example, a healthy patient expels 90% of FVC during the first second of forced exhalation, but a patient with asthma may expel only 50% of FVC during the first second.

Airway Protection

Our respiratory system needs to protect itself from material, both big and small, that enters the airways. When denser material, such as liquid or a piece of food, slips past the **epiglottis** and enters the space above the vestibular and vocal folds, it triggers the vestibular folds to quickly close. This prevents the material from entering the trachea, but it also blocks the exhalation and inhalation of air. Sometimes it is actually the vestibular folds, not the ingested material, that block the airways and create the sensation of choking. The **Heimlich maneuver** remedies this by creating sufficient pressure in the airways to push the vestibular folds open and eject the food or liquid. **Aspiration** occurs when material passes the vestibular folds and enters the lungs. The left bronchus passes above the heart, so it is more horizontal than the right bronchus. As a result, aspirated bodies are more likely to enter the right bronchus.

In addition to larger or denser material, our respiratory system also must protect itself from airborne particles and pathogens. Mucus is produced by the epithelium lining the airways and traps particulates. **Cilia** sweep the mucus up toward the larynx, where it exits the airways and is swallowed. However, problems occur when the respiratory system overreacts to the presence of particulate matter. This occurs in people who suffer from asthma, resulting in inflammation, overproduction of mucus in the airways, and bronchospasm (contraction of the muscles that line the bronchi, causing the airways to narrow and making it difficult to breathe). Problems also occur when the respiratory system is outmatched by more particulate matter (including cigarette smoke) than it can clear away. This results in occupational or environmental lung diseases, such as emphysema, chronic bronchitis, silicosis, and black lung disease. Last, the respiratory system must rely on the immune system to protect itself from pathogens, including bacteria, viruses, and fungi.

14.8 Summary

The body's metabolic processes necessitate intaking oxygen and expelling carbon dioxide. Beyond very small body sizes, the physics of gas exchange becomes unfavorable quickly. To overcome these challenges, vertebrates have evolved several different mechanisms for optimizing gas exchange. There are two broad sets of respiratory organs: gills for gas exchange with water and lungs for gas exchange with air. Principally, both strategies increase their capacity and efficiency through three methods: (1) increasing surface area available for gas exchange, (2) maximizing the efficiency of flow through the respiratory system, and (3) maximizing the efficiency of the interaction of the respiratory system with the cardiovascular system. How these methods are employed varies across vertebrates, with evolutionary history and ecology playing a strong role.

Application Questions

1. Gills and lungs fulfill essentially the same job: extracting oxygen from the environment. What are the functional limitations that prevent a water-filled lung from working underwater?
2. When in development do most animals begin to have oxygen requirements?
3. What are some examples of respiratory evolution either constraining or being constrained by other organ systems?

4. How would life differ if breathing required skeletal muscles actively controlled by the brain and could not occur passively?
5. Why is it important for the ventilation rate to match the physiological need for gas exchange? What happens if the ventilation rate is too fast or too slow?
6. Countercurrent exchange is one way that gills continue to exchange gases, even in low-oxygen water. This form of exchange requires a specific orientation of fluid flows, and we actually see remarkably similar configurations across the tree of life, from penguin feet, to gas glands in many fish, to the nephrons in your own kidney. Why do you think these countercurrent exchange structures have independently evolved so many times?

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15. Circulatory System

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Focus Questions—to Guide Your Reading of This Chapter

1. How do changes in the circulatory system correlate to those that we also observe in the respiratory system?
2. How does a vertebrate's metabolic rate influence the structure of the heart?

15.1 Introduction

As we learned in the previous chapter, gas exchange is pretty darn important. All our cells need oxygen and nutrients, and they need waste products to be removed. It doesn't take long, developmentally speaking, for vertebrates to reach a size where simple diffusion is no longer sufficient and where a circulatory system is necessary to move things around the body. Those things are not just oxygen and carbon dioxide but also nutrients, hormones, chemicals, metabolic waste products, and cells. In other words, a blood draw can tell

your physician an awful lot about your health because blood does so much for your cells.

15.2 Structure and Function

At the most basic level, the vertebrate circulatory system is composed of three components: a fluid to carry everything we need to move around the body, the pipes that carry the fluid, and the pump that moves the fluid through the pipes. Even though they're part of the same system, we often divide the circulatory system into two components:

1. The **blood circulation**: blood (fluid), blood vessels (pipes), and heart (pump)
2. The **lymphatic circulation**: lymph (fluid), lymphatic vessels (pipes), and heart (pump)

This chapter will mostly focus on blood circulation, but we will discuss lymphatic circulation at several points.

The Fluid: Blood

Blood is composed of both solids and a liquid component called **plasma**. Plasma is largely water with a bunch of stuff dissolved in it, including gases, glucose, hormones, proteins, various clotting factors, and ions. In humans, plasma is responsible for just over half of your blood volume. The solid component of the blood is often called the **formed elements**, as they are cells that are formed in other organs, such as the bone marrow or spleen, and then make their way to the bloodstream via gaps in certain types of capillaries (see Box 15.1). We know these cells as **erythrocytes** (red blood cells),

leukocytes (white blood cells), and **thrombocytes** (platelets). The erythrocytes carry oxygen around the body. Leukocytes are broadly involved in the immune system, both as a first line of defense and as part of a body's long-term defense against potential pathogens. Thrombocytes form a key component of the clotting response, helping close wounds to prevent further loss of blood.

The Pipes: Vessels

Now we need a way to move that fluid around. Vertebrate blood circulation moves through a **closed circulatory system**, which means that the pipes never empty into anything but the heart—it's a closed system unless a vessel is damaged and you start bleeding. This is something that has independently evolved in a few other animals, including cephalopods (octopus, squid, and cuttlefish). Nonvertebrate chordates, like several other invertebrates, have an **open circulatory system**. A pump moves fluid through vessels that eventually empty into a cavity; the fluid then moves back to the heart thanks to the pressure of more fluid being added to the cavity. A major advantage of the closed circulatory system is the ability to preferentially direct blood flow to different tissues (by changes of vascular resistance) in response to changes in metabolic demand—for example, during digestion (during which blood flow can be prioritized to the gastrointestinal circulation) or during physical exertion, when blood flow is diverted to skeletal muscle.

Let's take a closer look at those pipes. We can first divide them up by size, into arteries, veins, and microcirculation (Figure 15.1). **Arteries** carry blood away from the heart, while **veins** carry blood toward the heart. Note that we did not connect either type of vessel to the oxygenation status of the blood; whether arteries (or veins) carry oxygen-rich or oxygen-poor blood depends on where in the circulation you are and which vertebrate you're examining. We'll get to that later.

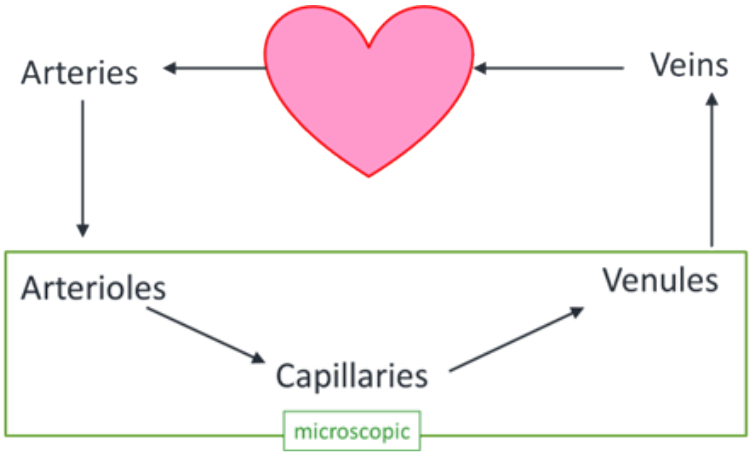

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Figure 15.1—A schematic of various blood vessels. The heart-shaped icon represents the anatomical heart, which is not actually shaped like a heart.

Both arteries and veins are made of the same layers (Figure 15.2). The outermost layer is called the **tunica adventitia** (sometimes also called the **tunica externa**), which is mostly connective tissue. The next layer is the **tunica media**, which is a combination of smooth muscle and elastic fibers. The ratio of these varies; veins tend to contain very few elastic fibers, and arteries tend to contain a lot of elastic fibers to deal with the pulse of pressure from the heart. The innermost layer is called the **tunica intima**, which is composed of a layer of endothelial cells. The open center of the vessel, where the blood flows, is called the **lumen**.

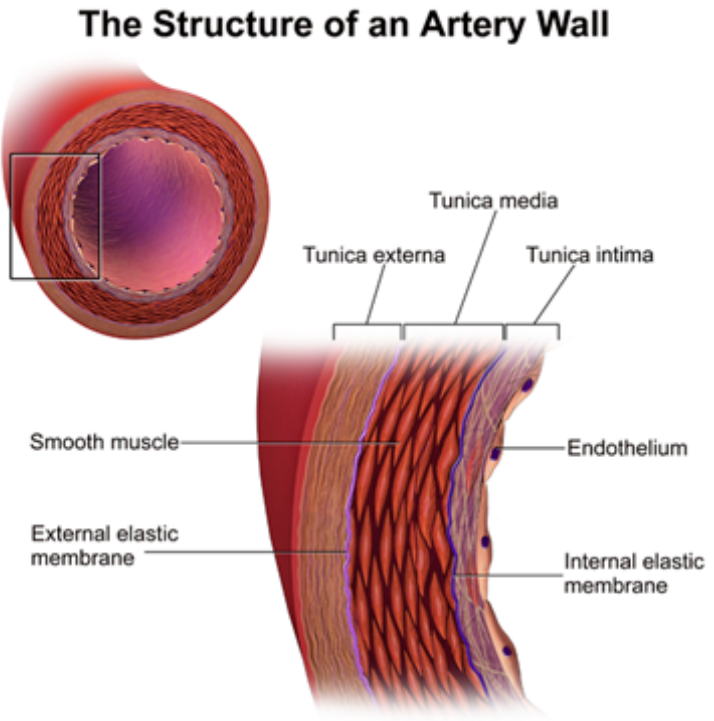

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Figure 15.2—The structure of a typical artery.

The microcirculation is composed of arterioles, capillaries, and venules, and as the name implies, these are the vessels that are not visible to the naked eye. **Arterioles** and **venules** are very small arteries and veins, respectively. **Capillaries** are the smallest blood vessels and only have a tunica intima and lumen. Having this single, thin layer is important because capillaries are the sites where materials can move in and out of the blood in exchange with the nearby cells.

Box 15.1—Leaky Capillaries

Capillaries are generally classified by how leaky they are (Figure 15.3). All capillaries are a little leaky because of the pressure of blood being pushed from the arterioles. This causes some of the liquid components of blood to leak out of the capillaries. When that liquid leaves the capillaries, it joins the **interstitial fluid** bathing your cells and tissues. **Continuous capillaries** (sometimes also called "closed capillaries") lose only a little bit of liquid and allow only small molecules to move through their walls. However, **fenestrated capillaries** have larger openings to allow some proteins to also move through. **Sinusoidal capillaries** have the largest openings and are thus the leakiest, allowing blood cells to pass. How leaky a capillary is, and thus the type of capillary you find at a particular organ, depends on the needs of the cells they serve. Knowing what you do about different tissues and organs so far, hypothesize about an organ that would need each type of capillary and why. Then do some research to find out if you were correct!

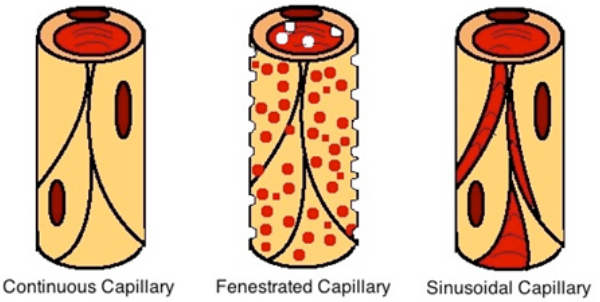

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Figure 15.3—The different types of capillaries.

Let's put our circulatory system together so far. Arteries move blood away from the heart. The largest arteries come straight off the heart and then split into smaller and smaller arteries, much as a building may have one pipe receiving water from a city, and then that pipe splits into smaller and smaller pipes to serve each room of the building. Eventually, the arteries divide into arterioles, which divide into capillaries. Capillaries merge into venules, which merge into veins, returning blood back to the heart.

The Pump: Heart

Now that we understand the pipes and the fluid within the pipes, we need to examine how that fluid is moved through the pipes. The **heart** is a ventrally located muscular tube that pushes the fluid into the pipes by contracting and creating positive pressure but also

brings fluid into the heart by creating negative pressure that pulls fluid in. As we examine different vertebrates later in this chapter, we will see that hearts vary in terms of the number of chambers in that tube and how they're arranged. However, regardless of the vertebrate, blood enters the heart cranially and leaves it caudally. The vessels are in charge of moving the blood in a different direction after it leaves the heart.

When the heart contracts and produces the highest positive pressure, it is in **systole**. When the heart muscle relaxes and produces its lowest pressure, it is in **diastole**. When a medical professional measures blood pressure, they give two numbers. The higher number is the **systolic pressure**, and the lower number is the **diastolic pressure**. These are often reported together (e.g., "120 over 80") and are reported in mm Hg as the unit.

The positive pressure created by the heart as it contracts is at its highest in the artery (arteries) immediately leaving the heart (Figure 15.4). In humans, the **aorta** is the artery that sends blood out of the body to drop off oxygen to its cells and pick up carbon dioxide waste; the pressure is about 130 mm Hg (about 2.5 psi) within the aorta. As we move through the arteries as they split and split into smaller and smaller vessels, blood pressure drops the farther we move away from the heart. Part of this pressure drop is due to the friction between the blood vessel walls and the blood. However, we're also distributing that blood volume among many small pipes/vessels. Our building water main analogy is useful here again. The pressure coming out of a single faucet is much lower than that out of the pipe bringing water to the building.

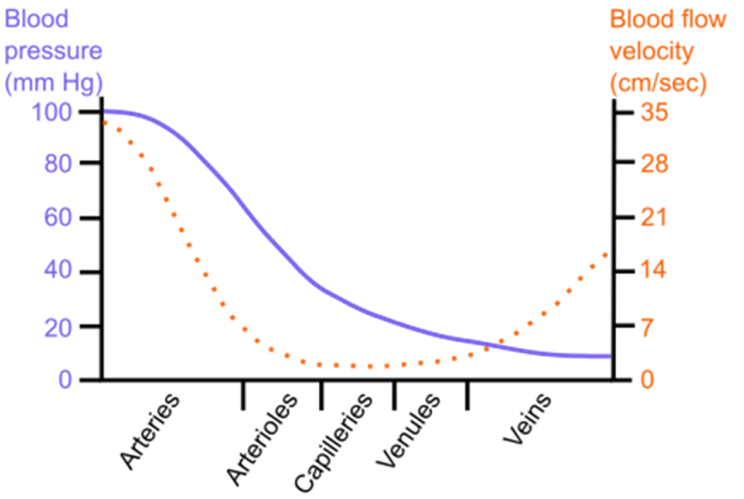

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Figure 15.4—Blood pressure (purple solid line) drops with distance from the heart. Notice that blood velocity (orange dotted line) does not recover completely until it leaves the heart again.

The blood vessels, however, are not rigid. Arterioles and veins can change much in diameter due to the contraction or relaxation of smooth muscle cells in their walls. If arterioles in the skin expand in diameter, as can happen when you are heating up, then the resistance to blood flow decreases in this part of the circulatory system, and blood flow increases to the skin. This can facilitate cooling down. Heating up the body can happen as a consequence of exercise. During exercise, a high number of arterioles in the active musculature dilate while veins may constrict under the influence of the nervous system. The cumulative effect of this is that a substantial fraction of the total blood volume is shifted from the veins to other elements of the systemic circulation. This helps maintain blood pressure, if not increase it.

By the time we get to the capillaries, the pressure has dropped to about 20 mm Hg (about 0.4 psi) in humans. Pressure

continues to drop as we move through the venules (12 mm Hg in humans) and veins (5 mm Hg in humans, to almost 0 mm Hg in some large veins) and head back to the heart. Pressure in these large veins can sometimes drop below 0 mm Hg; to prevent blood from moving backward during negative pressure, veins are equipped with one-way valves that prevent backward flow. Some large veins also receive an assist from the adjacent skeletal muscle. When those muscles contract, they squeeze the veins, providing a boost to the blood back toward the heart. This is why during long periods of sitting, such as a long airplane flight, it is important to move your legs to help that venous blood along.

Lymphatic Circulation: Our Second Set of Pipes

We have one more set of pipes to worry about. Recall that all capillaries are leaky and that some plasma will join the interstitial fluid. This fluid needs to be returned to the vascular system. Much of that happens right at the capillaries thanks to osmotic pressure. However, not all the lost fluid is returned, and this is where the lymphatic circulation contributes.

Lymphatic capillaries are often found in conjunction with the blood capillaries (Figure 15.5). Like blood capillaries, lymph capillaries are very small and thin walled. Unlike blood capillaries, they are blind-ended. The interstitial fluid enters through openings called **buttons** between the endothelial cells to allow fluid and immune cells to enter. When interstitial fluid enters the lymphatic vessels, it is called **lymph** and has a similar composition to blood plasma.

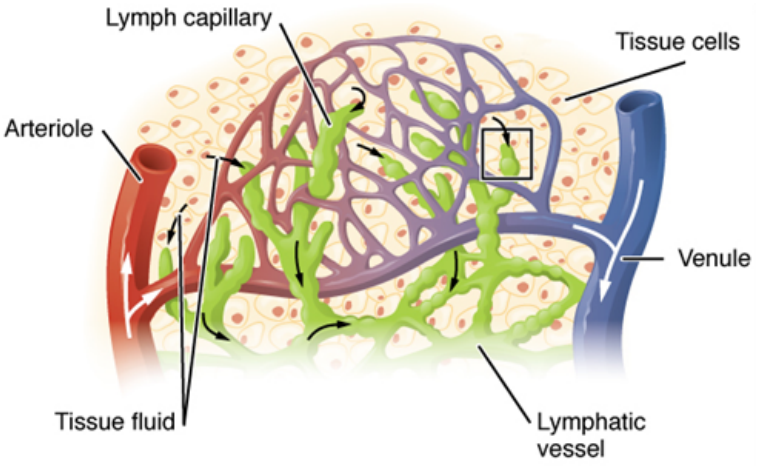

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Figure 15.5—Lymphatic capillaries are interwoven between blood capillaries, which allows them to capture interstitial fluid.

Lymphatic capillaries have a small layer of smooth muscle that can contract to help move lymph from the capillaries into the lymphatic vessels. Lymphatic vessels have a similar organization to blood vessels—they have a tunica intima, media, and adventitia. Like veins, the lymphatic vessels operate under low pressure and need some help. Pressure is generated by that smooth muscle as well as the surrounding skeletal muscles and nearby pulsing arteries. And like veins, lymphatic vessels have unidirectional valves that prevent backflow. So how does that lymph eventually make it back into the blood? Eventually, the large lymphatic vessels connect to veins near the heart and return the filtered fluid to the blood. Some vertebrates (amphibians and nonavian reptiles) have **lymphatic hearts** that help create more pressure to get that lymph back into the bloodstream.

In addition, the lymphatic system is also part of the immune system. **Lymphatic tissue**, also known as **lymphoid organs**, are sites of immune cell production and maturation (Figure 15.6).

These include the bone marrow, thymus, spleen, appendix, tonsils, lymph nodes, and lymphatic cisterns or sacs. Not all vertebrates have all these structures. For example, lymph nodes are strictly a mammal and occasional bird feature. **Lymph nodes** are in line with the lymph vessels, receiving lymph from lymph vessels. The interior of the lymph node is composed of a series of sinuses that allow macrophages to remove antigens and dead cells from the lymph before the lymph returns to blood circulation.

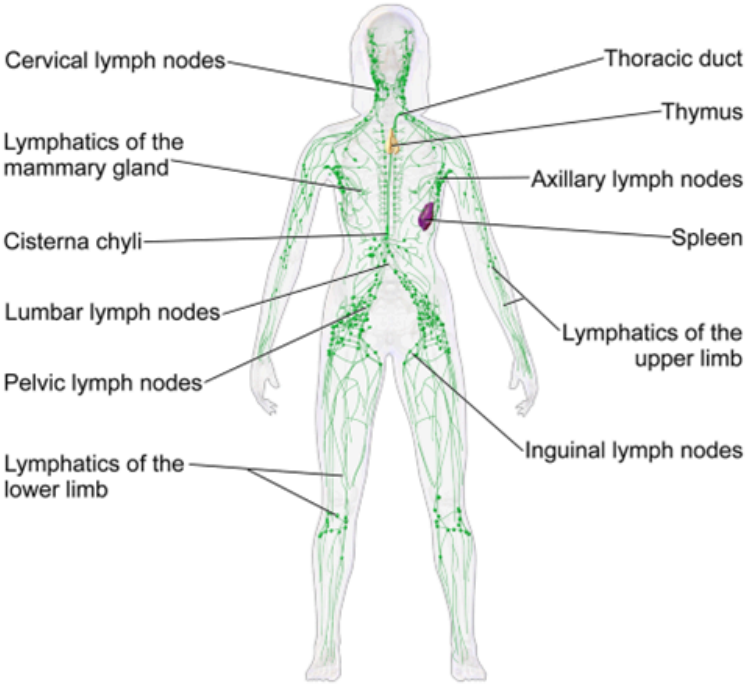

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Figure 15.6—Some of the lymphoid organs present in humans.

15.3 Morphological Development

The development of the heart and the blood vessels is inherently

intertwined. They are among the first structures to form during development, as their role in moving materials around the body is crucial for the development of other body systems. Consider the entire circulatory system as one large tube, interconnected with many different subdivisions and mergers. Once the general "tube" is in place, then it is just a matter of how the tubes grow, twist, and shape themselves that will help distinguish the different structures that come together to form the entire system.

Most circulatory system elements are derived from mesoderm and cardiac neural crest cells. The original pieces of the blood vessel network are stem-endothelial cells, cells that will eventually line the interior of all blood vessels and the heart. Now, these early stages are quite complicated, but we can simplify things for our purposes. At the same time, these endothelial cells are forming and migrating, and precursors of red blood cells are also forming. Through genetic and chemical signaling, the endothelial cells wrap around pockets of the developing blood cells. Imagine there are many of the pockets forming in the developing embryo. As they encounter one another, they will merge (*anastomose*, in blood vessel terminology), gradually forming larger and larger networks. At this point, we can now refer to these growing networks as capillaries. New vessels forming this way can be referred to as **vasculogenesis**, the origin of blood vessels without other preexisting blood vessels. As the networks grow larger and larger, they will connect to one another. Larger vessels will differentiate into arteries and veins; smaller vessels will remain as capillaries. When these different vessels have formed, new vessels will develop and may connect them together. The formation of new vessels (throughout the entire life of an individual) from existing vessels is known as **angiogenesis**.

The embryological development of the heart provides an informative background to understand the structures in the adult heart and the blood vessels. This process takes place within the **cardiopharyngeal field**. In addition to the heart, and as implied by

its name, the cardiopharyngeal field also gives rise to the lungs and craniofacial features.

The part of the cardiopharyngeal field that develops into the myocardium folds in on itself and forms a **heart tube**. The heart tube is a straight tube oriented parallel to the embryo's long axis and has a solitary venous pole and a solitary arterial pole (Figure 15.6). Some species, such as zebrafish, deviate from this pattern and first form a **cardiac disk**. The disk then extrudes itself to become a tube. In doing so, it comes to lie at a skewed angle relative to the embryo's long axis, and this process is referred to as "jogging." Jogging leaves the venous pole of the heart tube to the left of the body midline, and this is the earliest example of a morphological break with left-right symmetry in zebrafish development. The **endocardium** lines the inside of the heart tube and separates the lumen from the **myocardium**. The endocardium also connects with the endothelium of the developing arteries and veins. In this sense, the heart of the early embryo appears as a modified blood vessel, and blood vessels are phylogenetically much older than the heart.

Later in development, the straight heart tube loops, and its topology now becomes asymmetrical relative to all three principal anatomical planes. By default, the heart is looped in a counterclockwise fashion, if we were to view it from a caudal position. Importantly, the looping sets the stage for where chambers develop and thus the topology of the formed heart (Figure 15.7). In humans and chickens, the processes of heart tube formation and subsequent looping are distinct, whereas they occur simultaneously in mice. Also, the length of the heart tube and its degree of looping vary between clades. While modest looping occurs in zebrafish and Adriatic sturgeon, in lungfishes, looping well in excess of 360 degrees occurs. The formed lungfish heart has a very long outflow tract that turns some 270 degrees, and the embryonic heart tube formation and looping appear to set the stage for this.

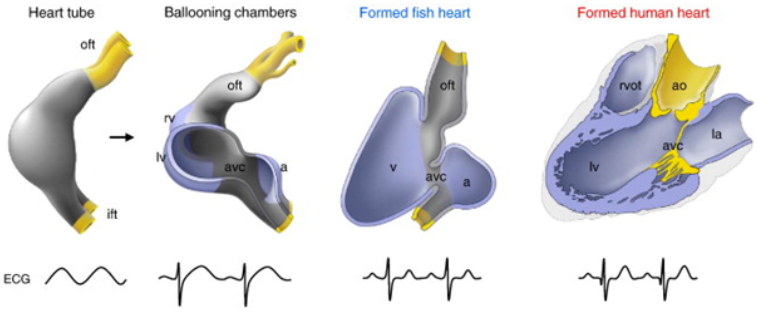

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Figure 15.7—The development of the heart. The curvature of the looped heart tube (far left) sets the stage for where chambers develop (center left). Chambers have the capacity to spread the activating electrical impulse faster than heart tube myocardium. This explains that the presence of chambers coincides with the appearance of sharp deflections on the electrocardiogram (EKG). These features are evolutionarily conserved, and quite some similarity can be found between fish hearts and the human heart in terms of both structure and (electrical) function. Image from Jensen et al., 2013, provided by the authors.

Activity of transcriptional networks is required for the formation and looping of the heart tube as well as the development of chambers. Transcription factors (proteins that influence gene activity) are key components of these networks, and so are growth factors and more. These also induce the formation of cushions of mesenchyme, or connective tissue. Cushions form versatile scaffolds that give rise to the atrioventricular and arterial valve leaflets. They can also set the stage for atrial and ventricular septation and contribute to the final closure of interatrial and interventricular communication in tetrapods. In addition, two long cushions or ridges can contribute to separate oxygen-poor and oxygen-rich blood, as in the African lungfishes. In amniotes the outflow tract cushions merge to separate the pulmonary trunk from the systemic channel. This particular division requires not only mesenchymal tissues but also immigrating neural crest cells.

After the heart has taken its final shape, it will continue to grow as the individual does. Parts of the myocardium may become

so thick that a dedicated vasculature is required. This could be the conus arteriosus or the ventricular wall. There, **coronary vessels** develop via angiogenesis, and they grow across these parts of the heart, becoming conduits for oxygenated blood to the myocardium. Thin parts of the myocardium, which could be trabeculations or walls of veins, remain homeostatic for the most part by exchange by diffusion from luminal blood.

15.4 Evolution and Diversity

Before we walk through the details of the evolution of the various circulatory system components, we should first get a general idea of the blood circulatory pathways in various extant vertebrate groups.

The cyclostomes, chondrichthyans, and teleosts follow a similar general circulatory plan (Figure 15.8). Oxygen-poor blood travels from the heart to the gills via arteries, where oxygen enters the blood and carbon dioxide leaves the blood across the gill epithelium. In Chapter 14, we discussed that gills are also responsible for the movement of ions in or out of the blood plasma. The newly oxygenated blood is distributed through arteries after the gills to all cells of the body, where a second capillary bed enables gas exchange in the tissues. Oxygen-poor blood then returns to the heart to undergo another circuit.

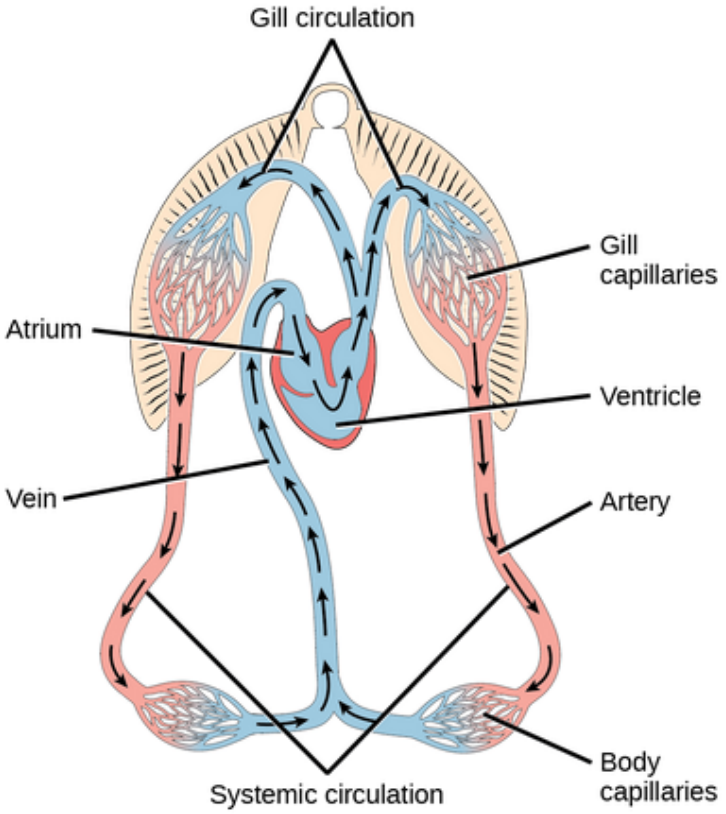

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Figure 15.8—Generalized fish circulation.

During the transition from water to land, there are a few major changes that occur in lineages leading to the tetrapods as well as throughout the tetrapods. The respiratory and circulatory systems are tightly coupled. Changes in the mode of respiration necessitate changes to the circulatory system. To varying extents, this means that instead of one single circuit of blood in the fish heart (Figure 15.8), we get a double circulatory system where oxygenated blood from the lungs is returned to the heart (Figure

15.9). The **pulmonary circuit** refers to the pulmonary arteries that bring blood from the heart to the lungs and the pulmonary veins that return oxygenated blood to the heart. The **systemic circuit** consists of all the blood vessels that supply the remainder of the body. The transition from a single circulatory circuit to a double circuit is going to require us to walk through some developmental and functional changes.

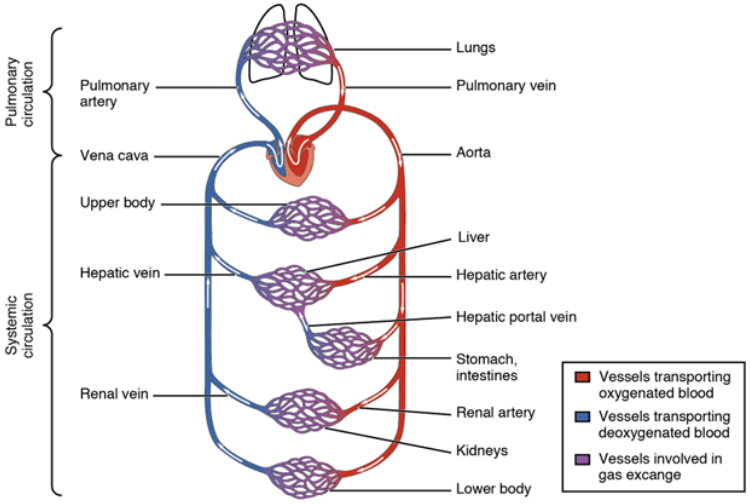

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Figure 15.9—The pulmonary and systemic circuits in humans, as an example of a vertebrate without gills.

The structure of the heart and major arteries shows the most taxonomic variation across the vertebrates and so will be the focus of the sections below, although peripheral vascular adaptations will also be highlighted where relevant.

Arches and Vasculature

All blood leaving the heart, across all vertebrates, leaves through one large vessel that we refer to as the **aorta** (Figure 15.9). However, once that blood enters the aorta, the path it travels varies considerably in our vertebrate groups. Some of these changes are concurrent with changes that are occurring with the already mentioned changes in respiration (see Chapter 14—Respiratory System) and the changes to the structure of the heart (see the subhead Hearts). To begin, let us build a schematic that will serve to orient us to the arches (Figure 15.10). There are six **aortic arches** (AA) in this developing embryo, with the first pair being the most cranial. These six arches arise from the ventral aorta and then travel to the dorsal aorta. If we return to the general plumbing schematics above, we can already get a feel for how blood moves through this system. Blood enters the ventral aorta from the heart. As the blood travels through the ventral aorta, some of the volume will be diverted at each passing arch. As the blood passes through each arch, in a vertebrate like the dogfish shark, the blood will be oxygenated through the gills. It will then pass into the dorsal aorta and through to the rest of the body. By working our way through the vertebrate lineages, we can track the fate of the six aortic arches and closely examine how these changes correspond to body-wide changes in other systems.

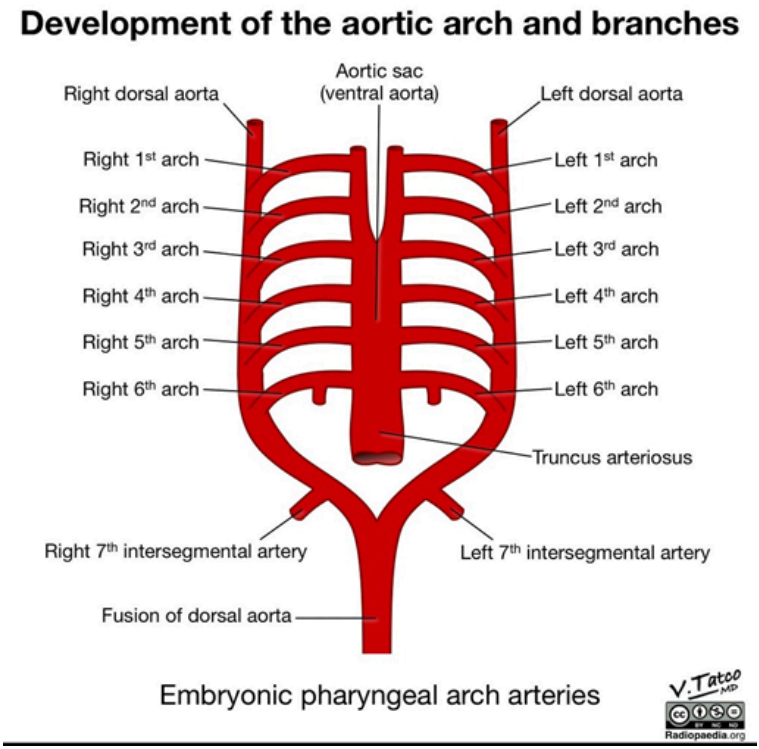

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Figure 15.10—A schematic of the aortic arches. The heart would be positioned just below the truncus arteriosus in this image.

The branchial arches in invertebrate chordates primarily serve for filter feeding and/or ion regulation, but they play a pivotal role in gas exchange in cyclostomes and probably also in the early vertebrates.

Cyclostomes, the jawless hagfish and lamprey, while having a similar schematic of aortic arches as described above, vary widely in the number of arches. Lamprey may have 6 or 7 arches, and hagfishes have anywhere from 6 to as many as 15. In all cases, the aortic arches are associated with the pharyngeal arches, which

contain the gill arches. The cartilaginous fishes widely share a similar pattern, although the fate of the arches now begins to change. In the sharks, the skeletal elements of arch 1 are going to become the jaws (Figure 15.11). The blood vessels that would be associated with that arch (aortic arch 1) are also going to be modified. While not completely traveling with the skeletal elements, aortic arch 1 (AA 1) is now going to be associated with the spiracle, an accessory respiratory structure, but is not a site for gas exchange. AA 2–6 are still going to be associated with gill arches, going through gas exchange and providing oxygen to the other body tissues.

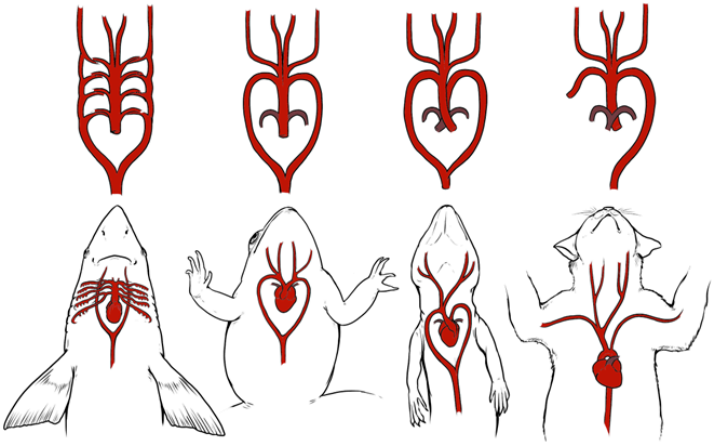

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Figure 15.11—Aortic arches in vertebrates. The darker purple represents the pulmonary circuit. Species represented from left to right are the spiny dogfish (Squalus acanthias), the European common frog (Rana temporaria), the green iguana (Iguana iguana), and the domestic cat (Felis catus).

It is in the bony fishes (Osteichthyes) that the aortic arches start to become more heavily modified. Developmentally, all six arches appear in a sequential pattern, seemingly unmodified. However, as development progresses, changes to the

musculoskeletal framework (and respiratory system) are tightly correlated with the changes that we will observe in the arches. In the teleost fishes, AA 1 and 2 are lost functionally as sites of gas exchange. They instead contribute to the mandibular arch (AA 1) and the hyoid arch (AA 2). AA 3–6 remain associated with gills and are therefore sites of gas exchange (Figure 15.11). The lungfishes (Dipnoi) are an interesting comparison to the teleosts, as they provide insight into the closeness of the relationship between the arches and the respiratory system. As a group, lungfishes can use both gills and lungs for gas exchange (the efficiency and use of each are for a different chapter). They are equipped with gill filaments in the Australian lungfish, but only the two posterior pairs (and to a limited extent the most anterior pair) retain the capacity for gas exchange in African and South American species. This is functionally important because this design prevents oxygen loss to the hypoxic water when oxygen-rich blood from the lungs traverses the gills. AA 2, 5, and 6 interact with the gills for the purposes of gas exchange. AA 1 degrades during development, and AA 3 and 4 move blood directly to the dorsal aorta without engaging with the gills. These are lungfishes, so how do the lungs get their blood supply? A branch of AA 6, which we will call a **pulmonary artery**, directs blood to the lungs (Figure 15.12). The blood will then return directly to the heart instead of to the dorsal aorta. The blood will return to the heart via the pulmonary vein. A small shunt vessel, the **ductus arteriosus**, controls whether blood from these branchial arteries enters the dorsal aorta (systemic circulation) or pulmonary circulation. This configuration resembles that of embryonic tetrapods, where the pulmonary arteries develop from the sixth pharyngeal arch. In lungfishes, the oxygen-rich blood from the lungs bypasses the respiratory circulation by traversing the anterior gill arches and entering the systemic circulation. At this point, we have set the stage for further exploration of two significant themes: the loss/degradation of aortic arches during development and the evolution of the pulmonary circuit.

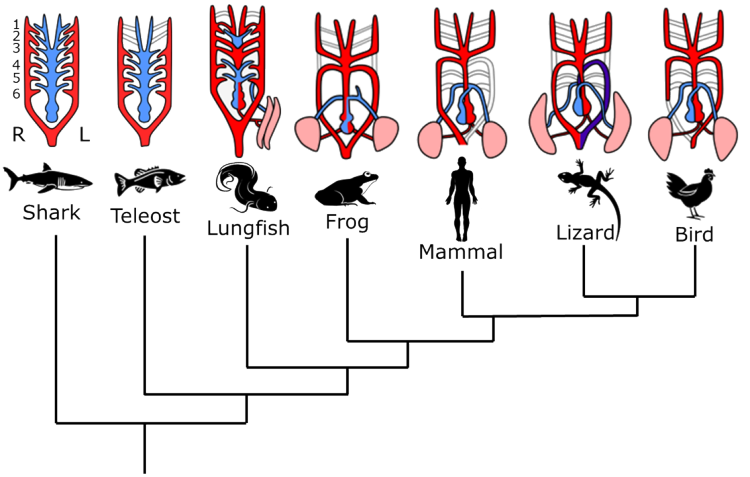

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Figure 15.12—Evolution of aortic arches and their connection to lungs (ventral view) in representative vertebrates. Cranial is at the top of the diagram, R = right, L = left. Air sacs are not shown for the bird.

In bony fishes the gastrointestinal circulation has also received considerable study, as it is central not only to digestion but to other physiological processes such as ion/water regulation and acid-base homeostasis. In many cartilaginous fish, in common with other vertebrates, the gastrointestinal circulation is made up of three major vessels (i.e., **celiac**, **mesenteric**, and **lienogastric arteries**) that branch from the dorsal aorta. However, lamnid sharks (including mako sharks and great white sharks) show an unusual vascular arrangement associated with "regional endothermy"—that is, their capacity to maintain metabolic heat generated in swimming muscles. In lamnid sharks, the gastrointestinal arteries emanate from the third and fourth efferent branchial arteries and proceed to branch into a network (**rete mirabile**) that forms a countercurrent heat exchanger with blood returning from swimming muscles, allowing blood destined for the gastrointestinal tract to be warmed to aid digestion. Ray-finned fishes exhibit an unusual (among

vertebrates) circulatory anatomy in that the major gastrointestinal arteries branch from the dorsal aorta in a common "coelomesenteric" artery before branching to supply different organs.

The invasion of land by the ancestors of the tetrapods represents a pivotal moment in transitioning from gills as the major organ of respiration to using the lungs. The modern amphibians are a useful illustration of how this transition occurred. The earliest diverging lineage, the salamanders, has some variability depending on the environment they live in (Figure 15.11). The fully aquatic salamanders (e.g., the mudpuppy *Necturus*) retain four aortic arches, similar to the fishes. Terrestrial salamanders have transitioned to three arches, having lost AA 1, 2, and 5. Critically, the pulmonary artery in terrestrial salamanders is now the direct branch of AA 6 from the ventral aorta (and AA 6 is now just referred to as the pulmonary artery). There is the ductus arteriosus that connects the pulmonary artery to the dorsal aorta, but it is much smaller than the pulmonary artery. The small vessel is also critically important in human development, which we will discuss toward the end of this chapter. The frogs and toads (Anurans) have taken the transitions even further. AA 3 and 4 have lost their connection to each, effectively severing AA 3's role in supplying blood to the dorsal aorta. The ductus arteriosus is also lost; blood from AA 6 is now completely directed to the lungs. At this stage, the pulmonary circuit is completely separated from the systemic circuit. The nonavian reptiles will show a very similar setup to that of the anurans. In some groups (snakes), small ducts will remain to connect AA 3 and 4. There are two important distinctions that we need to make note of now. The first is that AA 4 is now simply referred to as the **aortic arch**. This will continue to be the case in birds and mammals. The second is that the blood supply for the aortic arch is divided in the reptiles. Changes in the structure of the heart (discussed below) will change which "type" of blood enters the right and left aspects of the aortic arch. For the right aortic arch, it will

receive only blood that has been oxygenated. The left aortic arch will receive a mix of oxygenated and deoxygenated blood.

As we conclude our journey with the birds and mammals, there are only a few (but important!) changes that are going to occur. The **truncus arteriosus** is what will eventually be named the **ventral aorta** in many vertebrates or simply the **aorta** in humans. AA 3 will be unchanged, and it will be referred to only by the names of its major branches: **external carotid**, **internal carotid**, and subclavian (birds only). The aortic arch (AA 4) will lose one of its major vessels, no longer being a paired vessel. In birds, the right side of the aortic arch is retained and is no longer always referred to as the dorsal aorta, instead simply being referred to as the aorta or **descending aorta**. The mammals will retain the left side of the aortic arch but keep the names "aorta" and "descending aorta." The abdominal aorta will also sometimes be used, particularly in humans. The right side of the aortic arch is partially retained in humans, becoming the **subclavian artery** that we identified as belonging to AA 3 in birds. It is important to note that the subclavian artery in birds is not homologous to that of mammals, as they have different developmental origins despite their shared probable function (bringing oxygenated blood to the forelimb).

Hearts

Let's take a look at the nonvertebrate chordates first. The circulation of cephalochordates relies on multiple contractile vessels and a single heart that can be difficult to find on specimens. For a long time, that difficulty spurred the widespread belief that they lack a heart, but a ventral linear heart tube that is homologous to the heart of vertebrates can be defined by molecular markers and developmental genetics. The cephalochordate heart is fairly simple—it is composed of a valveless and uncompartmentalized contractile tube made up of a single layer of nonstriated muscle,

which resembles the linear heart tube during the early developmental stage of vertebrates (see Figure 15.13).

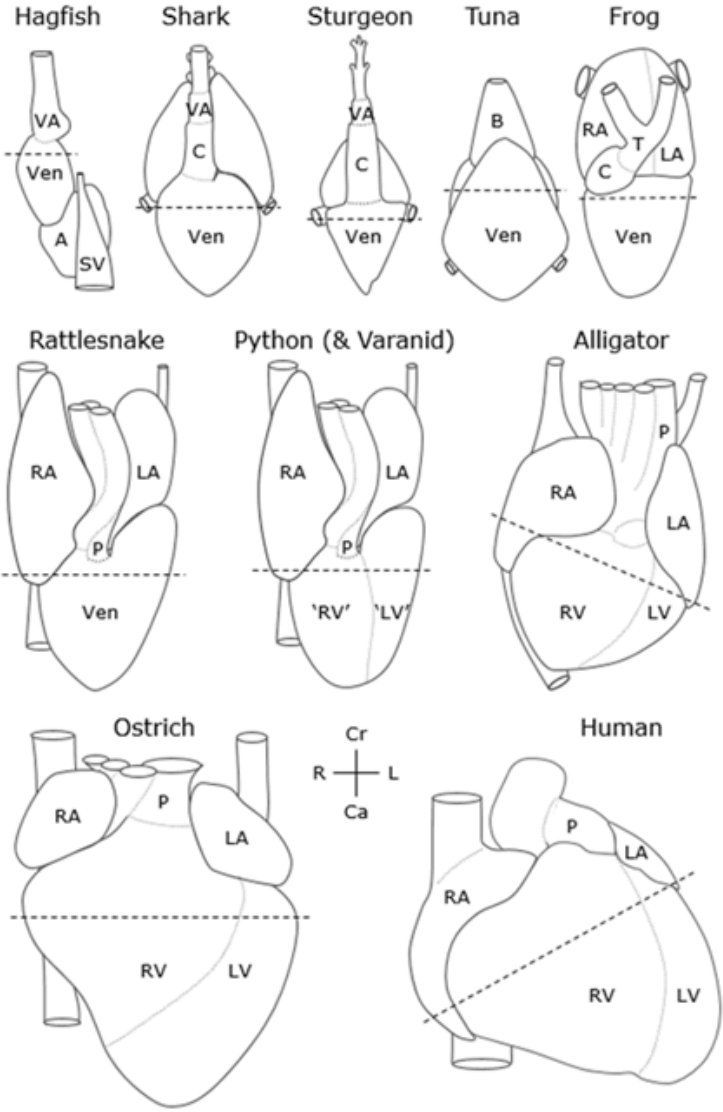

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Figure 15.13—Hearts in a representative sampling of vertebrates. A, *atrium*; B, *bulbus arteriosus*; C, *conus arteriosus*; Ca, *caudal*; Cr, *cranial*; L, *left*; LA, *left atrium*; LV, *left ventricle*; R, *right*; RA, *right atrium*; RV, *right ventricle*; P, *pulmonary trunk*; SV, *sinus venosus*; T, *truncus arteriosus*; Ven, *ventricle*; VA, *ventral aorta. Dashed lines represent where cross-sectional views in Figure 15.13 are taken.*

The heart in urochordates is much easier to find. Like the heart of cephalochordates, the single-chambered tunicate heart lacks valves. The heart is enclosed within a rigid pericardium, and experimental puncturing of the pericardium greatly disturbs cardiac function, indicating that it is important for the heart to work properly. Both cephalochordates and tunicates lack nervous innervation of the heart.

Cyclostomes

Cyclostome hearts are three chambered. Chambers are separated by leaflet-shaped valves, which provide for a unidirectional circulation. Poorly oxygenated blood enters the sinus venosus, followed by the atrium, and the more muscular ventricle (Figure 15.7). The heart is encased in a pericardial sac. These hearts are homologous to other vertebrate hearts; however, like cephalochordates and tunicates (and unlike most other vertebrates), the hearts of cyclostomes are devoid of a coronary circulation to provide blood to the heart muscle itself. The lamprey heart is innervated by the vagus nerve, but innervation differs from the jawed vertebrates by being excitatory—that is, it increases heart rate. The hagfish heart lacks cardiac innervation altogether.

Hagfishes have several **accessory "hearts,"** which are not homologous with other vertebrate hearts; their job is to return blood toward the heart. The **caudal heart** is located posteriorly and moves blood from the sinuses near the caudal trunk and kidneys back to the main (**systemic** or **branchial**) heart. The abdominal **portal heart** is responsible for pumping blood from the intestines to the liver; it is an enlarged portion of the hepatic portal vein. The **cardinal hearts** in the anterior part of the body move blood from sinuses in the head back to the systemic heart via the inferior jugular vein. All the accessory hearts are capable of contraction and give an extra boost to the venous blood pressure to get blood back to the heart. Interestingly, the hagfish accessory hearts, except for

the caudal heart, do not appear to be under the control of nerve signals. Lampreys have no accessory hearts, instead relying on a fairly large systemic heart.

Chondrichthyes and Actinopterygii

The other fish hearts are four chambered (but not in the same configuration as the human heart). Blood passes through, in series, the sinus venosus, atrium, ventricle and **outflow tract**. The compositional nature of the outflow tract varies and is broadly defined as a muscular **conus arteriosus** in Chondrichthyes and nonteleost Actinopterygii or, in teleosts, a **bulbus arteriosus** mainly composed of elastin and smooth muscle. While the bulbus arteriosus is the most obvious structure of the teleost outflow tract, a small conus arteriosus persists between the ventricle and the bulbus arteriosus (Figure 15.13).

Cartilaginous and ray-finned fish often exhibit complex and variable ventricular architecture. The ventricular wall can be divided into an outer "compact" layer and an inner trabeculated "spongy" layer of cardiac muscle (**myocardium**). Cartilaginous and at least some ray-finned fishes both have coronary arteries to bring blood to the cardiac muscle itself, but the layers they serve differ between fish groups. In cartilaginous fishes, the coronary arteries are widespread and nourish both the compact layer and spongy myocardium. In ray-finned fishes, the coronary arteries are confined to the compact layer, and the spongy layer relies on oxygen supply from the oxygen-poor venous blood that returns to the heart. However, teleosts have lost the compact myocardium and exhibit an avascular heart composed of only spongy myocardium. Surprisingly, this does not necessarily limit cardiac performance; some teleosts with spongy hearts exhibit comparable capacity to those with compact hearts. However, the most athletic teleosts, such as tuna, exhibit greater proportions of compact myocardium.

Nervous innervation of the heart is well developed in these

groups of fishes. Ray-finned fishes, like other jawed vertebrates, exhibit a characteristic double innervation of the heart from the autonomic nervous system composed of inhibitory parasympathetic innervation from the vagus nerve and excitatory sympathetic innervation (Chapter 19—Peripheral Nervous System). Cartilaginous fishes lack sympathetic innervation, although it is unclear if this represents an ancestral state or secondary loss.

Box 15.2—Plumbing for Air-Breathing Organs

As mentioned in Chapter 14, the ability to breathe air through specialized gas-exchange organs (**air-breathing organ, ABO**) has evolved many times in osteichthyes, and the ABO must be accommodated into the circulation. While the vascular anatomical arrangement varies depending on the precise nature of the ABO (which may be represented as simple modification of the buccal cavity to specialization of the swim bladder or even co-option of the gastrointestinal organs), blood always returns to the veins en route to the heart and thus the gills before it reaches the systemic tissues (Figure 15.14).

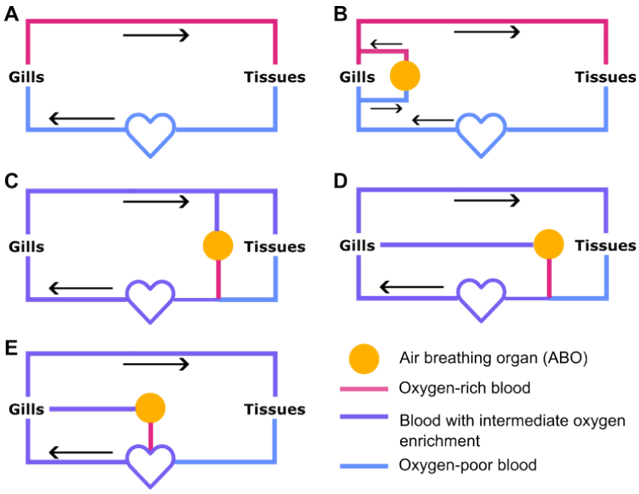

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Figure 15.14—Schematic of cardiovascular system in various air-breathing fishes. The heart shape represents the heart, and the gold circle represents the air-breathing organ (ABO) when present. Line colors represent how oxygenated the blood is, with pink as oxygen rich, purple as intermediate, and blue as oxygen poor. (A) Fish with no ABO (e.g., a goldfish); (B) Fish with an ABO made from gills, buccal cavity, or opercular cavity (e.g., Clarius catfish); (C) Fish with an ABO from the gastrointestinal tract (e.g., Plecostomus catfish); (D) Fish that use their swim bladder as an ABO (e.g., the bowfin Amia); (E) Fish with lungs (e.g., lungfish).

Indeed, it has been suggested that air breathing may have evolved in fishes primarily for cardiac oxygenation during exercise. Remember that the coronary arteries only reach the spongy layers of most actinopterygian hearts. It is possible that adding some oxygenated blood prior to reaching the heart helps deliver some oxygen to the heart. However, high

vascular resistance means the ABO could not be placed elsewhere in circulation. A more likely possibility may have to do with **hypoxic** (low-oxygen) environments, another situation where having some extra oxygen in the bloodstream might be helpful.

Actinopterygians with no ABO simply increase their gill ventilation rate in hypoxic conditions, which allows them to uptake more oxygen, even if it may not be enough. For fishes with an ABO, it has long been believed that when fish breathe air in hypoxic water, they risk losing oxygen as blood traverses the gills (down an oxygen concentration gradient). However, recent studies indicate that little oxygen is lost in hypoxic conditions, even though carbon dioxide excretion is still maintained at the gills. How does this work? ABO-bearing fishes decrease their gill ventilation rate in hypoxic waters, which makes the ABO the primary site of gas exchange, not the gills.

Remarkably, at least in one teleost species, the northern snakehead *Channa argus*, it has been shown their undivided ventricle can maintain separate streams of blood flow and keep the poorly oxygenated blood from the systemic tissues separated from the more oxygenated blood from the ABO. This is likely a consequence of having not one but two aortas. Well-oxygenated blood returning from the gills is primarily directed to the systemic circulation via the posterior gill arches, which have poorly developed gill filaments that therefore won't lose much oxygen. Oxygen-poor blood enters the anterior gill arches before reaching the ABO for aquatic and aerial gas exchange, respectively.

Sarcopterygii

As implied by the name, lungfishes (Dipnoi) are characterized by well-developed lungs. These are homologous to those of tetrapods (see Chapter 14—Respiratory System). As we mentioned above, the shift to breathing with lungs resulted in a lot of replumbing of the circulatory system. This includes the heart (Figure 15.15). Lungfishes give us a good glimpse into how the heart adapted to this new circulatory pattern. The atrium is partially divided by a small sheet of connective tissue called the **pulmonalis fold**. Oxygen-rich blood returns from the lungs in a distinct pulmonary vein, which passes over the sinus venosus dorsally. From here, the blood enters the atrium to the left of the **pulmonalis fold** and therefore into the left part of the atrium. The oxygen-poor blood from the systemic circulation enters the right side of the atrium.

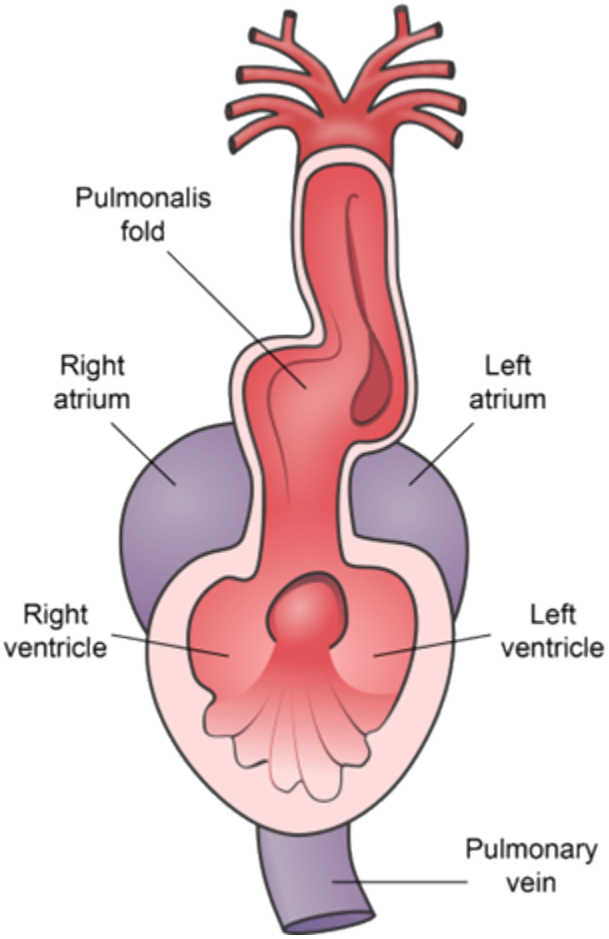

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Figure 15.15—Illustration of the African lungfish heart.

The ventricle is partially divided by a septum to keep these two streams of blood separated. The septum does not reach the orifice of the conus arteriosus. In South American and African lungfish, the conus arteriosus maintains separate streams of blood flow by virtue of a spiral fold of connective tissue. There is no

spiral fold in the Australian lungfish, but this species has numerous valves in the conus arteriosus. Lungfishes have the most unusual **atrioventricular valve** (the valve between the atrium and ventricle), as it is basically a ball of connective tissue rather than leaflets. Closure of the atrioventricular canal is achieved by the myocardium contracting against the "ball."

The lobe-finned fishes also include the coelacanths. Their hearts resemble those of other actinopterygii nonteleost fish, but the chambers are somewhat stretched out on the caudo-cranial axis. The most pronounced deviations are the presence of trabeculations in the sinus venosus and the presence of a pulmonary vein that opens into the sinus venosus but is filled with fat and thus nonfunctional with regard to blood flow. The atrium, ventricle (with well-developed coronary arteries), and outflow tract are all undivided. Given the famously elusive nature of these fish, there has unfortunately been little opportunity for detailed molecular and physiological studies of their cardiovascular system.

Box 15.3—Change in Shape: How?

A major theme of this chapter is the differences in form. Differences in form are often associated with differences in function. But how do these differences come about? Very often, "new" structures are not as much new as they are dramatically increased or reduced versions of evolutionarily older, and developmentally earlier, structures; for example, the wings of a bat are clearly modified versions of the front limbs that all mammals share. Therefore, what we perceive as "new" will often merely be a "preexisting" structure that has grown relative to its surrounding structures. Growth is mostly about adding more cells, so a key mechanism of

morphological changes is the control of cell proliferation, including the onset, duration, and cessation of proliferation. One example of this is the difference in chamber walls between ectotherms (highly trabeculated) and endotherms (compact-dominated). In humans, a major factor in shaping the ventricular wall is a three-week period at the end of embryonic development in which the compact wall is highly proliferative and the cavities expand, while the trabecular layer has lower rates of proliferation and its growth is less fast. Then the fetal period begins, and the trabecular layer sees greater rates of proliferation again. But this three-week lull in trabecular growth, which does not occur in ectotherms, effectively changes the morphology of the ventricular chamber walls from ectotherm-like to endotherm-like. Such a change in growth rate of one structure in relation to its surrounding structures is a crucial mechanism of shape change in general, and it is often referred to as allometry, meaning that one structure has an "other" (allo-) growth. Conversely, with isometric growth (iso-, meaning "same") an organ grows while the proportions of its constituent parts do not change. From the late embryo to adult stages, the ventricles of both ectotherms and endotherms largely grow isometrically.

Tetrapods

Amphibians and nonavian reptiles retain a sinus venosus, like other ectothermic vertebrates (i.e., the fishes), which receives the

systemic venous blood prior to the right atrium. The sinus venosus contracts prior to the atrium and contributes to the filling of the atrium. Interestingly, in both mammals and birds, the myocardium of the sinus venosus retains its function, but its contraction now occurs simultaneously with that of the atria. In this way, the sinus venosus has been lost as a separate chamber in these groups. The functional significance let alone importance of this evolutionary convergent evolution is elusive. In terms of the cardiac outflow tract, amphibians retain a conus arteriosus, while in amniotes (reptiles and mammals) it is largely assimilated into the ventricle, or "ventricularized" (in Figure 15.13, notice how the structure labeled "C"—for conus—is gone in reptiles and mammals). Experimentally, this process has been shown by labeling the outflow tract of young chicken embryos, and days later the labeled tissue is within the right ventricle.

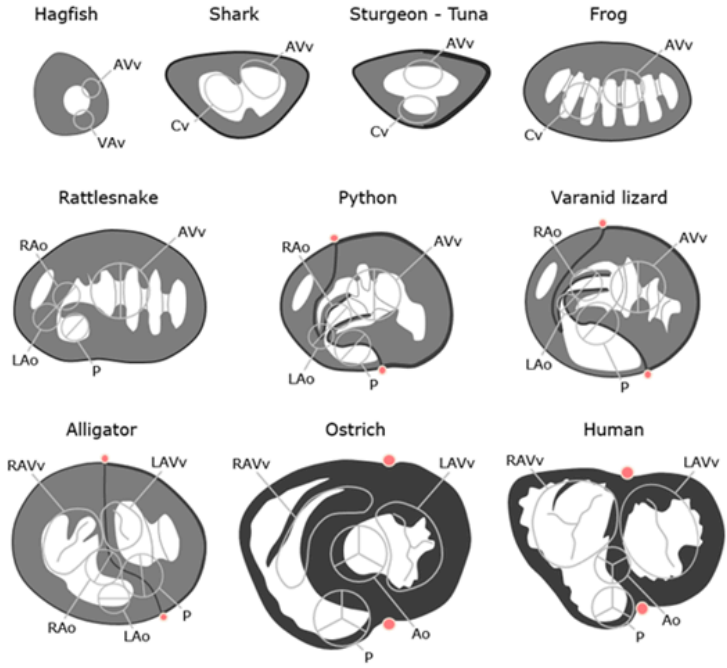

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Figure 15.16—Comparative ventricular morphology illustrated by sections in the transverse plane close to the ventricular base (see dashed line in Figure 15.13). Notice how various manifestations of ventricular septation occur in reptiles. In the instances of a full ventricular septum (alligator, ostrich, and human), the atrioventricular junction is expanded rightward and a dedicated right atrioventricular valve is present. Notice also that the proportion of trabeculated and compact myocardium is coded in light and dark gray, respectively, showing that it is in endotherms only that the ventricular myocardium is dominated by compact myocardium. A, *aorta orifice/valve*; AVv, *atrioventricular orifice/valve*; Cv, *conus arteriosus orifice/valve*; LAo, *left aorta orifice/valve*; LAVv, *left atrioventricular orifice/valve*; RAo, *right aorta orifice/valve*; RAVv, *right atrioventricular orifice/valve*; P, *pulmonary trunk orifice/valve*; VAv, *ventral aorta orifice/valve.*

For the vast majority of amphibians, the atrium is completely divided, with the left atrium receiving pulmonary blood and the right atrium receiving systemic blood (Figures 15.13 and 15.17). We see an exception to having completely divided atria in

salamanders who lack lungs as adults, such as those in the family Plethodontidae. Without lungs, there is no need for a pulmonary artery. Thus, these salamanders have either a missing atrial septum or an incomplete one. Tiny frogs sometimes also lack or have an incomplete atrial septum.

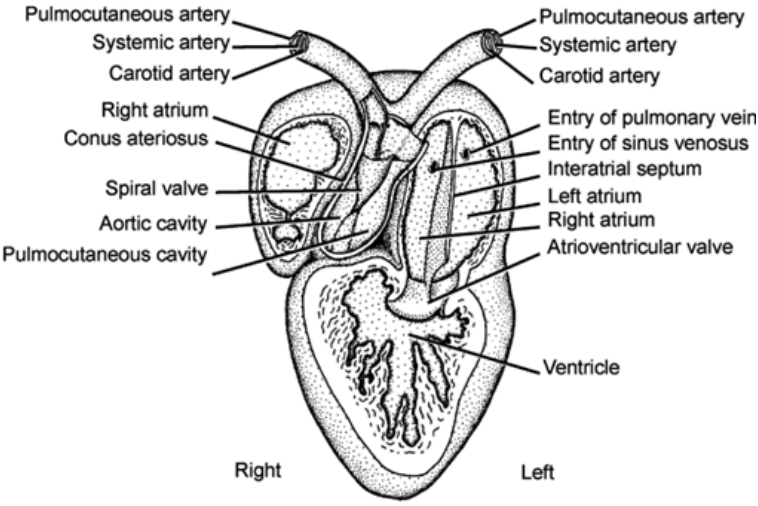

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Figure 15.17—Anatomy of a general amphibian heart.

The amniotes have complex hearts that achieve separation of oxygen-rich and oxygen-poor blood in several different ways. The atria are completely separated into left and right atria (Figure 15.13). As we saw in amphibians, the left atrium receives pulmonary blood, and the right atrium receives systemic blood. Much of the variation across amniotes instead has to do with ventricle structure.

Unlike the lungfishes, many amphibian hearts have an undivided ventricle (Figures 15.16 and 15.17). Yet the oxygen-rich and oxygen-poor blood are kept largely separate by well-developed trabeculae. While the heart is filling with blood during diastole, it pools in the large pockets created by the trabeculae. Blood entering the ventricle from the left atrium tends to stay in the left trabecular

pockets, and blood from the right atrium tends to stay on the right. When the heart contracts during systole, blood stays separated as it moves into the conus arteriosus. However, some salamanders, like the hellbender *Cryptobranchus alleganiensis*, have ventricles with a partial septum to assist. Regardless of whether there is a septum in the ventricle or not, the conus arteriosus contains a spiral valve, similar to some species of lungfish, which keeps the blood from each side of the ventricle separated.

In most nonarchosaur reptiles, the ventricle is not fully divided by a septum, although oxygen-rich and oxygen-poor blood deriving from the left and right atria, respectively, are kept well separated (Figure 15.16). This is aided by discrete subchambers; the **cavum venosum**, the **cavum pulmonale** and the **cavum arteriosum** (Figure 15.18). Oxygen-poor blood from the right atrium is received in the cavum venosum and overflows into the cavum pulmonale, from where it is mainly ejected into the pulmonary circulation during systole. When the well-oxygenated blood returns to the left atrium via pulmonary veins, blood passes into the cavum arteriosum from where it is ejected into the systemic circulation (left and right aortic arches) after crossing the cavum venosum during ventricular contraction. Because oxygen-poor blood and, later in the cardiac cycle, oxygen-rich blood both pass through the cavum venosum, it is a site of limited mixing of blood.

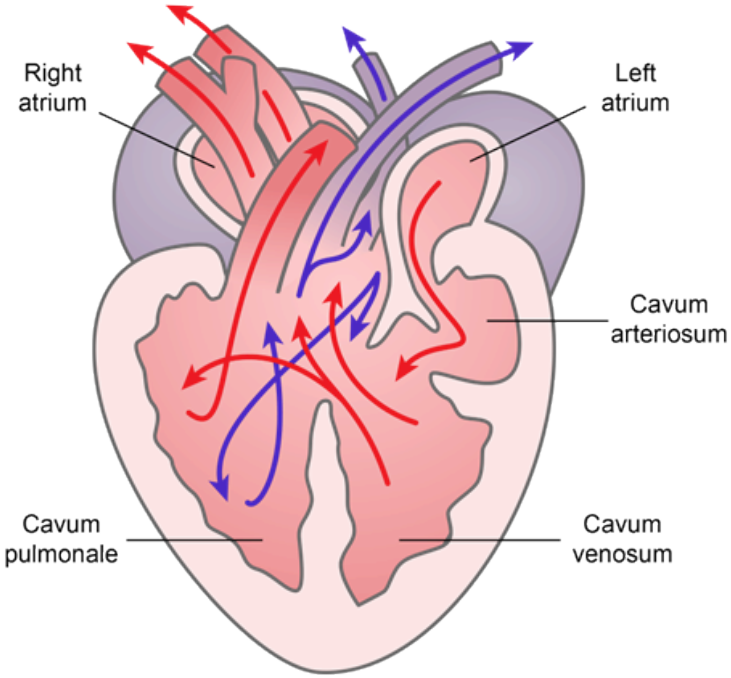

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Figure 15.18—A generalized reptile heart showing the different cavae in the ventricle.

Many reptiles (as well as some amphibians and fishes) have the ability to change their circulatory patterns. These **cardiac shunts** (Figure 15.19) occur when changes in systemic or pulmonary artery resistance make passage into one circulation less favorable, and so blood is diverted for a short period of time. For example, pulmonary artery vasoconstriction, stimulated by the vagus nerve, increases pulmonary resistance and diverts a greater proportion of oxygen-poor blood toward the systemic circulation. This situation is typical of resting states or during diving in aquatic species such as sea turtles. This action is known as a **right-to-left** or **pulmonary**

bypass shunt. The "right-to-left" indicates that systemic oxygen-poor blood from the right atrium is moving directly back to the systemic circulation (associated with the left side of the ventricle)—it's bypassing the pulmonary circuit. **Left-to-right** or **systemic bypass shunts** may also occur where well-oxygenated blood is shunted from the left atrium back to the respiratory organs, bypassing the systemic circulation. For example, the freshwater red-eared slider turtle *Trachemys scripta* uses a left-to-right shunt when it returns to the surface after diving.

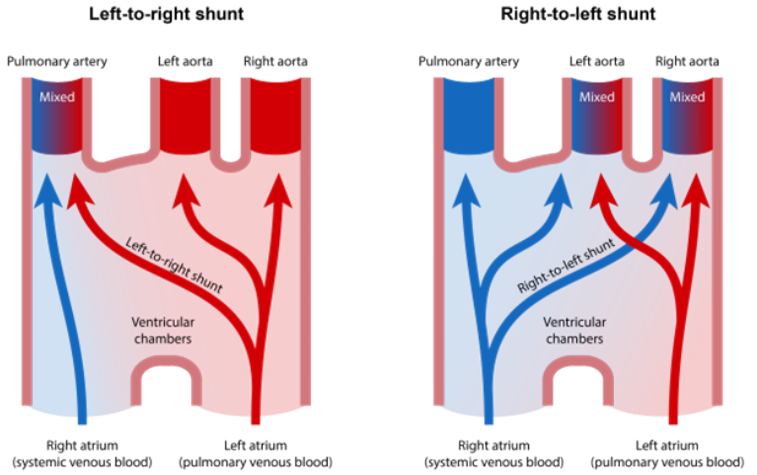

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Figure 15.19—Schematics of blood flow in left-to-right and right-to-left shunts in reptile hearts.

The archosaurs (including birds) and mammals share a generally similar plan. Both the atria and the ventricles are completely divided into left and right (Figures 15.13 and 15.16). Oxygen-rich blood from the pulmonary circulation is received by the left atrium and then moves into the left ventricle and out to the systemic circulation (Figure 15.9). Oxygen-poor blood is returned to the heart from the systemic circulation and into the right atrium,

followed by the right ventricle, and then exits to move to the pulmonary circulation. In all three groups, the left ventricle is larger and more muscular, reflecting the need to generate higher blood pressure to move the blood throughout the entire body.

Crocodylians, unlike the other archosaurs, have a complete ventricular septum dividing the right and left ventricles (Figure 15.16). It very much resembles that of mammals by being almost exclusively myocardial except for a small membranous septum immediately below the base of the arteries. In birds, the configuration is the same, but the connective tissue of the membranous septum differentiates to myocardium in late development.

Crocodylians, like other nonavian reptiles, retain two aortic arches, wherein the right aortic arch exits the left ventricle, and the left aortic arch exits the right ventricle along with the pulmonary artery (Figure 15.20). The left and right aortae communicate via an opening, the foramen of Panizza, in their shared vessel wall, close to the ventricular outflow valves. This anatomical arrangement means that, in common with other nonavian reptiles, crocodylians are capable of central shunts despite the ventricular septum. The left aorta can receive oxygen-rich blood from the left ventricle via the right aortic arch and the foramen of Panizza, or when pulmonary vascular resistance is high, oxygen-poor blood from the right ventricle is ejected into the left aorta (a right-to-left shunt as described above, Figure 15.19). This mechanism is partially supported by the "cog-teeth valve" at the base of the pulmonary artery, which gates blood flow to the lungs. Like other reptiles, the ensuing right-to-left shunt is typical of resting submergence. Crocodylians are incapable, however, of left-to-right shunting. Mammals and birds cannot shunt at all.

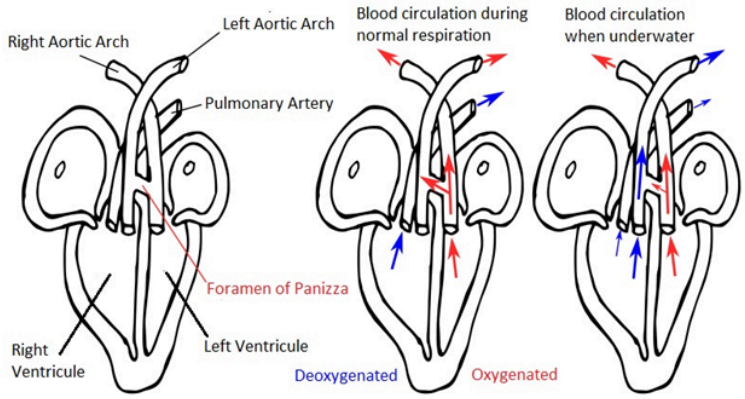

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Figure 15.20—Crocodilian heart anatomy and shunting mechanism.

Endothermy and the Amniote Heart

Remember that archosaurs and mammals represent two different evolutionary lineages and that the general anatomy of these hearts represents convergent features. If we leave nonavian archosaurs aside, we can see a possible reason for the convergence: endothermy. Endothermy (the capacity to maintain a high stable body core temperature) likely evolved at least twice in amniotes: in mammals and in birds (although there is evidence of endothermy within the nonavian dinosaurs as well). The higher metabolic demands of endothermy are associated with cardiovascular changes including faster heart rates, higher systemic blood pressures, and lower pulmonary blood pressures. Both birds and mammals are characterized by complete ventricular division and both only have one aortic arch with no shunting. The completely separated pulmonary circulation allowed the evolution of lower blood pressures and a thinner blood-gas barrier (see Chapter 14 on the respiratory system). There is also a clear transition between the mixture of compact and spongy myocardium seen in ectotherms.

Hearts of ectotherms are often dominated by a spongy trabeculated wall with extensive lumens and only a thin compact layer, whereas endotherms tend to have a highly compact wall, which may have only a few large lumens (Figures 15.16 and 15.21, Box 15.3). Presumably, this is an important adaptation to facilitate faster ventricular filling required for the higher heart rates of endotherms. The thick compact wall requires an extensive coronary artery supply, whereas ectotherms derive much of their cardiac oxygenation from venous luminal blood. While forms of coronary arteries are found across jawed vertebrates, they have likely evolved independently in different lineages, although the coronary arteries of amniotes are likely homologous.

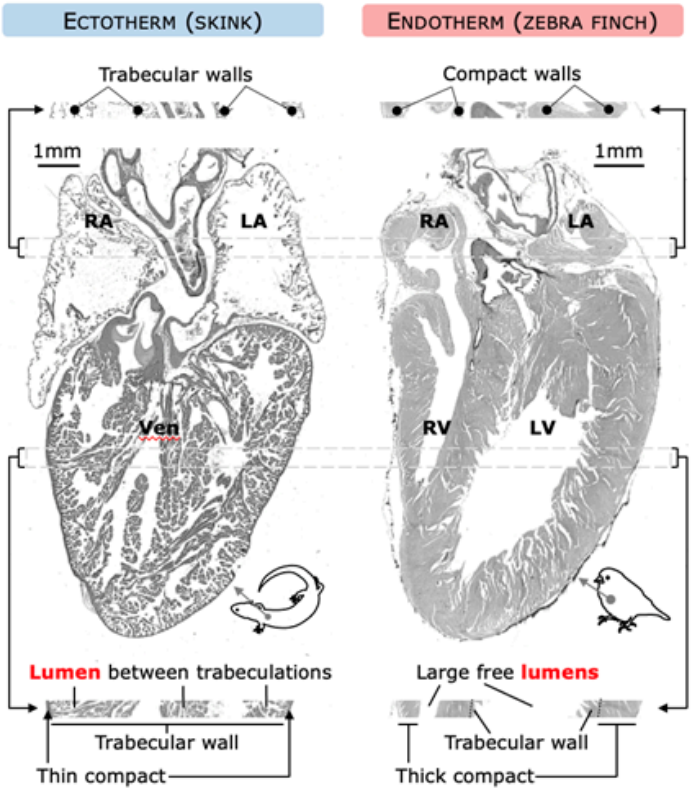

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Figure 15.21—Main differences in cardiac architecture between ectothermic and endothermic amniotes.

Another potential convergent link to endothermy has to do with the processes involved in the initiation of cardiac contraction. Recall from Chapter 11 that muscles contract when an action potential is reached. The hearts of most mammals and birds have a **cardiac conduction system** that starts each heartbeat and the electrical activation of the chambers, but hearts of ectotherms are generally without this system. The cardiac conduction system is

composed of heart muscle cells with specializations for impulse generation (**pacemaking**), fast electrical propagation, or both. Most upstream is the **sinus node** (**SA node**), the dominant pacemaker of the heart (Figure 15.22). If present, it sits in remnants of the embryonic sinoatrial junction. A somewhat similar structure sits in the remnants of the embryonic atrioventricular junction; this is the **atrioventricular node** (**AV node**). This node can also spontaneously generate action potentials, but this normally occurs at a slower rate than the SA node. The primary function of the AV node is to delay electrical propagation because it gives the time it takes for atrial contraction to finish the filling of the ventricles.

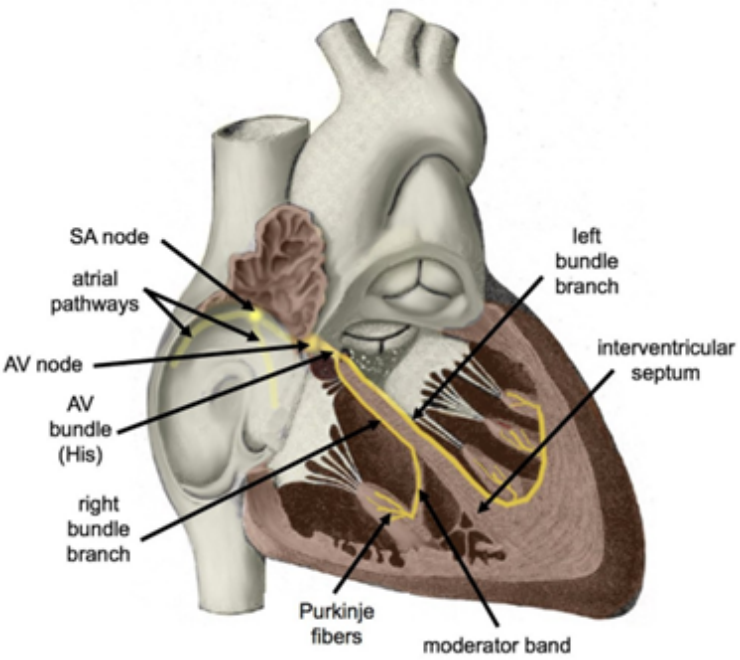

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Figure 15.22—The cardiac conduction system of the human heart.

From the AV node extends the **bundle of His** (named after

a Swiss anatomist and also called the AV bundle), which is the fast and only electrical connection between the atrial and ventricular myocardium. It reaches the crest of the ventricular septum, then drapes over the ventricular septal surfaces as the left and right bundle branches. From there it terminates into the **peripheral conduction system** of **Purkinje cells** (also called Purkinje fibers). In mammals, the peripheral conduction system is mostly restricted to the ventricles, while it can occur in a less developed state in the atria. By contrast, in birds the peripheral conduction system is very extensive in both the atria and the ventricles, and for reasons that are poorly understood, the sinus and atrioventricular nodes can be quite inconspicuous and in this sense underdeveloped relative to mammals.

We just finished examining the evolution of the blood circulatory system in terms of tracking changes across various vertebrates. There is a lot to keep track of! We have provided a visual summary in Figure 15.23.

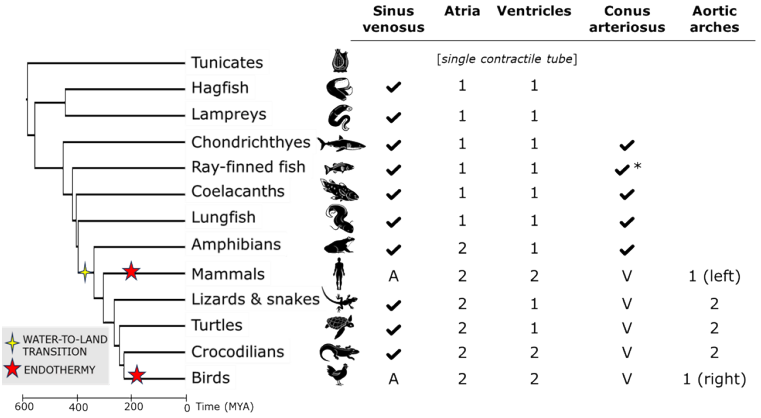

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Figure 15.23—Vertebrate cladogram annotated with major evolutionary events and changes in gross cardiac anatomy. A = *atrialized*, V = *ventricularized*, * = *reduced in teleosts.*

Lymphatics

Remember that we have a second circulatory system to follow—the lymphatic system. Unfortunately, the evolutionary story of the lymphatic system is murky. While scientists have a good understanding of the tetrapod lymphatic system, that of the fishes is still up for debate thanks to the presence of the **secondary vascular system** (**SVS**) in many extant fishes (Figure 15.24). The SVS is composed of vessels that contain blood, but do not receive it directly from the heart. Instead, there are special connections called **interarterial anastomoses** that are so large that even erythrocytes can enter the SVS, at least under some circumstances. Importantly, these SVS vessels serve the external-most parts of the fish body, the fins and body surfaces, and run in parallel to the primary blood circulation. While this means that the fluid in the SVS is not filtered fluid as the lymph of tetrapods is, the structure of the SVS capillaries is very similar to tetrapod lymphatic capillaries. Moreover, both systems operate under low-pressure regimes. Perhaps the SVS could be considered a lymphatic system precursor. Studies on zebrafish (a teleost and model organism) over the last two decades have suggested that zebrafish potentially have a proper lymphatic system. This makes sense if you recall that the lymphatic system has an important role to play in immune function—that's a good function to have! However, two recent studies on zebrafish have suggested that some aspects of the SVS transition into lymphatic vessels, meaning that zebrafish may have some sort of hybrid SVS-lymphatic system. For now, we can say that ray-finned fishes have some sort of lymphatic system that is somewhat similar to mammals, but it remains to be determined how the SVS fits into the evolutionary story, and much may be learned from reexamining cyclostomes or chondrichthyans.

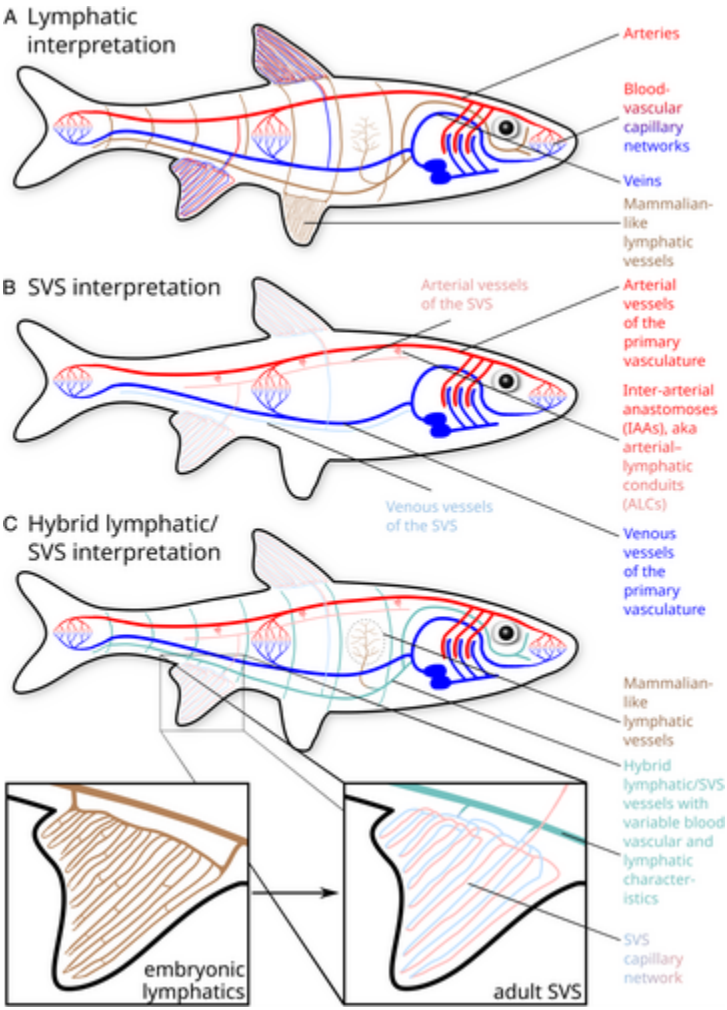

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Figure 15.24—The secondary vascular system (SVS) and lymphatic vascular system (LVS) in ray-finned fishes. (A) The lymphatic interpretation considers the fish's cardiovascular and lymphatic vascular systems to be parallel but separate networks being functionally and anatomically largely identical to the mammalian-like LVS. (B) The SVS interpretation redefines all lymphatic vessels as secondary blood vessels that receive their flow from interarterial anastomoses, which can regulate the influx of red blood cells (RBCs). (C) In the hybrid lymphatic/SVS interpretation, a subset of lymphatic networks maintains their lymphatic nature in the adult fish (termed here "mammalian-like lymphatic vessels").

If we look at our closest extant fish lineage to tetrapods, the lungfish, we see the lack of the SVS, and the lymphatic system looks more similar to that of mammals than that of the zebrafish. There is no input directly from the blood into the lymphatic capillaries (just interstitial fluid), and the structure of the lymphatic capillaries is similar to mammals. However, lungfish lymphatic capillaries do not merge into lymphatic vessels. Instead, lymph is immediately pumped into the adjacent blood capillaries by **micropumps**, tiny lymphatic hearts that are unique to the Dipnoi.

Once we get to the tetrapods, the lymphatic system follows the same general plan described in Section 15.2. Pressure in the lymphatic system is generated by adjacent skeletal muscle and, when present, lymphatic hearts. As we see in blood vessels, lymphatic hearts have three layers: an endothelial tunica interna, a muscular tunica media, and a tunica exterior composed of connective tissue. The number of lymphatic hearts varies widely across tetrapods and within tetrapod groups (Figure 15.25). Within the amphibians, caecilians can have up to 200 lymphatic heart pairs, whereas different salamander species have anywhere from 8 to 23 pairs. Lymphatic hearts are also found in all extant reptiles (including Aves). As with the amphibians, their location and number vary. Mammals are the only tetrapods that do not have lymphatic hearts.

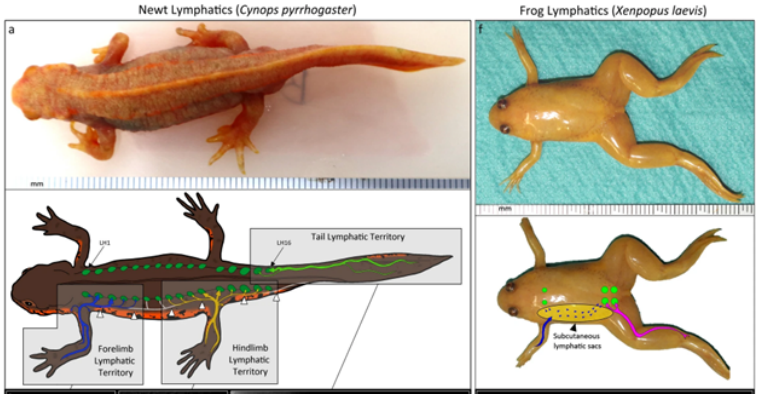

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Figure 15.25—The location of lymphatic hearts and lymphatic sacs in two representative amphibians.

Anurans (frogs and toads) only have two pairs of lymphatic hearts. However, like just about every other part of their body, anuran lymphatic systems are highly specialized. Anurans have a series of subcutaneous **lymph sacs** that are connected via muscularly controlled unidirectional valves (Figure 15.25). The lymph sacs themselves are not contractile. Compression of the sacs forces lymph toward the lymphatic hearts, which in turn provides that extra boost of pressure to get the lymph returned back to the veins. Compression happens in two ways, through contraction of surrounding skeletal muscles and through increased pressure from the lungs as they inflate during breathing. Exhaling is also useful for the movement of lymph in frogs—as the lungs deflate and volume decreases, the subvertebral sinus expands due to sharing a pleural membrane with the lungs. The increase in subvertebral sinus volume creates a drop in pressure, which draws lymph into the lymphatic sacs. When the frog inhales again, that new lymph is moved toward the lymphatic hearts.

15.5 Human Circulatory System

The human heart has four chambers, a solitary pulmonary trunk, and an aorta, as does any mammal (Figure 15.26). But there is substantial variation between mammals in the number of veins that connect to the atria, the configuration of the right atrioventricular valve, and more. Perhaps the human vasculature also has unique features—for example, as an adaptation to bipedalism—but such features may be subtle, and their impact could be widely distributed in the vast circulatory network. Either way, what is unique about the human circulatory system is most readily seen and investigated at the level of the heart.

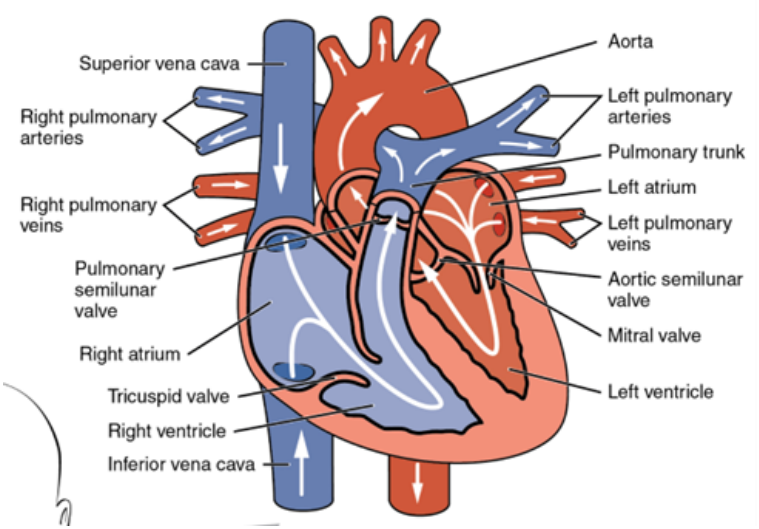

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Figure 15.26—The human heart diagrammed to visualize all four chambers and the major vessels. The white arrows indicate the direction of blood flow.

In humans and eutherian mammals generally, the gestational formation of the atrial septum is particularly complicated. It involves the formation of a primary septum that is

later perforated such that a large foramen ovale forms. The **foramen ovale** enables blood to cross to the left atrium and bypass the pulmonary circulation of the developing embryo that does not use the lungs for gas exchange. The foramen is closed after birth because the perforated atrial septum butts against a part of the atrial roof that folds into the right atrial cavity. The remnant of this process is the circular depression, the **oval fossa**, on the right atrial side of the atrial septum. Approximately one out of four humans will have an imperfectly closed atrial septum, or so-called **patent** foramen ovale. Because the pressure in the left atrium is higher than in the right atrium, the patent foramen ovale is effectively closed in most circumstances. In humans and eutherian mammals generally, the right atrioventricular valve comprises leaflets coming off from the right ventricular free wall and the ventricular septum. In contrast, in monotreme and marsupial mammals, the right atrioventricular valve is dominated by a large leaflet coming off the free wall.

A variable feature of mammal hearts is the number of systemic veins that connect to the right atrium, which is typically three but only two in primates (these veins are the so-called **caval veins**). The number of pulmonary veins connecting to the left atrium is particularly variable; it may vary between one and seven depending on the species, and it is typically four in primates. In humans, these veins undergo remodeling only in the fetal period, whereas the vast majority of morphogenetic processes have already taken place in the embryonic period. Only in the second trimester will the left superior caval vein begin to regress, or about two months after it has initially formed. A remnant of it always persists as the coronary sinus into which the coronary circulation drains. The regression leaves the (right) superior caval vein as the sole conduit for the systemic venous blood returning from the upper limbs and the cranial circulation. The regression proceeds to a different extent between individuals, and a few can be born with a persistent left superior caval vein. Around the same time the left superior caval vein regresses, the pulmonary venous tree will be

incorporated into the posterior wall of the left atrium up to around the second branching point. In this way, over the course of a month, one pulmonary venous orifice becomes two and then four (and five in about 20% of us).

Growing evidence suggests that the primate left ventricle is relatively rich in trabeculation and that the human left ventricle, while still quite trabeculated, may be less trabeculated than in great apes. The functional implications of the degree of trabeculation in the left ventricle are not clear, but a lower degree of trabeculation appears to set the human heart apart from that of our closest relatives.

15.6 Circulatory System Integration

The circulatory system is tightly integrated with several other systems. The reproductive system, endocrine, digestive, and more all rely on the circulatory system to transport their products across a body. However, as you may have gathered from this chapter, the evolution of the circulatory system is most tightly correlated with the respiratory system. As the respiratory system has changed over evolutionary time (see Chapter 14—Respiratory System), the circulatory system has changed alongside it. Blood vessels have vanished, and new ones have evolved to adjust to the changes in primary mode of respiration. The changes to the aortic arches very closely mirror the changes to the respiratory system. The size and complexity of the heart have also adjusted across the history of vertebrates. Chambers have been added and others subsumed. Changes to metabolic rate and the nervous system have altered the heart's structure.

Box 15.4—When Form and Function Are Not Related

Circulation of blood requires energy in the form of blood pressure. Pressure is imposed on blood by contractions of the heart. Generally speaking, the more heart muscle there is, the greater the pressure that can be generated (in proportion to the size of the chamber cavity, as dictated by the law of Laplace). This is much like skeletal musculature, where larger muscles generate more force, but there is considerable variation. Among mammals, for example, the extent of the trabecular layer varies between the two ventricles (the trabecular layer of the right ventricle relative to that of the left ventricle can be anywhere from shallow to very extensive) as well as between species (great apes have quite extensive trabecular layers, whereas many ungulates have relatively sparsely trabeculated ventricles). It is not clear, however, that such pronounced differences in trabeculation are related to differences in function. In mammals, key functional parameters such as mean systemic arterial blood pressure and stroke volume relative to body mass do not vary much between species; they are typically around 90 mm Hg and 0.1%, respectively. Also, when thousands of healthy humans are surveyed, a very substantial variation in left ventricular trabeculation is found, but again functional parameters are uncorrelated to this variation. In this case, then, one might infer that the substantial variation in form (trabeculation) is not related to function.

The number of pulmonary veins that connect to

the left atrium is another example of the lack of direct relationship between form and function. In three out of four humans, there are four veins, but some 20% of us have five veins; again, this variation does not have any obvious impact on the function of the left atrium or left ventricle.

15.7 Summary

The circulatory system works to move blood around the body and in doing so transport oxygen and nutrients to the tissues that need them. The circulatory system must continue to function as long as the individual is to remain alive. The physics of fluid flow is paramount to linking the structure of the circulatory system to the function. The heart is muscular with multiple chambers, directing blood flow to large vessels that carry the blood to where it is needed. Thick-walled arteries will resist intense pressures as blood leaves the heart. Capillaries are extremely small and thin walled to allow for the diffusion of oxygen, carbon dioxide, and nutrients across tissues. The circulatory system is most tightly connected to the respiratory system, their shared responsibility of gas exchange greatly influencing the evolution of both systems. Beginning with a basic pump and series of tubes, the many vertebrate groups have tinkered with and tweaked these structures to optimize the delivery of blood. In examining these tweaks and changes, there is real insight into the evolutionary history and ecology of these vertebrates.

Application Questions

1. How does the number of chambers relate to whether a vertebrate is ectothermic or endothermic?
2. Why does the human heart have four chambers only? (Why not an embryonic/reptilian sinus venosus, with five chambers? Why not an embryonic/amphibian myocardial outflow tract / conus arteriosus, with six chambers?)
3. Although coronary atherosclerosis (and the associated ischemic heart disease) is the major killer of humans worldwide, rattlesnakes do not die from coronary arterial ligation—why not?

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16. Urogenital System: Excretion

LISA B. WHITENACK

Focus Questions—to Guide Your Reading of This Chapter

1. How does kidney structure change as vertebrates transition from water to land?
2. What organs do various vertebrates use for maintaining osmotic balance other than their kidneys?

16.1 Introduction: Why "Urogenital"?

It may seem odd to consider "uro-" and "-genital" together. After all, we might see different medical providers for issues with kidneys versus reproductive anatomy. However, the "uro-" and "-genital" organ systems share a common developmental and evolutionary history, and parts of the urinary system have been co-opted into the reproductive system. Necessarily, it is difficult to talk about one without the other.

The plan for this chapter is to focus just on the excretory parts—the uro- part of urogenital. The next chapter will cover the reproductive part. Note also that when people think about waste leaving the body, they also typically think about pooping—after all, everybody poops. This is part of the digestive system, and as such it is covered in Chapter 13. When we say **excretion**, we really are talking about kidneys and the associated tubes.

16.2 Structure of the Excretory System

The main functions of the excretory system are waste management and dealing with water and solute balance. This is primarily done through large, paired structures called **kidneys**. However, each kidney is composed of up to 1 million microscopic, tubular structures called nephrons (Greek for "of the kidney"; Figure 16.1). **Nephrons** are the functional units of the kidney, in that all nephrons within a kidney do not need to be in working order to produce urine.

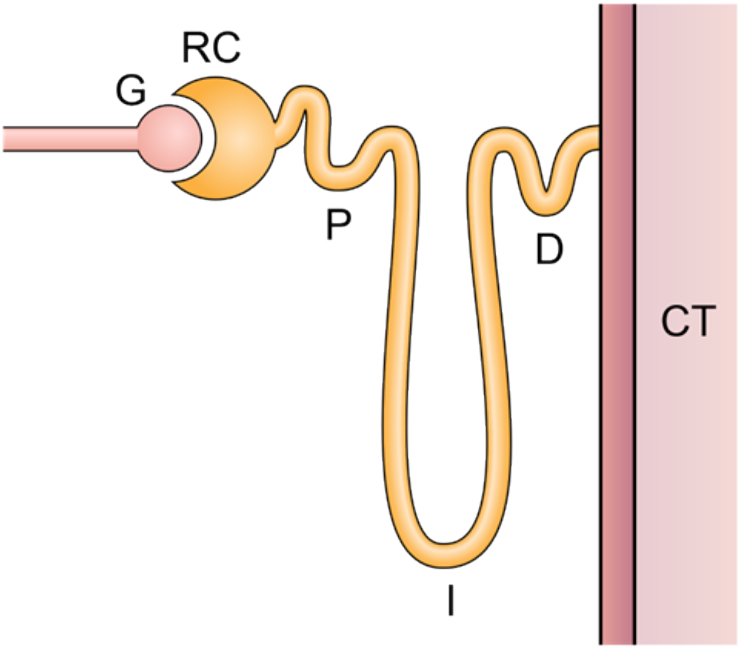

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Figure 16.1—Nephron structure. G = glomerulus; RC = renal capsule; P = proximal tubule; I = intermediate segment; D = distal tubule; CT = collecting tubule. Together, the glomerulus (G) and renal capsule (RC) make the renal corpuscle. The nephron is composed of the renal capsule (RC), proximal tubule (P), intermediate segment (I), and distal tubule (D).

Nephrons get their fluids (filtrate) from the **renal corpuscle**, which is in turn composed of the **glomerulus** (a ball of capillaries that receive blood from the renal artery; plural: glomeruli) that is covered by a pouch-like part of the nephron called the **renal capsule** (sometimes known as Bowman's capsule, glomerular capsule, or Malpighian capsule; Figures 16.1 and 16.2).

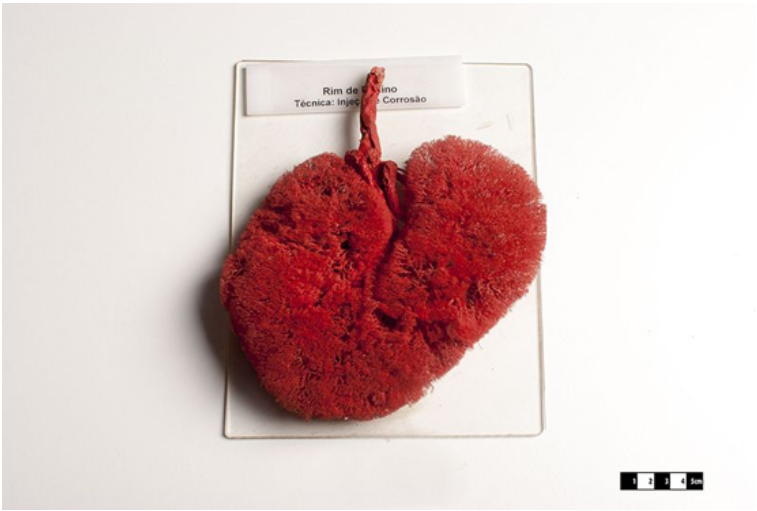

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Figure 16.2—Blood vessels in a cow kidney.

The renal corpuscle collects fluid, cations (positively charged ions), and small molecules (e.g., glucose and amino acids) that have filtered out of the capillaries of the glomerulus. This filtrate then makes its way into a tubule. The structure of the tubule depends on the vertebrate, but we will use an amniote nephron as our example here. In tetrapods, the tubule is divided into three regions—the **proximal**, **intermediate**, and **distal tubule**—and filtrate moves from the renal corpuscle through the tubules in that order (Figure 16.3). As filtrate passes through these three regions of the nephron, both active and passive transport are used to move molecules and water in and out of the filtrate, depending on the needs of the organism and the concentration of solutes in the surrounding kidney tissue. Each part of the nephron is responsible for moving different types of ions and molecules. Filtrate then leaves the nephron through the distal tubule and enters the collecting tubule. Collecting tubules collect filtrate from many nephrons, and final adjustments to the filtrate content are made before it is passed

on for excretion. Once the filtrate reaches the end of the collecting duct and no further adjustments are made, we call it urine.

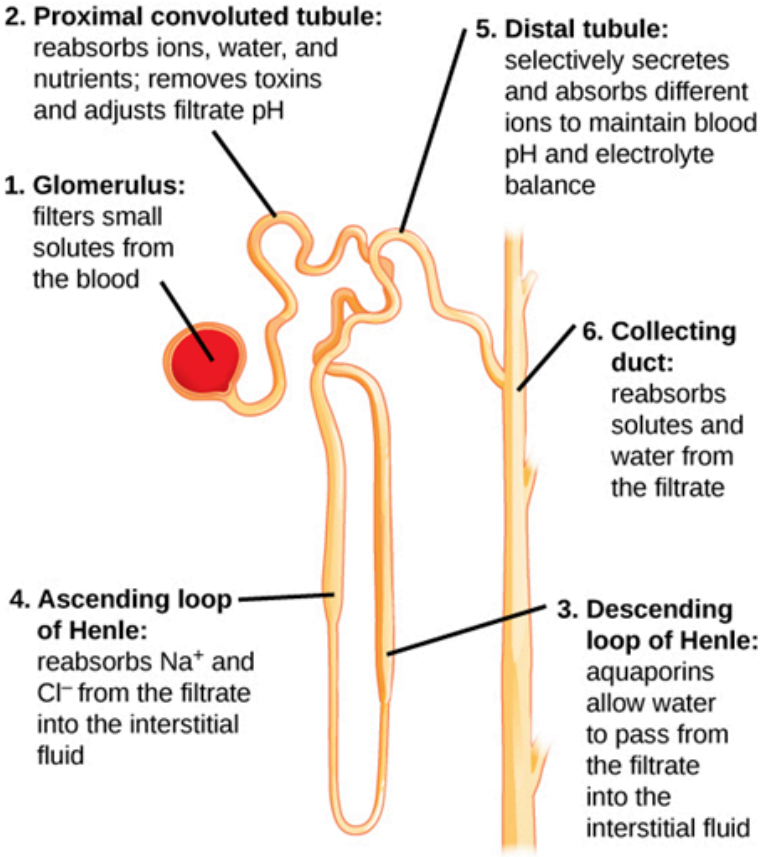

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Figure 16.3—Each part of the nephron has its own job with regard to filtering waste and maintaining the right concentration of ions and molecules in the body. Note that the loop of Henle is the intermediate tubule in this mammalian nephron.

For such a tiny organ, nephrons do a lot! They are responsible for maintaining the pH of blood. They are responsible for removing toxins from the blood, such as nitrogenous waste,

and releasing them through urine. Importantly, nephrons are responsible for **osmoregulation**, the process that maintains the water and electrolyte (solute) balance in body fluids.

16.3 Development of the Kidney

The kidneys, and therefore the nephrons, develop from mesoderm, which forms paired **nephric ridges** in the left and right walls of the dorsal coelom down the length of the coelom. Each ridge is divided into several segments called nephrotomes, which will eventually develop into nephrons (Figure 16.4). The medial portion of each nephrotome develops into the renal capsule, and glomeruli will develop within the capsule. The lateral portion of each nephrotome lengthens and merges with similar regions of adjacent nephrotomes to form a single nephric duct (or nephric tubule).

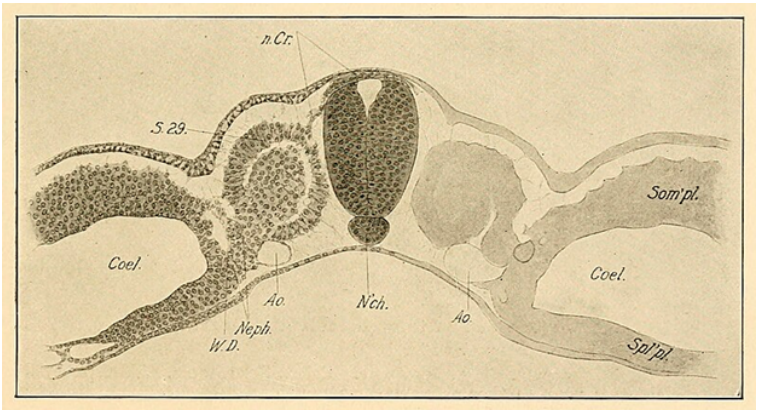

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Figure 16.4—A transverse section through the 29th somite of a chick embryo. N. Cr. = neural crest; Neph. = nephrotome; N. ch. = notochord; W. D. = Wolffian duct; Coel. = coelom; Ao. = aorta. Note that the nephrotomes are not in the coelom but rather outside it.

Understanding the embryonic development of the kidney gives us important clues to how kidneys have continued to change through vertebrate evolution. Vertebrate kidneys follow a (three-part) development sequence, where the nephron development previously described happens in three distinct phases (Figure 16.5).

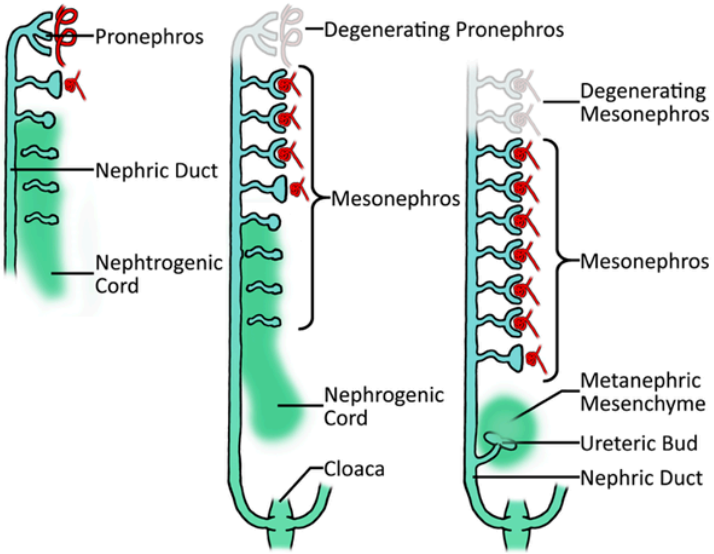

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Figure 16.5—The tripartite kidney development hypothesis.

The first stage is the formation of the **pronephros**, which happens in the anterior part of the nephric ridge. The pronephric tubules join to form a single **pronephric duct**. The pronephric duct develops posteriorly until it connects with the cloaca. In most vertebrates, this is a transient stage of development, and the pronephros does not persist for long. However, a small number of vertebrates retain the pronephros, including hagfishes, lampreys, lungfishes, and a few teleost species such as salmon and trout. This is sometimes called a "**head kidney**," referring to its anterior position, and is additional to other kidney tissue that develops later.

The **mesonephros** develops next, just posterior to the pronephros. Mesonephric tubules develop as described above, but instead of merging into a brand-new common duct, they join the preexisting pronephric duct. At this point, the name of the duct is now called the **mesonephric duct** to reflect this developmental change.

What happens next depends on which vertebrate you're considering. In amniotes, the caudal-most portion develops into the **metanephros** and the mesonephros disappears, and a gap occurs between the metanephric and mesonephric regions of the nephric ridge. As the metanephros develops, an outgrowth of the posterior mesonephric duct called the **uteric bud** develops and joins with the metanephric tubules. This small portion of the mesonephric duct goes through another name change to become the **metanephric duct** (also known as the **ureter**), and the metanephros becomes the adult kidney.

In vertebrates that are not amniotes, we have a different path to follow. The metanephros does not develop, nor does the uteric bud. Nephrons still develop in the posterior part of the nephric ridge that is adjacent to the mesonephric region, but there is no gap. The newly formed tubules join the mesonephric duct, and this longer mesonephric kidney is now called the **opisthonephros** to reflect that it is more extended than a typical mesonephric kidney. As you might have guessed by now, we also change the mesonephric duct to be called the **opisthonephric duct** in this case.

It is worth noting that we have painted a very broad, general picture of kidney development here. Like many areas of comparative vertebrate anatomy, this is still an active area of study. As anatomists and developmental biologists study the nephric ridge and its development in more detail and across more organisms, exceptions to the tripartite story become apparent. In many cases, there is not a neat boundary between each of the three regions discussed above. This has led to an alternative hypothesis called the **holonephric concept**. The Greek root holo- means "whole" or "entire"; thus holonephric indicates that the entire kidney is one

organ (the holonephros, sometimes called the archinephros), and pro-, meso-, and metanephros are all regions of that holonephros that develop in sequence. The boundary between pronephros and mesonephros in particular seems to be extra fuzzy; some larvae never develop a functional pronephros (including humans, potentially), and nonfunctional pronephric cells look very similar to degrading mesonephric cells. Experiments show that the differentiation of nephric ridge cells is largely dependent on location; for example, moving cells in the cranial region of the nephric ridge to the caudal region produces cells that look just like metanephric cells. In other words, these cells lack specificity. To date, there are no genes that are specific to the development of the pronephros, for example. Rather, the same genes are active in the development of all three regions of the holonephros. However, while some vertebrates (e.g., lampreys, hagfishes, and the occasional caecilian species) develop what looks like a holonephros during their larval stages, no adult vertebrates retain a holonephros. And there are organisms, such as hagfishes, that have a clear, demarcated pronephric region.

For now, we will continue to use the tripartite concept in our discussion of the evolution of the renal system (nephrons, kidneys, and associated urinary structures). However, as with all areas of anatomy, it is possible or even likely that our future definitions and prevailing hypotheses will change as scientists continue to learn more.

16.4 Excretory System Evolution and Diversity

The story of the evolution of the excretory system is largely an environmentally driven one. Recall that one of the main purposes of this system is to manage water and solute balance. Based on what we know about the osmoregulatory strategies of nonvertebrate deuterostomes, these early vertebrates were likely

osmoconformers. This means that the **osmolality** (the amount of solute dissolved in a solution) of the cells and fluids of these early vertebrates was likely similar to that of the surrounding salt water. The fact that nonvertebrate deuterostomes are all osmoconformers has also been used as evidence that vertebrates arose in at least a brackish environment. Among the extant vertebrates, only hagfishes, lampreys, chondrichthyans, and coelacanths still use this strategy.

However, as vertebrates expanded into freshwater and terrestrial habitats and back to the marine realm, osmoconforming was not possible. Instead, most vertebrates are **osmoregulators**—the osmolality of their body fluids does not match that of the environment. As such, osmoregulator excretory systems must fight against constantly losing or gaining water and electrolytes, depending on the surrounding environment.

Cyclostomes

Both hagfishes and lampreys develop a pronephros during larval development followed by a mesonephros, as we would expect from the tripartite concept we discussed. Unlike most other vertebrates, both hagfishes and lampreys retain their pronephros into adulthood. This "head kidney" is no longer functional from an excretion standpoint, although it may be active as a lymphoid organ. Instead, the opisthonephros (remember, this is an elongated mesonephros) is the functional kidney (Figure 16.6).

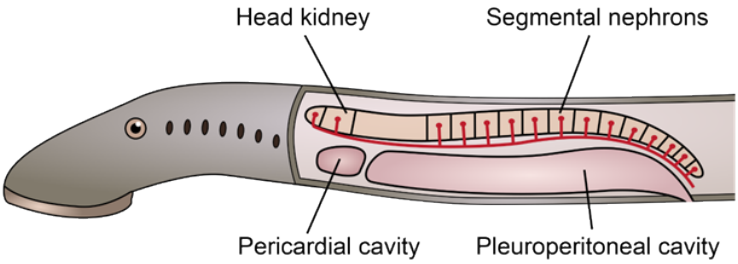

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Figure 16.6—Illustration of a lamprey kidney.

While the pronephros has no glomeruli, each hagfish opisthonephros (remember, this is a paired structure!) has approximately 35 large glomeruli connected to the opisthonephric duct. In fact, the glomeruli are 10 times larger than mammal glomeruli, making them particularly useful for studying renal physiology and toxicology. Once filtrate leaves the nephron, urine empties out of the opisthonephric ducts into the cloaca. As in many fishes, kidneys are not the only excretory organ that hagfishes use. Hagfishes also excrete large amounts of nitrogenous waste (ammonia, in this case) through their gills and skin. Usually, this would be a case of simple diffusion, as there should be less ammonia in the surrounding environment than in the body fluids of the hagfish, allowing ammonia to move down its concentration gradient. However, hagfishes are scavengers that eat large rotting marine animals on the seafloor. As part of the decomposition process, these carcasses are giving off large amounts of nitrogenous compounds, like urea and ammonia, that are toxic in high concentrations. Not only can hagfishes tolerate these high concentrations both internally and externally; their skin can use active transport to excrete ammonia against the concentration gradient.

Lampreys spend the majority of their lives in the larval stage, where they hang out in sediments and filter feed. The

pronephros in larval lamprey have a bundle of capillaries (**glomus**) that act similarly to a glomerulus but serve multiple tubules that empty into a common pronephric duct. The pronephros is functional while the lamprey remains a larva, but for most lamprey species, it largely degenerates to be nonfunctional by the time they shift to the adult stage. Before the metamorphosis to an adult, the opisthonephros develops and then serves as the functional adult kidney. As we saw in the hagfish, the gills and skin are also active sites of osmoregulation and excretion.

While most lampreys are osmoconformers, 9 of the over 30 different lamprey species are **anadromous**—they hatch in freshwater, migrate to salt water until they're mature enough to breed, then return back to freshwater to breed (Figure 16.7). This creates an osmoregulation problem. Lamprey body fluids, like those of any freshwater-dwelling vertebrate, have a far higher concentration of solutes than the surrounding freshwater. They are constantly taking on freshwater and losing electrolytes. Then when they get to salt water, the problem reverses—they are losing water and gaining electrolytes. That means that these anadromous lampreys have to be osmoregulators. Given the complexity of their environmental challenges, the nephrons of these anadromous lamprey are more complex than what we've seen in the other cyclostomes. Their collecting ducts are more complex and subdivided into proximal, intermediate, and distal sections; these sections are flexible in how they handle moving water and solutes in and out of the filtrate. The gills also need to be nimble. In freshwater, they uptake sodium and chloride ions to fight against the loss of electrolytes, but in salt water, the gills excrete sodium and chloride to keep the osmolality of the body fluids in the right place. In addition, the lamprey's gut joins in on osmoregulation. The esophagus is capable of absorbing ions, and the intestine acts as a site of water resorption while the lampreys reside in marine environments.

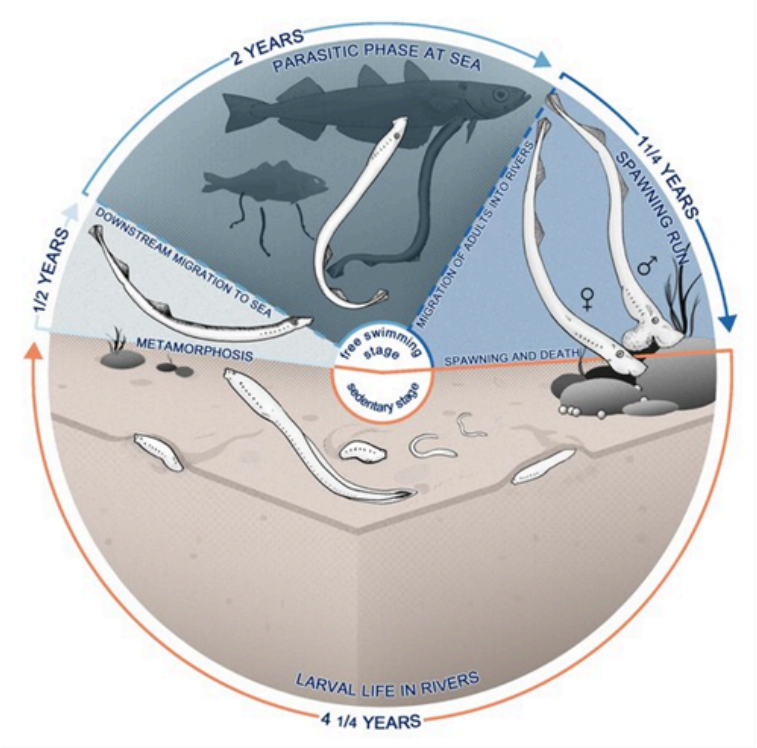

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Figure 16.7—The life cycle of a parasitic anadromous lamprey, the pouched lamprey Geotria australis.

Chondrichthyans

Chondrichthyan kidneys are opisthonephric, and their general shape depends on what kind of chondrichthyan you are examining, although they are generally composed of a series of irregularly shaped lobes. For example, sharks, with their more elongated bodies, have elongated kidneys that run along the length of the abdominal cavity and get wider toward the caudal end. Batoids (rays and skates) tend to have short, wide kidneys only toward the

caudal end of the body. Examined in cross section, chondrichthyan kidneys are divided into two regions—a dorsolateral **bundle zone**, and a ventromedial **sinus zone** (Figure 16.8). The tubules of the nephron are even more complex than those of mammals, with at least four loops and turns and at least five different segments that meander back and forth across both the sinus and bundle zones. The sinus zone contains loops 2 and 4 of each nephron, while the bundle zone contains loops 1 and 3. The sinus zone is named after large blood sinuses that hold blood drained from the large glomeruli that sit at the boundary between the bundle and sinus zones as well as other blood vessels. The nephron loops in the sinus zone are quite separated from each other, possibly allowing room for diffusion between the filtrate and the blood in the blood sinus. The nephron loops in the bundle zone, on the other hand, are very tightly packed (thus the name "bundle zone"). Eventually, the distal tubule will grade into a collecting tubule, which will add its filtrate to a shared collecting duct. The collecting ducts pass the filtrate into the opisthonephric duct (often referred to as a ureter in sharks), which then empties urine into the cloaca.

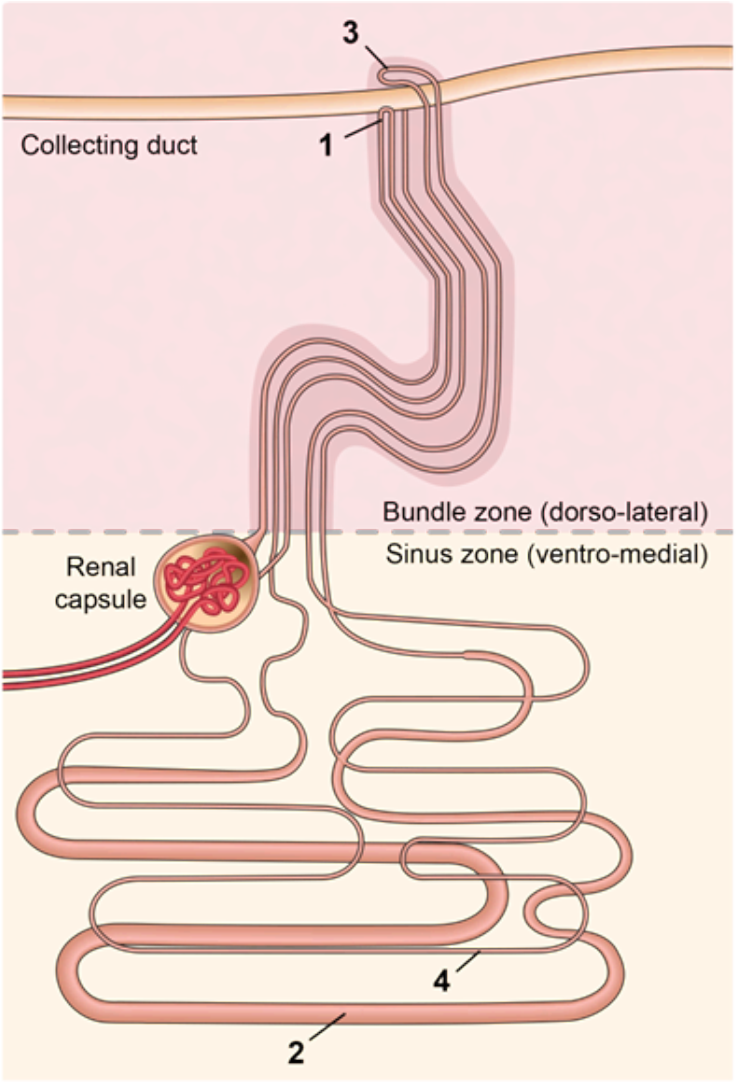

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Figure 16.8—Schematic of a shark nephron. The nephron's tubule has four different loops (labeled 1 through 4) that wind through two different zones. The proximal tubule extends into the bundle zone, where it loops back (1) toward the sinus zone. It then makes another turn (2) and heads back toward the bundle zone. Just prior to reaching the bundle zone, this tubule transitions into an intermediate tubule. The intermediate tubule continues only for a short distance into the bundle zone before it transitions into a distal tubule

and reaches a turn (3) to head back into the sinus zone. The distal tubule makes one more turn (4) toward the bundle zone to connect to the collecting duct.

Marine chondrichthyans are generally considered to be osmoconformers, although they tend to run just slightly hyperosmotic compared to the surrounding ocean. Unlike the hagfishes, which generally use the same ions as the ocean to maintain their internal osmolality, just under half of the osmolality of chondrichthyan fluids are due to urea and a molecule called **TMAO** (trimethylamine-N-oxide). Both of these molecules are readily available, as chondrichthyans produce urea instead of ammonia as a nitrogenous waste product. TMAO is also produced and counteracts urea's tendency to destroy proteins. Interestingly, TMAO in high concentrations is toxic once digested down to trimethylamine in our digestive system. There have been cases of people eating raw Greenland shark meat and exhibiting signs of TMAO toxicity, which includes gastrointestinal distress and neurological symptoms similar to alcohol intoxication.

As we've seen in other fishes, kidneys are not the only osmoregulatory organ in chondrichthyans. Chondrichthyans also osmoregulate with the **rectal gland** (also known as the digitiform gland and the salt gland, although it is not homologous with amniotes' salt glands). This large gland is located near the caudal portion of the digestive tract. In marine chondrichthyans, the rectal gland plays a much bigger role in salt secretion compared to the kidney and gills (in fact, the gills in marine chondrichthyans do not secrete salt). The rectal gland receives blood from the dorsal aorta via the posterior mesenteric artery. Inside the gland are thousands of tubules that are surrounded by capillary beds that receive blood from the artery. The tubules contain a series of protein channels that create the electrochemical gradient necessary to pull salt ions from the blood into the tubules, which then flow into a central lumen. The accumulated salty liquid flows from the central lumen

to the rectal duct, which joins with the intestine and dumps its contents there.

When discussing the rectal gland, we purposely mentioned that the rectal gland is important in marine chondrichthyans. While most extant chondrichthyans are indeed marine, there are fully freshwater elasmobranchs, such as the rays of the Amazon River, and euryhaline species, such as bull sharks, that can tolerate a wide range of salinities from freshwater to fully marine. In both fully freshwater species, the rectal gland tends to be much smaller, as the osmoregulatory challenge is to hold on to electrolytes instead of gaining too many. However, the structure of the rectal gland is generally similar to their marine counterparts. Unlike the marine species, gills take on a much more active role in osmoregulation in freshwater and euryhaline elasmobranchs. As we would expect to see, gill tissues actively take up sodium and chloride to make up for the passive loss of these electrolytes.

Box 16.1—Bull Sharks and Migrating Salmon

Often aspects of renal physiology and anatomy are linked to where the animal lives, which largely determines whether water and ions are more likely to passively move in versus out of the animal. For example, we would expect an animal living in the desert to have anatomical and physiological adaptations to help combat water loss. However, not all animals stay within one environment for their entire lives. Some animals shift between environments depending on their life stage. We call animals that can tolerate a wide range of salinities **euryhaline**. A great example is the Atlantic salmon *Salmo salar* (Figure 16.9).



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Figure 16.9—The Atlantic salmon, Salmo salar.

Salmon begin to mate and hatch in freshwater, and the young salmon live in freshwater for a few months before heading out to sea. As they head down rivers into estuaries, salinity gradually increases. They spend much of their adult lives in fully marine environments. As you may have gathered, this poses some challenges. Remember, freshwater fishes tend to lose salts and gain water, whereas saltwater fishes have the opposite problem. Salmon change their behavior as they move between environments—they drink water when in the ocean but do not when they're in freshwater. Their physiology also changes. The kidneys make a lot of very dilute urine while they are in freshwater but produce far less urine that is very concentrated when the salmon is in salt water. Most incredibly, the cells in the gills (**mitochondria-rich cells**, also known as chloride cells) that are in charge of dealing with salts can shift the direction that they send

those salt ions. In freshwater, they can use ATP to actively transport salt ions into the blood plasma; in salt water, they switch to pumping salt ions into the surrounding salt water.

Other animals may shift between aquatic environments frequently over their entire lifetime. One particularly famous example is the bull shark *Carcharhinus leucas* (Figure 16.10), although we should note that there are about 40 chondrichthyan species that can do this. Bull sharks are found in warm oceans across the globe and can tolerate a range of salinities from salty oceans to freshwater rivers and lakes. This means that they need to be particularly nimble as they move across these very different environments. Recall that in salt water, chondrichthyans need to retain water and export salts. However, in fresh water, they ended up gaining too much water. Bull sharks retain approximately one-third to half as much sodium, chloride, and urea in fresh water than when they are in salt water, lowering the concentration of their body fluids to slow down water gain. Urine output also increases in euryhaline sharks living in freshwater. This indicates that the gills, rectal gland, and kidneys are shifting their physiological actions to account for the change in environment.



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Figure 16.10—The bull shark, Carcharhinus leucas.

Osteichthyans

In general, osteichthyan kidneys are opisthonephric. However, there is a lot of variation in structure and function when you compare bony fish kidneys. We will consider actinopterygian kidneys for most of this section. If we take a look at the teleosts, we see that a number of fishes—such as tilapia, salmonids, and rockfish—retain a distinct pronephros past the larval stage. These "head kidneys" generally do not participate in excretion (Figure 16.11). For example, head kidneys can have a hematopoietic function (producing blood components) or an endocrine function (see Chapter 21). Thus, it should not be a surprise that the head kidneys tend not to have

nephrons. However, there are exceptions—rockfish head kidneys do contain nephrons. For those fishes that have a nonexcretory head kidney, the mesonephric portion of the opisthonephros performs the excretory duties. Thanks to their aquatic environment, most bony fishes secrete their nitrogenous waste as ammonia, as the surrounding water quickly dilutes this toxic substance.

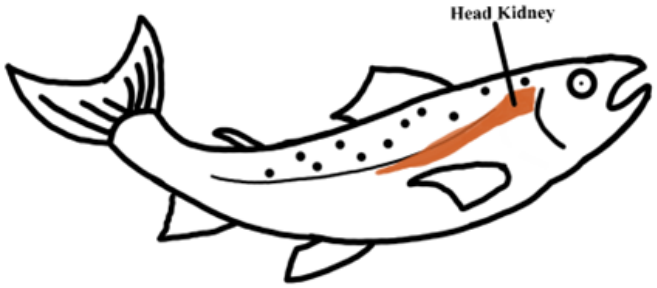

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Figure 16.11—Location of the head kidney in a trout.

The details of the nephron differ across species as well but are largely dependent on what environment the fish lives in, as fishes have different osmoregulatory and water-balance challenges in fresh versus salt water (Figure 16.12).

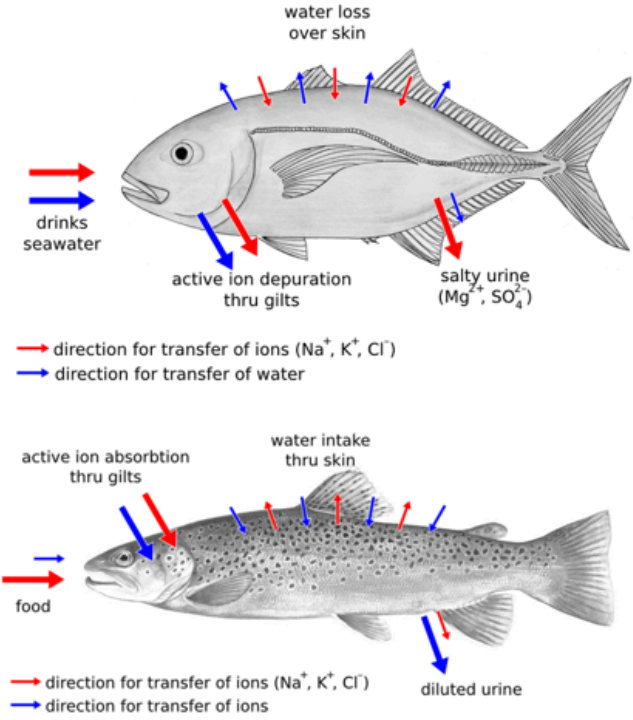

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Figure 16.12—Sites of osmoregulation in fishes. Top: A saltwater fish, the yellow jack Carangoides bartholomaei. Bottom: A freshwater fish, the brown trout Salmo trutta.

In freshwater fishes, the glomerulus is well vascularized, and the nephron is generally large because freshwater fishes typically need to excrete a lot of the water that is passively taken in due to being hyperosmotic to their environment (Figure 16.13). On the other hand, the nephrons of marine fishes tend to be smaller and less complex, and the parts that deal with inputting water into the filtrate are reduced or absent. Interestingly, there are several marine teleosts with aglomerular kidneys—they have no glomeruli! These include gulper eels, toadfish, anglerfish, and seahorses. For

these species, the tubules do all the work, and the loss of glomeruli means that the tubules do not have to reabsorb as much water from the filtrate later.

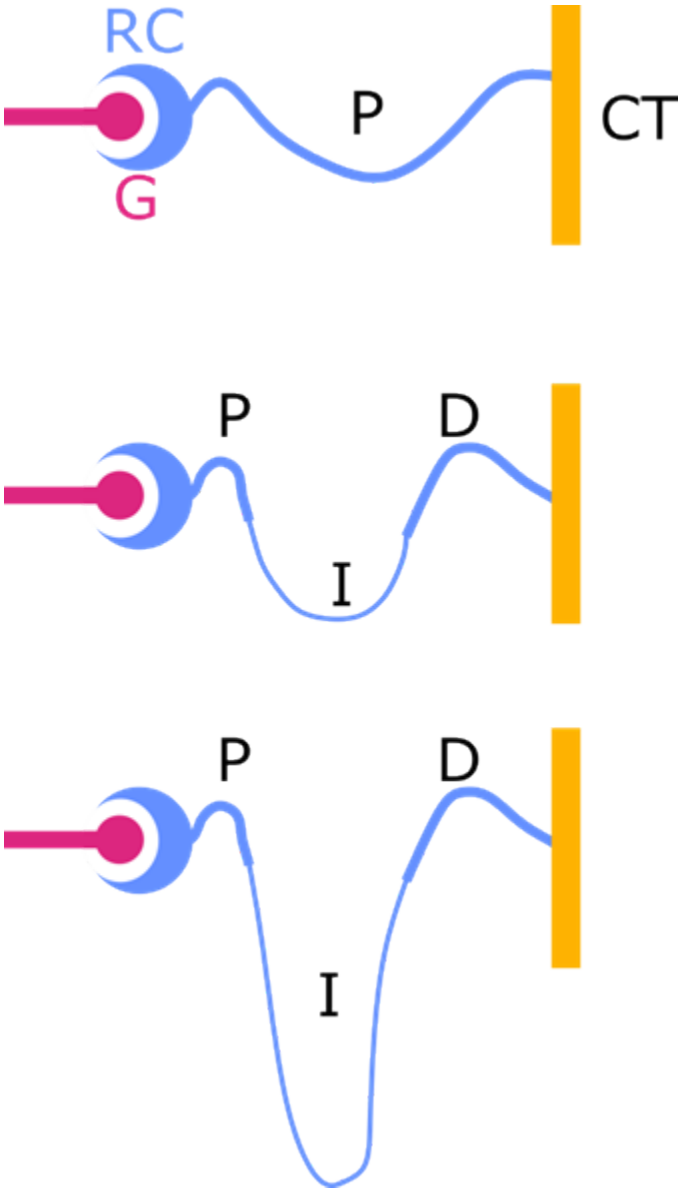

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Figure 16.13—General nephron structure for various osteichthyans (including tetrapods). Top: marine teleosts; Middle: freshwater teleosts and nonmammal tetrapods, including Aves; Bottom:

mammals and Aves. G = glomerulus; RC = renal capsule; P = proximal tubule; I = intermediate segment; D = distal tubule; CT = collecting tubule. Together, the glomerulus (G) and renal capsule (RC) make the renal corpuscle. The nephron, indicated in blue, is composed of the renal capsule (RC), proximal tubule (P), intermediate segment (I), and distal tubule (D).

Regardless of what variety of nephrons teleost fishes have, filtrate makes its way from the collecting ducts to the mesonephric duct. Teleosts also have urinary bladders that form from the mesonephric duct. Unlike human urinary bladders, teleost urinary bladders can resorb water, sodium, and chloride from urine. The intestines of teleosts are also active in osmoregulation.

Gills are an incredibly important excretory and osmoregulatory organ as well. Remember that freshwater fishes are hyperosmotic to their environment. They have to deal with the passive loss of ions and gaining of water. While freshwater fishes do pass large quantities of very dilute urine, they also have mitochondria-rich (MR) cells (also called chloride cells) in the gills that take up ions to counter those they have passively lost. Those chloride cells are also active in the gills of marine fishes, albeit in a different way. Marine fishes drink a lot of water to regain what they passively lose to the environment. However, the water they drink is salty and adds to the ions they are passively taking in through their skin. The MR cells actively excrete those extra ions.

As demonstrated here, and as you may recall from previous chapters, the bony fish story is a complex one. While actinopterygians (the ray-finned fishes) have a marine evolutionary origin, those extant actinopterygians that live in the marine realm stem from freshwater ancestors. In other words, they reinvaded the marine environment. Similarly, when we look at the history of the sarcopterygians (lobe-finned fishes), we see that while extant coelacanths stayed in salt water, the extant lungfishes are freshwater residents, but their ancestors also originated in salt water. What does all this mean? It means that we need to be careful

when we are making evolutionary inferences about excretory system evolution using extant osteichthyans. Many of our living osteichthyan kidneys reflect how marine fishes made the transition to freshwater or, in some cases, how they retransitioned back to salt water.

However, there is also substantial evidence that the rhipidistians, the sarcopterygians that gave rise to the tetrapods, lived in high-salinity environments. When one considers that both terrestrial and marine osmoregulators have similar challenges—water loss to the environment—it makes sense that excretory systems from marine rhipidistians were poised to handle the similar dehydrating challenges of the terrestrial environment.

So while lungfish are not a good window into the past kidney-wise, they are interesting sarcopterygians in their own right. The kidneys of dipnoans are similar to other freshwater osteichthyans in that they have large nephrons with glomeruli and they secrete ammonia. However, the posterior nephrons are sometimes modified for sperm transport (more on that in the next chapter!). Lungfishes live in lakes and rivers that occasionally dry out, but they can handle it like champs. African lungfish will enter a dormant state called **estivation**, where they burrow into the mud and form a mucus cocoon around their bodies, leaving a tiny hole in the cocoon to breathe through. This stage can last for up to four years. This cocoon allows them to avoid dehydration while drastically decreasing their metabolism. How do lungfish hold on to their water, since they're not drinking anything? Their muscle proteins produce both urea and water, they stop secreting ammonia, and the renal capsule collapses and thickens to almost shut down filtration. When they're ready to leave estivation, the renal capsules return to normal.

Amphibians

Extant amphibian kidneys are considered to be opisthonephric, and the nephrons in all amphibians follow the general tetrapod plan described previously, which looks very similar to freshwater fishes (Figure 16.12). However, kidneys vary in morphology depending on which type of amphibian you're examining. Amphibian kidneys are elongate, although the degree of elongation and lobation varies. For example, kidneys of salamanders and caecilians tend to be far more elongated than those in anurans. Kidneys can also differ by life stage within a species. For example, amphibians with aquatic larvae have functional pronephros and mesonephros in the larval stage. During metamorphosis, the pronephros breaks down, and the mesonephros persists as the functional kidney.

Salamander kidneys, unlike other amphibian kidneys, are usually divided into a cranial portion (the "**genital kidney**," as nephrons are used for sperm transport—more on that in the next chapter; note that this is different from the kidneys of lungfishes) and a caudal portion (the "**pelvic kidney**"), which does the excretory work. However, testes-bearing plethodontid salamanders do not have the cranial portion at all but retain the sperm-transporting nephrons. Regardless of which salamanders you're looking at, urine moves from the nephrons into the collecting ducts, as they do in other amphibians. The collecting ducts merge into a common collecting tube (often referred to as a ureter), which dumps urine straight into the cloaca in some families of salamanders or into a Wolffian duct that connects to the cloaca in other families.

Amphibians also possess a urinary bladder; however, it is not homologous to other urinary bladders (Figure 16.14). Amphibian urinary bladders form from a ventral outpocketing of the cloaca. Thus, urine moves from the kidneys to the cloaca to the urinary bladder, which is unique. As we saw in teleosts, the urinary bladder is used to store water and can resorb sodium and water for

osmoregulation. Amphibians, like their osteichthyan ancestors, secrete ammonia as a nitrogenous waste product.

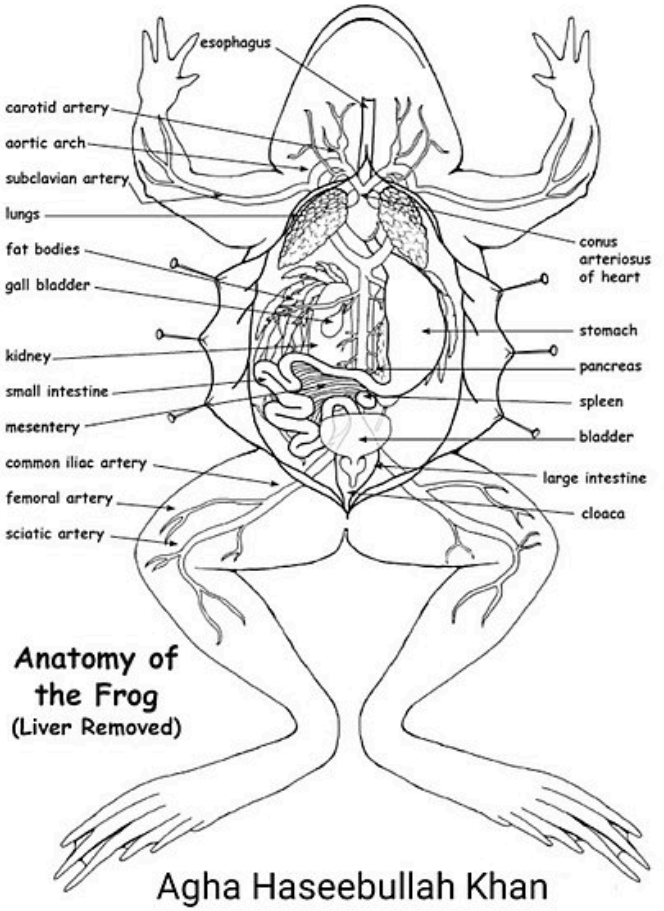

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Figure 16.14—General internal anatomy of a frog. Note the urinary bladder (labeled simply as "bladder") and kidney.

In fishes, we saw that the gills are a major osmoregulatory

organ. While many adult amphibians do not have gills, amphibians that go through an aquatic larval stage do. Similar to freshwater fishes, MR cells in larval amphibian gills are primarily responsible for active transport of ions into the blood, which are lost through the skin to their freshwater environment. For the occasional salamander that does retain gills as an adult, such as mudpuppies and axolotls, it is not clear whether the gills retain their osmoregulatory function. The integument of amphibians also acts as an osmoregulatory system. As discussed in Chapter 6, the amphibian integument is quite permeable, which allows it to function in gas exchange. That same permeability also means that water and ions easily pass between the environment and amphibian bodies, although the direction of water movement depends on whether the amphibian is in a freshwater or terrestrial environment. MR cells are also active in the integument. Interestingly, the epithelium in the colon can also regulate ion balance.

Amniotes

Amniote kidneys are metanephric; the pronephros is transient and nonfunctional when it forms (it does not always form), and the mesonephros is a functional but transient embryonic kidney. The nephron is as described previously, containing a glomerulus and multiple tubule sections. In case you haven't yet picked up on this theme, there is a lot of variation among the amniotes in terms of kidney morphology, even within specific amniote groups. Across broad groups of amniotes, this partially reflects differences in renal function, as mammals tend to make urea as their major nitrogenous waste, whereas reptiles of all sorts tend to make uric acid.

Humans (and Pigs and Rodents)

Even within a broad group of amniotes, we see variation (Figure 16.15). You are likely familiar with the bean-shaped kidneys that humans have. Several other mammals (but not other primates!) share this kidney shape with us, including rodents, pigs, and canines. The internal organization of their kidneys is similar as well, and we will use the structure of the human kidney as our example (Figure 16.16). The outermost layer of the kidney is called the **renal cortex**, and it surrounds the interior layer called the medulla that is responsible for urine concentration. The **medulla** is composed of several lobes (**multilobar**), which in turn are divided into **renal pyramids** and surrounding connective tissue called **renal columns**. At the narrow portion of each pyramid are bundles of collecting ducts called **renal papillae**. The papillae serve as a conduit to transport urine from the nephrons to the **calyces** (singular: calyx) of the kidney. Human kidneys have two types of calyces—**minor calyces** about the renal papillae, whereas the **major calyces** are where the minor calyces merge. The major calyces fuse into a single renal pelvis, and urine flows from there to the ureter. The ureter from each kidney joins the **urinary bladder**, and the **urethra** takes urine from the urinary bladder to exit the body.

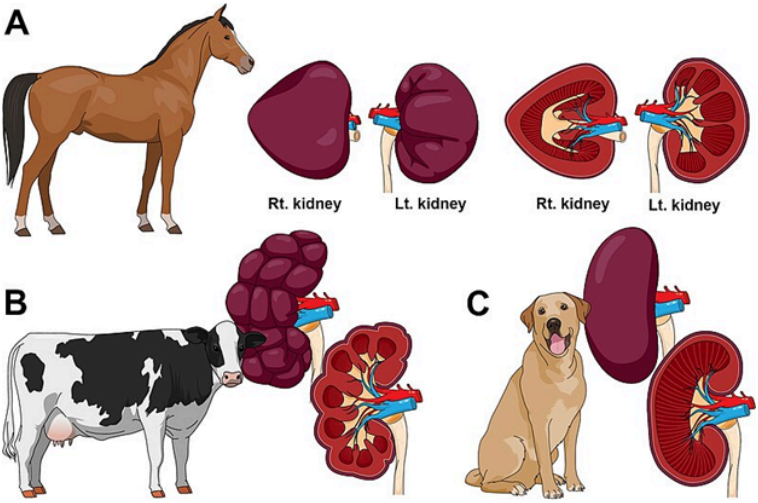

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Figure 16.15—Variation in kidney structure among domesticated mammals. (A) Horse, showing that the right and left kidneys also differ in morphology; (B) Cow; (C) Dog.

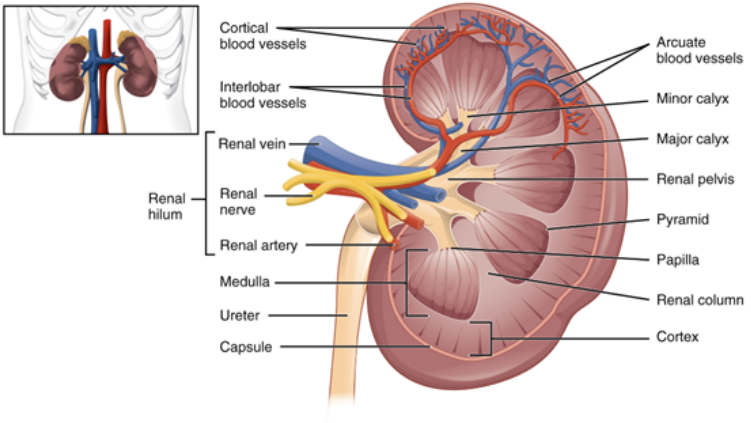

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Figure 16.16—Structure of the human kidney.

Humans, like other mammals, have very convoluted nephrons (Figures 16.3 and 16.17). The nephron begins in the cortex, where the glomeruli are situated. As described previously, the filtrate next enters the proximal convoluted tubule, and intermediate tubule, and then the distal convoluted tubule. However, the intermediate tubule has morphologically and physiologically distinct parts, and so we give it a special name—**the loop of Henle**. The thin **descending limb** of the loop of Henle receives filtrate from the proximal convoluted tubule and extends down into the medulla; this part of the loop is highly permeable to water but not to ions or urea. After the lowest point of the loop, we move into the **thin ascending limb** of the loop that is only permeable to ions and moves filtrate toward the cortex. The ascending limb grades into the **thick ascending limb** as it continues to move toward the cortex; physiologically, its job is to remove ions from the filtrate and return them back to the plasma. Finally, the **cortical thick ascending limb** is where the limb enters the cortex and connects to the proximal convoluted tubule. The final components of the loop of Henle are the **vasa recta** and **peritubular capillaries**. Together, this net of arterioles, capillaries, and venules surrounds the tubule and helps maintain the concentration gradient in the kidney that allows ions and water to move across the walls of the tubules. The loops of Henle are arranged in parallel, which contributes further to the ability to make urine more concentrated than plasma.

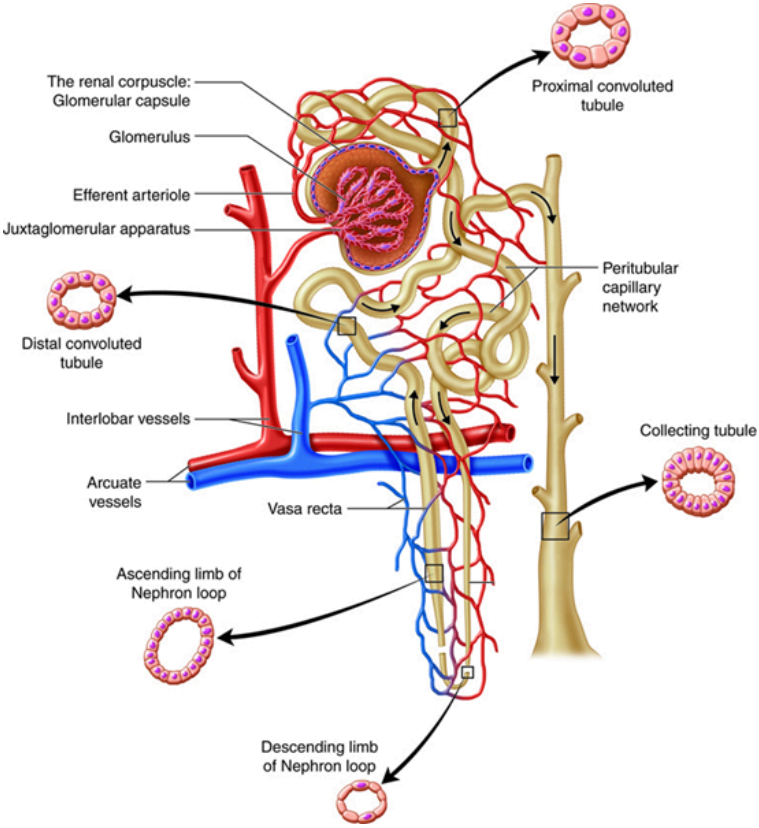

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Figure 16.17—Structure of the human nephron.

The long loops of Henle are what allow mammals to retain water and produce very concentrated urine, which is helpful in a terrestrial environment where water loss is constant. While the loop of Henle may not dip terribly far into the medulla in many mammals, desert rodents such as kangaroo rats and spinifex hopping mice have some of the longest loops of Henle for their body size among the mammals. Their loops of Henle extend far into the medulla and have several physiological modifications, allowing them to recoup

more water from the filtrate than other rodents. These desert rodents also have a whole other suite of adaptations beyond the kidney to conserve water, including being able to produce and harvest more water during cellular respiration; storing digested carbohydrates as glycogen instead of lipid, which takes less water; and producing fecal pellets that are far drier than other rodents.

Other Amniotes

Not all multilobar kidneys look like human (or pig or rodent) kidneys. Bovines have multilobar kidneys that have prominent lobes and no renal pelvis. The multilobar kidneys of cetaceans, pinnipeds, bears, hippos, and otters are even more extreme—they are **reniculate**, resembling a cluster of grapes (Figure 16.18). Each "grape" is called a **renicule**, which has its own distinct cortex, medulla, and calyx. On the other side of the spectrum, cats have **unilobar** kidneys, where the papillae are fused into one renal crest and there is only one pyramid per kidney. Unilobar kidneys also lack calyces, and urine passes directly into the renal crest.

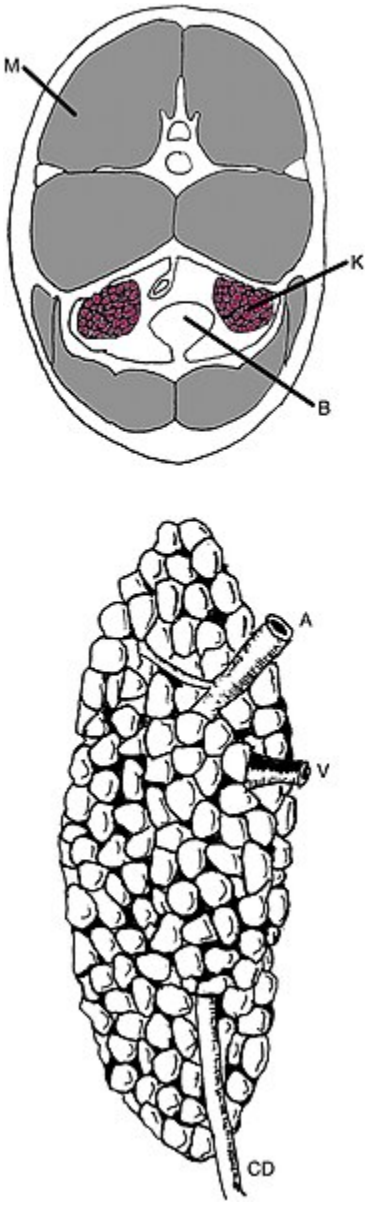

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Figure 16.18—The kidney of a bottlenose dolphin, Tursiops. Top: cross-sectional view of the torso with

the kidneys highlighted in red; Bottom: the kidney itself. M—axial musculature; K—ventrally located kidneys; B—urinary bladder; V—renal vein; A—renal artery; CD—common collecting duct that becomes the ureter.

We can also think about kidney shape in terms of the structure of the renal pyramids. Small mammals (smaller than rabbits) tend to have **unipapillary kidneys**, where the renal pyramids fuse into one papilla. Mammals that are in the size range between rabbits and kangaroos tend to have **crest kidneys**, which have single cortex and multiple medullary and papillary regions. However, it's worth noting that there are some larger mammals (giraffes, camels, horses) that still have crest kidneys, and there are animals larger than rodents, such as dogs, that have unipapillary kidneys.

What has driven the diversity of mammalian kidneys, then? Unilobar kidneys are the ancestral state for mammals, and multilobar kidneys have evolved independently, multiple times. Many of the mammals with multilobar kidneys have ancestors that were either fully or semiaquatic. As we've already seen, the salinity of an organism's environment (and the food in it!) affects how osmoregulation works. For mammals that are marine or have marine ancestors, having a lot of machinery (nephrons) to handle that salt intake is necessary. In addition, multilobar kidneys tend to be in larger mammals. If we consider that metabolic needs increase as mammal size increases, the number of nephrons needed also will increase. There are only so many nephrons you can stuff into one place; a multilobar kidney may have provided that extra space.

Nonavian reptiles have an even wider variety of kidney shapes than mammals, which is tied in part to body shape as we saw in the amphibians. As you may expect, snakes tend to have very long, thin kidneys, whereas lizard kidneys are shorter and more

compact (Figure 16.19). Unlike mammal kidneys, and we will see next in birds, nonavian reptile kidneys are not subdivided into a medulla and cortex.

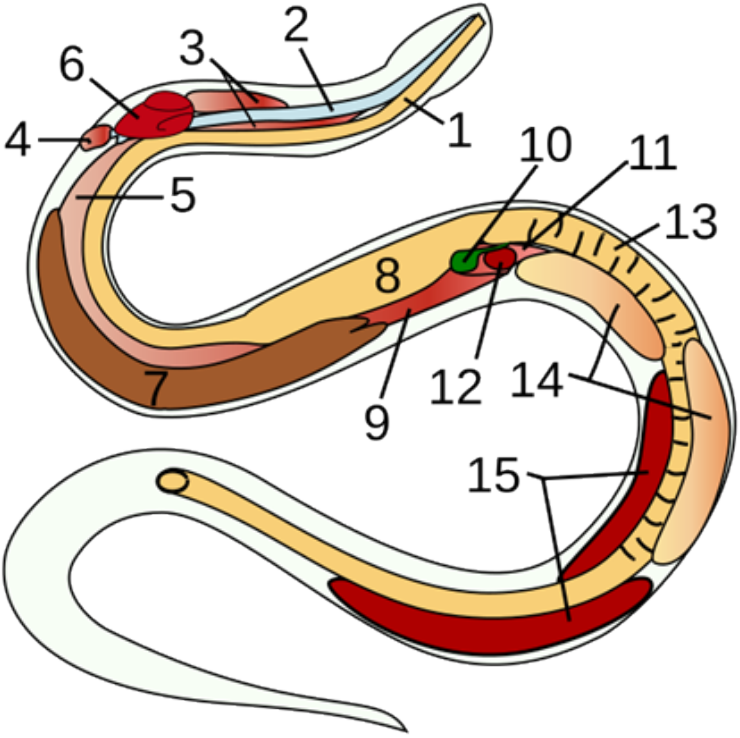

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Figure 16.19—Generalized snake anatomy. (1) esophagus, (2) trachea, (3) tracheal lungs, (4) rudimentary left lung, (5) right lung, (6) heart, (7) liver, (8) stomach, (9) air sac, (10) gallbladder, (11) pancreas, (12) spleen, (13) intestine, (14) testicles, (15) kidneys.

Avian kidneys are generally uniform in shape across different species, but still structurally complex (Figure 16.20). They are located in the renal fossa of the synsacrum but are still **retroperitoneal** like other vertebrate kidneys—they lay external to the peritoneum. Most avian kidneys can be divided into three

regions—cranial, middle, and caudal—although the distinctness of each region may vary across species. Each region is composed of lobes, and each lobe has a large cortex and small medulla, as we saw in mammals.

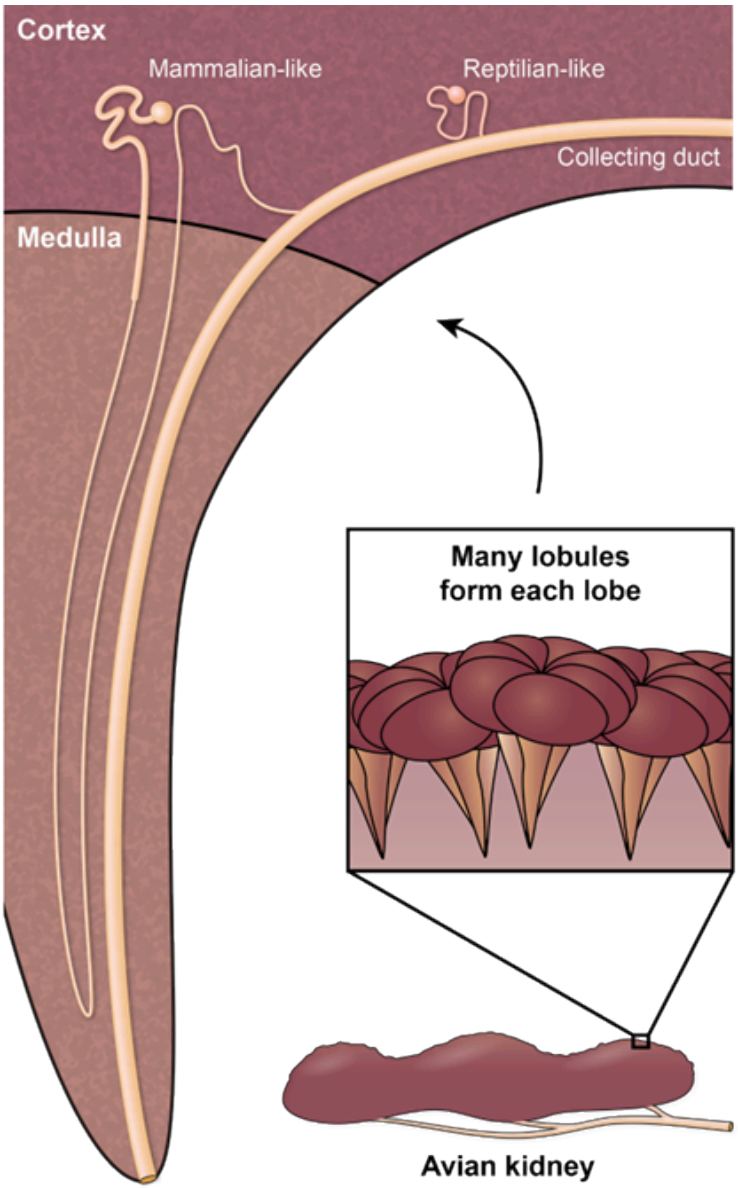

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Figure 16.20—Different levels of organization in the avian kidney. Note that the kidney on the bottom right of the diagram can be divided into three lobes: cranial (region on the left side of the image), middle, and caudal (region on the

right side of the image).

Two different types of nephrons are found within each lobe: nephrons that more closely resemble those of nonavian reptiles, and nephrons that more closely resemble those of mammals (Figure 16.20). The nephrons that are "reptilian-type" are located in the cortex and are relatively simple with no intermediate segment, very few loops and folds, and a small glomerulus. The medullary "mammalian-type" nephrons look strikingly similar to mammalian nephrons, with a larger glomerulus and a portion that looks very similar to a loop of Henle with a thinner descending limb, thicker ascending limb, and vasa recta. And like mammalian kidneys, the mammalian-type nephrons are arranged similarly, in parallel, allowing birds to be the only other vertebrate besides mammals to make urine that is more concentrated than their plasma (although mammals can make more concentrated urine than birds).

There are also often nephrons that are somewhat intermediate between the two types of nephrons, and these are located in the transition between the cortex and medulla. All types of nephrons pass their filtrate to collecting ducts, which are located in a structure called a **medullary cone** that also contains the loops of Henle, and then on to the ureter. Note that there is no renal pelvis, as we saw in mammals. The ureter discharges urine into the cloaca.

Reptiles of all kinds have other means of osmoregulation alongside the renal system. The cloaca can reclaim water, sodium, and chloride. Similar to amphibians, the urinary bladders of Gila monsters are able to resorb water back into the body when food and water are not available. Birds who spend a long time at sea without access to freshwater—as well as sea turtles, sea snakes, and marine iguanas—have similar osmoregulatory challenges as marine fishes. They need to drink salt water because they're constantly losing water to their environment, which comes with taking in extra salts. Since these amniotes do not have gills and chloride cells, they

have to rely on salt glands to excrete the extra salts. These cranial glands are capable of secreting fluid that is far more concentrated than the urine's concentration. It's not just marine reptiles that use salt glands. Many lizards also have salt glands to keep their plasma electrolytes balanced; for example, desert iguanas use their salt glands to excrete high concentrations of potassium with minimal water loss.

16.5 Integration with Other Systems

We have already seen that several other systems work alongside the renal system to accomplish excretion and osmoregulation. In the next chapter, we will also see how tightly reproductive systems have evolved and integrated with renal systems.

We can look to humans for another function of the renal system—participating in maintaining and adjusting blood pressure. When blood pressure is too high, renal perfusion (the amount of blood flow that passes through a mass of renal tissue within a given time) rises. Simultaneously, stretch receptors in the right atrium of the heart are activated, causing the release of **atrial natriuretic peptide** (also known as ANP) from the right atrium of the heart. Together, these actions cause the nephrons to keep more sodium and water in the filtrate, which depletes the volume of extracellular fluid and moves blood pressure closer to the baseline. When blood pressure is low, kidneys secrete **renin**, an enzyme that sets off a series of physiological reactions that result in the tubules reabsorbing more water and sodium as well as vasoconstriction in blood vessels. Together, these activities raise blood pressure back to the baseline. The nephrons are also induced to increase water retention by the secretion of **vasopressin** (also known as antidiuretic hormone, ADH), which is secreted by the hypothalamus in response to feedback from **baroreceptors** (pressure sensors) in the aortic arch and carotid sinus.

16.6 Summary

Kidneys as well as gills, integument, and various glands are all important for maintaining both water balance and ion balance in the vertebrate body. Vertebrates show a variety of adaptations and variations of these organs to meet the specific challenges of their particular environments.

Application Questions

1. In the parts of the US that are subject to icy conditions in the winter, a common de-icing practice is to spread salt on roadways to lower the freezing point of the ice. However, the salt has to go somewhere and ends up running into local freshwater waterways. Given what you learned about osmoregulation and anatomy, how would the fish and amphibian faunas in those waterways be impacted and why?
2. Urine analysis, or urinalysis for short, is one of the most useful fluids for medical diagnosis. Medical professionals can use a dipstick to measure levels of salts, proteins, sugars, and acids in the urine. Urine can also be spun in a centrifuge to isolate cells and other bits of debris for identification. In the words of Dr. Jonathan Reisman, "More than any other fluids, urine gives information about not only the troubles

within its own exit route through the urinary tract but also the body as a whole." Given what you've learned about the renal system, why can we learn so much about "the body as a whole" from urine?

3. Even though blood pressure is often associated with the heart, high blood pressure is a common thing for nephrologists (kidney medical specialists) to treat. Why would a kidney specialist be an appropriate person to treat high blood pressure?

16.7 Further Reading

Check out the hashtag #nephmadness online. Not only did it inspire some of the fun, unusual examples used in this chapter; there are a lot of amazing human renal physiology facts that you can learn about!

You can read more about Dr. Jonathan Resiman's love of urine (and anatomy in general) in his book *The Unseen Body*, 2021, Flatiron Press.

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17. Urogenital System: Reproduction

LISA B. WHITENACK

Focus Questions—to Guide Your Reading of This Chapter

1. What adaptations in reproductive organs and strategies occurred to support the shift from aquatic to terrestrial lifestyles?
2. How did the evolutionary changes in the urinary system affect changes in the reproductive system, particularly in testes-bearing vertebrates?
3. How do teleosts differ from other gnathostomes in terms of reproductive anatomy?

17.1 Introduction: Thinking About Terminology

This chapter will focus on the other half of the urogenital system—the reproductive part. As we alluded to in Chapter 16, these are tied together through the occasional co-opting of various ducts initially associated with the kidneys. Before we start our

conversation about reproduction, it is worth pausing and considering the language that we use. In this case, we are not talking about the many colorful turns of phrase that people use to refer to their reproductive parts but rather discussing sex and gender.

If you were to briefly and randomly survey scientific papers across time that study animal reproduction, mating behavior, or any similar thing, you would see the terms "sex" and "gender" used interchangeably, regardless of the animal, for much of that history. In the last several years, there has been a shift in terminology, and more studies use "sex" when studying nonhumans. The current general definition of **sex** is that it is a classification based on the structural and functional characteristics of an organism, and there are several more specific definitions of sex that focus on particular characteristics. These could be chromosomes, the presence or absence of particular reproductive organs, or hormone levels, for example (there are plenty more characteristics that could be used). In humans, sex is often assigned at birth on the basis of external genitalia. Often the categories of sex used for a particular species (including humans) are binary, male or female. However, when it comes to nonvertebrate organisms, there are other systems. We use + or − for some green algae. The unicellular ciliate Tetrahymena thermophile has seven sexes, and they are numbered with Roman numerals (I–VII). Relying on a binary classification system also tends to ignore the many organisms (including vertebrates) that are hermaphroditic (possessing both "male" and "female" genitalia at some point in their lives, whether that is simultaneous or sequential). For humans, we instead use the term **intersex** to refer to people who are born with genital or gonad anatomy or chromosomal patterns that do not fit into the binary boxes of "male" and "female."

Gender is a human social construct that denotes identities such as woman, man, agender, nonbinary, two-spirited, and more. Because gender is an identity and a social construct, it is human-specific. Nonhuman animals have a sex, but not a gender.

We also want to remind our readers that the human

spectrum is diverse, as is the variation we see within many other species. In humans, sex and gender intersect in complex ways. To honor that, we are going to use "egg producer" or "ovary-bearing" and "sperm producer" or "testis-bearing" instead of "female" and "male," respectively, as not all egg producers are "female," and not all sperm producers are "male."

17.2 Structure and Function of the Reproductive System

At its most basic, reproductive systems generally consist of **gonads** (which are responsible for making gametes), some sort of **genital duct** (a path for the gametes to travel through), and some sort of opening for gametes (or other reproduction-related things) to enter or exit the body. There is an incredible amount of variability across sexes, species, and higher taxonomic levels (Figure 17.1). Variation is tied to lifestyle (e.g., aquatic vs. terrestrial), fertilization behavior (external vs. internal), parental care, and evolutionary history, among other things. We will begin by describing some of the general components of the vertebrate reproductive system here, and then we will save our discussion of the fascinating variation of these systems for after we've talked about development.

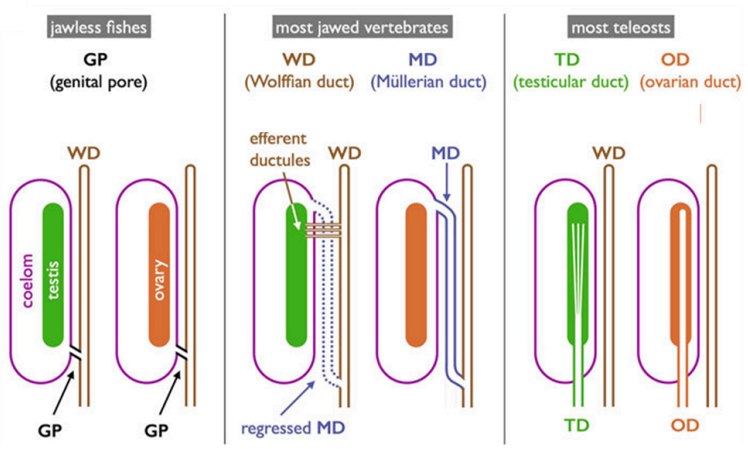

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Figure 17.1—Three types of gamete-exporting organs in vertebrates. In jawless fishes, genital ducts are absent, and both sperm and ova are released into the coelom and exported through the genital pores (GPs). In most jawed vertebrates, sperm and ova are exported through the Wolffian ducts (WDs) and Müllerian ducts (MDs), respectively. In most testes-bearing gnathostomes, sperm are exported through the efferent ductules to the WDs; the MDs are developed but later regress in many cases. In most ovary-bearing gnathostomes, mature oocytes are shed into the coelom and then exported through the opening of the MDs to the urinary sinus. Finally, most teleosts develop genital ducts as the posterior elongation of gonads: testicular ducts (TDs) and ovarian ducts (ODs).

Components of the Egg-Producer Reproductive Systems

Ovaries are the gonads that produce eggs, or **ova** (singular: ovum). This is not the ovary's only job, as it is also an active component of the endocrine system (see Chapter 21 for a discussion of endocrine function). However, the production and maturation of ova (**oogenesis**) are closely tied to the endocrine function of the ovary as a gland. The general structure of the vertebrate ovary can best be described as a bundle of **follicles** held together with some connective tissue (Figure 17.2). These follicles are composed of two

things—oocytes and follicle cells. **Follicle cells** form a layer around the oocytes and help provide the yolk within the developing ovum. **Oocytes** (literally, "egg cells") can be thought of as precursors to mature ova (see Section 17.3, Morphological Development). The follicles and associated connective tissue are enclosed by a capsule of connective tissue called the **tunica albuginea**, which is overlaid by a layer of epithelium. The ovary is suspended from the coelom wall by a ligament called the **mesovarium** (mesos = middle, ovo = egg; Figure 17.3). You may recall that we saw similar sheets of suspensory tissue called mesenteries in the digestive system (Chapter 13), and the similarity in name is no accident.

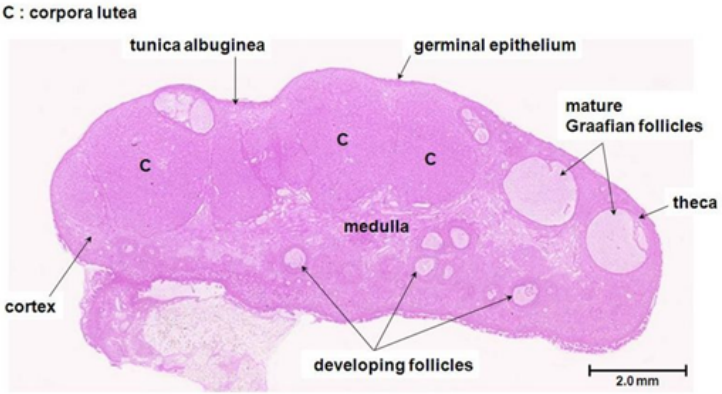

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Figure 17.2—Histological section of a rabbit ovary.

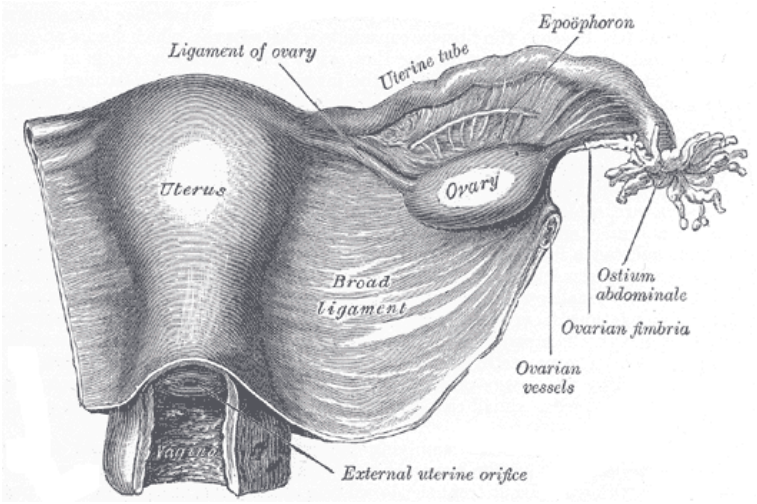

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Figure 17.3—This view of the human uterus and ovary is from the dorsal side. The mesovarium is the portion of the broad ligament that connects to the ovary. Note that there are several other sheets of tissue that suspend the ovary, including the ovarian ligament.

When ova are released from an ovary, a process called **ovulation**, the eggs are shed into the coelom (Figure 17.1). In the vast majority of vertebrates, the **oviduct** is close by and collects the eggs. The oviduct is also referred to as the **Müllerian duct**, which we will talk about when we get to development. The oviduct can include a wide variety of structures, depending on which vertebrate you're examining, including the **uterus**, **vagina**, **cervix**, and **shell glands**. Not all vertebrates have all these regions of specialization.

While you may assume that the ovaries and oviducts are paired structures as they are in many humans, this is not the case in several vertebrates. Almost every vertebrate class has species that have only one functional ovary or oviduct; this can be due to the fusion of gonads, the atrophy of one gonad or oviduct, or the failure of one gonad to develop at all. Birds, in particular, tend to only have

a left functional ovary, while the right ovary atrophies while the bird is still an embryo.

Components of Sperm-Producer Reproductive Systems

Testes (singular: testis) are the gonads that produce **sperm**, a process called **spermatogenesis**. Similar to ovaries, testes also have an endocrine function (see Chapter 21). In fact, we're going to see a lot of parallels in structure as well! Recall that the ovaries are generally made up of connective tissue and follicles. Similarly, the testes are made up of **somatic tissue** and **seminiferous tubules**, the former of which includes connective tissue, nerves, blood vessels, and **Leydig cells** (Figure 17.4). Leydig cells (also known as interstitial cells) are responsible for producing androgen hormones. Spermatogenesis takes place within the seminiferous tubules. Sertoli cells, a major structural component of the seminiferous tubules, are in contact with the germ cells and aid the production of sperm (see Section 17.3, Morphological Development). The seminiferous tubules merge together into collecting ducts, which then meet up with the **ductus deferens**. For most vertebrates, the testes are internal and suspended from the coelom wall by a ligament called the **mesorchium** (literally, "middle testicle" in Greek). Mammals are the exception; many mammals instead have external testes contained within a pouch called the **scrotum**. The migration of the testes from within the coelom to an external position will be discussed later in this chapter. The testes are covered by an epithelium, and beneath that is a layer of connective tissue called the tunica albuginea, just as we saw in the ovaries. Also as we saw with ovaries, testes are not always paired; they can be completely fused into one testis, they can be partially fused, or just one testis may develop fully.

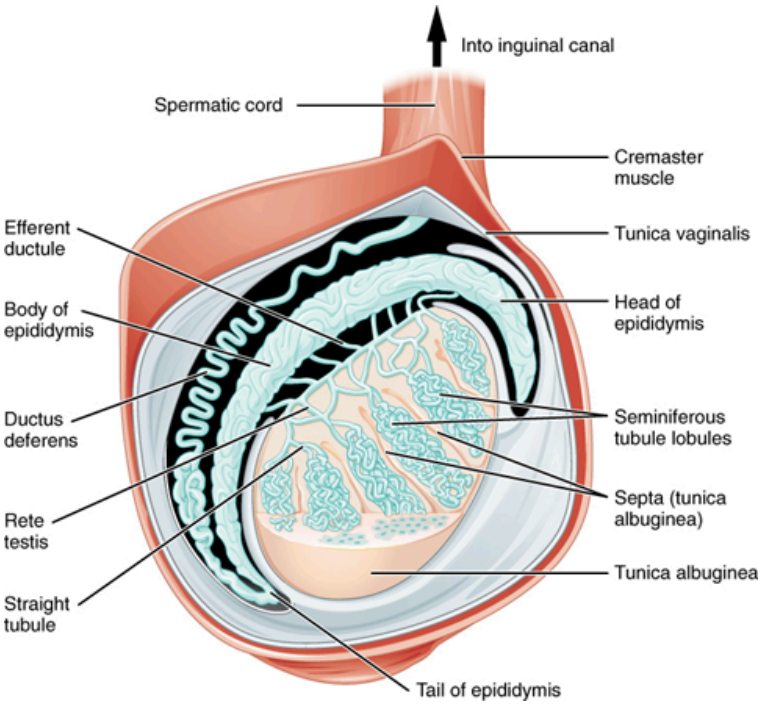

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Figure 17.4—Structure of a human testis.

Unlike eggs, sperm are generally not released into the coelom. Instead, for most vertebrates, the archinephric duct (opisthonephric duct) from the renal system is co-opted for sperm transport (Figure 17.1). In some vertebrates, this duct may carry urine and sperm (although not simultaneously), but recall from the previous chapter that amniotes instead rely on a metanephric duct for urine transport. As we saw with the oviduct, the archinephric duct may include regional specializations, depending on which vertebrate you're examining.

Sperm-producing vertebrates may also possess **intromittent organs**, which are external organs that help transmit sperm in internal fertilizing species. In mammals, this is known as

the **penis**, and we will see other intromittent organs when we dive into the diversity of reproductive systems.

17.3 Morphological Development

Regardless of whether a vertebrate develops gonads capable of making ova or sperm, the gonads arise from the **genital ridge**, specifically as mesoderm with contributions from the mesenchyme. At this initial stage, the paired masses of tissue do not show any particular sperm- or egg-related features; as such we call it an **indifferent gonad** (Figure 17.5). The germ cells that eventually will make gametes originate outside of the genital ridge in the extraembryonic endoderm. Once the germ cells arrive at the indifferent gonad, we start to see some differentiation. For many vertebrates, germ cells will migrate to the **cortex** of the indifferent gonad if the gonad will be producing eggs, but they will migrate to the **medulla** of the gonad if the gonad will be producing sperm. However, cyclostomes and teleosts do not follow this pattern, and germ cells migrate to the cortex regardless of what the gonad develops into.

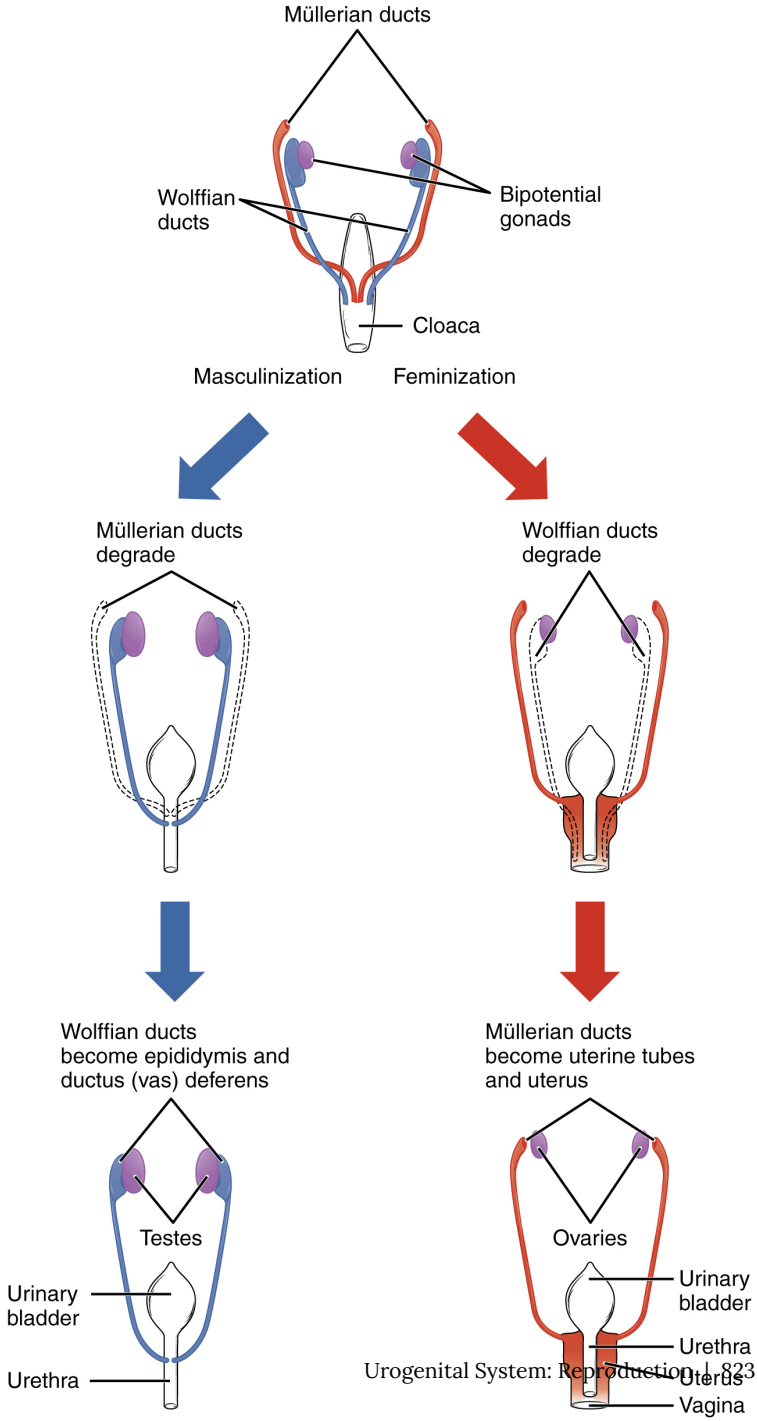
Müllerian ducts
Wolffian ducts
Bipotential gonads
Cloaca
Masculinization
Feminization
Müllerian ducts degrade
Wolffian ducts degrade
Wolffian ducts become epididymis and ductus (vas) deferens
Müllerian ducts become uterine tubes and uterus
Testes
Urinary bladder
Urethra
Ovaries
Urinary bladder
Urethra
Uterus
Vagina

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Figure 17.5—The process of sex differentiation in a human embryo.

When germ cells reach the ovary, we change their name to **oogonia**. These oogonia go through several steps during oogenesis. Briefly, the oogonia carry two complete sets of chromosomes, making them diploid. They divide via mitosis, and those products of cell division are called **primary oocytes** (Figure 17.6). The primary oocytes then proceed through Meiosis I, producing a small **polar body** and a larger **secondary oocyte**. If you remember your meiosis, these are haploid. During Meiosis II, we split the sister chromatids between two cells so that a haploid ovum is created from the secondary oocyte, along with another small polar body (also haploid). The haploid polar body produced by Meiosis I may or may not go through Meiosis II. It's worth noting that the process of oogenesis is extremely variable across vertebrates in terms of developmental timing and sometimes is not completed until the end of reproductive maturity or even until fertilization takes place.

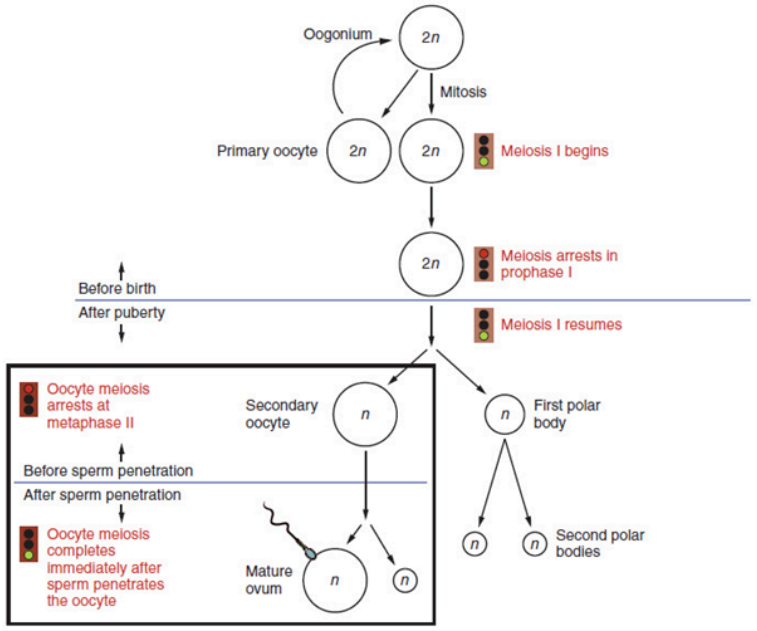

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Figure 17.6—The process of oogenesis. Note that the first polar body doesn't always go through Meiosis II and divide.

Spermatogenesis is a process that is similar to oogenesis (Figure 17.7). When germ cells reach the testes and the Sertoli cells, we change their name to **spermatogonia**. Like the oogonia, they are diploid. As these develop during spermatogenesis, they undergo rounds of mitosis, and those products are called **primary spermatocytes**. Those diploid cells will undergo meiosis, forming haploid **secondary spermatocytes** at the end of Meiosis I and **spermatids** after Meiosis II. Unlike oogenesis, no polar bodies are formed during this process. Instead, we make four haploid spermatids. Spermatids undergo a final step where the chromosomes are condensed and excess cytoplasm and organelles are removed from the cell. When this is complete, we have sperm (or more properly, **spermatozoa**).

To sum up all of this, both eggs and sperm are produced via meiosis. However, the products differ. One diploid oogonium will produce a single egg that can be fertilized and two to three polar bodies that will not. One diploid spermatogonium will produce four sperm that can potentially fertilize an egg.

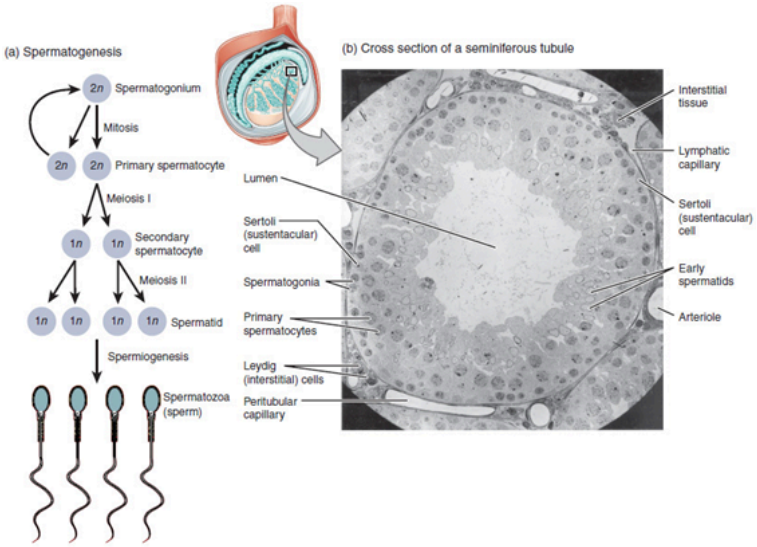

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Figure 17.7—Spermatogenesis (a) Mitosis of a spermatogonial stem cell involves a single cell division that results in two identical, diploid daughter cells (spermatogonia to primary spermatocyte). Meiosis has two rounds of cell division: primary spermatocyte to secondary spermatocyte, and then secondary spermatocyte to spermatid. This produces four haploid daughter cells (spermatids). (b) In this electron micrograph of a cross section of a seminiferous tubule from a rat, the lumen is the light-shaded area in the center of the image. The location of the primary spermatocytes is near the basement membrane, and the early spermatids are approaching the lumen (tissue source: rat). EM × 900. (Micrograph provided by the Regents of University of Michigan Medical School © 2012)

The processes governing whether a vertebrate ends up with ovaries or testes (sex determination) are complex and variable. As one might expect, there is often a genetic component. We call this **genetic sex determination** (**GSD**). At the chromosome level,

many vertebrates have some sort of chromosomes that are tied particularly to sex determination. In mammals and some lizards, we see X and Y chromosomes, where the XX condition (**homogamous**, because we see the same chromosomes) is usually associated with egg producers, and XY (**heterogamous**) is associated with sperm producers. However, in birds, some lizards, and snakes, we see Z and W chromosomes and the opposite condition, where the ZZ homogamous condition is associated with sperm producers, and the ZW heterogamous condition is associated with egg producers. Amphibians and fishes can be either XY or ZW, depending on the species. Interestingly, the XY chromosomes are not homologous to the ZW chromosomes, and they act in very different ways. For example, the mammalian Y chromosome contains a gene called **Sry**, which is not found on the X chromosome. Sry is the gene that causes the indifferent gonad to develop into a testis by stabilizing the SOX9 protein, which in turn jump-starts the development of both Sertoli cells and testicular cords. The absence of Sry expression causes the indifferent gonad to develop into an ovary. It's worth noting that there is a whole series of gene expression and inhibition events that are required to move forward with gonadal development that is beyond the scope of this chapter, and there are genes other than Sry in nonmammalian vertebrates that perform similar functions. There are resources at the end of this chapter if you are interested in learning more.

Genes are not the sole determinant of sex in some vertebrates. There are several vertebrates that rely on **environmental sex determination**. Some reptiles, such as the tuatara, crocodylians, and many turtles do not have sex chromosomes. Instead, they have **temperature-dependent sex determination** (**TSD**). High versus low incubation temperature is not firmly tied to sex across all TSD species. Many turtle hatchlings tend to be ovary-bearing if they are incubated at high temperatures, whereas high-temperature incubation tends to lead to crocodilian hatchlings developing testes instead of ovaries. In still other reptiles, such as the loggerhead sea turtle, there is a more complex

TSD regime, where both high and very low incubation temperatures produce hatchlings with ovaries, and more intermediate temperatures produce hatchlings with testes. How exactly TSD works with respect to temperature affecting gene expression is an active area of research with no clear answers yet.

Another form of environmental sex determination uses social cues. Roughly 450 teleost species are **sequential hermaphrodites**, meaning that they transition between functional ovaries and functional testes (or vice versa) at some point in their lives (Figure 17.8). Most are **protogynous**, initially possessing functional ovaries. Wrasses, which include parrotfish, are wonderful examples. Bluehead wrasse, which can be found on reefs throughout the Caribbean, have initial GSD; most start off bearing ovaries, but a small number will have testes. In this initial phase, both sexes have similar yellow, green, and white coloration. Initial-phase wrasses participate in **group spawning**, gathering in large groups, and releasing gametes into the water. A small number of initial-phase bluehead wrasses will transition from ovary-bearing to testis-bearing. When this happens, the initial ovaries redifferentiate into testes, followed by the body changing color to include the bright-blue heads that give these fish their common name. Interestingly, these terminal-phase testis-bearers also change their spawning behavior, only mating in pairs. The social cue that initiates these changes is a decrease in the number of large sperm-producing wrasses in the population, and transition happens within two weeks.

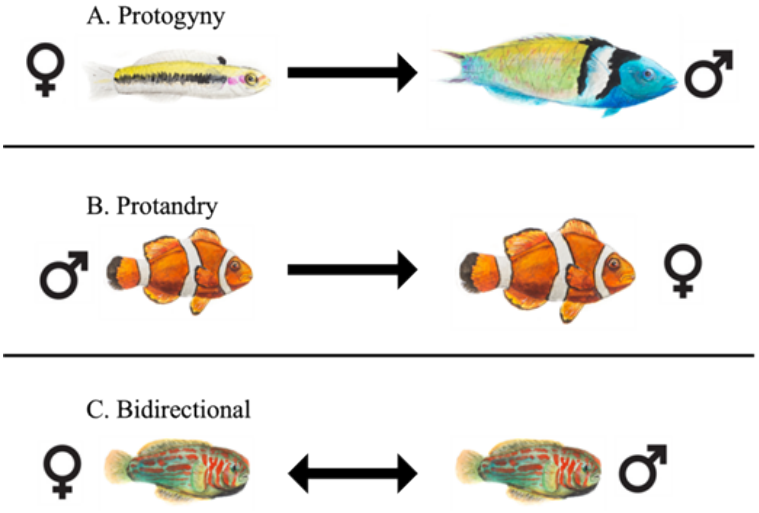

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Figure 17.8—Sequential hermaphroditism in teleosts. (A) Protogyny in the bluehead wrasse Thalassoma bifasciatum; (B) Protandry in the ocellaris clownfish Amphiprion ocellaris; (C) Serial bidirectional change in the broad-barred goby Gobiodon histrio.

A smaller percentage of hermaphroditic fishes are **protoandrous**, and my favorite example is the clownfish of *Finding Nemo* fame. This Indo-Pacific reef fish tends to live in groups consisting of one breeding pair (the largest fish has functional ovaries and the next largest has functional testes), plus up to four smaller nonbreeding fish. These fish start their lives with both testicular and ovarian tissues, and as they mature, only the testicular tissue becomes functional. If the largest ovary-bearing fish dies or is removed from the group, the second largest fish will transition from having functional testes to having functional ovaries—the testicular tissue regresses and the ovarian tissue matures. If you're thinking back to the beginning of *Finding Nemo* when (spoiler alert!) Marlin's mate dies…yes, Marlin should have transitioned.

An even smaller percentage of teleosts, mostly a few coral-dwelling goby species and the cleaner wrasse *Labroides dimidatus*, can engage in **serial bidirectional sex change**. This is a relatively recent discovery, and most of the species identified were previously assumed to be protogynous. Some of the gobies can repeatedly switch back and forth, which is thought to be an adaptation to limited mating opportunities on coral reef heads that are fairly isolated. Generally, these gobies have **ovotestes**, with a portion of the gonad being composed of ovarian tissue and the other portion composed of testicular tissue. Keeping this gonad bisexual allows the gobies to switch sexes more rapidly.

The development of the genital ducts has been partially covered already in the previous chapter. Recall that the archinephric ducts have been co-opted for sperm transport; the development of these ducts has been discussed in Chapter 16. However, we haven't talked about the Müllerian ducts yet. For the vertebrates that have them at some point in development, these ducts form from mesonephric epithelial tissue surrounded by mesenchyme and more epithelium. As the embryo develops, most vertebrates that develop testes lose the Müllerian ducts; in mammals, this is thanks to the production of **anti-Müllerian hormone** (**AMH**) by the testes.

17.4 Reproductive System Evolution and Diversity

With the exception of the cyclostomes, we are going to follow the evolution of the sperm-producing vertebrates first. We will then circle back and discuss the evolution of the ovary-bearing vertebrates. This will let us first follow a trend that we saw in the previous chapter (the co-opting of the archinephric duct to carry sperm), and then we can focus on the many evolutionary changes that ovary-bearing vertebrates accrued as they became more terrestrial (egg-laying is ancestral, as is external fertilization).

This means that we will be covering each group of vertebrates twice (except cyclostomes). As a reminder, there is a basic schematic of these reproductive systems in Figure 17.1.

Cyclostomes

Lampreys and hagfishes have relatively simple reproductive systems. They have gonads and that's about it—there are no genital ducts at all (Figure 17.1). Instead, the ovaries and testes shed their gametes directly into the coelom (Figure 17.9). Gametes leave the body by moving through the coelom and through **genital pores**, which develop only after these fishes reach reproductive maturity. The gametes pass through the pores into the **urinary sinus** and then leave the body through an opening called the **cloaca** (Latin for "sewer" or "drain"). Lampreys and hagfishes are **external fertilizers**, meaning that they shed their gametes into the water. It's worth noting that this relatively simple reproductive system is not limited to our jawless friends. There are several basal actinopterygians—such as tarpons, moray eels, and arowanas—that utilize genital pores instead of genital ducts.

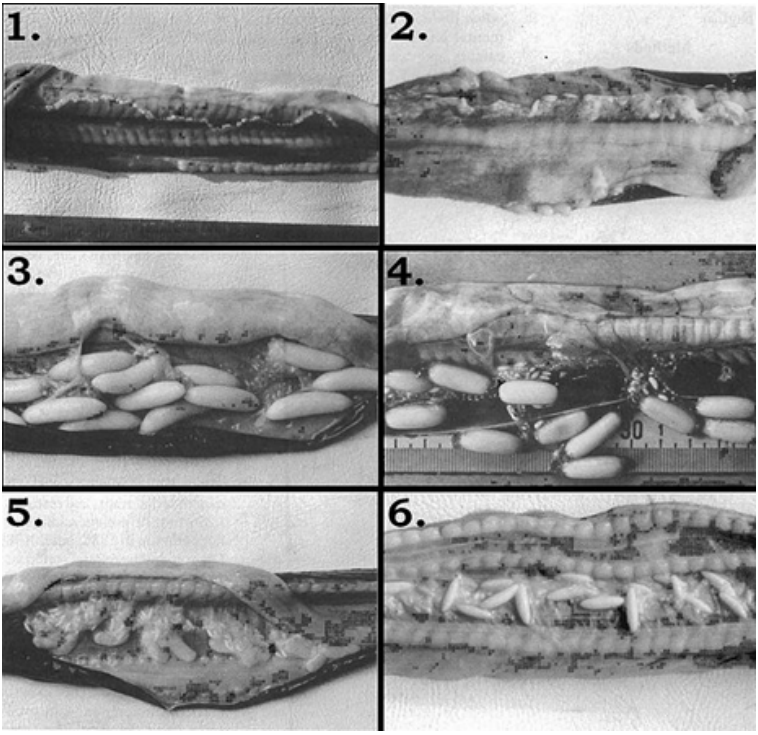

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Figure 17.9—Black hagfish Eptatretus deani ovaries. (1) Gonad stage 1, immature individual. All eggs are small (≤1 mm in length), round, and located all along the length of the gonad. (2) Gonad stage 2, maturing individual. There are a few oblong eggs >1 mm but <5 mm in length. (3) Gonad stage 3, mature ova, developing individual. Here, some eggs are >5 mm in length. (4) Gonad stage 4, mature ova, developed individual. Hooks are present at both ends of each large egg. (5) Gonad stage 5, mature, spent individual. Large empty ovarian capsules are present (as well as some small oblong eggs). (6) This gonad contains three different size groups of eggs.

Remember that when we introduced ovaries, we noted that these are not always paired. Hagfishes only have the right ovary, as the left one degenerates. Lampreys also have only one ovary, but this is a case of fusion—the right and left primordia fuse early

in development, resulting in a single ovary located on the midline. Similarly, lampreys and hagfishes have single testes.

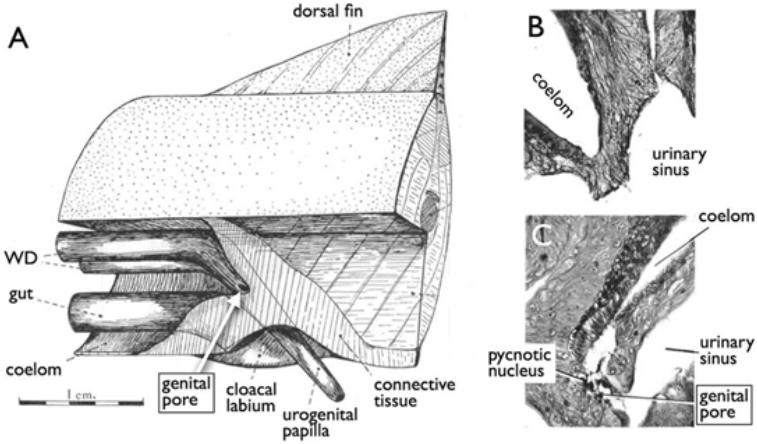

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Figure 17.10—Induction of the genital pores in river lamprey by gonadotropins (reproduced from Knowles [1939], with permission from the Company of Biologists). (A) View of the cloacal region of a matured adult Lampetra fluviatilis, which has been dissected from the left side. (B) Section through the cloacal region of a testes-bearing adult. (C) Section through the cloacal region of an ovary-bearing adult, which had been injected with gonadotropins.

Testes-Bearing Gnathostomes

Chondrichthyans

Sperm is produced in paired testes, located at the anterior end of the coelom (Figures 17.11 and 17.12). Sperm pass into a series of ducts called the **efferent ducts** (also known as vasa efferentia and ductus efferens); these are derived from the nephric tubules.

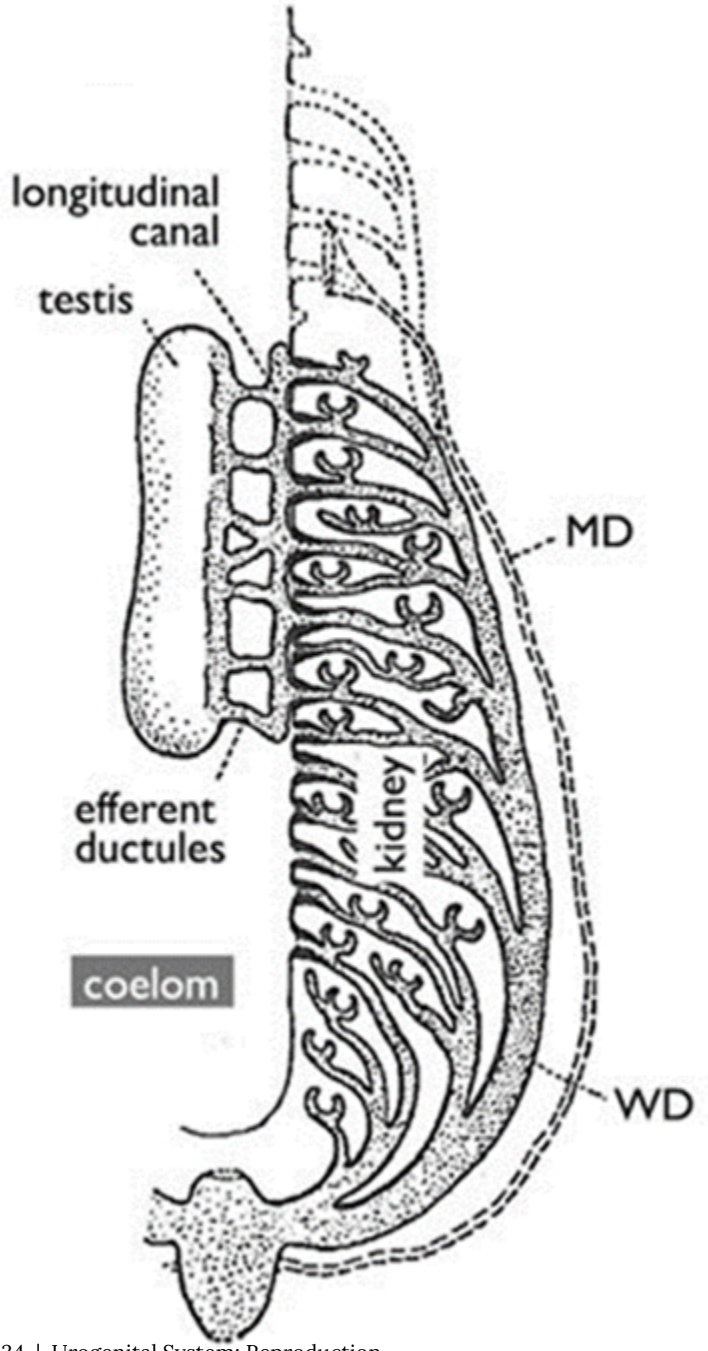
longitudinal canal
testis
MD
efferent ductules
kidney
coelom
WD

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Figure 17.11—A generalized jawed vertebrate urogenital system (adapted from Goodrich [1945], with permission from the Company of Biologists). MD = Müllerian duct, WD = Wolffian ducts.

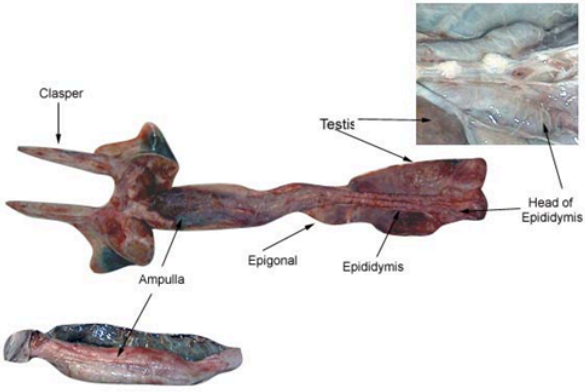

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Figure 17.12—The reproductive system of a sperm-producing porbeagle shark Lamna nasus.

For each testis, the efferent ducts serving it merge into a single duct called the **vas deferens**, which is also known as the opisthonephric duct, the ductus deferens, or the Wolffian duct (see Table 17.1 for all the duct names). The vas deferens does not carry urine in the chondrichthyans—remember from the previous chapter that they have accessory urinary ducts to drain the kidneys and pass urine to the ureter. The anterior part of the vas deferens is highly convoluted and referred to as the **epididymis**. The sperm travel from the epididymis, down the vas deferens, to the enlarged posterior portion called the **seminal vesicle** or ampulla ductus deferens. **Leydig glands** contribute seminal fluid at the epididymis and middle portion of the vas deferens; sperm plus seminal fluid is

what we call **semen**. Both the middle portion of the vas deferens and the seminal vesicle can store sperm and seminal fluid. The seminal vesicles empty their contents into a **urogenital sinus**; the "uro" in the name indicates that this cavity also receives urine from the ureter (though not usually at the same time). Both urine and semen leave the urogenital sinus through projections called **urinary papillae** (singular: urinary papilla; also known as urogenital papilla) that extend into the cloaca.

Table 17.1—A guide to the different duct names in the urogenital systems of vertebrates

Term that we're using	**Other names for that duct**
efferent ducts	vas efferentia, ductus efferens
Wolffian duct	opisthonephric duct, ductus deferens, pronephric duct, archinephric duct, vas deferens (if only carrying sperm),
oviduct	Müllerian duct, Fallopian tube (in humans only)
ovarian duct	oviduct (in teleosts only; likely not homologous with the Müllerian duct type of oviduct), gonoduct

Chondrichthyans are **internal fertilizers**. That means that the sperm need to find their way inside the ovary-bearing shark, skate, or ray. Sperm-producing chondrichthyans have **secondary sex organs** (i.e., not gonads and ducts) called **claspers** and **siphon sacs** (Figure 17.13). Claspers are modifications of the medial part of the pelvic fins and are composed of several pieces of cartilage that are joined to the metapterygium and one another. Each clasper has a dorsal **groove** that runs longitudinally down the clasper. During copulation, a single clasper is rotated and inserted into the cloaca of the egg-producing shark. Sperm is ejected from the urinary papilla down the groove, where it can make its way toward the oviduct of

the other shark. Sperm gets a boost from the seawater in the siphon sacs, which are internal muscular bladders that open at the proximal opening of the clasper.

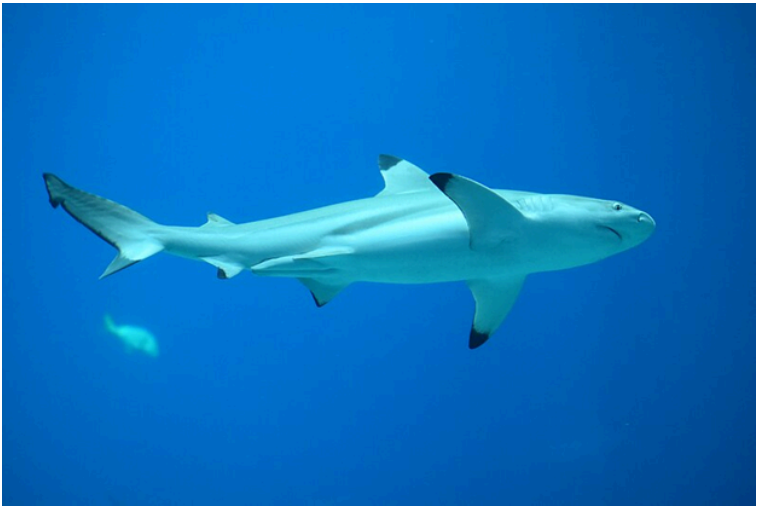

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Figure 17.13—A blacktip reef shark Carcharhinus melanopterus. Note the paired claspers on the pelvic fins.

Osteichthyan Fishes

Most bony fishes have paired testes that are fairly elongated. However, we also see cases where only one testis develops, partially fused testes (e.g., perch), and completely fused testes (e.g., guppies). Bony fish testes tend to fall into one of two categories: **tubular** (contains highly branched spermatic tubules within the testis) or **lobular** (less branched spermatic tubules; Figure 17.14). As you might guess, those tubes contain the Sertoli cells responsible for spermatogenesis, as well as other epithelial cells.

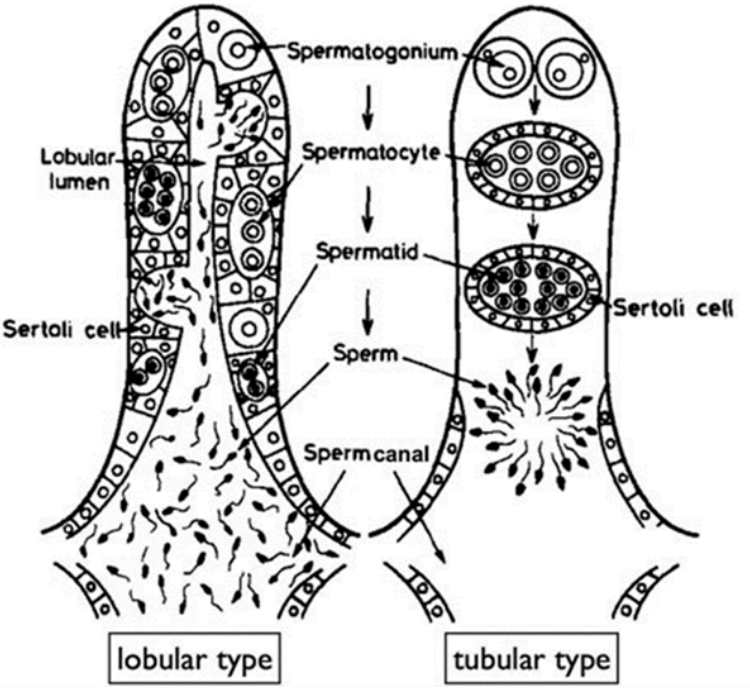

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Figure 17.14—In both lobular-type (spermatogonia distributed at various locations along spermatogenic tubes) and tubular-type testes (spermatogonia localized at the tips of tubes), spermatogenesis occurs within cysts surrounded by Sertoli cells. Mature sperm are released into spaces (sperm canals) covered by epithelial cells, which are continuous with the Sertoli cells of ruptured spermatogenic cysts (reproduced from Nagahama [1983], with permission from Elsevier).

How the sperm leave the testes largely depends on what type of bony fish you're looking at. Some have no ducts and shed sperm directly into the coelom and utilize genital pores, as we mentioned in the cyclostomes section. Other nonteleost bony fishes (sturgeons, paddlefish, lungfishes) have co-opted the opisthonephric duct, just as we saw in the chondrichthyans. However, unlike the chondrichthyans, their opisthonephric duct still carries urine as well as sperm. In sturgeons, efferent ducts

connect the testes to canals within the kidneys. The kidney canals can also carry urine and will carry both urine and sperm to the opisthonephric duct. The connections work differently in the sarcopterygian lungfishes—the efferent ducts connect the testis with the opisthonephric duct, not the kidneys. The paired ducts may or may not merge at the urinary papilla, which empties into the cloaca.

The teleosts do not follow either of these plans (Figure 17.1). The opisthonephric duct is only used to transport urine, not sperm, and we can call it a ureter in this case. Instead, teleosts develop a **testicular duct** to transport sperm. The testes contain small **spermatogenic tubes**. The spermatogenic tubes merge as they exit the testes, forming the testicular duct. Note that this means that the testicular duct is not homologous with the opisthonephric duct! The testicular duct passes through the coelom and ends at the point where sperm leave the body. Usually, this takes place through an opening between the anus and the urinary aperture. Other teleost species have testicular ducts that connect with the urethra, and sperm and urine will leave through a urogenital papilla (Figure 17.15).



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Figure 17.15—The urogenital papilla of the teleost Neogobius melanostomus, the round goby.

The majority of bony fishes are external fertilizers and release their gametes into the water in a process called **spawning**. Spawning can happen in large groups or in pairs, in the open ocean or near the sea floor, and so forth. There is a lot of variation in fish spawning behavior. The timing of spawning is not random! Fishes time their spawning events using cues such as seasonal changes of light, seasonal changes of temperature, and the phase of the moon (as a proxy for tidal movement). Approximately 3% of bony fishes are internal fertilizers, and this strategy has arisen independently at least 12 times in bony fish evolution, including in the coelacanths, rockfishes, and guppies. While 3% may seem small, remember that there are approximately 28,000 osteichthyan species; just within the teleosts, this strategy is spread across 21 families and somewhere between 500 and 600 species! How do they accomplish this? In general, there is some sort of structure that a sperm-producing fish can insert into an egg-producing partner. For

example, sperm-producing guppies have developed a **gonopodium**, long extensions to the third through fifth rays of the anal fin, which functions similarly to claspers in sharks in that it is inserted into the ovary-bearing fish and used to transfer sperm (Figure 17.16). Other fishes, such as rockfishes and surfperch, will insert their urogenital papilla into the reproductive partner.



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Figure 17.16—Trinidadian guppies Poecilia reticulata. Note that the upper guppy has a gonopodium, making this a testes-bearing individual.

Amphibians

All amphibians have paired, lobular testes composed of seminiferous tubules, which are the site of spermatogenesis and Sertoli cells, as well as connective tissues, nerves, blood vessels, and Leydig cells. Amphibians also possess a pair of **fat bodies**, one adjacent to each testis (Figure 17.17). As you might guess, fat bodies are full of lipids. In terms of reproduction, they are capable of synthesizing steroid hormones and thus are important to gamete formation. Sperm

travels from the seminiferous tubules to the efferent ducts, as we have seen in other vertebrates. However, unlike other vertebrates, the efferent ducts connect to nephrons within the kidney. In salamanders, this is relegated to only nephrons in the anterior "genital kidney," as mentioned in Chapter 16. Once sperm leave the kidney, they head into the Wolffian duct to head to the cloaca and exit the body. That means this duct carries both sperm and urine to exit the body in the majority of amphibians. Interestingly, plethodontid salamanders ("lungless" salamanders) are the exception to this rule, as the testes do not connect to the kidney. Thus, the Wolffian duct is solely a sperm-carrying duct until it is close to the cloaca and urinary ducts merge with it.

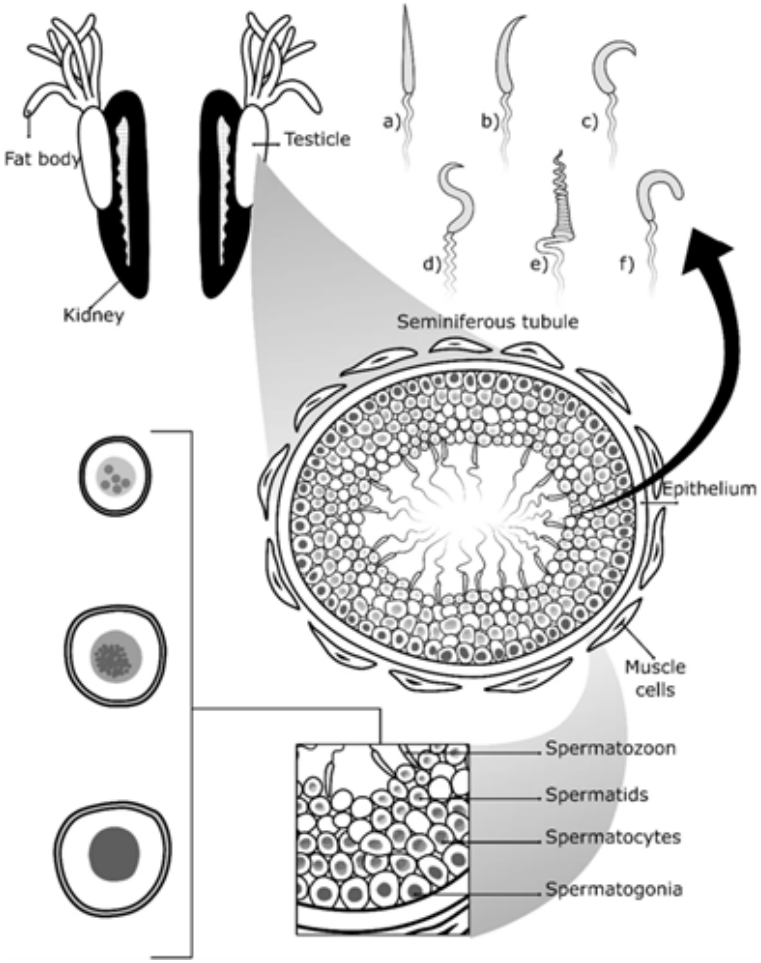

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Figure 17.17—The reproductive system of testes-bearing anurans. a–f illustrate the variety of spermatozoa morphologies across different anurans. The lower part of the figure shows a cross section of a testis.

The gymnophionans (caecilians) have an additional feature—the Müllerian duct remains intact in sperm-producing caecilians and empties into the cloaca. In egg-producing caecilians,

this duct is the oviduct, as we would expect. But in our adult sperm producers, this duct functions as a gland, producing a variety of products that mix with sperm in the anterior part of the cloaca to help with sperm motility and physiology. The caecilians are notable for another reason: The cloaca of sperm-producing caecilians can evert itself into the **vent** (opening) of the ovary-bearing caecilians and thus acts as an intromittent organ during internal fertilization. Specifically, the posterior part of the cloaca called the **phallodeum** is shaped like a tube. When it's time to mate, muscles evert the phallodeum; when mating is done, another set of muscles retracts it.

Most salamanders are also internal fertilizers, although this is a very different process than that of the caecilians (Figure 17.18). Sperm-producing salamanders that practice internal fertilization package their sperm in gelatinous masses called **spermatophores**; these are produced in cloacal glands. Often, they will deposit spermatophores on a substrate, and the ovary-bearing salamander will walk over it and uptake it into their cloaca. Other salamanders will directly place the spermatophore into the other salamander's cloaca.



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Figure 17.18—Mating in the alpine salamandra Salmandra atra.

Anurans (frogs) are largely external fertilizers, although they do not generally spawn as bony fishes do. Instead, the sperm-producing frog will get on the egg-producing frog's back and secure themselves by grasping the head, waist, or armpits with their forelimbs. This behavior is called **amplexus** (Latin for "embrace"; Figure 17.19). As eggs are being extruded by one frog, the other releases sperm directly on top of the eggs. Internal fertilization is rare and seems to only occur in the two species of "tailed frogs" (*Ascaphus*; Figure 17.20). The common name is misleading, as the "tail" in question is actually an extension of the cloaca in sperm-producing tailed frogs. This part of the cloaca is highly vascularized. When it is time to mate, the tissues become engorged, and the "tail" is inserted into the egg-producing frog. Sperm runs from the cloaca down a groove in the "tail," much as we saw in shark claspers.



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Figure 17.19—Mating in the American green tree frog Hyla cinerea.



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Figure 17.20—The "tailed" frog Ascaphus truei.

Amniotes

The biggest evolutionary trend we've followed throughout this book is the shift from a fully aquatic lifestyle to a fully terrestrial one. In terms of reproduction, we have now seen hints of what this looks like from a reproductive perspective in the amphibians: internal fertilization, the copulatory organs that go along with that, and (preview for the section on egg producers) fewer, larger eggs. All amniotes are internal fertilizers and have some sort of organ for

copulation. This is generally called a penis, but we will see that some reptiles have hemipenes, and most extant birds have either greatly reduced or totally lost their penis. We will talk more about these shortly.

Aside from the penis (or hemipenes, or lack thereof), the general layout is the same regardless of which amniote you're examining (Figures 17.1 and 17.11). Sperm are produced in the paired testes and travel through the efferent ducts, epididymis, and then Wolffian ducts to leave the body. The other major shift that occurs in sperm-producing amniotes is related to the kidney evolution we talked about in the previous chapter—the separation of urine and sperm into different tubes becomes the rule. Recall that our Wolffian ducts are just another word for opisthonephric ducts, and these end up being used entirely for sperm transport in amniotes, whereas a new duct forms (the metanephric duct or ureter) to handle urine transport. Because these Wolffian ducts solely carry sperm, we often refer to them as the **vas deferens** to distinguish them from Wolffian ducts that carry both sperm and urine.

Reptiles, Including Aves

Testes-bearing reptiles, including birds, generally only differ once you get down to the point where sperm are leaving the body. Turtles and crocodylians have single, unpaired penises that are tucked into the cloaca until it's time to copulate. However, they work in very different ways. Turtles have two ridges of erectile tissue called **seminal ridges** that run down the length of the penis. A **seminal groove** lies between the two ridges. Turtle penises have a single erectile region composed of a **corpus fibrosum** and the **corpus cavernosum** (Figure 17.21). As the corpus cavernosum fills with blood, the penis is erected. Once inserted into the other turtle, sperm travels down the seminal groove into the other turtle's reproductive tract. Turtles have a **retractor muscle** to pull the penis back into the cloaca, which originates on the sacral ribs and inserts

onto the ventral corpus fibrosum. Penis morphology varies quite a bit among turtles. Several species have a **sulcus** and **glans** (tip or head of the penis) that **bifurcate**, or split in two. One genus of soft-shelled turtles, *Trionyx*, has a five-lobed glans, each lobe with a separate opening for the split urethra.

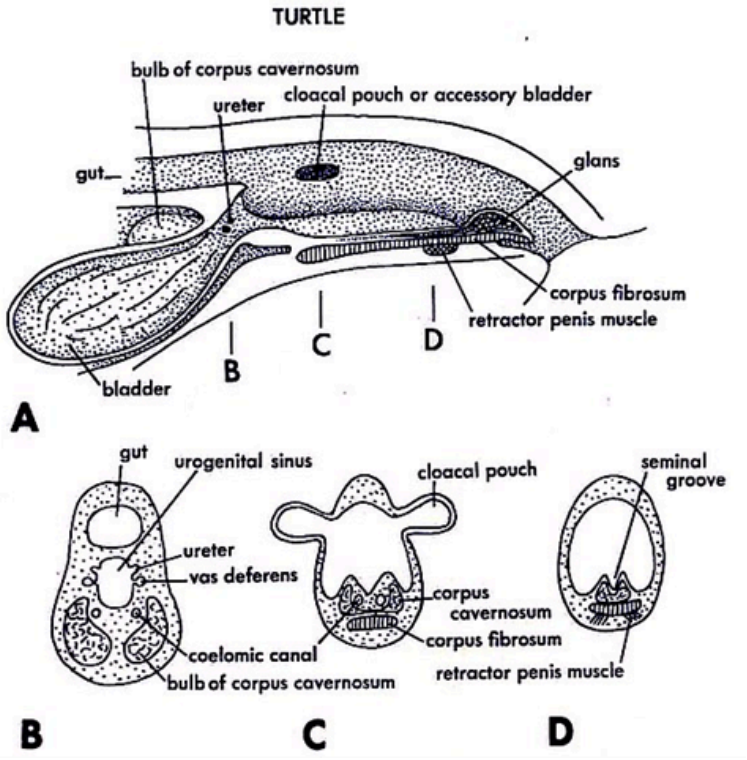

Figure 17.21—The penis of a turtle. (A) Sagittal section through the cloaca. (B–D) Cross sections at the points indicated in part A.

Crocodylians, on the other hand, do not have erectile tissue in their penises. Paired **levator muscles**, which originate at the anterior cloaca and insert on the penis, rotate the penis out of the

cloaca. This causes strain on a ligament that connects the base of the penis to the ischia, which serves to erect the penis. Crocodylians also have a central groove, called the **sulcus spermaticus**, for sperm to travel down. When those levator muscles relax, the strain on the ligament eases and the penis returns back into the cloaca.

Squamates (lizards and snakes) do something totally different—they have **hemipenes**. The paired hemipenes are tucked away just caudal to the cloacal vent when they are not in use (Figures 17.22 and 17.23).

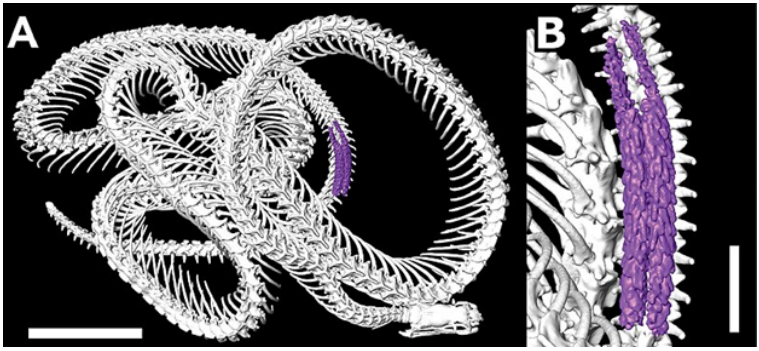

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Figure 17.22—A μCT scan of Toxicocalamus longhagen showing the (A) whole body (scale bar 20 mm) and the (B) hemipenes (scale bar 5 mm) highlighted in purple.



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Figure 17.23—The everted hemipenes of a western diamondback rattlesnake Crotalus atrox.

When it's time to copulate, sperm travels through the Wolffian ducts and exits into the cloaca at the base of the hemipenes. Just one hemipenis is everted straight into the ovary-bearing squamate's cloacal vent via contraction of the **transversus penis** muscle (Figures 17.24 and 17.25). Each hemipenis has a sulcus spermaticus for the sperm to travel down from the cloaca. As we saw with the turtles, there is a retractor muscle to pull the hemipenes back into the cloaca. Some varanid (monitor) lizards have some erectile help from a **hemibaculum**, or hemipenis "bone," located in the glans of the hemipenis. Note that the hemibaculum is composed of cartilage, not bone tissue. We will see that some mammals have something similar made of bone.



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Figure 17.24—The everted hemipenes of a common lizard Zootoca vivapira.



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Figure 17.25—Common house geckos Hemidactylus frenatus mating. The hemipenis of one gecko is everted directly into the cloaca of the other gecko.

Approximately 3% of extant birds have external(ish) genitalia. Most of the time, they're tucked away in the cloaca within a pocket, and you don't see them until postcopulation. This is because, unlike many other amniotes, they are erected with lymph and not blood. As you may recall from Chapter 15, the lymphatic circulation operates at a much lower pressure than the blood

circulation. Thus, bird penises are erected into the other bird's vaginal canal. The most basal living birds, paleognaths (e.g., ostriches, emus, tinamous, and rheas), retain them. Given that we also see penises in crocodylians, this suggests that extinct dinosaurs likely did as well. A few neognaths retain them, particularly ducks and a few other fowl. The shape of these penises is variable, from penises that veer to the left (ostriches, kiwis) to highly coiled penises (ducks, tinamous; Figure 17.26).

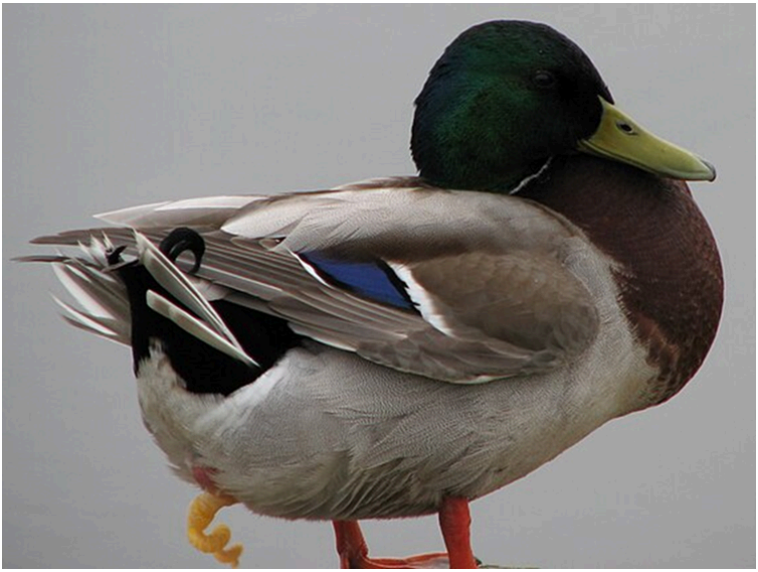

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Figure 17.26—A mallard Anas platyrhynchos with its penis visible.

So why did birds lose their external genitalia? Like for many other bird features, one of the reigning hypotheses is to lighten the body as much as possible for flight. Yet ducks and other fowl are long-distance migrators. Other hypotheses that show promise include minimizing sexually transmitted infections and preventing unwanted copulation. We will return to this when we get to ovary-bearing birds.

If there is no intromittent organ in most extant birds, how does internal reproduction take place? Copulating birds simply line up their cloacas and sperm is transferred into the other bird's cloaca. This behavior is called a "**cloacal kiss**," as fleshy lips surround each bird's cloaca (Figure 17.27).



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Figure 17.27—A pair of spotted doves Spilopelia chinensis mating.

Mammals

So far, we've seen that the testes are located cranially in the coelom in most vertebrates. For most terrestrial mammals, the testes are located externally. This shift is called "the descent of the testes" and occurs during development (Figure 17.28). The testes are attached to a ligament in the scrotum. This ligament, called the **gubernaculum** (this author's favorite anatomical word, for the record), shortens through development, pulling the testes caudally through the **inguinal canal** and finally into the scrotum. The gubernaculum persists in anchoring the testes to the scrotum.

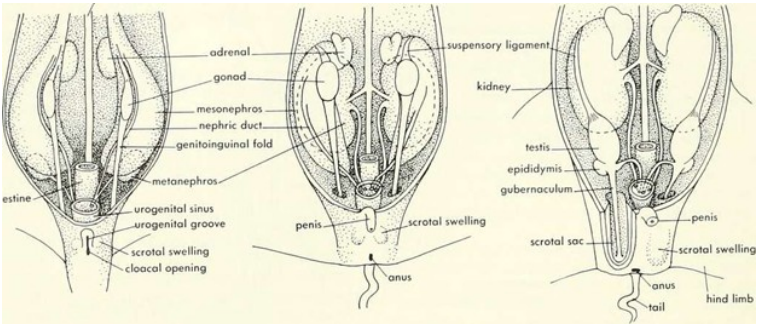

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Figure 17.28—The route of the vas deferens and testes from the coelom to the scrotal swelling in a pig.

There are two main hypotheses for why this change occurred. The first considers temperature control for the developing sperm. Mammals tend to keep their internal body temperature rather high, which may be too high for optimal sperm production. Externally, temperatures are cooler. Some mammals, such as rats and other rodents, can retract their testes and scrotum into their abdominal cavity with the **cremaster muscle**. This same muscle is used to draw the human scrotum closer to the body. The second hypothesis concerns locomotion, specifically galloping. The muscles activated during galloping can produce large fluctuations in abdominal pressure, which in turn could cause some leaking of sperm, since there are no muscular sphincters to keep semen where it belongs. Interestingly, the majority of galloping mammals have external testes. On the other hand, elephants have internal testes (and do not gallop), as do marine mammals. However, they are still somewhat descended. Elephants retain their testes within the abdominal cavity, whereas marine mammals such as pinnipeds and whales send their testes through the inguinal canal but not into a scrotum. It is unlikely that there is a single, one-size-fits-all answer for all mammals.

After sperm leave the testes (Figure 17.1 for a general

schematic), what happens next depends on the mammal in question. Monotremes have a urogenital sinus at the base of the bladder, which receives both urine from ureters (which does eventually end up in the bladder) and sperm from the testes (Figure 17.29). In echidnas, urine leaves the urogenital sinus through the urinary papillae. Semen, on the other hand, leaves through the penis. As such, the penile urethra only carries sperm. Echidna penises have a "**rosette glans**," where the glans is split into two sections, and each of those forms a bifurcated structure called a **rosette**. Each rosette has its own urethral opening and epidermal spines, which means that the urethra splits into four parts as well. When not in use, the penis is stored above the cloaca. Erection causes the penis to pass through a sphincter and then the lumen of the cloaca before exiting. Initially, all four rosettes are erect, but only two of the four rosettes remain erect and ejaculate. Similarly, the platypus has a bifurcated urethra and glans with epidermal spines.

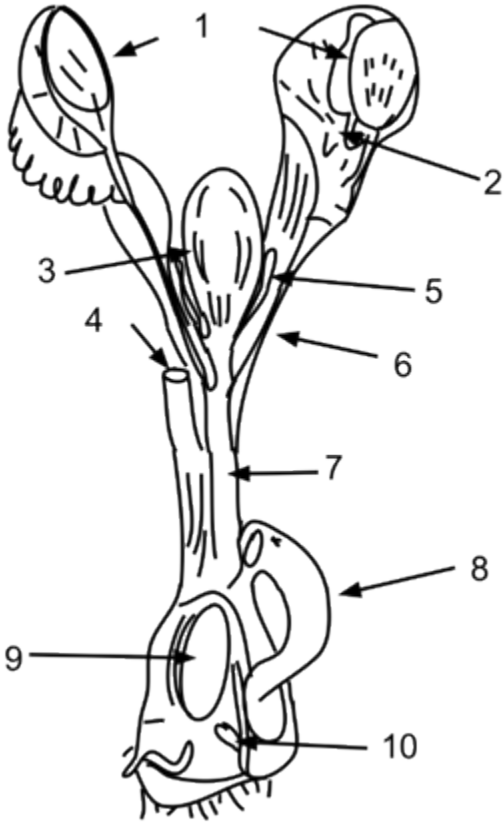

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Figure 17.29—The reproductive system of a testes-bearing platypus: (1) testes; (2) epididymis; (3) bladder; (4) rectum; (5) ureter; (6) vas deferens; (7) urogenital sinus; (8) penis (enclosed in fibrous sheath); (9) cloaca; (10) the opening in the ventral cloacal wall for the penis.

In therians (marsupials and "placental" eutherian mammals), the vas deferens connects to the urethra, which thus

carries both sperm and urine (Figure 17.28). The urethra is centrally located within the penis, which is surrounded by erectile tissue—a paired **corpora spongiosum** and a ventral erectile tissue called the **corpus cavernosum**. Some mammals, such as walrus, raccoons, ground squirrels, and bears, have further support by a **baculum** (os penis; Figure 17.30). These shapes range from a simple long rod (bears, sea lions) to spoon shaped (ground squirrels) or trident shaped (rice rats, voles). The external morphology of therian penises is also quite variable.



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Figure 17.30—A store display of a taxidermized raccoon Procyon lotor holding a bowl of raccoon bacula. People often use raccoon bacula as a "drip tube" to aid in distilling alcohol, as its curved shape is helpful in funneling alcohol into containers.

The surface of the penis may include structures such as

keratinous spines, hooks, flaps, and papillae. The location of these structures is also variable across species, whether they're located on the glans, shaft, or base of the penis. It's not clear what all these structures are for, although spines have been hypothesized to induce ovulation by stimulating the vaginal tract or to aid in sperm competition. Most eutherians have a single glans and shaft; marsupial penises are bifurcated in some species. The latter has been hypothesized to correspond to the ovary-bearing marsupials that have two vaginal openings (see the Mammals section of Ovary-Bearing Gnathostomes).

Ovary-Bearing Gnathostomes

Chondrichthyans

Sharks and their relatives can have either paired ovaries or a single ovary, with the second ovary either completely absent or underdeveloped. A number of sharks belonging to the Galeomorphii, such as hammerheads and bull sharks, have a fully formed right ovary and an underdeveloped left ovary (Figure 17.31). A small number of rays instead retain the left ovary.

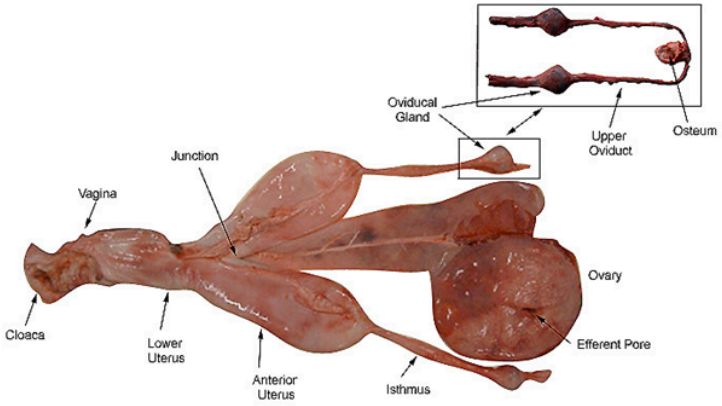

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Figure 17.31—The reproductive system of an ovary-bearing porbeagle shark, Lamna nasus.

As we will see with many other vertebrates (including us!), the ovaries are not directly connected to the oviducts. **Ovulation**, the process of an ovum leaving the ovary, instead dumps the ova into the coelom. However, the funnel-shaped opening of the oviduct, the **ostium**, is right next to the ovary, so the ova are unlikely to go astray. As the oviducts curve away from the ovaries and move posteriorly toward the cloaca, there is an enlarged section in the anterior portion of the oviduct. This is the **shell gland** (sometimes also referred to as the oviducal gland or nidamental gland). This gland is responsible for secreting an egg capsule or membrane over the ovum after the ova is fertilized; it is also sometimes used for sperm storage after copulation. After an egg (fertilized or not) leaves the shell gland, it enters the **uterus**, which is just an enlarged section of the oviduct (as it is in us!). Remember that many sharks and their relatives have two functioning ovaries and thus two functioning oviducts. That means there are also two uteruses. In elasmobranchs, the two uteruses will merge posteriorly, which then leads to the cloaca. In holocephalans (chimeras and ratfish), the

uteruses do not merge before reaching the cloaca and have separate openings.

Whether the embryonic chondrichthyan develops in the uterus or not depends on the species of chondrichthyan. We see some that are **oviparous**—such as horn sharks, cat sharks, and many skates—which means they lay eggs and their embryonic development mostly takes place outside of the parent's body. This is likely the ancestral condition for chondrichthyans, based on some fossilized egg cases from the Devonian period. Other chondrichthyans are **viviparous**, giving "live birth," and this can take many different forms (see Box 17.1). No chondrichthyan is known to care for their offspring once they've left the parent's body.

Box 17.1—The Wonderful World of Chondrichthyan Reproduction

Sharks and their relatives have a fascinating array of strategies in terms of how embryos are nourished during development. We have listed some terms and chondrichthyans to research below. For each example, describe what happens in each strategy. Then as you continue reading this chapter, keep an eye out for similar strategies. Which vertebrates do something similar? Which are unique to chondrichthyans?

1. **Aplacental viviparity** (formerly called ovoviviparity) and the spiny dogfish *Squalus acanthias*
2. **Histotrophy** (a form of matrotrophy) and the round stingray *Urobatis halleri*
3. **Placental viviparity** and the bull shark *Carcharhinus leucas*

4. **Intrauterine cannibalism** and the sand tiger shark *Carcharias taurus*

Osteichthyes

As we saw in the sperm-producing Osteichthyes, there is a lot of variation in reproductive anatomy in the bony fishes. When we discussed the cyclostomes, we mentioned that some bony fishes (tarpons, moray eels, and arowanas) do not have oviducts to catch those eggs that are ovulated into the coelom; instead, they utilize genital pores. Ova then enter the urogenital sinus and leave through the cloaca. They tend to have solid ovaries. Most teleosts have hollow ovaries, where ovulation releases ova into ovarian cavities that are connected to an **ovarian duct** or gonoduct (Figure 17.32). This tube essentially functions as an oviduct does, catching the ovum and sending it on its path toward leaving the body. Therefore, you will still see it called an oviduct in other papers and books. However, the ovarian duct forms from the dorsal visceral peritoneum and peritoneum lining the ovarian cavity and not the mesonephric epithelial tissue. Thus, the teleost ovarian duct is likely not homologous to the oviduct we see in other vertebrates, and we have chosen not to use "oviduct" for this structure to indicate that. Regardless of whether the ovaries are solid or hollow, bony fishes may have paired ovaries with one larger than the other, partially fused ovaries, or fully fused ovaries.

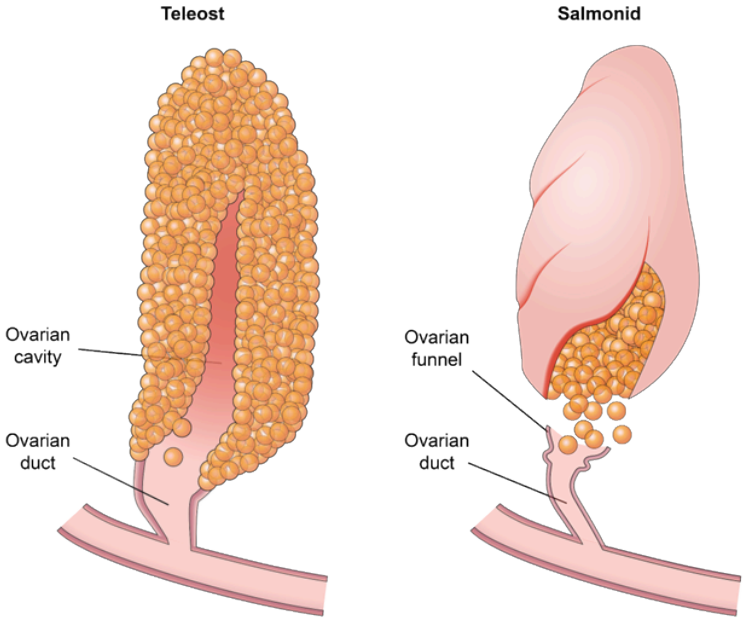

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Figure 17.32—Examples of actinopterygian ovaries.

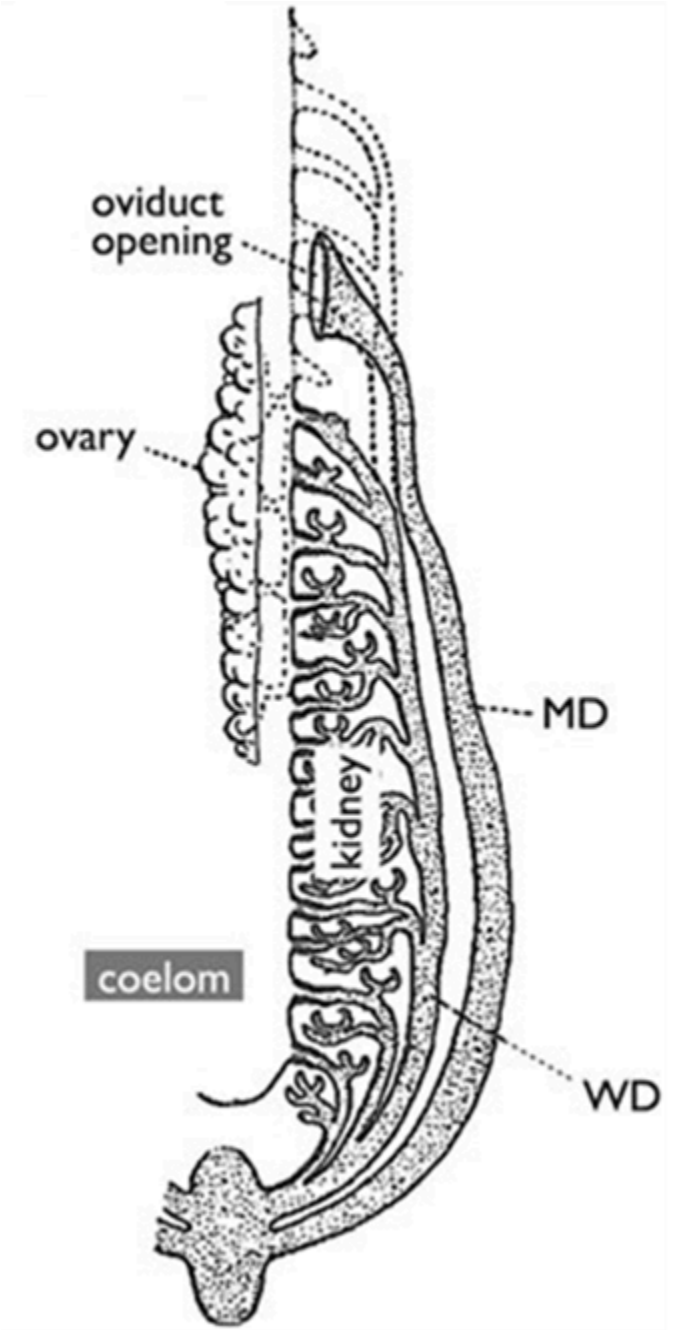
oviduct
opening
ovary
MD
kidney
coelom
WD

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Figure 17.33—A generalized jawed vertebrate urogenital system (adapted from Goodrich [1945], with permission from the Company of Biologists). WD = Wolffian ducts, MD = Müllerian duct.

When we look at tetrapods, lungfishes, and coelacanths, we see that their oviducts are indeed true oviducts, derived from that Müllerian duct (Figure 17.33). There are actinopterygians that have true Müllerian ducts—gar, bowfins (*Amia*), sturgeons, and bichirs all do, which means that some of the Neopterygii (gar, bowfin) have them, while the teleosts (also a subdivision of the Neopterygii) do not. Remember, teleosts are (1) highly derived fishes and (2) not the closest ancestors of the tetrapods. In other words, they have done their own thing when it comes to reproductive anatomy. So where did the gonoducts evolutionarily come from? We don't know! Scientists have been looking at genetics, histology, and of course, anatomy to try to figure this out. It's not clear if they are derived from the genital pores, if they are somehow still homologous with the Müllerian duct, or even whether they are derived from the testicular ducts; these are all hypotheses that scientists are currently sorting through.

Recall that the vast majority of bony fishes are external fertilizers; thus they are oviparous. However, unlike the chondrichthyans, there are several species that practice some sort of parental care. This may include behaviors such as hiding eggs, nest guarding, **mouth brooding** (carrying fertilized eggs in the oral cavity), or something as unusual as the testes-bearing seahorse *Hippocampus* carrying developing eggs in a ventral brood pouch. The egg-producing seahorses deposit their unfertilized eggs into the brood pouch, which is located ventral to the gonopore. Sperm are released above the pouch and make their way into the brood pouch's lumen (cavity) to fertilize the eggs. The new zygotes implant in the brood pouch, which triggers the tissues of the pouch to

change, including increasing vascularization to help regulate osmolality, gas exchange, and nutrient supplementation.

Box 17.2—What Do We Know About Fossil Fishes?

As we've said before, the soft and squishy bits do not fossilize particularly well. So our knowledge of extinct organisms' organ systems beyond the skeletal system is spotty, at best. However, as we will see when we talk about dinosaur reproduction later in this chapter, we can use both direct and indirect evidence to make some hypotheses about reproduction. While we don't have anything to help us figure out what was going on with our ancient jawless fishes, there are some fossils that can help us out with the placoderms. For example, there are some placoderm fossils from Latvia and the US that are clearly juveniles and occur in areas that appear to be potential nursery sites. While there haven't been any egg cases found, the current hypothesis is that these particular placoderms were oviparous. However, other placoderms were internal fertilizers with viviparity. A beautifully preserved placoderm from Australia, aptly named *Materpiscis* (literally, "mother fish"), was preserved with an embryo (Figures 17.34 and 17.35). Soon after, two other genera were also found with preserved embryos inside their bodies. Further evidence for internal fertilization comes from preserved clasper-like pelvic fins for a few genera of placoderms.

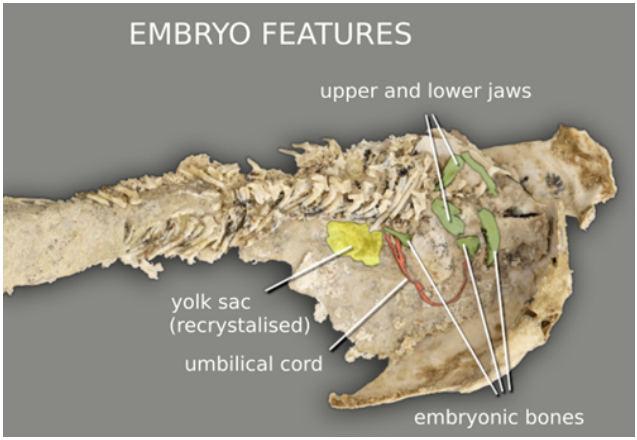

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Figure 17.34—A fossilized embryo of the placoderm Materpiscis attenboroughi.



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Figure 17.35—A reconstruction of the placoderm Materpiscis attenboroughi.

Amphibians

Generally, the anatomy of the egg-producing amphibians is as we would expect: Paired, sac-like ovaries produce ova, which are released into the coelom (Figure 17.36). The ova are picked up by the oviducts, which eventually connect to the cloaca. As we saw in the sperm-producing amphibians, finger-shaped fat bodies lie adjacent to the ovaries and are important for supporting gamete production via the hormones they secrete. The oviducts are divided into three parts:

1. The **pars recta**, also known as the infundibulum, is where the ova enter the oviduct.
2. The **pars convulata**, also known as the ampulla, tends to be full of twists and turns. It is responsible for adding the gelatinous layers to ova via oviductal glands and is the longest portion.
3. The **pars utera**, also known as the uterus or ovisac, is the most posterior part of the oviduct and ends at the cloaca. Ova can be held here until the amphibian is ready to mate. Some amphibians have tubules to store sperm in this portion of the oviduct.

Salamanders can also store spermatophores and fertilized eggs in their cloacal glands.

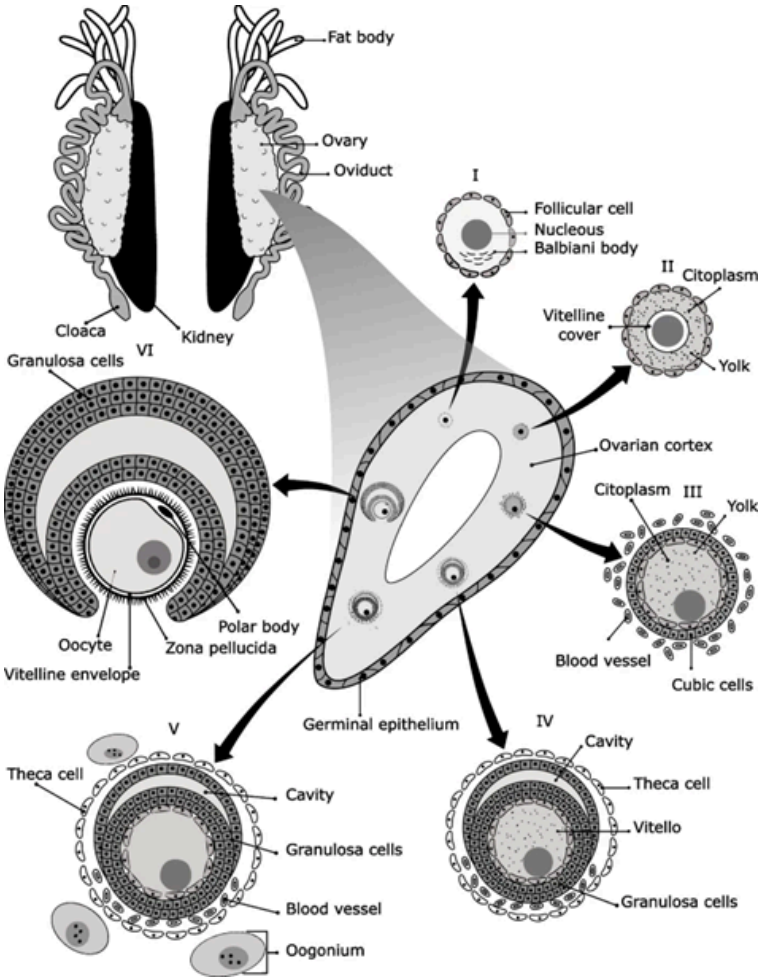

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Figure 17.36—Reproductive anatomy in ovary-bearing frogs. Stages I–VI of follicular development are illustrated.

Terrestrial adults with aquatic breeding habits, which are the likely ancestral mode of life, are oviparous. This includes external fertilizers, like most frogs, which lay their eggs in water and leave them to develop without any parental care. This is probably

the most familiar form of amphibian reproduction—eggs are laid in an aquatic environment where larvae (such as tadpoles) hatch and later metamorphose into adults. Aquatic adults tend to only have aquatic breeding habits and follow a similar plan. However, amphibians are not limited to this reproductive strategy! Terrestrial adults with terrestrial breeding habits might be oviparous but do not lay eggs in water. Instead, they may lay fewer, larger eggs to protect against desiccation (Figure 17.37). These amphibians, roughly half of all salamander species and one-third of frog and caecilian species, have no larval stage and just hatch looking like miniadults.



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Figure 17.37—A Cheat Mountain salamander Plethodon nettingi guarding its eggs.

Other terrestrial breeders are viviparous. Viviparous caecilians are particularly interesting. They retain fertilized eggs in **pars utera**. Once the eggs hatch, the larvae need to eat, and the only food available is the tissue lining the pars utera. While that may be an extreme example of parental care, roughly 75% of the amphibian

families have evolved some sort of parental care including nest building, egg tending, and egg brooding in various anatomical parts (including dorsal sacs, vocal sacs, and stomachs). In amphibians with external fertilization, parental care most often comes from the sperm-producing sex. The egg-producing amphibians tend to provide parental care if they use internal fertilization.

Amniotes

Again, we remind you of our big evolutionary trend—the shift from aquatic to terrestrial habitats—and that reproduction also had to change to accommodate that shift. However, one particular major evolutionary innovation helped vertebrates completely divorce themselves from relying on the aquatic realm for reproduction: the **amniotic egg** (also known as the cleidoic egg; Figure 17.38). This is also one of the synapomorphies for amniotes and the feature that gives amniotes their name.

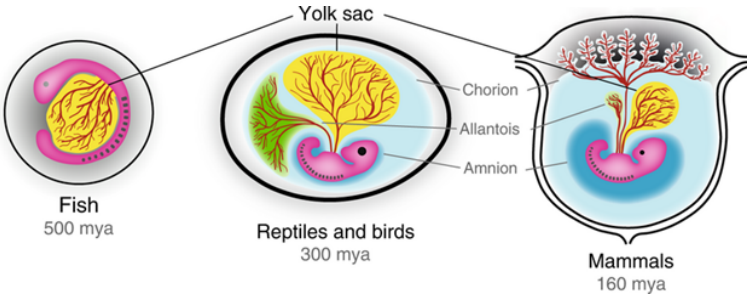

Figure 17.38—Extraembryonic membranes in fish, birds and mammals. MYA = million years ago.

Fish and amphibian eggs do share some features with amniotic eggs. The parts of the egg that we are interested in are called **extraembryonic membranes**—"extra" meaning "outside," so

these are things that are not the embryo itself. The **yolk sac**, which contains the yolk, is an extraembryonic membrane that we see in most vertebrate eggs. Because fish and our ancestral amphibian eggs were laid in water, the embryos could stay hydrated, and gas exchange and waste removal could easily happen in this aqueous medium. However, a move to land meant that eggs could dry out and waste had nowhere to go. A more watertight shell and three new extraembryonic membranes aided this transition to land. The **chorion** and **allantois** (Greek for "sausage," as it looks when it first develops) are both derived from the ectoderm and mesoderm, and in many amniotes, they eventually merge to store waste and participate in gas exchange (sometimes referred to as the **chorioallantois**). The **amnion** not only produces **amniotic fluid**, which helps prevent desiccation, but it also contains contractile fibers that can move the embryos early in development to ensure they do not adhere to any of the extraembryonic membranes. We will revisit these membranes again once we get to ovary-bearing mammals.

We also see another new reproductive feature in the ovary-bearing amniotes, the **clitoris** (Figure 17.39). This structure is homologous to the penis of testes-bearing amniotes. During development, there are three paired structures. The **anterior** and **posterior cloacal swellings**, as the name suggests, give rise to the cloaca. The third structure, the genital tubules, forms either a penis or clitoris; the fate is determined by the relative amounts of testosterone and estrogen. Structurally, the two organs are also similar.

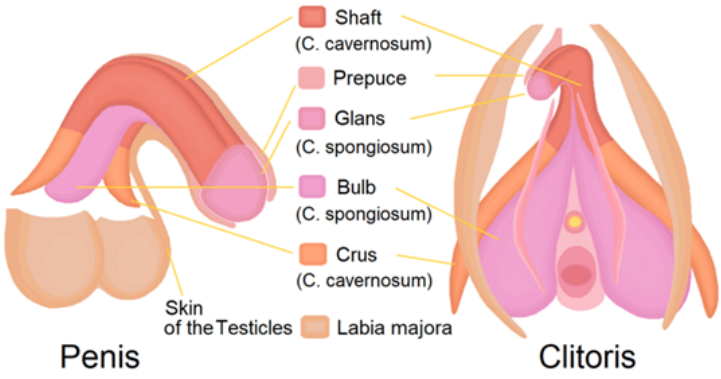

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Figure 17.39—The penis and clitoris are homologous structures. Corresponding parts are labeled.

Adult crocodylians and turtles have a well-developed clitoris, and they have corresponding tissue to penises. The clitoris has been studied in some mammals, such as various primates, rodents, and bottlenose dolphins (see Box 16.3 for more!). Just as testis-bearing snakes and lizards have hemipenes, ovary-bearing snakes and lizards have a **hemiclitoris**. Given the homology between the clitoris and penis and that most extant testes-bearing birds have either a reduced or absent penis, it probably isn't surprising that a bird clitoris has only been found early in development and not in adults. However, the function of this organ and why it is retained is not well studied. Some hypothesize that it functions primarily for sexual pleasure, which is difficult to study in animals. However, we also know that in some mammals such as rats, cattle, and pigs, stimulation of the clitoris during copulation can lead to increased pregnancy rates. Other hypotheses include that the clitoris serves to increase vaginal lubrication and to prepare the reproductive organs for sperm.

Reptiles, Including Aves

Reptilian oviducts are often divided into five sections: The ovum enters the **infundibulum**, **uterine tube** (also known as the **magnum** in birds, as it's the largest section of the oviduct), **isthmus**, uterus (also called a shell gland in birds), and **vagina** (where the egg will leave the oviduct; Figure 17.40). There is a considerable amount of variation in how well differentiated these five regions are. For example, many squamates (lizards and snakes) have infundibula and uterine tubes that are not particularly different from each other. On the other hand, not only do the extant archosaurs (crocodylians and birds) have well-differentiated sections, but the infundibulum and uterus are further subdivided into anterior and posterior portions that have different functions. While it is clear that the oviduct as a whole is homologous across reptiles, the individual sections may not be.

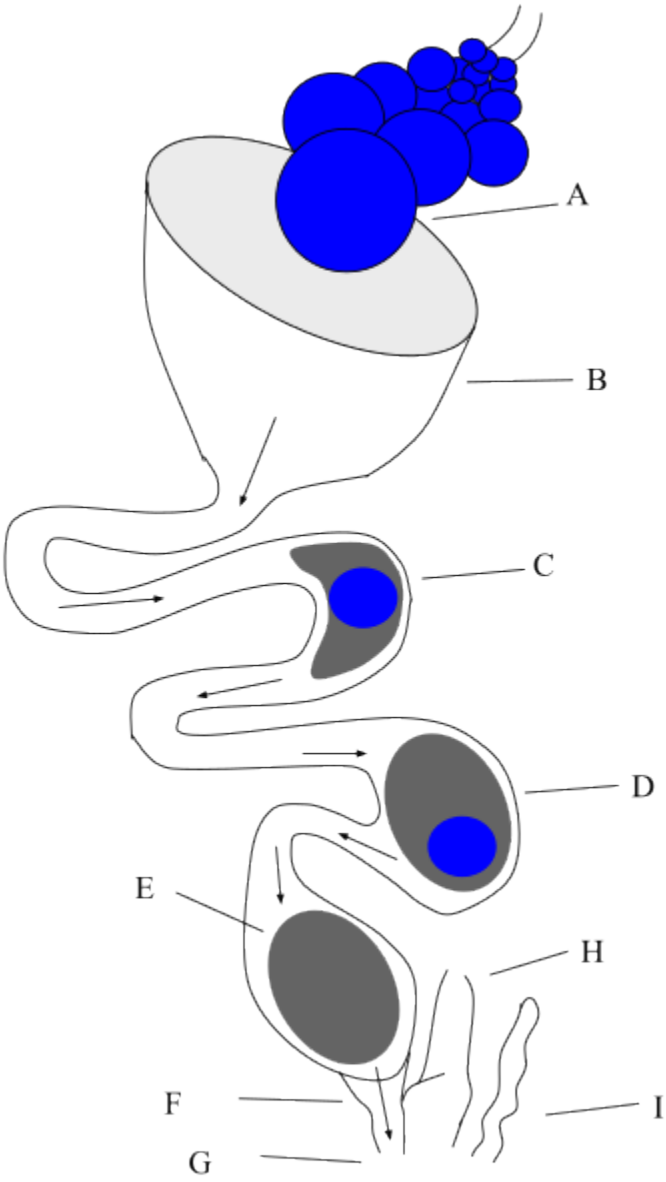

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Figure 17.40—The reproductive system of an ovary-bearing chicken: (A) left ovary; (B) infundibulum; (C) magnum; (D) isthmus; (E) uterus; (F) vagina; (G) cloaca; (H) large intestine; (I) rudiment of right oviduct.

In turtles, crocodylians, and birds, the uterine tube / magnum is responsible for forming much of the **albumen** (what we know as "egg whites" in our kitchens). However, albumen-producing glands have not been found in squamate oviducts at all, and squamate eggs don't contain much of this fluid either. The isthmus is also quite variable across reptiles in terms of what tissues and glands it's composed of. As such, it's not clear if this section is homologous across all reptiles. For example, we know that in birds, the inner and outer shell membranes are formed on the egg here. In crocodylians, the anterior portion of the uterus is structurally and functionally similar to the isthmus of birds, producing those membranes. Similarly, the posterior portion of the crocodilian uterus lays down the eggshell, and the section immediately prior to the vagina in birds (often called the shell gland) does the same. These are likely also homologous with each other. There are also sperm storage tubules located at the junction between the vagina and posterior uterus in both birds and crocodylians. Turkeys can store their sperm for up to 10 weeks! Note that crocodylians and birds are all oviparous. We will come back to this.

In squamates and turtles, the uterus does something different. For oviparous squamates (and all turtles are oviparous), all layers of the eggshell including their membranes are formed in the uterus. Viviparity has evolved independently in various squamate groups over 100 times, and uteri of viviparous species have incubation chambers and do not make eggshell material. Note that for all our oviparous reptiles, including birds, fertilization of the ovum has to happen prior to the eggshell being laid down. Viviparous squamates utilize a **placenta** to feed their embryos, just as many mammals do. However, with the many independent origins of viviparity in squamates, we get six different types of placentas made of different tissues. All squamate placentas are composed of a combination of extraembryonic membranes from the embryo and uterine epithelium from the parent. The variation comes from which extraembryonic membranes are integrated into the placenta. Most

often it's both the chorion and allantois, but some placentas use the yolk sac or other single membranes.

Bird vaginas are wildly diverse in morphology (Figure 17.41). They can be a simple folded tube, or they can be full of spirals with blind pouches. Waterfowl are particularly well studied, as there is a lot of variation in vaginal morphology across different species. It seems that the complexity of vaginal morphology is correlated with the commonality of forced extrapair (outside of a mated partner) copulation within a species as well as penile shape. For example, African geese *Anser sygnoides* do not practice forced copulation and have short penises; correspondingly, vaginal tracts are relatively simple and straight. On the other hand, mallards *Anas platyrhynchos* frequently force ovary-bearing mallards into copulation. Their vaginas are full of blind pouches and are quite convoluted. Studies have shown that successful fertilization is drastically lower in waterfowl subjected to forced copulation by nonmates, and the explosive penile eversion we saw earlier in the chapter is actually stopped by those vaginal twists and turns.

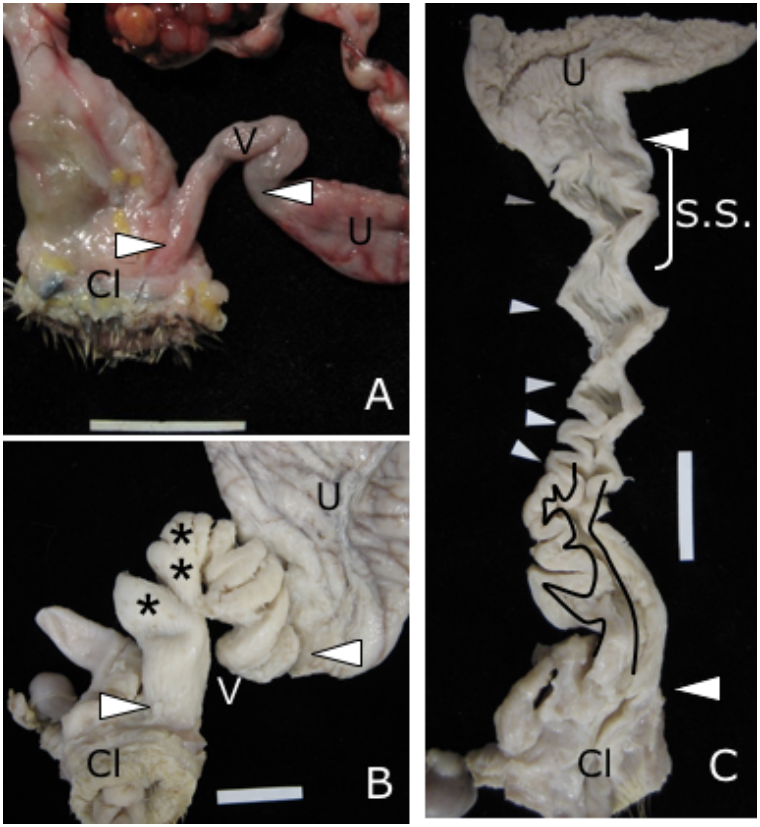

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Figure 17.41—Variation in avian vagina morphology. (A) *Tubular vagina from a pheasant Phasianus colchicus*; (B *and C) Complex vagina of Pekin duck Anas platyrhynchos.* V = *vagina*, *Cl = cloaca*, *white arrow = spirals*, *S.S. = sperm storage tubules location*, * = *pockets.*

Squamates, turtles, and crocodylians have paired ovaries and oviducts. However, squamates also often show some asymmetry. In lizards, it's just slight, but in many snakes, the right oviduct is much longer than the left (Figure 17.42). In some squamates, the left oviduct is either vestigial or lost completely.

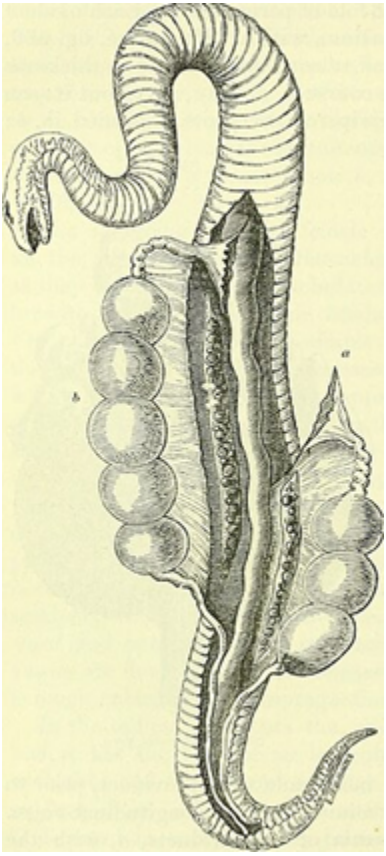

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Figure 17.42—The right and left oviducts of a viper. Note that the right oviduct, which is full of eggs, is longer than the left.

On the other hand, many extant birds only develop a functional left ovary and oviduct (Figure 17.40). The right oviduct and ovary develop early in development but quickly regress thanks to the secretion of AMH by the ovaries, which triggers apoptosis (cell death). The left ovary produces enough estrogen to protect itself from the effects of AMH. Again, this has been hypothesized

to have something to do with making the body lighter for flight. However, we have to be careful not to generalize this to all birds! We know the most about birds that have been domesticated—chickens, geese, and ducks. The brown kiwi, some sparrows, the occasional pigeon, and some falcons retain both the right and left ovaries, although they are often asymmetrical. Note that all these birds except the kiwi are excellent fliers.

The oviducts, regardless of whether there are one or two, each open into the cloaca. This is also where we find the clitoris (or hemiclitoris, in the case of our squamates). Crocodilian clitorises lie ventrally in the cloaca and have the same general parts as crocodilian penises: paired crurae, a shaft, and a glans. Lizards have evertable hemiclitorises, much like lizard hemipenes. Like hemipenes, they lie caudal of the cloaca and have a sulcus and similar eversion/retraction mechanisms. Unlike some lizard hemipenes, there is no cartilaginous "bone." Snake hemiclitorises are understudied at best, and until recently scientists weren't sure whether they had one or not. We do know that they cannot be everted but do have erectile tissue that can be filled with blood.

We previously mentioned that all extant birds are oviparous, as are the other extant archosaurs (crocodylians). Does this mean that we should expect the nonavian dinosaurs to be oviparous as well? Probably! We know that there are fantastic fossils of dinosaur eggs, some including embryos in the eggs of theropods (carnivores), sauropods (long-necked), and hadrosaurs ("duck-billed"). What about the reproductive tracts? Birds and crocodylians have very different ones. Soft tissues rarely fossilize, so there are very few fossilized ovaries and oviducts in any bird, nonavian dinosaur, or reptile. However, evidence from fossilized nesting behavior and egg arrangement within dinosaur nests give us some clues. Unless the dinosaurs are moving their eggs within the nest in particular patterns, the pattern that eggs are arranged in the nest should reflect where they were oviposited. Some patterns are more likely to occur with two functional oviducts and others with one. Coupled with eggshell structure and particular types of egg

pathologies, and comparing those to our extant archosaurs, it seems that sauropods may have had two functional oviducts, while theropods that are more closely related to birds likely only had one.

Mammals

As we should expect by now, much of the reproductive tract in ovary-bearing mammals is the same as other amniotes. Ovaries come in many shapes and sizes, and they vary with life stage. For example, an adult blue whale's ovary weighs 30 kg while pregnant but a mere 7 kg when not pregnant. Mammals tend to have paired ovaries, although in the platypus (a monotreme), only the left is functional (Figure 17.43). Meanwhile, the other extant monotremes, the echidnas, have two functional ovaries.

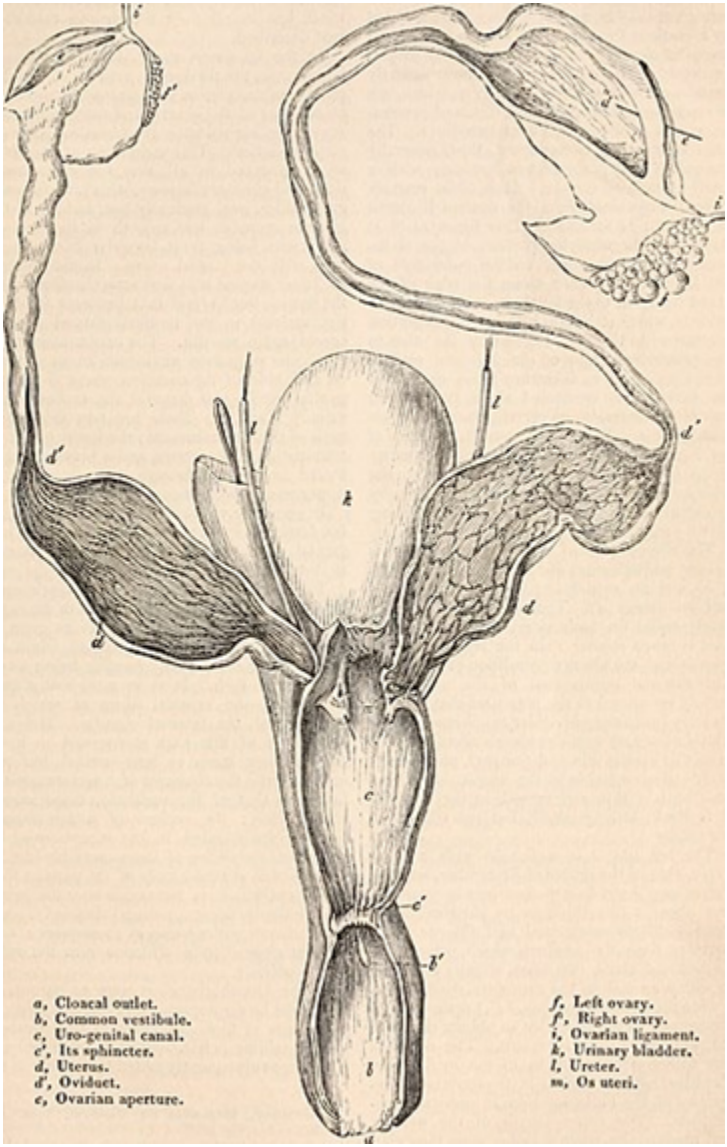

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Figure 17.43—The reproductive tract of an ovary-bearing platypus. (a) Cloacal outlet, (b) Common vestibule, (c) Urogenital canal, (c′) Its sphincter (cervix), (d) Uterus, (d′) Oviduct, (e) Ovarian aperture, (f) Left ovary, (f′) Right ovary, (i) Ovarian ligament, (k) Urinary bladder, (i) Ureter, (m) Os uteri (part of the cervix).

Mammalian oviducts are generally divided into four regions (but hold that thought until you get to the next paragraph). Ova enter the oviduct at the infundibulum, then travel to the ampulla, isthmus, and **utero-tubal junction** (the border between the uterus and oviduct). The ampulla and isthmus are not always differentiated, and in humans, fertilization tends to happen in the ampulla. The oviduct is usually folded into **rugae**, especially near the utero-tubal junction; those rugae sometimes are used for sperm storage. Oviducts also come in many different shapes, including looped (bears and squirrels), coiled (pigs), bent (kangaroo rats, mink, pocket gophers), and straight (rabbits and humans).

Note that the uterus was left out of the oviduct divisions described above. The uterus is, as we've seen in other vertebrates, the fifth division of the oviduct. Yet it's often treated as a separate organ completely. The rationale here is not clear—perhaps this is a holdover from naming organs and parts in humans without understanding the evolutionary history. Or perhaps this is because the uterus is where embryos develop in therian mammals, so it warrants its own consideration. Regardless, at this point in the chapter, you know that the uterus is indeed homologous to the uteri we've seen before, and as such it is part of the oviduct.

The uterus is more than a site of embryonic development in therians. It's an organ that is active in just about everything happening with that embryo, from communication with other organs, to placenta formation (which we'll get to later), to expelling both the fetus and placenta, and even maintaining microbial health. Like the other parts of the oviduct, there's a lot of variation in uterine shape (Figure 17.44). Some mammals, such as primates and nine-banded armadillos, have a **simplex uterus**, where the halves are totally fused and there is only one **cervix**, the muscular boundary between the uterus and vagina. Other mammals have a **duplex uterus**, where the right and left uteri are totally separate, and each has their own cervix. This includes rabbits, rats, monotremes, and metatherians. We also see something in between, a **bicornate** (biconvex) uterus, where the right and left halves are

partially fused. Where that fusion happens varies. Most commonly it's the internal lumen, and we see that in ungulates of all sorts, cetaceans, and carnivorans. But wildebeest have separate lumens, two separate cervical canals, and then just one cervix. And narwhals and xenarthrans have no cervix at all.

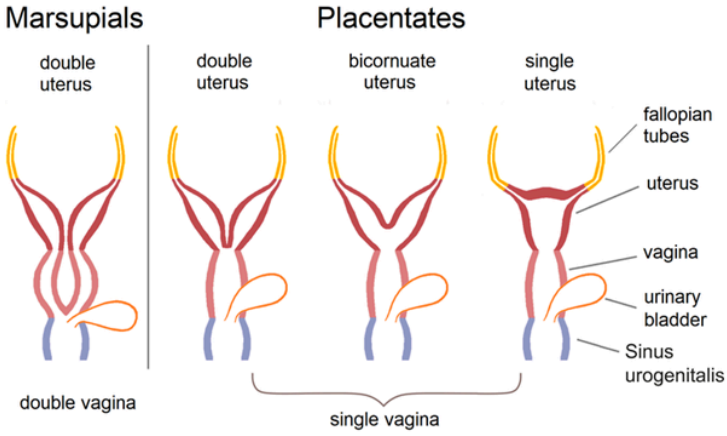

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Figure 17.44—Simplified schematics of therian reproductive tracts. Note that "Fallopian tubes" is the same as "oviducts," and the sinus urogenitalis (urogenital sinus) is only present during development in most eutherian (placental) mammals.

After we leave the cervix and start heading out of the reproductive tract, there is usually a vagina that contains the same layers as the uterus except for an endometrium. However, monotremes do not have a vagina, and there is considerable variation in terms of what embryonic tissues contribute to which portions of the vaginal canal across mammals. Vaginas come in a lot of lengths, shapes, and sizes, although not as extreme as some of the birds we previously learned about. Metatherians have two separate vaginas to go with their duplex uteri with separate cervixes, with

the exception of opossums that have two cervixes with three vaginas.

What happens next depends on which mammal you're looking at. Some mammals—including the monotremes, tenrecs, porcupines, and pikas—retain a cloaca. Other mammals keep only a portion of the cloaca, called the urogenital sinus, a common opening for urinary and reproductive tracts. This opening is separated from the anus by a **urorectal septum**. This is seen in a wide range of mammals, including hippos, xenarthrans (anteaters, sloths, armadillos), rabbits, marsupials, and elephants. Still other mammals, including the primates, have three completely separate openings—the anus, the **urethral meatus**, and the **vaginal vestibule** (vestibulum).

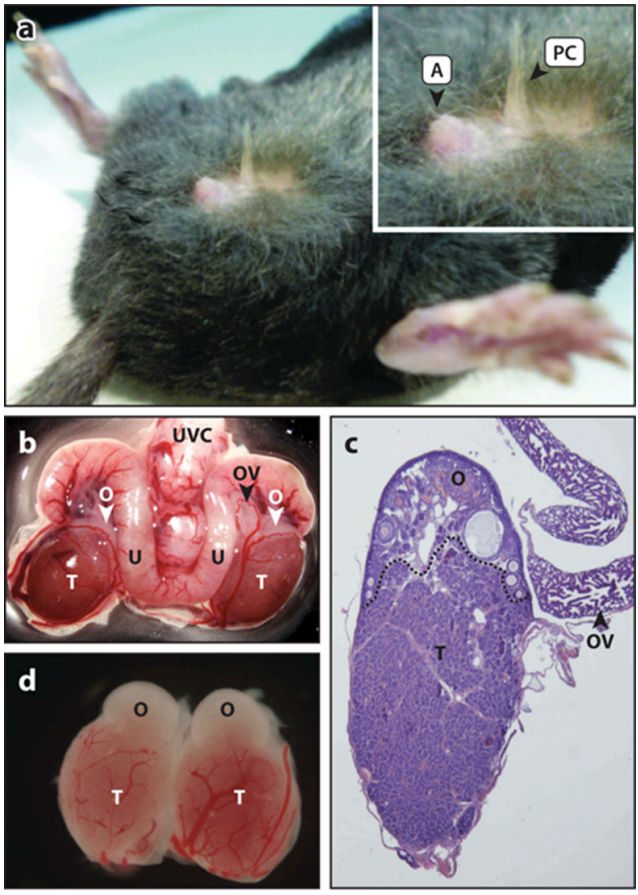
Most mammals also have a clitoris, and we see just as much variation here as we have seen in mammalian penises (Figure 17.39). Most mammal clitoral erectile tissues are composed of paired corpora cavernosa and a **corpus clitoridis** that lies ventral to the urethra. Some mammals also have paired or fused **crurae**, which are structures that provide attachment to muscles that run to the pelvis. Much of the clitoral structure is internal and not externally visible (although see Box 17.3), but that does not mean it is a small organ. For example, elephants can have a clitoris up to 37 cm long! The external portion of the **corpus clitoridis** (also called the glans clitoris) varies in position. Sometimes it is in or on the rim of the vestibulum; other times it may be in the lumen of the urogenital sinus. Some mammals also have a bone or cartilage called the **baubellum** (or os clitoridis), similar to the baculum.

Box 17.3—Blurred Lines: Spotted Hyenas and Talpid Moles

At the beginning of this chapter, we discussed how

sex is often assigned based on chromosomes or, in the case of humans, at birth based on the morphology of the external genitalia. You also just read about the general anatomy of ovary-bearing mammals. Just as we saw in other vertebrate groups, there is a lot of variation in anatomy across species (and within species!), and mammals are no exception. There are several mammal species where the ovary-bearing sex has external genitalia that more closely resemble that of the testes-bearing sex. This is often an enlarged clitoris, as we see in spider monkeys, bonobos, elephants, and various viverrids (civets, genets). Both spotted hyenas *Crocuta crocuta* and talpid (true) moles have both penis-like external genitalia and territorial, aggressive behavior. As such, they've been very well studied.

Moles have an elongated clitoris that encloses the urethra, and a **prepuce** that covers the urethral opening (Figure 17.45). The vaginal opening is separate and stays firmly shut unless it is breeding season. Interestingly, the moles have **ovotestes** instead of ovaries! These gonads are composed of both testicular (medullary) and ovary (cortical) tissue. The testicular tissue cannot make sperm, but it does make testosterone and has an underdeveloped epididymis. The rest of the reproductive tract is typical of ovary-bearing mammals, complete with oviducts, a bicornate uterus, and a uterovaginal canal.



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Annu. Rev. Anim. Biosci. 11:141–62

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Figure 17.45—Anatomy of ovary-bearing moles. (a) External genitalia of an adult Talpa occidentalis. The inset shows the anus (A) *and the peniform clitoral prepuce* (PC; *reproduced with*

permission from Carmona et al., 2008). (b) Reproductive tract of an adult Iberian mole showing the uterovaginal canal (UVC), uterine cornua (U), oviducts (OV), and two ovotestes composed of a large mass of testicular tissue (T) and a small portion of normal ovarian tissue (O). (c) Haematoxylin- and eosin-stained histological longitudinal section of an ovotestis from an adult mole (breeding season) showing a testicular portion (T) containing abundant Leydig cells and the ovarian portion (O) with maturing follicles. The adjacent structure is the oviduct (OV). (d) Ovotestes from a juvenile showing profuse vasculature in the testicular portion (T) but not in the ovarian one (O). Panels b–d adapted with permission from Jiménez et al., 2023.

Ovary-bearing spotted hyenas have an entirely different setup (Figures 17.46 and 17.47). Unlike moles, their clitoris (often referred to as a **pseudopenis**) can actually be erected. Hyenas also have a **pseudoscrotum** formed by the fusion of the urogenital folds—in many other mammals, these form the **labia**. In what may seem like a throwback to other vertebrates, hyenas have a urogenital sinus that opens at the tip of the clitoris. There is no separate vaginal opening. That means copulation, urination, and birth all happen through the same opening. The internal reproductive organs are fairly typical of mammals: two ovaries, two oviducts, a bicornate uterus, and a vaginal canal. However, the vaginal canal makes a hairpin, 180-degree turn such that successful birth of pups is very difficult.



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Figure 17.46—A spotted hyena with a pseudopenis.

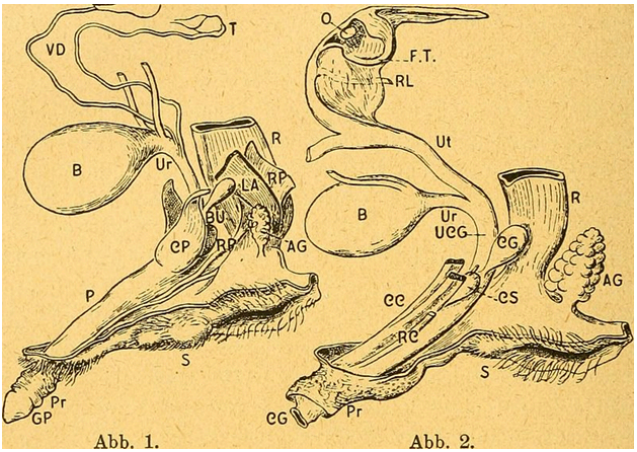

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Figure 17.47—Diagram of the reproductive tracts of a testes-bearing spotted hyena (left) and an ovary-bearing spotted hyena (right). AG: *anal gland*; B: *urinary bladder*; BU: *bulbus urethrae*; CC: *corpus clitoridis*; CG: *Cowper's gland*; CP: *corpus penis*; CS: *corpus spongiosum*; F.T.: *Fallopian tube (oviduct)*; GC: *glans clitoridis*; GP: *glans penis*; LA: *musculus levator ani*; O: *ovary*; P: *penis*; Pr: *praeputium*; R: *rectum*; RC: *musculus retractor clitoridis*; RL: *ligamentum uteri*; RP: *musculus retractor penis*; S: *scrotum*; T: *testis*; UCG: *urogenital canal*; Ur: *urethra*; Ut: *uterus*; Vd: *vas deferens.*

As biologists have studied these two mammals, the inevitable question is, Why did this evolve? We tend to be skewed toward assuming that everything is an adaptation—a feature that was favored by natural selection for its current function. However, there are other possible explanations. Perhaps this is an **exaptation**, which is a feature that evolved for a particular use but is now used for something else. Or

this could be a **nonadaptation**, in which the feature was not favored by natural selection at all. Stephen Jay Gould considered these concepts in his 1979 paper "The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme."

For either our moles or spotted hyenas, pose a hypothesis that explains why these particular reproductive features evolved for each of our three concepts (adaptation, exaptation, and nonadaptation). How would you test each of your three hypotheses?

As you've now read earlier in this book, we divide our mammals up based on reproductive strategy. Monotremes, whose extant forms only include a few species of echidna and the platypus *Ornithorhynchus anatinus*, are oviparous. Theria includes the eutherians (placentals) and metatherians (marsupials), and both groups are viviparous. The term *placentals* for the Eutheria incorrectly implies that other mammals do not have placentas. In fact, while monotremes do not, marsupials do!

Placentas are pretty interesting organs. In mammals, they have a variety of functions, including nutrient transfer, immune system transfer, in utero communication, waste exchange, and gas exchange. As we saw in our viviparous squamates, both the embryo and the parent gestating said embryo contribute to the development of this organ. However, mammalian and squamate placentas are not homologous—the mammalian placenta arose independently from squamates, as squamates are diapsids and mammals are derived from synapsid reptiles. In mammalian placentas, the gestating parent's endometrium contributes to the structure of the placenta, and for the embryo, all four extraembryonic membranes are involved. The chorion fuses with

either the yolk sac (**choriovitelline placenta**, as "vitelline" means "yolk sac") or the allantois (**chorioallantoic placenta**). In both eutherians and metatherians, the choriovitelline placenta is what is initially formed, and it persists to be the functional, final placenta in metatherians. In eutherians, the final placenta ends up as chorioallantoic (Figure 17.48). How much these extraembryonic membranes integrate with the parental endometrium is variable, ranging from just a little to enough integration that the parent's blood vessels are eroded enough that parental blood ends up accumulating within the chorioallantois.

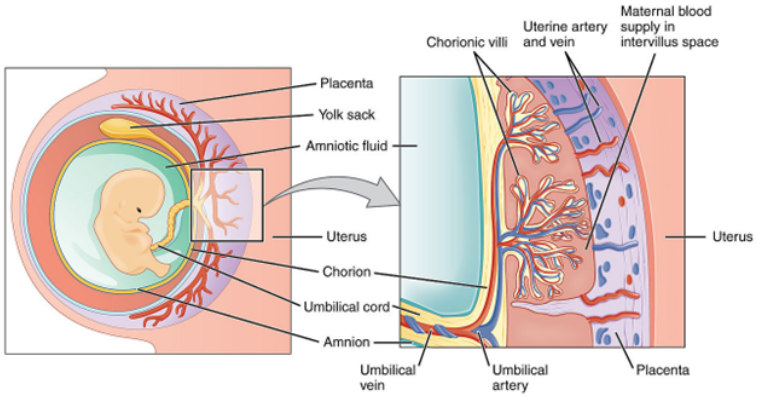

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Figure 17.48—Structure of the human placenta, showing the contributions of the fetus and the parent.

Marsupials are also known to have a short intrauterine gestation period (2–5 weeks), followed by young making their way to the marsupium (pouch) when present. It has been hypothesized that perhaps the choriovitelline placenta is less efficient than the chorioallantoic placenta, necessitating a quick exit from the uterus. However, that does not seem to be the case. First, there are rodents that have 3- to 4-week gestation periods. Second, if we focus on the **neonates** (recently born), we see wonderful adaptations associated with **lactation** (milk production), including highly developed

olfaction and very functional forelimbs. Both of these allow the neonates to make their way from the vestibulum up to the mammary glands without parental help. It's also worth remembering that the most recent common ancestor of metatherians and eutherians was sometime in the Jurassic or Early Cretaceous period. Our extant marsupials are pretty darn derived and shouldn't be considered "primitive" to eutherians.

Speaking of lactation, this is something shared across mammalian species and is considered a synapomorphy. **Mammary glands** are composed of milk-secreting cells that line spaces called **alveoli** (not to be confused with those alveoli in your lungs). Alveoli are grouped together into **lobules**, and muscle fibers squeeze the alveoli to send milk to **collecting ducts** (Figure 17.49). These ducts send milk to pores (in monotremes), to nipples, or to storage organs called **teats** (think of cows and their udders). The number and placement of mammary glands vary across mammals, but they tend to occur in pairs. Unfortunately, mammary glands are soft tissues that do not fossilize particularly well. Thus, paleontologists use associated skeletal elements to indicate when this feature may have evolved. Specifically, we can look at the evolution of the secondary hard palate, which is associated with creating suction, and the development of deciduous (primary, baby) teeth. We see these for the first time in the Late Triassic and Early Jurassic stem mammal *Morganucodon*.

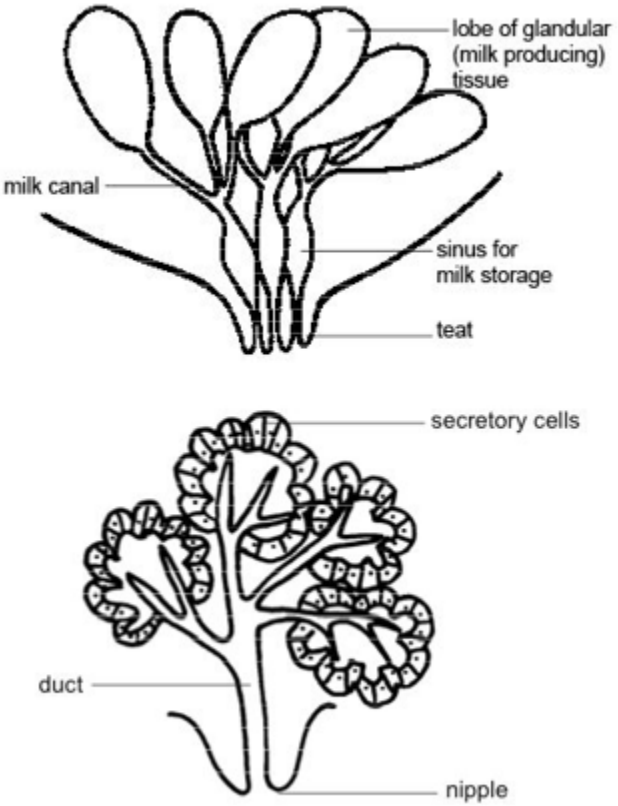

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Figure 17.49—Mammary gland structure. Top: mammary gland in a mammal with teats; Bottom: mammary gland in a mammal with nipples.

Let's return to this idea of metatherians as lactation specialists. Extant marsupials make two types of milk. The first is called teat attachment milk, which is for when the neonates are attached to the teat 24 hours per day, starting when they first emerge from the vagina. The second type of milk is called

intermittent sucking milk, for when the neonates are not constantly attached to the teat and may even leave the marsupium. Kangaroos can make both kinds of milk at the same time but at different teats. This isn't something that eutherians can do.

17.5 Humans

Truthfully, human reproductive systems are not very different from those of other eutherians. As with many other systems, some components have special names because the Western scientists who named them did not understand their homology to other organisms' anatomy (such as Fallopian tubes, which are just oviducts). Thus, ovaries shed their eggs into the coelom, where the fingerlike fimbrae of the oviduct are ready to sweep the egg into the tube. Like sloths, some bats, and our fellow primates, humans have a simplex uterus with a cervix at the boundary between the uterus and vagina (Figure 17.50). The cervix is composed of a ring of cartilage encased in tissue. Humans have a straight, rugose vagina composed of connective tissue, muscle, nerves, and blood vessels. Several glands, such as the Bartholin's glands and lesser vestibular glands, secrete mucus that serves to keep the vagina moist to support the extensive microbiota and to protect the vagina from trauma.

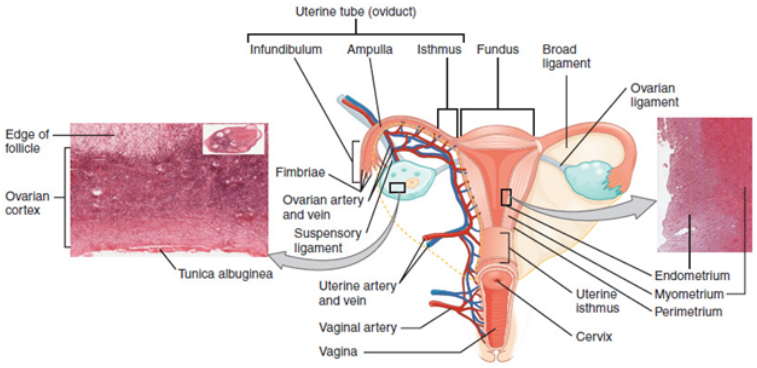

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Figure 17.50—Anterior view of the human uterus and associated organs. From left to right, LM × 400, LM × 20. (Micrographs provided by the Regents of University of Michigan Medical School © 2012)

Collectively, the external genitalia of ovary-bearing mammals are called the **vulva** (Figure 17.51). In humans, the external opening to the vagina is enclosed in folds of skin called the **labia** (singular: labium); there are two sets of folds, the **labia majora** and **labia minorum**. The labia also enclose the visible portion of the clitoris. Also included in the external genitalia category are the **mons pubis**, a pad of fatty tissue that sits over the pubis, and a **hymen**, which is a thin membrane that may partially cover the vaginal entrance. Ovary-bearing humans also have a pair of **greater vestibular glands** (also known as Bartholin's glands), which are homologous to bulbourethral glands in testes-bearing humans; these glands are located lateral to the vaginal opening and similarly produce a mucous-like secretion that acts as a lubricant. The **lesser vestibular glands** (also known as Skene's glands) are homologous to the prostate gland found in testes-bearing mammals. The function of these glands is less clear; it is hypothesized that they also produce ejaculate and act as an antimicrobial. We did not mention these glands previously, as they are very poorly studied in any mammalian taxa.

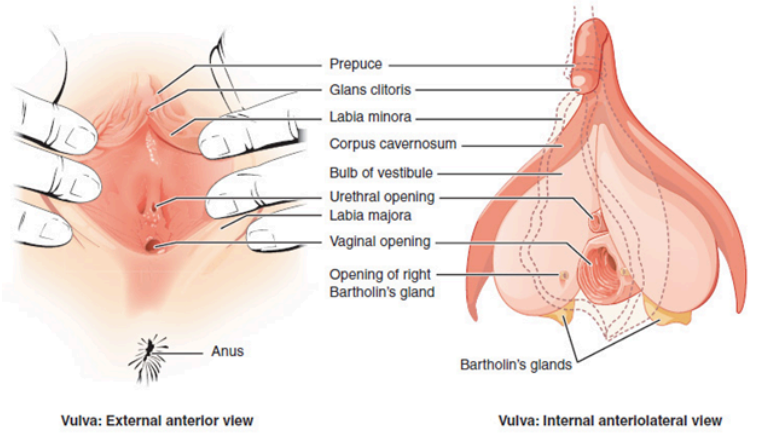

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Figure 17.51—The external genitalia of ovary-bearing humans.

Many ovary-bearing humans menstruate (**eumenorrhea**) during a portion of their lifetimes, shedding the endometrium and growing a new one in 21- to 50-day cycles—the 28-day cycle is a statistical average, but few people actually have a cycle of this exact length, and it can vary within one's lifetime. The shed endometrium passes through the cervix and vagina and then out. Again, humans are not unique here, as eumenorrhea occurs in primates, hedgehogs, carnivorans, quolls, and some groups of bats.

For sperm-producing humans, per usual, we start with the testes, which are encased in an external scrotum (Figure 17.52). Sperm move from the seminiferous tubules to the rete testes, a network of tubules, and then the efferent ductules, and then to the epididymis. Sperm are stored in the epididymis until ejaculation, when smooth muscle contractions push the sperm into the vas deferens.

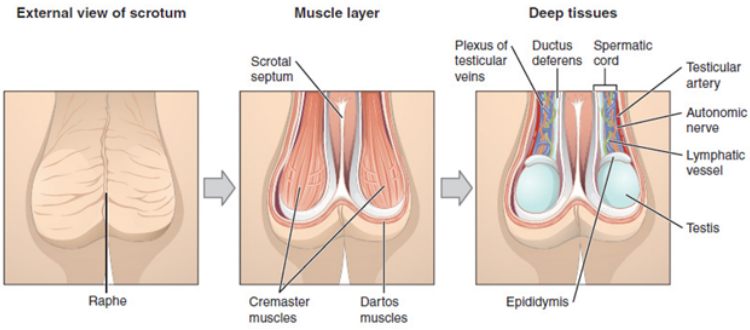

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Figure 17.52—The anatomy of the human scrotum and testes.

Remember what we learned from the descent of the testes—that those testes were pulled through the inguinal canal in the abdominal wall. Thus, the vas deferens passes through the inguinal canal and heads to the pelvic cavity, where each vas ends in an **ampulla**, a small bladder-like sac (Figure 17.53). Seminal vesicles secrete fructose-rich fluid into the ampullae. The ducts of the seminal vesicles and ampullae combine to form a short ejaculatory duct, which then leads to the **prostate gland**. The prostate in humans is about the size of a walnut and secretes more fluid to the semen. Prior to ejaculation, a pair of **bulbourethral glands** (also known as Cowper's glands) release preejaculate fluid that removes urine and lubricates the penis. Human penises are largely identical to other mammalian penises in terms of their structure and erectile tissue. Unlike many other mammals, humans do not have a baculum, spines, or other hard materials.

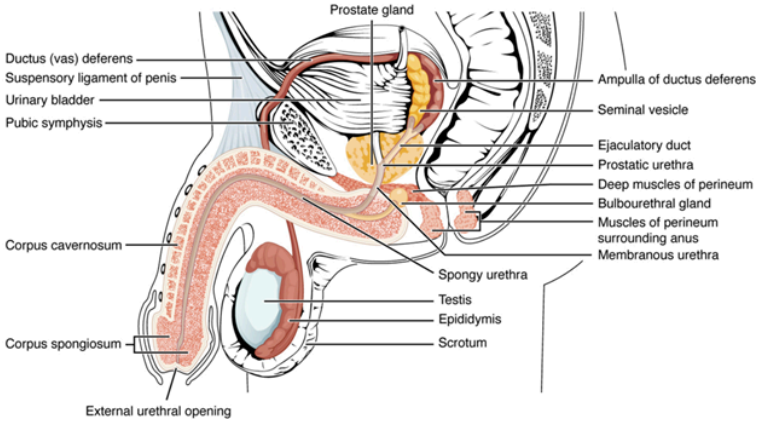

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Figure 17.53—The anatomy of the human testes-bearing reproductive system.

17.6 Integration

The gonads serve as endocrine organs as well and interplay with a whole host of things in your body. You can read more about this in Chapter 18. However, we want to draw your attention to another site of integration: mammalian pelvises. As you learned in Chapter 10, the evolution of the pelvic girdle had big implications for locomotion. However, once that pelvis is in place and the limbs articulate with it, various tubes and nerves have to pass through it to accomplish urination, defecation, and in ovary-bearing tetrapods, **oviposition** (egg-laying) or **parturition** (birth). This means that the pelvis itself is subject to numerous selective pressures.

Human parturition is complex in large part due to the shape of the pelvis. As the fetus passes from the uterus through the vagina to its hopeful exit, it undergoes a complex rotation. Assuming it is entering the vagina headfirst, the head rotates, then the shoulders, then the rest of the body. This is due to the changing shape of the pelvis as the fetus passes through the **inlet**, **midplane**,

and **outlet** (also known together as the **birth canal** or pelvic canal)—the inlet's longest axis is oriented mediolaterally, while the outlet's longest axis is oriented anteroposteriorly (Figure 17.54). Meanwhile, the midplane is narrower than either the inlet or outlet. The shape changes are largely due to ischial spines, ischial tuberosities, and sacrum all being in the way. The fetus barely fits through these openings, and this can cause injury or death to the fetus or the birthing parent. However, if we look at the great apes, our closest relatives, the birth canals of their ovary-bearing individuals are roomier, and the long axis remains in the anteroposterior direction. This is true of other primates, including both New- and Old-World monkeys as well.

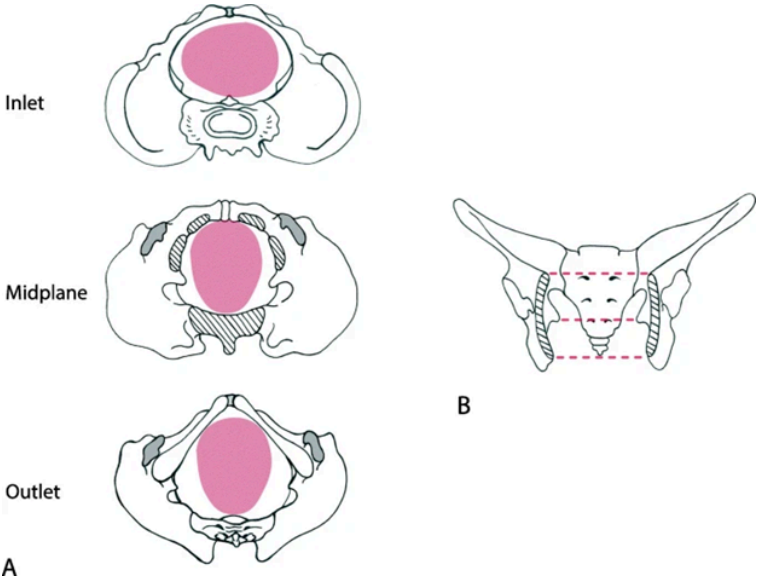

Figure 17.54—Rotational birth in humans. (A) Shape of various points in the birth canal, as illustrated in (B), which shows the pelvis with the pubis and ischium removed.

Unfortunately, a large birth canal, associated with larger

pelvic floor muscles, is not ideal for bipedal locomotion. As we shift to a bipedal posture, more of our weight is stacked above the pelvis. The larger pelvic floor cannot handle the stress and strain associated with this shift in force distribution. In addition, some studies have shown that a shorter distance between hip joints is more energy-efficient for bipedal locomotion and that a shorter anteroposterior length in the inlet allows for less pelvic tilt and a more stable posture, as it better supports the vertebral column.

The shape of the pelvis also has implications for locomotion-related injuries in humans. Often we see differences in pelvis shape between ovary-bearing and testes-bearing humans—something called **sexual dimorphism** (= two shapes). Not only do the pelvises of human egg producers have a wider pelvic canal, but they also tend to have a sacrum that is shorter and oriented more posteriorly, a smaller acetabulum, a larger distance between acetabulae, and a larger subpubic angle (Figure 17.55).

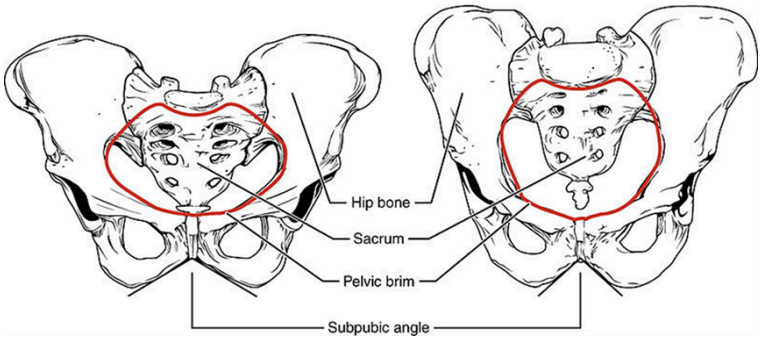

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Figure 17.55—Sexual dimorphism in the human pelvis. Left: pelvis from an ovary-bearing person; Right: pelvis from a testis-bearing person.

That leads to sex differences in the muscles of the lower limbs. For example, recall from Chapter 12 that the quadriceps femoris (what you may know as your quads) originates on the ilium, just superior to the acetabulum. The heads of your quads insert

on the patella via a common tendon. Given the difference in pelvic shape, we should expect differences in the "**Q angle**," or the angle that the line of action of the quadriceps makes with the patellar tendon (Figure 17.56). Indeed, we see that ovary-bearing humans have a higher Q angle than testes-bearing humans by just a few degrees (a mean of 17 vs. 14 degrees, respectively, albeit with a lot of variation). However, those few degrees make a big difference, corresponding to an increased prevalence of anterior cruciate ligament (ACL) injuries in ovary-bearing humans, as it puts a bit more torque on the knee joint.

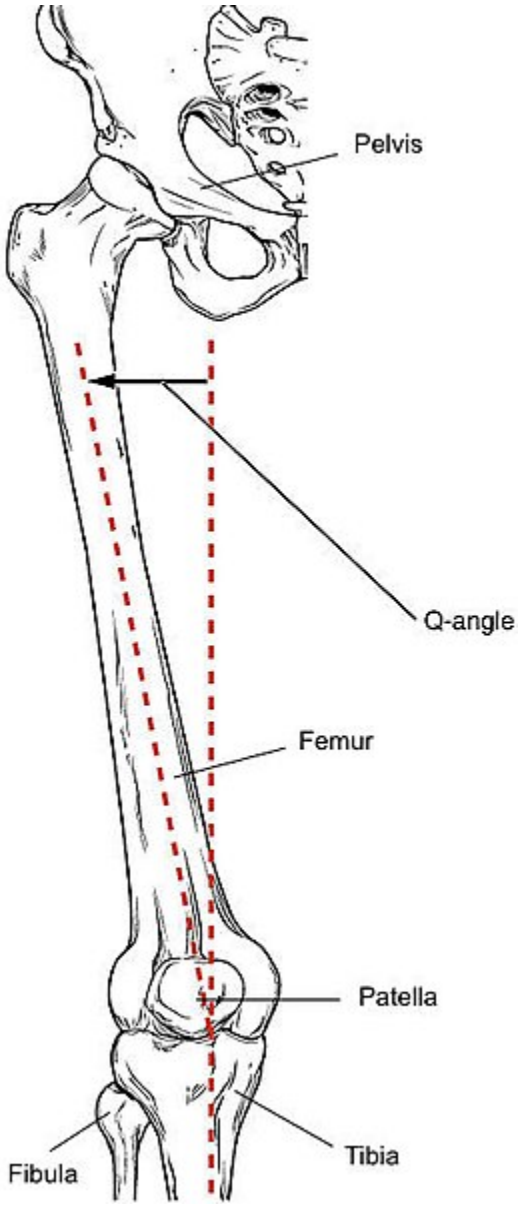

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Figure 17.56—Human leg demonstrating how to measure Q angle.

17.7 Summary

The vertebrate reproductive system is integrated with the urinary system, with some ducts being co-opted to carry gametes and the shift from a fully aquatic lifestyle to a terrestrial one. Even with those shifts as driving forces in the evolutionary trends we see, there is tremendous variation within vertebrate groups.

Application Questions

1. Given what you learned about the reproductive system of mammals, why are sperm-producing humans more likely to develop inguinal hernias than egg-producing humans?
2. The move from water to land drives evolutionary trends in so many different anatomical systems. Summarize the ways in which this transition led to the main different reproductive features and behaviors we see in amniotes.
3. Why can't sea turtles, sea snakes, and marine iguanas lay their eggs in the ocean? What do they do instead? Given that, what do you think the reproductive strategies of ichthyosaurs, mosasaurs, and plesiosaurs—all marine reptiles from the Mesozoic—were?

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18. Central Nervous System

BILL RYERSON

Focus Questions—to Guide Your Reading of This Chapter

1. How do the sizes of different regions of the brain relate to a vertebrate's interactions with their environment?
2. The spinal cord, in many ways, reflects the structure and function of the brain. In what ways are they similar? Different?
3. Why is surface area so important for a cortex?
4. Is brain size an indicator of intelligence?

18.1 Introduction

The brain: The phrase alone carries a significant weight. Unlike many of the other structures we cover in this textbook, there is an incredible amount of outside influence and discussion about the structure and function of the brain. Whole fields outside of biology are focused on the workings of the human brain. To be existential, right now your brain is processing information about…your brain.

In this chapter, we will focus primarily on the anatomical, developmental, and functional elements of the brain across vertebrates. However, there will be times when we must consider how the broader world interprets this anatomy. This may include some misinterpretations of brain structure and function (some amusing and some negative) as well as how some of these happenings helped us better understand the brain. As the brain also influences and controls so many other elements, this chapter will also refer to several other chapters in this book. These are not necessary in our exploration of the brain, but they may help you put the structures and their related functions in context. There is a lot of specific terminology when referring to the brain and its myriad structures. Throughout this chapter, look for clues and hints that will provide insight into the terminology, and use them to help you further your understanding of the brain.

18.2 General Structure and Function

The brain and the spinal cord make up the **central nervous system** (**CNS**; Figure 18.1). Both structures arise from the **dorsal hollow nerve cord** that is a defining characteristic of all chordates. The brain forms at the anterior end of the nerve cord, and while it shares many characteristics with the spinal cord, it is unique in its development and structure.

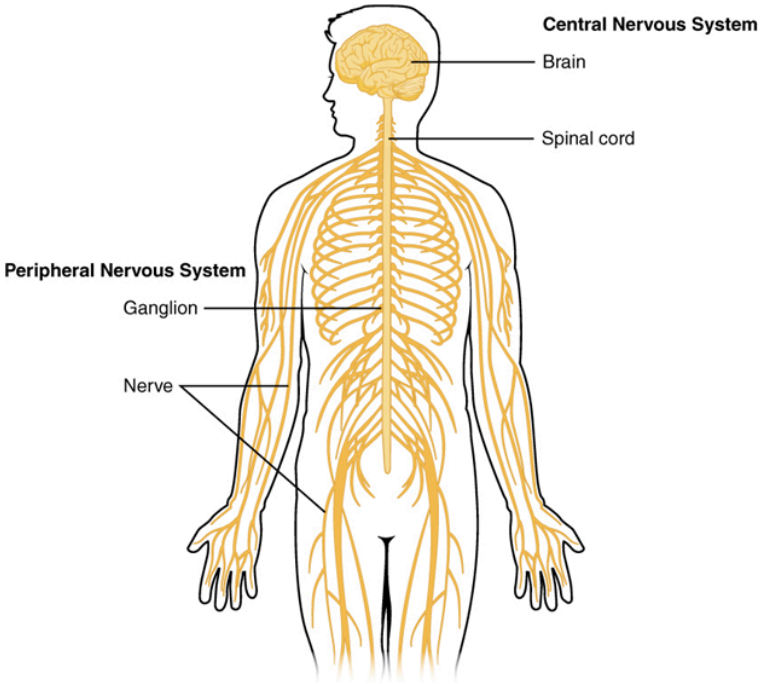

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Figure 18.1—The central and peripheral nervous system of a human.

To begin, let us examine the major cellular components of the nervous system. At the cellular level, the brain is predominantly composed of neurons, individual nervous system cells, and neuroglia. **Neurons** are the cells that make the nervous system work. A generalized neuron illustrates the various pieces of most neurons (Figure 18.2).

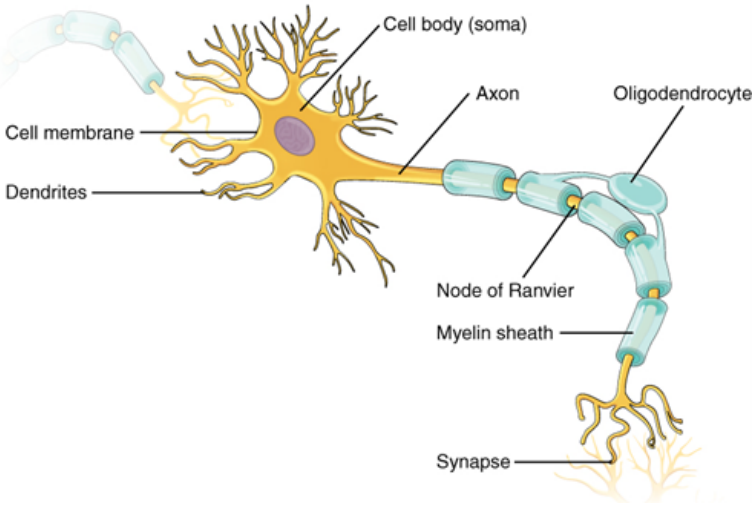

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Figure 18.2—A generalized neuron with relevant parts labeled.

Dendrites on neurons are where signals enter the cell, the **cell body** is the site of integration, and the **axons** are where nerve signals are sent out. There are many types of neurons, but they all share those same three components. The axons can vary greatly throughout the body and can be extremely long. The longest single axon in the human body runs from the spinal cord to the base of your big toe, almost a full meter in length. Not bad for a cell only a few micrometers in diameter. A key component of a neuron that will help us understand the brain is also found on the axons. In Figure 18.2, note that the axon is covered in a structure called a **myelin sheath**. The myelin sheath is an insulator and helps the signals move faster down the axon by jumping along gaps in the sheaths known as the **Nodes of Ranvier**. Myelin sheaths seem to be a vertebrate innovation for increasing the speed of a nerve signal. In invertebrates like the octopus, axons are much larger, the increased diameter helping increase the speed of signal transmission. The myelin-covered axons are quite numerous in the nervous system,

their presence able to be observed by the naked eye. In the brain and spinal cord we observe areas that are full of cell bodies and others that make up tracts of axons. The regions full of cell bodies (lacking myelin) appear as darker gray areas in the brain, subsequently called **gray matter**. The regions with myelin sheaths appear much lighter, and we call those areas **white matter** (see the spinal cord cross section in Figure 18.3).

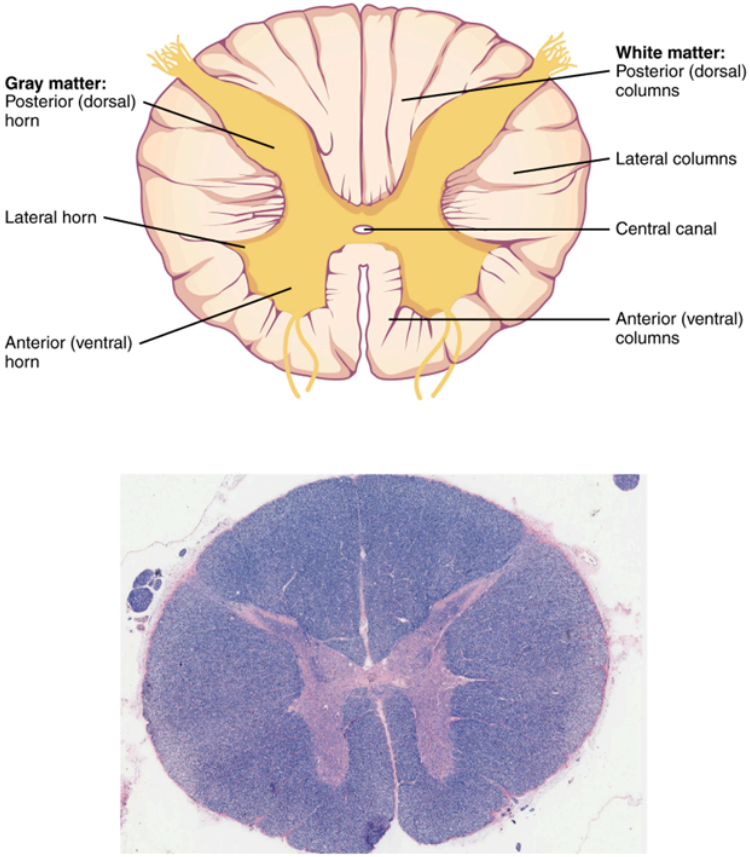

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Figure 18.3—Cross section of the spinal cord drawn and via histological section.

This will be one of the ways in which we can differentiate the functions of the different regions of the CNS. If the white matter is full of axons, then we know that these particular regions are responsible for communicating signals from one part of the brain to another, or from the brain to the rest of the body. Gray matter, with the majority of the cell bodies, is the area where incoming and outgoing signals are integrated and processed. As we observe in the spinal cord, the gray matter remains closer to the center of the spinal cord, with the white matter closer to the edges (Figure 18.3). The brain will originally evolve the same way, with the gray matter toward the center and the white matter along the periphery. This distinction will become more important as we explore the evolution of the brain through the vertebrates. While much of our discussion will focus on the nature, location, function, and amount of gray matter in the brain, it would be negligent to ignore the role of the white matter.

While neurons are the majority of the cells in the brain and very important for the function of the nervous system at large, they are not the only cells active in the brain. **Neuroglia** (or glial cells) are complementary cells that are responsible for monitoring the environment around neurons and responding accordingly. There are many different types of neuroglia: Some function in the immune system response, and others monitor the chemical environment (Figure 18.4).

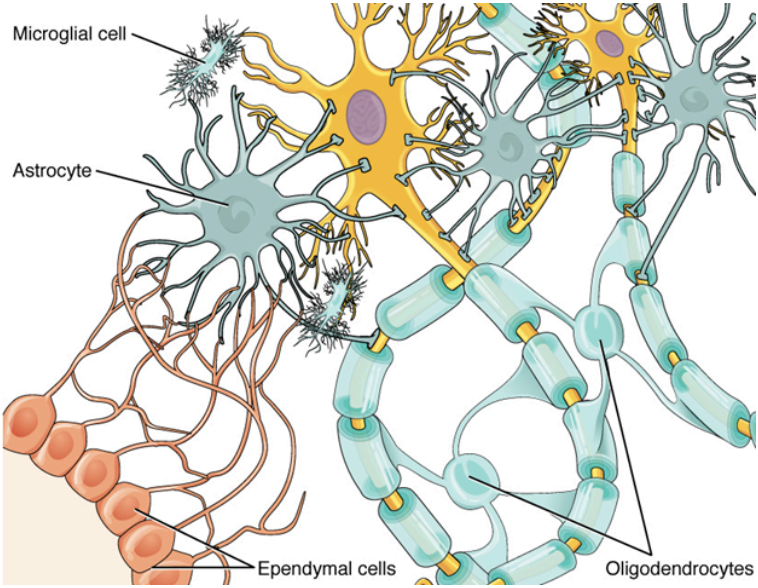

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Figure 18.4—Glial cells in the central nervous system.

One type of neuroglia, the **astrocytes**, uses cellular processes to pull capillaries and neurons closer together, making the movement of oxygen and carbon dioxide more efficient. The **oligodendrocytes** produce the myelin and wrap it around the axons of the neurons. **Ependymal cells** line the **ventricles** of the brain (spaces that form during development) and produce cerebrospinal fluid. There are other glial cells, providing immune system support, regulating the chemical environment, and monitoring the health of neurons.

You will also note in Figure 18.3 an opening at the very center of the spinal cord. Recall from Chapter 2 that one of the chordate characteristics was the presence of a **dorsal, hollow nerve cord**. While in many vertebrates this space has been filled in quite a bit, the hollow characteristic remains, in the form of a small structure known as the **central canal**. The central canal is a

consequence of the neural tube formation during development (see Chapter 4). Through the central canal passes **cerebrospinal fluid** (**CSF**), an essential component of a healthy CNS. The gray matter forms a butterfly-like shape in cross section, and the distinct areas of this butterfly help us separate different regions. Each distinct region is referred to as a horn, and the resulting structures are known as the **dorsal and ventral horns**. In the dorsal horns, sensory information from sensory receptors is received and processed. In the ventral horns, motor responses leave the spinal cord on their way out to the body through the spinal nerves. Some regions of the spinal cord will also have **lateral horns**, which are the origin of information for the sympathetic division of the peripheral nervous system (PNS; see Chapter 19).

To understand the general structure of the brain, we have to first examine its structure early on in development. At the earliest stages, the brain can be divided into three regions, anterior to posterior: **forebrain**, **midbrain**, and **hindbrain** (Figure 18.5). Classically, they are also referred to (in order) as the **prosencephalon**, **mesencephalon**, and **rhombencephalon**. From there, the forebrain can be divided into the **telencephalon** and **diencephalon**, the midbrain stays the mesencephalon, and the hindbrain divides into the **metencephalon** and **myelencephalon**.

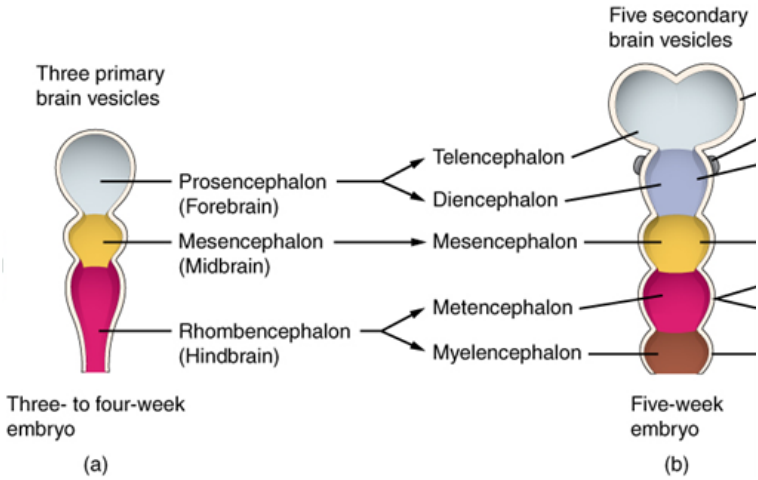

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Figure 18.5—The major regions of the brain as they appear in a developing human embryo.

Note that anywhere you see "enceph" or "ceph," it is referring to the brain or head region. As we progress through the evolutionary sections, we will see that many other structures will be derived from the major regions, and their size/shape has been implicated in better understanding of their function. This will include structures like the **cerebellum**, derived from the metencephalon of the rhombencephalon/hindbrain. We will introduce new structures as we go, but these are all derived from the previously aforementioned regions. Using the brain of the dogfish shark, *Squalus acanthias*, as a model, we can explore these concepts (Figure 18.6). The forebrain, midbrain, and hindlimb are color coded and compared with that of a human brain. Note how regions of the brain in the shark appear in a direct line.

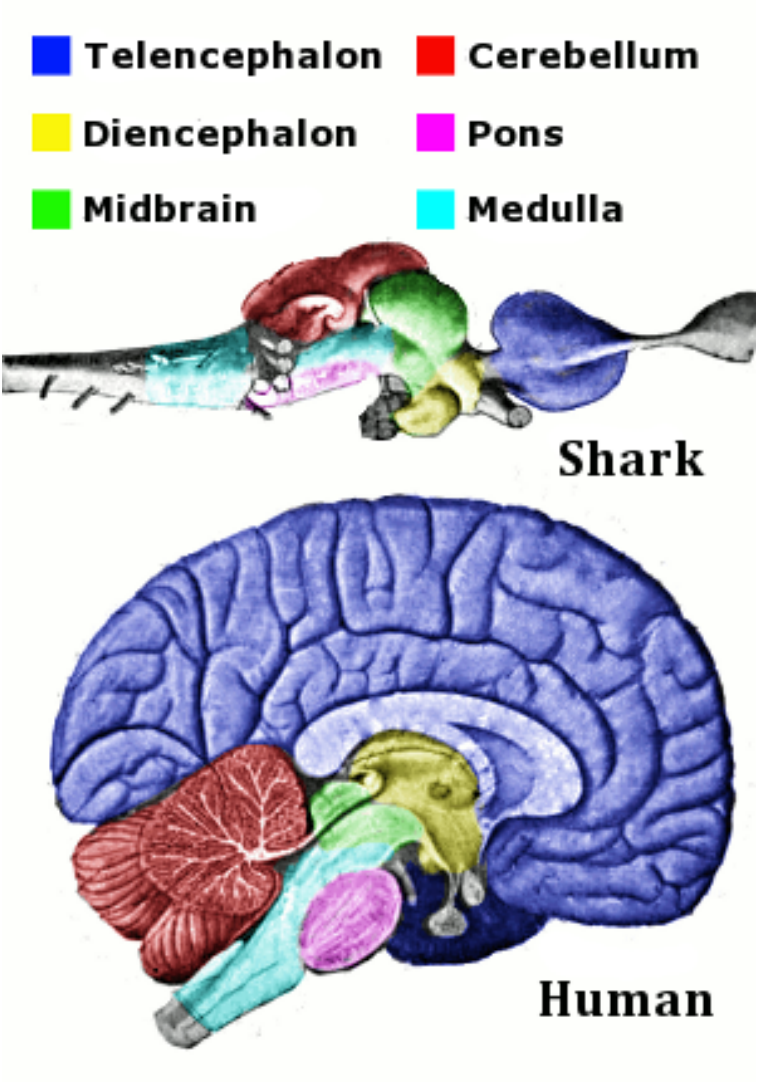

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Figure 18.6—The brain of the spiny dogfish, Squalus acanthias , as it compares to that of a human, with anterior being on the right. Homologous regions are color coded for comparison. Brains are not scaled to size.

We will find that mammals are the exception to this rule. There are two structures in Figure 18.6 that have not been introduced yet but are labeled because of their importance to vertebrates: the **cerebellum** (part of the hindbrain) and the **pons** (part of the midbrain). The midbrain and hindbrain together form a region that is known as the **brain stem**. The reasons for calling these regions the brain stem are twofold: (1) all information passing through the brain must go through at least a portion of the brain stem, and (2) many of the functions that are critical for life (heart rate, breathing) are regulated, monitored, and controlled by structures within the brain stem. Damage to the brain stem is likely fatal to most vertebrates.

Three structures that will help us orient ourselves on most brains are the **olfactory bulbs**, **cerebrum**, and **cerebellum** (Figure 18.6; the olfactory bulbs are to the far right on the shark brain and not visible on the human brain). There are often large structures, and changes in their size and shape are useful for understanding changes to the animal itself. Both the olfactory bulbs and cerebrum are involved in the sensory biology of an individual, while the cerebellum helps with the control of movements (more on this in a few sentences). These three structures are useful because they are typically visible from both the lateral and dorsal views across vertebrates and also form a nearly straight line from the anterior/cranial part of the brain to the posterior/caudal. The olfactory bulbs are typically the most anterior of the three. The cerebrum is slightly caudal to the olfactory bulbs, and the cerebellum is the most caudal.

Now that we have set up a brain and oriented ourselves, it is time to introduce a lot of the structures that are common across vertebrates. The most anterior region is the telencephalon, a subdivision of the forebrain/prosencephalon (and host to the olfactory bulbs, the most anterior of the most anterior). The cerebrum is also part of the telencephalon, receiving many different types of sensory input and integrating from multiple senses together. In mammals, the derived cerebrum is also referred to as the **cerebral hemispheres**, with further differentiation of both

structure and function. Posterior to the telencephalon is the diencephalon (still a member of the forebrain). The diencephalon is the home to many structures that direct and regulate many of the body's "subconscious" functions: heart rate, respiratory rate, hunger, thirst, blood pressure, sleep/wake cycle, body temperature, and more. Additionally, the diencephalon acts as a relay station for signals coming to or from the telencephalon, including motor activation patterns and sensory signals. There are three major structures in the diencephalon: **epithalamus**, **thalamus**, and **hypothalamus**. In addition to the functions already described, it is believed that the thalamus played an important role in the evolution of consciousness, a vertebrate feature that is the subject of intense debate. The hypothalamus is an important connection between the nervous system and the endocrine system, with a physical connection to the pituitary gland. In humans (more on this later), the hypothalamus is the beginning point for the production of the sex hormones testosterone and estrogen (among others). The mesencephalon, or midbrain, also serves as a relay station for many signals originating in different areas. In particular, the midbrain is associated with alertness (in mammals), sleep/wake cycles, relaying visual and auditory information, and body temperature. Last, there is the hindbrain, made up of the metencephalon and myelencephalon. The metencephalon is the host to two major brain structures: cerebellum and pons. The cerebellum is an important gateway from the brain to the rest of the body. The cerebellum is known to be involved in movement or motor patterns. However, it does not generate the motor patterns involved in movement. Instead, what the cerebellum is doing is coordinating the movements in response to other signals that are being sent and received by the brain. This includes the timing and precision of movements. Imagine throwing darts at a dartboard. As you line up your shot, you are receiving visual signals from your eyes about the target. You are also receiving information about the position of your hand relative to the rest of your body. Then you have to coordinate all the muscles involved in the actual throwing of the dart. The

cerebellum is playing a large role in coordinating all this information to make sure you hit the bull's-eye. The pons is home to several cranial nerves (Chapter 19—Peripheral Nervous System) but is also one of the centers of respiration. In humans, the pons plays a role in switching from inhalation to exhalation. The myelencephalon is home to the medulla oblongata. The medulla oblongata is the last major structure in the brain. It has quite a few different functions, but it is mostly associated with regulating elements of heart rate, blood pressure, and respiration. In mammals, it is also known to play a role in sneezing and vomiting. Sneezing and vomiting are good examples of complex behaviors that are involuntary in nature.

With all these elements working together, the brain functions as the control center of the body. Information from the senses is received by the brain, and coordinated responses to stimuli originate from the brain. The brain is also the primary center for the regulation of the other body systems. We will focus on these systems and their regulation primarily in the section on humans to better understand how the different elements of the brain come together. However, that does not mean we cannot glean a few helpful elements now to help elucidate the names, structures, and function of those structures. Large regions of the brain are given one of a few names: **cortex**, **hemisphere**, or **lobe**. These lobes or cortexes are subsequently identified by either location or function. For example, the optic lobe is the location where signals from the eyes are received in the brain, while the parietal lobe is found directly under the parietal bone in humans. Whenever we introduce a new location or structure, take a moment to consider why it has that name and what its function may be.

One last element in this discussion on the general structure of the brain is the fate of the central canal. You may recall the central canal from the general structure of the spinal cord. The central canal is a small channel at the center of the spinal cord that is the direct result of the formation of the neural tube. As the brain grows, turns, and twists during its development (more on this in the next section), the central canal will also get twisted,

contorted, and altered as well. Within the brains of vertebrates, the result of this twisting and growing is the formation of **ventricles**. Ventricles are cavities within the brain that allow for the circulation of cerebrospinal fluid (Figure 18.7). The number of observed ventricles and their location will vary within vertebrates. However, one consistent fact is that all the ventricles are connected to each other and the central canal! This allows the cerebrospinal fluid to circulate throughout the entire CNS.

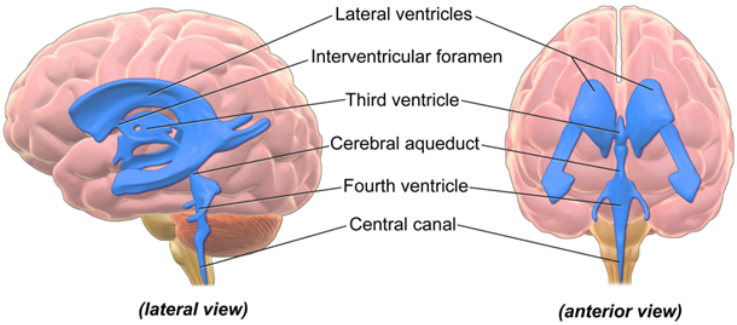

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Figure 18.7—A *visual representation of the ventricles within the human brain. All the blue regions are open spaces filled with cerebrospinal fluid.*

In addition to the physical structure of the brain itself, there are several different levels of protection that are fundamentally part of the structures we must take note of. The skull is the most obvious layer of protection, a bony case that completely surrounds the brain. However, the skull receives its own chapter in this textbook (Chapter 8—The Skull). Between the skull and the brain are several additional layers of protection. Three connective tissue layers form an almost impenetrable barrier around the brain, including preventing the passing of blood and extremely small microbes through their layers (Figure 18.8).

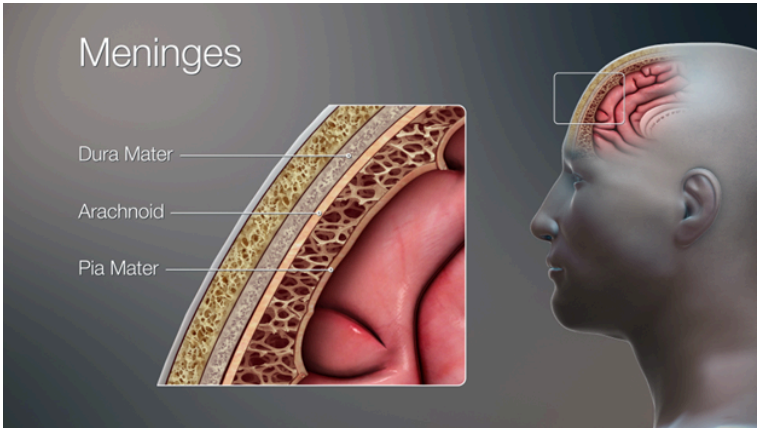

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Figure 18.8—The three meninges deep to the skull and superficial to the brain.

These are the **meninges** (from the Greek word for "membrane"). Each layer of the meninges is known as a "mater" from the Latin word for "mother." The three meninges are quite different in their structure and their function. The outermost layer is known as the **dura mater** ("tough mother"). The dura mater is the thickest of the meninges, composed of multiple layers of connective tissue. It serves as the physical barrier between the brain and its outside environment. The middle layer is known as the **arachnoid mater**, named for a web of filaments that stretch from the arachnoid mater closer to the surface of the brain. Underneath the arachnoid mater, these filaments result in a relatively open space known as the **subarachnoid space**. Within this space are the large arteries and veins that are the basis of the blood supply for the brain. The brain is an extremely energy-hungry organ, with an incredible supply of blood to provide the nutrients and oxygen required to keep it functioning. The web of filaments help secure the blood vessels and keep them closely associated with the surface of the brain. The last layer, the **pia mater**, is an ultrathin layer that is in direct contact

with the brain. Capillaries pass through the pia mater as they enter and leave the brain.

Along with the meninges, there is a special fluid known as **cerebrospinal fluid** (**CSF**) that circulates throughout the brain. Produced by the aforementioned ependymal cells, CSF moves through the open spaces within the brain and in the subarachnoid spaces. CSF provides many support functions for the brain. It helps regulate blood flow via pressure changes. It helps pull wastes as it circulates and also serves as an additional level of immune system protection for the brain (in humans, we sample CSF from the spinal cord to look for infections). Critically, it also acts as a chemical and physical buffer. In the case of trauma, CSF serves as a bit of cushion to prevent potential brain damage. CSF circulates around the brain but also through specialized open regions known as ventricles. Ventricles form during development as the brain grows and folds over itself. They are all interconnected, allowing the CSF to flow through the ventricles to the subarachnoid space and vice versa.

18.3 Development

The brain originates as part of the dorsal hollow nerve cord, one of the characteristics shared by all chordates. The cord itself forms during neurulation (see Chapter 4—Embryology for more on the process of neurulation). The tube remains just that, a tube that terminates at the anterior end of the body. Early on in development, the most anterior portion of the tube begins to enlarge (Figure 18.9).

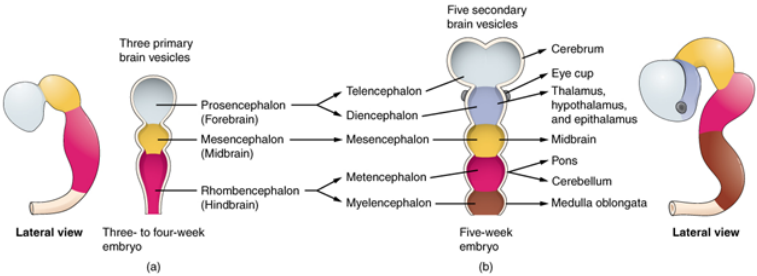

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Figure 18.9—The early developmental stages of the human brain.

Three small "bubbles" begin to differentiate themselves, and at this stage we can refer to them as the primary vesicles. The primary vesicles correspond to the three regions of the brain: forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). At this point, it is easy to still think of the neural tube as forming a straight line, but the reality is that the developing brain begins to fold and bend at this stage. Ultimately, in many vertebrates, the brain will resemble a more linear structure, but several of these steps will still occur during development. During the development of the primary vesicles, the brain will "fold" in two locations (both on the ventral portion), then in a third dorsal location. These folds will cause the three primary vesicles to subdivide into the five secondary vesicles: the telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon we have already met. We call these folds **flexures**. The first flexure is the cephalic flexure, occurring in the region of the mesencephalon. Shortly after the appearance of the cephalic flexure, the cervical flexure will appear. The cervical flexure is an especially useful landmark for monitoring the development of the brain, as it occurs near the separation of the hindbrain from the spinal cord. A short time later (2–3 weeks in humans), a third flexure will appear on the dorsal side of the developing brain. This is the pontine flexure, separating the metencephalon from the

myelencephalon. A small layer of tissue will remain, creating a roof for the developing fourth ventricle.

Speaking of the ventricles, this is now a good point to reintroduce them and discuss their development. The developing neural tube is hollow at its center. With the formation of the vesicles and future brain regions, the hollow part of the tube does not disappear. Instead, it may get shifted, turned, or twisted around, but the space remains. These will eventually become the ventricles, meaning that every brain region should be associated with at least one ventricle. There is some variation in the naming of the ventricles, so we will be using the human ones as an example for this section. The telencephalon, as it subdivides and grows more laterally, becomes the home of the lateral ventricles. The diencephalon is host to the third ventricle. The cerebral aqueduct passes through the mesencephalon running into the fourth ventricle in the metencephalon and myelencephalon. As we would move through the hindbrain into the spinal cord, the ventricles narrow into the center of the spinal cord, and we refer to it as the central canal. It is important to note that all the ventricles/aqueducts/canals are linked to one another, forming one continuous path that moves throughout the entirety of the brain. Now you might be asking yourself, Why are there no first or second ventricles? These are the lateral ventricles. It is difficult to refer to one or the other as "first," as they align quite closely from front to back, and they just happen to have moved laterally during development.

After the first few weeks of development, the brain really begins to take its individual shape across the vertebrates. In the interest of space, we will not cover the last developmental stages of all the vertebrates. Instead, we will focus on one group of vertebrates with a fascinating story on brain development, the mammals. The developing mammal brain begins as every other vertebrate brain, following the steps we discussed in Section 18.3, Development. Once the five regions have begun to differentiate and

start to shift in place, the different regions begin to expand and grow in size.

For the majority of the vertebrates, the remaining stages of development are rather unextraordinary from a bigger anatomical perspective. At the cellular and genetic level, there are still many things that must divide, migrate, get turned on, and more before the brain can be fully functional. However, there is one special case that we should consider in fuller detail. The mammalian brain undergoes quite the transformation even after the development of the secondary vesicles and five major regions. If we look at our shark brain (Figure 18.6), we can roughly see all the major brain regions directly in a line. Compare this to what you see in the human brain, also in Figure 18.6. From the external viewpoint, you cannot see too many regions of the brain beyond the cerebrum and cerebellum. You can only see the diencephalon and mesencephalon in a midsagittal view or by removing the cerebral hemispheres. At first glance, you may think that these regions have shrunk or been lost in evolutionary time. In reality, it has nothing to do with the diencephalon and mesencephalon at all. The telencephalon in mammals grows fast, much faster than the other brain structures during the early stages of development. In addition to the telencephalon growing faster than the other regions of the brain, the telencephalon also grows faster than the developing skull. This is where the shift occurs. As the growing telencephalon pushes its way forward, it runs into the frontal bones of the skull. However, the telencephalon cannot stop growing. Instead, it pushes upward and then backward. In doing so, it ends up actually wrapping around the diencephalon and mesencephalon. These regions of the brain become almost completely encased by the telencephalon. As we will discuss later, some mammals (like humans) get even more complex. The telencephalon grows so fast that it will also almost fold over itself, with peaks, ridges, and valleys creating an extremely convoluted surface to the cerebrum.

The spinal cord is an extension of the developmental processes that form the brain. Recall the neural tube (Chapter 4)

and how differing growth in the anterior end of the neural tube eventually resulted in the formation of the brain. The posterior end of the neural tube becomes the spinal cord. The central canal remains, without all the folding that occurs with the ventricles.

18.4 Evolution of the Spinal Cord

The evolution of the spinal cord is particularly understudied in vertebrates. The most popular hypothesis is that the spinal cord is strongly conserved across the vertebrates, with only minor changes in the larger spinal cord structure across the vertebrate groups. However, this does not mean there are no changes—they are just beyond the scope of this book. For this discussion, we will separate the vertebrates into the fishes and the tetrapods to highlight the major differences.

Fishes

The fishes have a few minor changes in the spinal cord from the general structure highlighted earlier. The spinal cord is present throughout the entirety of the vertebral column from the head to the tail. In the hagfish and lamprey, the spinal cord does not have the distinct gray matter horns described earlier; gray and white matter are distributed throughout the entire spinal cord. Dorsal and ventral gray matter horns can be observed in Chondrichthyes and Osteichthyes. Lateral horns are not found in Chondrichthyes but can be found in the Osteichthyes. There is also a more subtle structural differentiation observed in the bony fishes. Using specialized staining techniques, it is possible to further differentiate the gray matter of the ventral horn into two subregions. The dorsomedial aspect of the ventral horn is most strongly linked with

the axial musculature. The ventral aspect is most strongly linked to the pectoral and pelvic fins. As you near the caudal (tail) end of the animal, the spinal cord diminishes in size. Intuitively, this should not come as much of a surprise. Individual nerves branch off the spinal cord, and as you move farther down the animal, there are fewer nerves to branch off. As such, the cord decreases in diameter but remains a solid cord throughout the entirety of its length.

Tetrapods

The spinal cord in vertebrates is observed to have a few more changes from the basal fish condition. The first is that the overall shape and size of the spinal cord changes along its entire length. In the fishes, we noted that the spinal cord continues the length of the vertebral column, with a decrease in size caudally. The spinal cord of tetrapods does not always continue the length of the vertebral column (Figure 18.10).

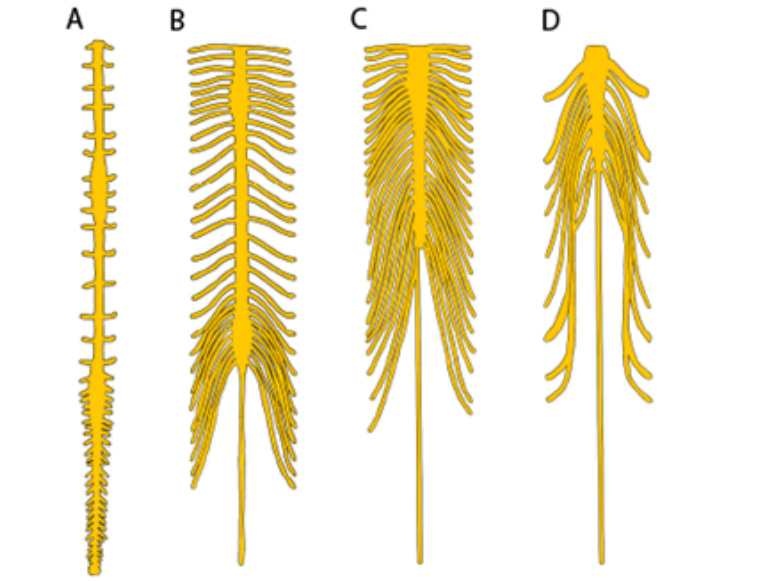

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Figure 18.10—The spinal cords of four different tetrapod species: (A) *red-eared slider, Pseudemys scripta elegans* ; (B) *harbor seal, Phoca vitulina* ; (C) *human, Homo sapiens* ; (D) *common toad, Bufo bufo.*

Some amphibians, reptiles, and birds will have spinal cords that abruptly end prior to the end of the vertebral column, and others may have just a few tracts continue onward. The mammals follow a different trajectory. As we saw in other vertebrates, the spinal cord decreases in diameter in mammals. Moving caudally, however, individual nerve fibers within the spinal cord separate and spread out caudally. Because of its appearance (as though the spinal cord has frayed apart), this area of the spinal cord is known as the **cauda equina** (Latin, "horse's tail") for this reason. This has several clinical implications for veterinary and human medicine (Box 18.1).

Note that the cauda equina is still contained within the meninges and still considered part of the spinal cord.

Along the spinal cord in the tetrapods, there are regions where the spinal cord is enlarged. In mammals, these can easily be observed (Figure 18.10B). The **cervical enlargement** and **lumbar enlargement** appear as thickenings in the spinal cord. These correspond roughly to where the major spinal nerves (Chapter 19) of the limbs will originate. Within the spinal cord, we can also see changes around these same regions. While the tetrapods retain the horns and gray matter / white matter distinction we observed in the cartilaginous and bony fishes, there is considerable variation among (and within) groups of vertebrates (Figure 18.11). Even within an individual, the shape of the horns will vary along the length of the spinal cord (Figure 18.12). The most supported hypothesis is that these changes coincide with the spinal enlargements and reflect the unique demands of terrestrial locomotion.

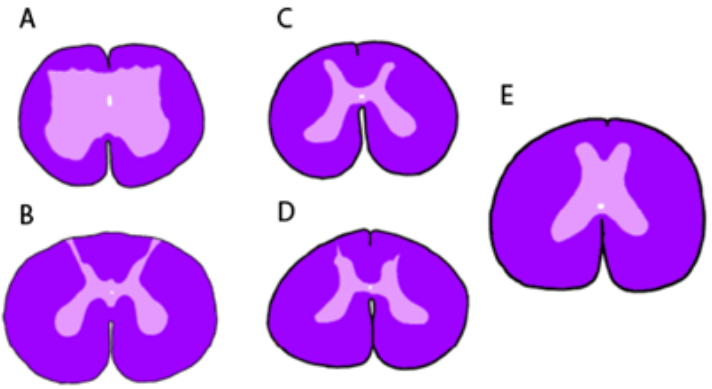

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Figure 18.11—Transverse sections of the spinal cord (stained as in Figure 18.3) at the level of the seventh vertebrae in an assortment of amphibian and reptiles: (A) common toad, Bufo bufo; (B) red-eared slider, Pseudemys scripta elegans; (C) Hermann's tortoise, Testudo hermanni; (D) Argentine black-and-white tegu, Tupinambris merianae; and (E) reticulated python, Malayopython reticulatus.

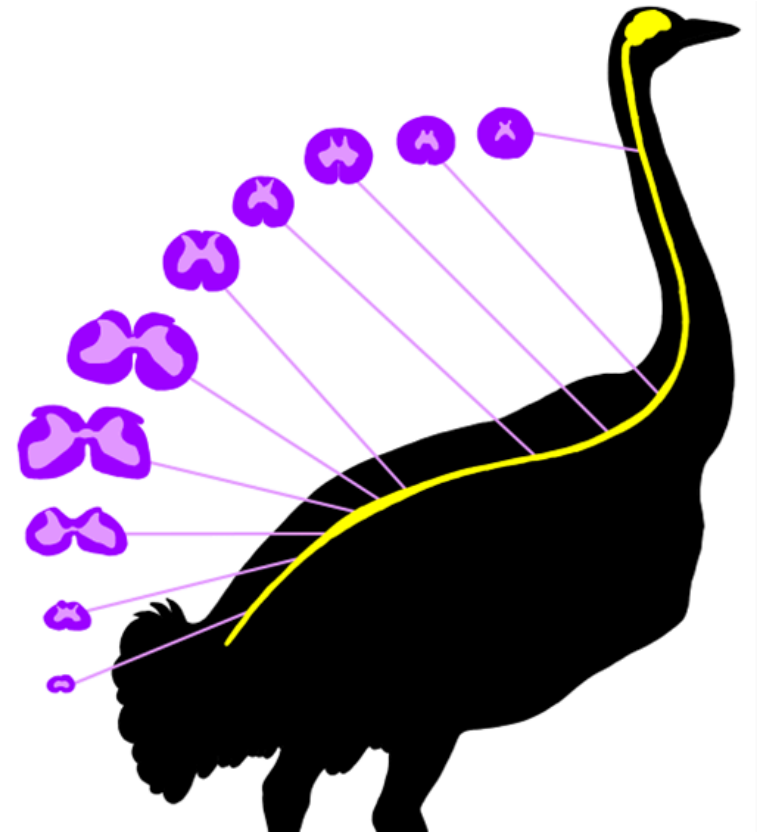

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Figure 18.12—Variation of spinal cord structure in an ostrich, redrawn from Streeter, 1904. The spinal cord cross sections have the same color pattern as they would stain histologically. The lighter pink regions are gray matter, and the darker purple ones are white matter.

Box 18.1—A Lumbar Puncture

There are many places where a disease can infiltrate the body. Viruses, bacteria, fungi, and parasites can hide out and damage just about every tissue in the body. However, detecting these disease-causing agents can be difficult if you do not know where to look. Some can be detected (for example) through a blood draw, and for others you can remove and examine a piece of the infected tissue (a biopsy). How would you test for a disease like bacterial meningitis, an infection that causes inflammation of the meninges? You would have to take a sample of the cerebrospinal fluid (CSF) to look for the bacteria responsible. However, sampling the CSF is a dangerous task; you do not want to accidentally puncture the brain or spinal cord. In human medicine, the safest way to sample the CSF is by doing a lumbar puncture (Figure 18.13). In a lumbar puncture, a hypodermic needle is passed into the subdural space between two lumbar vertebrae. As we just discussed, this is the location of the cauda equina. Without the distinct spinal cord, there is more space to safely sample the CSF.

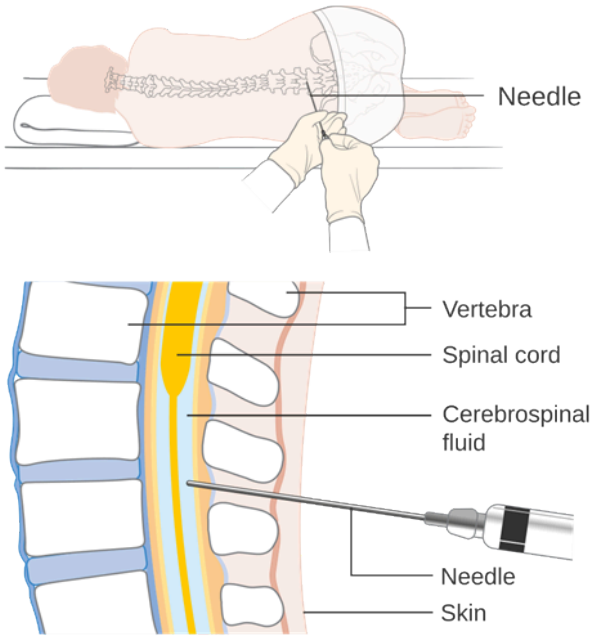

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Figure 18.13—A diagram of the appropriate sampling location for a lumbar puncture in humans.

18.5 Evolution of the Brain

Setting the Stage

Before we begin working our way through the evolutionary history of the brain, it is important to understand a little of the interactions between nerves, the senses, and organisms beyond the vertebrates. The neuron is the single unit of the nervous system. The more information that needs to be processed, dealt with, or sent out, the more neurons are necessary to handle that information. However, there is a word of caution to be had. The number of neurons in a brain and/or the size of the brain does not immediately equate with intelligence. Body size plays a large role as well in the size of the brain and number of neurons. The author of this chapter has been fooled by more than one mouse, with his bigger brain and everything.

Prior to the arrival of the vertebrates, an evolutionary novelty appeared that would drastically change how many animals, not just the vertebrates, are structured. In an introductory biology course, you would have learned about many of the characteristics of all animals that helped define lineages like the jellyfish (Cnidaria) from the lobster (Arthropoda). One of the more obvious traits that separates the jellyfish from the lobster is that lobsters are bilaterally symmetrical with a clear left and right side. Vertebrates are bilaterally symmetrical (Figure 18.14).



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Figure 18.14—A photo of a bald eagle, Haliaeetus leucocephalus. This photo illustrates both the bilateral symmetry of vertebrates as well as some evidence of cephalization.

At the same time we observe bilateral symmetry appearing in animals, another phenomenon known as cephalization appears. **Cephalization** is the grouping of the major sensory organs in a "head" region, along with the mouth. The majority of animals have an obvious head, with sensory organs and the mouth dominating the region. Referring to Figure 18.14, what senses can you observe on the head of the eagle? What does this mean for the evolution of the brain? The congregation of sensory organs in a relatively small area has consequences for the nervous system. All the senses need neurons to transmit and process their signals. In this small space, neurons come together in a large group to process all this information. Voilà, you have a brain. You can think of a brain as the largest collection of neurons in a body. An individual neuron is just a neuron. A small collection of neuron cell bodies is a **ganglion** (plural: ganglia). A large collection of cell bodies is a brain. As all vertebrates exhibit cephalization, we only find brains in the head region. The most favorable hypothesis currently is that as senses gain or wane in use, it should not come as a surprise that those regions of the

brain will grow or shrink across evolutionary time. The same will be true for the regions of the brain that coordinate movements or "higher-level" functions (emotions, memories, etc.). However, there has been no compelling evidence that contradicts this hypothesis, which we now refer to as Jerison's principle of proper mass, from Harry Jerison's groundbreaking textbook, *Evolution of the Brain and Intelligence*. So we will continue to assume that the size of a particular region of the brain is correlated with its importance to a species' biology. However, we should also take a second to remind ourselves that all these comparisons are relative to other parts of the brain or to the individual's body mass.

It is also important to note that there is considerable variation within groups as to the size and structure of brains. As we will frequently note throughout this next section, there is no "one size fits all" when it comes to defining patterns of brain evolution across large groups. We should also note that brain evolution is a rapidly changing field as more researchers seek answers to questions that have so far eluded us. As we begin this exploration, keep these elements in mind.

Cyclostomes

Extant hagfishes and lampreys represent highly specialized forms of vertebrates and not necessarily those of the common ancestor of all vertebrates. This is especially true when we consider the brain. The brain in the hagfish and lamprey is linear (as it is in most nonmammalian vertebrates). The hagfish brain is highly reduced with few surface distinctions that enable us to identify key structures. The forebrain, midbrain, and hindbrain are observable, and there are ventricles throughout. An olfactory bulb and cerebrum are present. The olfactory bulb is very large relative to the rest of the forebrain, indicating the increased importance of the olfactory system relative to the other senses. It should not be

surprising, given the lack of eyes, that the visual processing centers are highly reduced in hagfishes. However, beyond that, there is little to distinguish the hagfish brain from other vertebrates. Many of the structures we identified in our section on generalized vertebrate brains are difficult to locate and identify. The lamprey brain has been well studied for understanding the development of the brain and the changes that can happen over ontogeny, the lifetime of an individual. In many ways, the lamprey brain resembles the developing shark and fish brain (Figure 18.15).

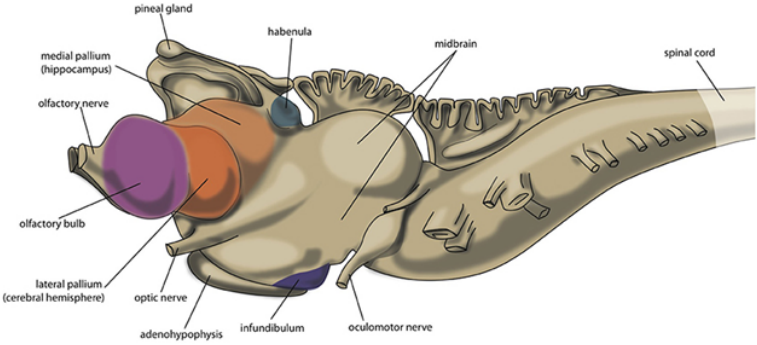

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Figure 18.15—The brain of an adult lamprey. The colored regions indicate different areas of the pallium.

One unique element of the brain of the lamprey is the extension of the **pineal gland**. The pineal gland is a small gland that is part of the diencephalon. In many vertebrates, the pineal gland is associated with sleep-wake cycles. In some vertebrates, including the lamprey, the pineal gland is associated with the parietal eye (sometimes also known as the pineal eye). This eye is covered in more detail in Chapter 20 on the sensory systems. However, for our purposes, a brief summary of its function will help us. The parietal eye is specialized to sense only light or dark. The pineal gland is often closely associated with the parietal eye, as having a light/dark sensor can greatly impact sleep/wake cycles for many vertebrates.

One additional structure that makes its first appearance in the lamprey and will remain through several vertebrate groups are **Mauthner neurons**. Mauthner neurons are large, individual cells found in the hindbrain. They are the driving force behind the C-start, a characteristic escape response from fishes. In a C-start, the body of a fish bends into roughly a C-shape. It then straightens its body quickly, escaping the situation. Stimulation of Mauthner neurons results in the quick response of muscles to activate in a coordinated pattern. For juvenile lamprey, this allows them to quickly disappear into the sand via activation of their axial musculature to avoid a potential predator.

Chondrichthyes

In most comparative anatomy classes and textbooks, the shark brain serves as the model for the ancestral vertebrate brain. Typically, as we ourselves have done here, that is the brain of the spiny dogfish, *Squalus acanthias*. By comparison to the lamprey brain, we find that there are several key differences between the two, relating to changes in the sensory and motor systems of the two groups (Figure 18.6). The telencephalon and cerebellum are larger relative to the rest of the brain than we observed in the hagfish. This is suggestive of increased sensory processing and more coordinated muscle movements. The increased size of the cerebellum is the product of increased mobility and control of mobility in the water column. The main side-to-side bending that is characteristic of sharks (and other fishes) is not controlled or relayed through the cerebellum, instead being directed by central pattern generators found within the spinal cord. However, any sharp turns or avoidance behaviors as a response to negative stimuli (an unwanted bump on the snout or contact too close to the eyes) would be smoothed out and coordinated by the cerebellum. Generally, the chondrichthyan brain is elongated and particularly linear, making it easier to identify

the major structures of the brain and recognize the changes. However, there is considerable variation among shark brains. With the large diversity of both ecologies and body size in sharks, we can begin to look at how differences within groups shed light on the evolution of the brain and the factors pushing that evolution. Mako sharks and great whites have considerably larger optic tecta (singular: optic tectum; also sometimes referred to as optic lobes) than the dogfish; the Greenland shark and other deep-water species have much smaller visual processing centers. Generally, as body size increases, so too does brain size, which is why we try to use other measures to understand changes to the brain over time. In sharks, relative brain mass (brain mass divided by body mass) has been used to try to tease apart differences across species. One might expect that while brain size does increase with respect to body size, the relative size of the brain would remain the same. However, a trend emerges in sharks and rays that will show up independently in the other major vertebrate lineages as well. Some lineages within groups will develop brains that are much larger relative to body mass and others that are much smaller. This includes changes that might be considered ontogenetic or incorrectly comparing the juvenile of one species to the adult of another. In the sharks, the Carcharhiniformes (tiger sharks, bull sharks, lemon sharks, and more) and Lamniformes (makos, great whites, salmon sharks, and others) share a larger brain-to-body-mass ratio than the other groups. The sharks closely related to the dogfish (squalomorphs) seemingly have smaller relative brains. However, even within the larger groups are species that buck the trend. Among the largest sharks, the basking shark (one of the Lamniformes) has a noticeably smaller relative brain mass than the other members of its group. This provides us with an opportunity to hypothesize why that might be. In the rays, it is the myliobatids (the stingrays and eagle/manta rays) that possess much larger relative brain masses. The skates have reduced relative brain mass.

It is also in the sharks where we first start to see a new type of structure we call a cortex. Earlier in this chapter, we discussed

the distinction between gray matter and white matter. Aside from their functional and structural differences, there was also a key difference in where they could be found in the brain. At the center or within the edges of the brain are areas of gray matter called nuclei. Surrounding the nuclei are tracts of white matter. This is true for most areas of the brain and differs across different vertebrate groups. Some sharks, like the great white shark, have an additional layer of gray matter around the outer edge of the cerebellum, known as the cerebellar cortex (Figure 18.16). Additional gray matter means additional cell bodies, which we know provide additional ability to process and integrate information. Our highly active, fast-moving sharks likely need this cortex to help coordinate the tight turns and quick movements in pursuit of prey or evasion of large predators.

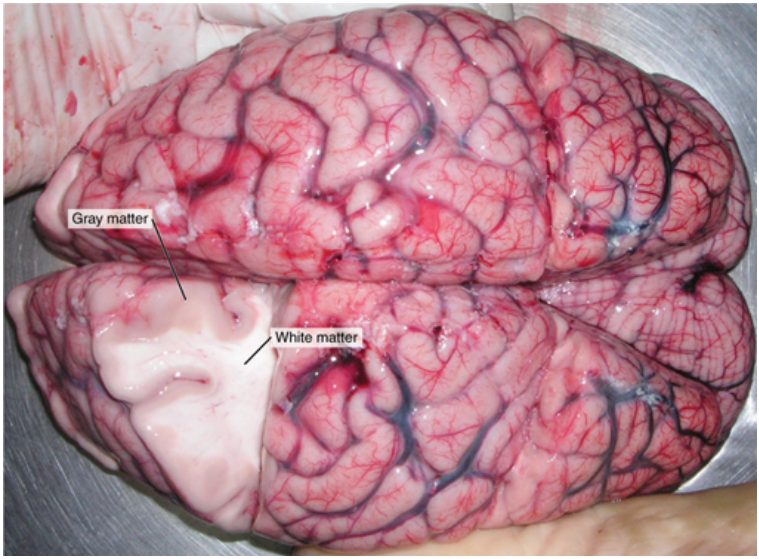

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Figure 18.16—The cerebral cortex of the human brain with the additional layer of gray matter on the external surface (easier to visualize than the aforementioned great white). Note that the location of the white matter and gray matter here is reversed from the general vertebrate condition.

Osteichthyes

Given the huge diversity of bony fishes, in their habits, habitats, and more, it is difficult to tease apart general trends in the evolution of the brain. To begin, let us consider how the bony fish move. Before, we stated that the cerebellum helps smooth complex movements. Lineages that use significant body bending in their swimming, like the eels and sturgeons, have a relatively smaller cerebellum compared to other lineages that use their pectoral, pelvic, and caudal fins independently for movement (e.g., freshwater sunfish). Overall, the relative brain mass in bony fishes is larger than the chondrichthyan fishes. Additionally, the size of the optic tectum and telencephalon as a whole is larger in the bony fishes than in the chondrichthyan fishes. Similarly to the chondrichthyan fishes, some lineages have evolved relatively larger brain sizes.

Another interesting pattern that is first observed in the bony fishes is that from a phylogenetic perspective, larger lineages have smaller relative brain mass than smaller lineages. We should stress here that the range of body masses is across a much larger span than that of the chondrichthyan fishes. While the largest fish in the world are sharks, the smallest shark in the world is still 20 cm in length (the dwarf lanternshark). However, there are many bony fish smaller than that size as adults (e.g., the dwarf goby at 7.9 mm) with juveniles only a few millimeters in length at birth. The constraints on brain size do not appear to be impacting the larger lineages. Instead, it is the ultrasmall lineages of bony fish that are pushing the lower limits for how small a functional brain can be. The smallest guppy still needs to see, hear, smell, catch food, evade predators, and find a mate. All these senses, movements, and behaviors require neurons to receive, integrate, and send signals.

One novel structure that we did not discuss in the section on the general structure of the brain is the **inferior lobe** (Figure 18.17). The inferior lobe of bony fishes occupies the same space as the hypothalamus in the tetrapods and was considered for some

time to be equivalent to the hypothalamus. However, there are a few key differences between the inferior lobe and the hypothalamus. Developmentally, there are separate origins for the inferior lobe and the hypothalamus, though there are several shared cell types between the two.

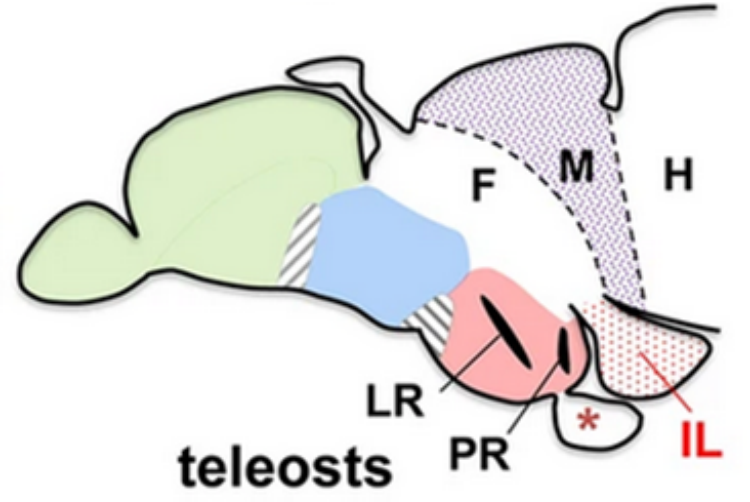

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Figure 18.17—A lateral view (anterior to the left) of the brain of a zebrafish, Danio rerio. Green is the telencephalon, blue is the thalamus, and red is the hypothalamus. F—forebrain, M—midbrain, H—hindbrain, LR—lateral recess of hypothalamus, PR—posterior recess, IL—inferior lobe.

Functionally, the inferior lobe seems to be involved in multimodal signal integration. Multimodal signal integration is where a part or parts of the brain take information from different senses and use them together to more completely assess the situation. For example, the sense of smell would be informative for the presence or absence of food, but add in the physical sensations from the lateral line column and you may be able to pinpoint the exact location of the food.

In addition to these other structures, we have to define

a new section of the cerebrum. The cerebrum, part of the telencephalon, can be subdivided into even further sections. Wrapping around the ventricle, we can refer to individual segments as **pallium**. This is specific to the upper layers of the cerebrum. The pallium can be broken further into dorsal pallium, medial pallium, subpallium, and so on (Figure 18.18). For now, the important distinction is that there is going to be a slight change in the structure of the pallium. Note in Figure 18.18 that the structure of the pallium follows our general principle for layering of gray matter and white matter. Gray matter (cell bodies) is found at the center with the white matter tracts (myelinated axons) around the outside of the gray matter. Beginning in the bony fishes and continuing onward, we will find select areas of the pallium where there will be additional gray matter on the outside edge.

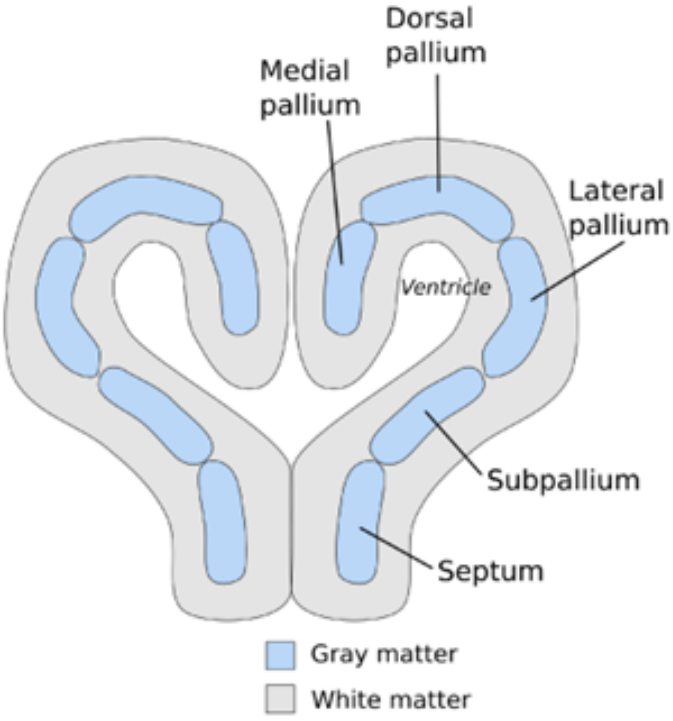

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Figure 18.18—A cross section of the forebrain of a teleost fish, highlighting the subdivisions of the gray matter.

What is the advantage of the additional gray matter? More cell bodies means more ability to process incoming information and direct responses. We will find that this is a recurring theme throughout the rest of the vertebrates. When space starts to become limited, where can we add neurons to increase the ability to process and integrate information? The pallium allows for additional neurons with a minimal increase in the overall size of the brain.

Amphibia

The transition to land produced evolutionary novelty across the body for tetrapods, but in the case of the brain, the impacts of the transition are unclear. As we will soon see, this may be because our models for the transition, the extant amphibians, are highly specialized for their lifestyles. Instead, we have relied on endocasts of the braincase in the "fishapods" (like *Tiktaalik*) and stem tetrapods to shed some light on the changes, but these data are currently sparse.

In extant amphibians, there are several broad trends and interesting differences between the different amphibian groups. Across the fishes, we found that at the phylogenetic scale, brain size relative to body mass increased in several lineages. In the amphibians, we find the opposite. Across the frogs and salamanders, there is a conspicuous reduction in the relative brain size. The functional/evolutionary reasons for this reduction in size are still unclear. One of the most striking reductions is that of the cerebellum in amphibians (Figure 18.19). In the previous section, we noted that as more complex movement behavior evolved in the fishes, the cerebellum increased in size relative to other regions of the brain, a correlation that made intuitive sense given our understanding of the cerebellum's role in smoothing and coordinating muscle outputs. A much smaller cerebellum would suggest a lack of complexity of movement in the amphibians, but we know that this is not the case. Frogs and salamanders both exhibit a large array of complex locomotor and feeding behaviors, even though the size of their cerebellum is reduced to other parts of the brain when compared with fish. In addition to the relative reduction in the size of the brain, the distinctions between regions are also less apparent in amphibians than in other vertebrate groups.

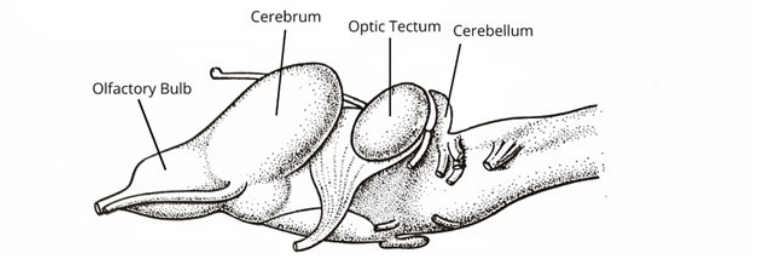

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Figure 18.19—A lateral view of the brain of the European grass frog, Rana temporaria.

Despite the general decrease in the relative size of the brain compared to body size across all amphibians from the fishes, there are a few interesting trends that emerge when comparing the different amphibian groups to one another. The anurans, our frogs and toads, have a relatively large optic tectum and increased auditory processing centers. Many frogs are visual hunters and also rely on sound to communicate between individuals of the same species. The salamanders have large olfactory bulbs, and many groups have an enlarged optic tectum as well. This is particularly true for the plethodontid salamanders, in which some species can capture their prey from quite a distance away using their tongue. The increased optic tectum is correlated with their targeting in prey capture.

One final item to note on the amphibian brain: The telencephalon is easily divided into two hemispheres (just like the cerebral hemispheres in humans). Across amphibians, the hemisphere has several different pallial zones. Without getting too far into the weeds, it is important to note that the lateral pallium is quite extensive. It is predominantly associated with olfactory input. Given what we have already discussed with amphibians, it should come as no surprise that these increased areas of gray matter are dedicated to the processing of olfactory information.

Nonavian Reptiles

The reptiles in many ways are quite similar to the amphibians (but larger) and fishes before them. Reptiles have a largely linear brain with a larger cerebellum and telencephalon (Figure 18.20).

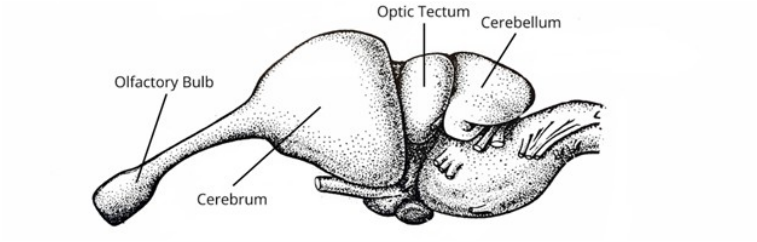

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Figure 18.20—Lateral view of the brain of an American alligator, Alligator mississippiensis.

However, a new structure can be observed, one that will change how we examine brains for the remaining vertebrate groups and in general how we as humans view the evolution of intelligence. In the section on chondrichthyan fishes, we introduced the cerebellar cortex, an additional layer of gray matter on the cerebellum that we hypothesize allows for greater maneuverability in the fishes. However, we will observe that a new region of the brain gains a layer of gray matter in reptiles: specifically, the neocortex, a layer of gray matter on the surface of the cerebrum (a part of the telencephalon), which is also referred to as a **cerebral cortex**. This is considered a distinct separate structure from the pallium we briefly discussed in the fishes and amphibians (though there is still some debate in this regard). Most in the field would consider the cortices to have been derived from the pallium. All parts of the neocortex are part of the pallium, but not all parts of the pallium are part of the neocortex. The cerebral cortex in reptiles is structurally very similar to what we will observe in mammals, although all evidence points

to it having arisen independently. The reptilian cortex has many fewer subdivisions than the mammalian one, only a medial, lateral, and dorsal region. The function of the lateral cortex is primarily olfactory, while the dorsal cortex receives some visual information.

The simplified function of the cerebral cortex is that it greatly increases the number of cell bodies available in the cerebrum for the processing of incoming sensory information and the generation of motor outputs. In particular, it would seem that the new cerebral cortex allows for the processing of multimodal information. Multimodal integration means using two distinct pieces of information and putting them together to solve a problem or provide context. Migrating sea turtles are well known for returning to the exact beach from which they were born. Using a combination of magnetic, visual, and olfactory cues, they can find their way back from thousands of miles away. It also allows for the generation of complex and learned behaviors. Monitor lizards can solve puzzles and snakes can cross large gaps between trees using a combination of sensory information and motor control.

Aves

In our exploration of the evolution of the brain in vertebrates, we found that different regions of the brain increase in size relative to others in response to changes in their sensory systems. As we focus here on birds, the most obvious change is the increased relative size of the telencephalon. The telencephalon is vastly enlarged in comparison to the other regions of the brain. There is also typically a very large optic tectum. There is also a relative increase in the size of the cerebellum compared to other vertebrate groups (Figure 18.21). However, we should begin outright by saying that the birds have some of the most drastic differences in the size of brain regions across species for any vertebrate group, a testament to the many different ecological niches they have been able to fill.

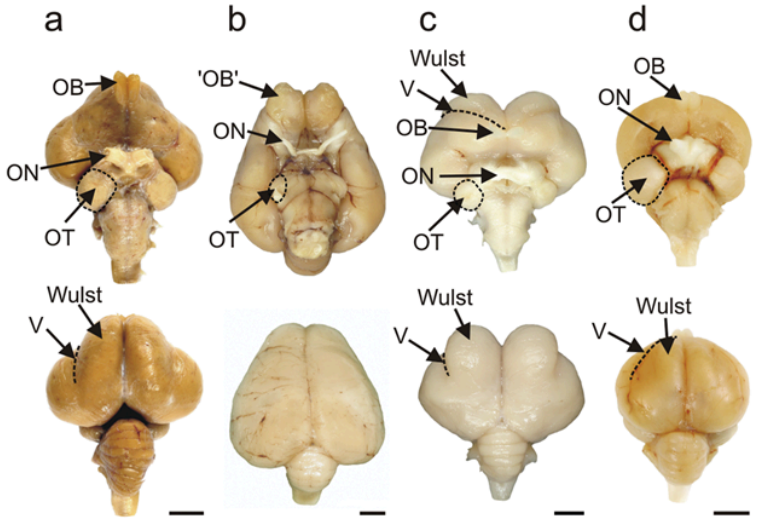

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Figure 18.21—Ventral and dorsal views of a few selected bird species. (A) *emu;* (B) *kiwi;* (C) *barn owl;* (D) *pigeon. Relevant regions have been labeled:* OB—*olfactory bulb;* ON—*optic nerve;* OT—*optic tectum;* V—*vallecula (border of the wulst). Anterior is toward the top of the page for each brain.*

The most obvious explanation for the increased telencephalon, optic tectum, and cerebellum is that they evolved in response to (or in tandem with) the evolution of powered flight. Similar increases in size of these three regions have also been identified from endocasts of pterosaurs, flying reptiles (not dinosaurs!) that are a distinct branch separate from the bird lineage. Intuitively, this should make sense. Take a quick look at the video of a kestrel hovering over an airfield in the United Kingdom (Kestrel Hovering). This individual bird is hunting for smaller birds and mammals below it. Birds, especially the hawks, falcons, and eagles, are well known for having exceptional vision. Exceptional vision requires exceptional processing power. Flying requires not just a lot of muscular power and energy but the ability to take into account all the information that it takes to remain in the air. For most terrestrial

tetrapods, knowing where they are in space is essentially a two-dimensional game. Birds have added a third dimension to this and must maneuver through that dimension accordingly. Changes in wind direction, height, and velocity all require sensory input and changes to be made via wing position and angle. Flight, it seems, has resulted in a very large cerebrum, balancing all the multimodal input that prevents a disastrous crash for the bird.

One thing to note that we have not discussed frequently to this point is how the brain sits within the skull. When we think of the brain in the skull, we probably do not imagine it as an incredibly tight fit but also understand that there is not a lot of extra space within the skull as well. There is a lot of variation (unsurprisingly) in how much space there is within the skull, but the birds make the most of that space. Bird brains are quite large for the brain cavity, leaving only a minimal amount of space between the brain and the inner surface of the skull. There is so little space that birds only have two meninges, and both layers are quite thin.

An interesting twist on brain anatomy in the birds is not so obvious from the surface. Despite not developing the ridges and valleys we will soon see in mammals, the avian brain has one major functional adaptation for increasing its ability to process large amounts of information, and that is by increasing the density of the cells in the cortex.

The bird telencephalon also has one new unique structure. Known as the **eminentiae sagittales** (or **wulsts**), these small lobes sit on the dorsal surface of the telencephalon. The wulsts are considered to be an additional area of visual processing for birds. In particular, they are considered essential for giving birds stereoscopic vision, allowing predatory birds to determine depth/distance based purely on vision. What's most interesting is that the wulsts are a site of considerable variation across bird groups (Figure 18.21). Owls have particularly large wulsts, while those of the kiwi are barely visible at all. This should make sense if we consider this fact in the context of their behavior and feeding ecology. Owls are nocturnal and fly (many species maneuver silently through dense

forests), while kiwis rely on mechanosensors in their beaks and don't fly.

Mammals

Much like we observed in the birds, there are several changes to the brain in mammals that require some investigation. However, in mammals there are quite a few changes that occur that on their surface do not seem as clearly defined as some of the previous changes we discussed. Like birds, the cerebrum is very enlarged, but in addition to the increased size, there are a few structural changes that occur. In the smaller mammals, like mice, there are comparatively fewer differences in the overall brain structure when compared with the brain structure we explored in the birds. However, as mammals get larger, an interesting phenomenon occurs that has wide-reaching impacts on the structure and function of the brain. Compare the mouse brain to that of the human (Figure 18.22).

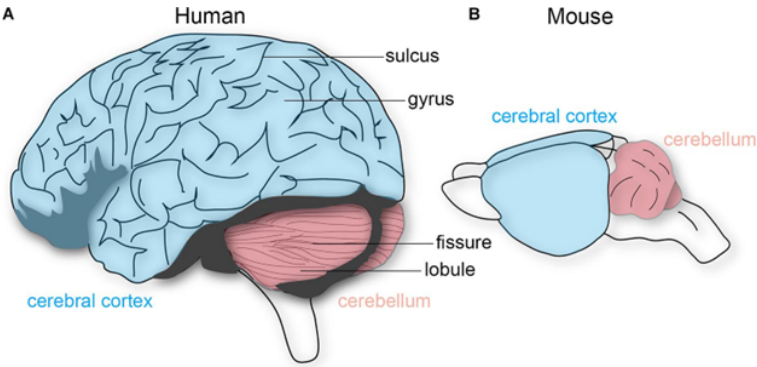

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Figure 18.22—Lateral views of a human and mouse brain. Note the "folds" visible on the human brain but not the mouse brain.

Take a closer look at the cerebral cortex in Figure 18.22. There is some size disparity, to be sure, but look more closely at the drawing. You may notice that there are ridges, lines, and grooves along the surface of the cerebral cortex. This is not a human trait but a significant component of many mammal brains, particularly as species get larger (Figure 18.23). The surface of the cerebral cortex is covered in small ridges and grooves. These are known as **gyri** (singular: gyrus) for the ridges and **sulci** (singular: sulcus) for the grooves. How they form requires us to take into account challenges in both physics and how brains develop during ontogeny. As we learned in the developmental section of this chapter (Section 18.3), the telencephalon grows fast in mammals, very fast. So fast that it ends up covering most of the diencephalon. However, this growth is not equal across the entirety of the telencephalon. During development, areas of the telencephalon that grow faster end up "pushing aside" other regions. As the differential growth continues, the folds and ridges become the gyri and sulci. Do not forget that some of these regions are also likely to be connected to each other already, further limiting the directions in which the growing brain can move.

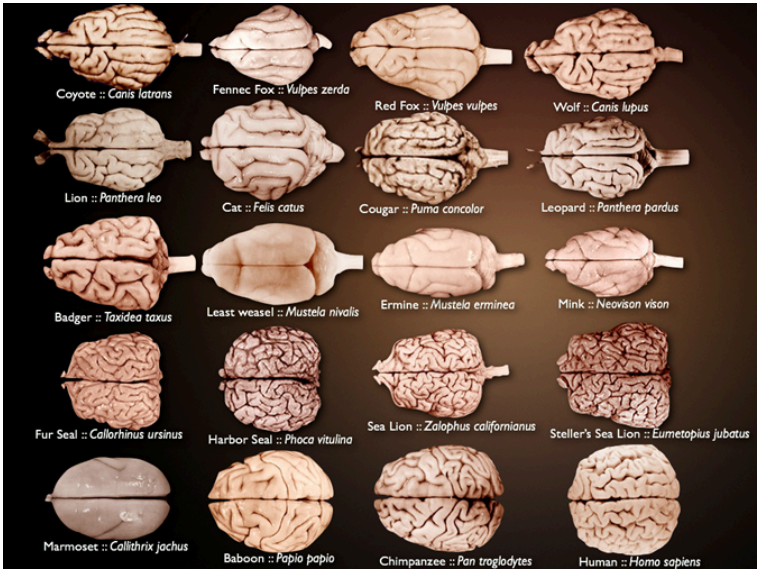

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Figure 18.23—Dorsal views of a broad array of mammal brains. Brain size is scaled for the image, not relative size of brain or individual species.

Why is it that we do not have spiky brains, with clear obvious peaks and valleys? Return to our section on development. As the brain continues to grow, the skull continues to act as a physical limit to growth, "flattening" the rising gyri. Sulci can continue to move inward, and we have names for those structures: **fissures**. Some fissures, like the longitudinal fissure in the primates, are very obvious. The **longitudinal fissure** is the deep groove that separates what we call the left cerebral hemisphere from the right cerebral hemisphere.

If the gyri and sulci arise from developmental and physical phenomena, do they have functional implications? We know from the reptiles that the cerebral cortex (or neocortex) is defined as more gray matter on the surface of the brain, which results in more cell bodies for the integration and processing of information. The increase in cerebrum size provides more surface for the gray

matter. The folds, therefore, are further increases in surface area, allowing for even more gray matter. Unfortunately, this is really the end of our understanding of the functional implications of the folding. As we will shortly see in humans, the cerebral cortex has many functions associated with it, but the question of those functions beyond humans is currently a hotly contested debate.

With all these different regions and all this growth that occurs in mammals, how do these different areas of the brain communicate with each other? To map this out, we will use the human brain as the model, though most of the tracts can be identified in other vertebrates. The first tracts are **association fibers**. Association fibers connect different parts of the same brain region. For example, looking at the right cerebral hemisphere in humans, fibers that connect the front of the hemisphere with the back would be association fibers. **Commissural fibers** connect different gray matter brain regions together, almost entirely those involved in the brain that have hemispheres (cerebrum and cerebellum). In humans, there is a structure known as the **corpus callosum**, a thick band of white matter that is found on the dorsal aspect of the **lateral ventricles**. The corpus callosum is the largest commissure, allowing communication between the left and right hemispheres of the cerebrum. Finally, there are **projection fibers**. Projection fibers connect different brain regions from front to back. While commissural fibers might connect different hemispheres, the projection fibers would be what connects the brain stem to the cerebral hemispheres, and vice versa. Projection fibers dissipate as the brain stem becomes the spinal cord and only become known as tracts beyond this point.

Humans

The human brain is arguably the most studied single structure in all of biology and medicine. In most regards, the human brain is quite

similar to that of other mammals. However, because of the intensive study that the brain has received over the years, the function of different regions of the brain is more well known in humans than it is in other vertebrates. With this in mind, we can spend more time discussing the function of various elements of the brain.

Broadly, the cerebral hemispheres are associated with the processing of sensory information and the onset of voluntary motor movements. We can break the hemispheres into five lobes: **frontal**, **parietal**, **temporal**, **occipital**, and **insula** (Figure 18.24).

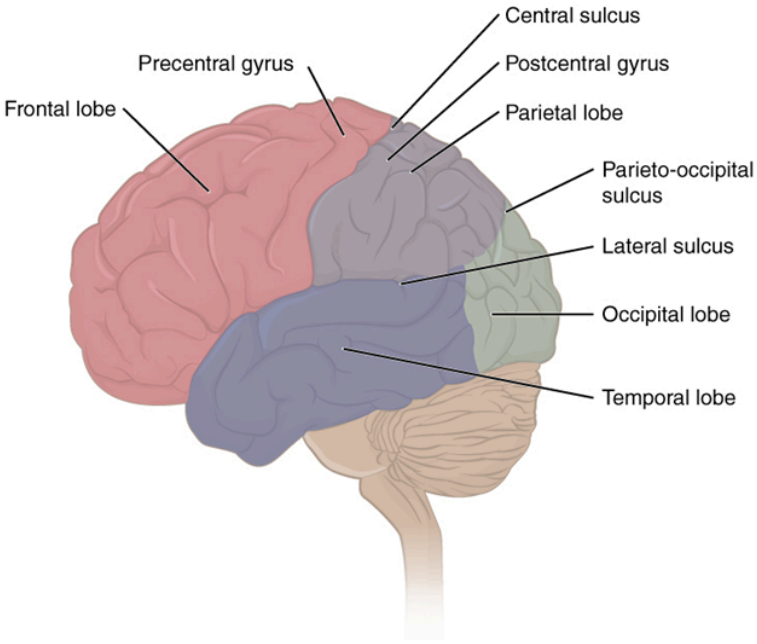

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Figure 18.24—The human brain with four of the five lobes of the cerebrum colored in.

Four of the five lobes are visible from the exterior surface of the brain; only the insula is not visible. Pioneering work in 1909 by Korbinian Brodmann, a German neuroscientist, found histological

differences all across the cerebral cortex (both within lobes and across lobes), and he used these differences to define more than 50 different regions. Many years later, we determined that those areas correspond quite well to functions within and across the lobes as well. To begin, let us explore the areas that control motor movement across the body. The **central sulcus** divides the anterior frontal lobe from the parietal lobe that is just posterior to it. The most posterior part of the anterior frontal lobe, just anterior to the central sulcus, is the **primary motor cortex** (Figure 18.25). You can think of the primary motor cortex as the beginning point for almost all voluntary movement. Just anterior to that is the **premotor cortex**. The premotor cortex is similar to the primary motor cortex, but it is understood to function predominantly in movements that require considerable practice or planning. Movements or patterns of the movement that you might refer to as "muscle memory" likely are controlled by the premotor cortex. Remember, all these movements are filtered through the cerebellum on their way out, where the different signals for the movements can be coordinated. There are two other regions of motor control, also nearby in the frontal lobe. The first is the **frontal eye field**, which controls voluntary eye movements. The second is **Broca's area**, which coordinates the muscles involved in speech production. It is named for the French physician Pierre Paul Broca, who first discovered that damage in that area resulted in the loss of the ability to speak coherently. The emphasis is on the coordination of the muscles involved in speech. To review, the muscles that contribute to speech include those of the face, tongue, and larynx. While all the muscles may operate just fine in their other tasks, the ability to produce speech (in the form of spoken language) requires significant coordination.

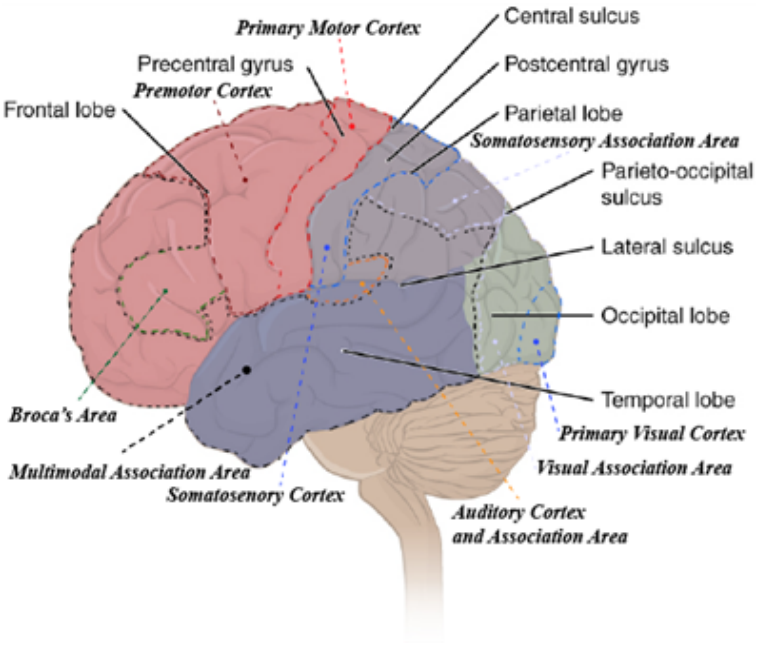

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Figure 18.25—The lobes of the brain with dashed lines indicating the cortices and association areas.

Overall, motor cortices are a relatively minor component of the cerebral cortex in humans and restricted to the frontal lobe. The remaining functional regions of the cerebral cortex may span across lobes and can be broken into three components: **primary sensory cortices**, **sensory association cortices**, and **multimodal association areas** (Figure 18.25). The differences between these different regions are quite important. The primary sensory cortices are where the raw signals come into the brain. The association areas are where those raw signals are given context. That context may be a memory or other information. We have all had an experience where someone we knew drastically changed their appearance. When you first saw them, perhaps you did a double-take and needed to verify that it really was them. In this case, the information you were

receiving from your primary visual cortex was not matching the context from your visual association area. The same is true for many of the other sensory systems as well. You can recognize particular voices, tastes, smells, and even body position. The sense of touch, housed in the primary somatosensory cortex, is also a key area to identify. It too has an association area, where memories can be informative for textures, shapes, and weight. For example, could you tell the difference between a dry-erase marker and a pencil with your eyes closed? What about a hardcover book and a paperback? Again, memories are providing context to raw signals. So if touch is working similarly to vision and other senses, why take the time to highlight it here? That is because unlike many of the other external senses, your sense of touch occurs across all your skin, but to different sensitivities. Your fingertips are especially sensitive, but the skin over your elbow is much less so. There is a broad correlation between how sensitive touch is in any particular region and how much of the primary somatosensory cortex is devoted to the region. This is often depicted as a 2D representation by something we refer to as the "**somatosensory homunculus**" (Figure 18.26). Not to be taken literally, as many of the regions overlap with each other, the homunculus nevertheless gives you a great working idea of the regions of the body that are more sensitive to touch.

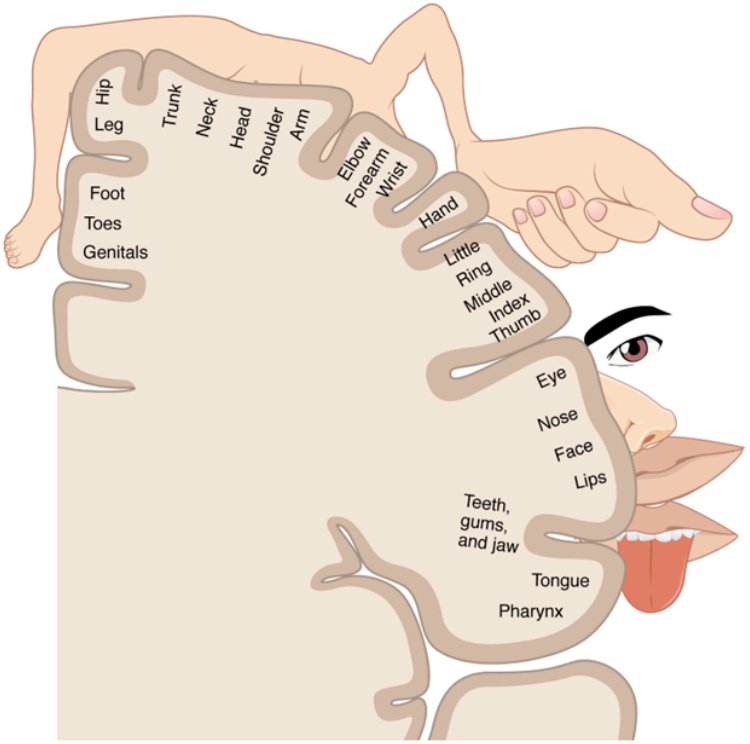

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Figure 18.26—The sensory homunculus as it is often depicted in the human brain. The relative size of drawn structures roughly correlates to the amount of area dedicated to interpreting sensory information from that area.

Finally, we come to the multimodal association areas. Their name likely gives away a bit of their function, but it only scratches the surface of what we consider the function of these areas to be. The multimodal association areas are large regions of the cerebral cortex that take different types of information (multimodal) and apply them with memories/context (association) to solve complex problems.

The multimodal association areas can be divided into three major regions: **anterior association area**, **posterior association**

area, and the **limbic system**. The anterior association area is arguably the most complex system in the cerebral cortex, from a human perspective. It is the home to almost all of what we might consider our "cognitive" processes: personality, learning, intellect, recall, planning, judgment, and reasoning. In humans, this area is incredibly plastic—that is, it will change based on experience over time. You may have heard the argument of "nature versus nurture" when it comes to positive and negative elements associated with a person's personality or behavior. This discussion originates from our understanding of the development and function of the anterior association area. This is also the area we associate most closely with the planning and handling of abstract ideas. Complex problems of logistics (I have three errands to run; which order do I do them in?) are handled in the anterior association area. Medically, the anterior association area has attracted the most attention, as damage to that area is correlated with "negative" behaviors and changes to a person's personality (Box 18.2).

Box 18.2—Phineas Gage, History's Most Famous (Damaged) Brain

How do we know so much about the regions of the brain that relate to personality? Much of our original understanding stems from a man named Phineas Gage. Phineas Gage was a railroad worker overseeing elements of the construction of a railroad in Vermont in 1848. An accident caused a tamping iron to run through Mr. Gage's brain. The tamping iron entered his left cheek, just under the zygomatic arch, and passed through his frontal lobe. Amazingly, he not only survived this ordeal but regained consciousness and sat upright in a cart being used to get him to a doctor. He survived

the accident, the surgery to remove the tamping iron, and extensive rehabilitation. However, many of those close to Mr. Gage noticed an abrupt change in his personality following the accident. He became brash and aggressive and had difficulties with impulse control, quite unlike the quiet, well-spoken, and thoughtful person they had known before. As his brain healed, his personality continued to change for another 12 years before he died.

The posterior association area controls pattern recognition in a variety of forms. Recognizing someone from their speech patterns as well as visual patterns is put together in the posterior association area. It is also the home to Wernicke's area, a small region that is considered vital in understanding (and using) spoken and written language. Last, the posterior association area also provides a sense of where we are in space. Combining pattern recognition with information from the somatosensory cortex gives us a sense of where we are in a location and how our body is positioned.

The limbic association area ties emotions to experiences and sensory information. The hippocampus is part of the limbic association area and a key component of it. The hippocampus is a region of gray matter; in humans it can be found in each hemisphere, as one of the walls of the lateral ventricles. This small, nondescript bump of tissue is a key area involved in our understanding of human memory (and one of the first regions to find physical degradation in diseases like Alzheimer's disease). The hippocampus is responsible for many elements, but there are two that we need to

discuss. The first is that the hippocampus helps turn short-term memories into long-term memories, taking sensory information, perception, and emotions and combining them into information that can be recalled at later points to be used in providing context to new information. The second element is that the hippocampus is vital to not just understanding where the body might be in space (that's the posterior association area) but more importantly how we navigate through that space.

Putting aside the cerebral cortex, the rest of the brain in humans functions quite similarly to that in other mammals and vertebrates. The thalamus connects the cerebrum with the rest of the brain and directs signals to different areas of the cerebrum. The hypothalamus regulates physiological functions, including heart rate, body temperature, water balance, and sleep/wake cycles. This includes physical responses to emotions (blushing when embarrassed, increased heart rate when excited or anxious). The hypothalamus is also a key player in the regulation of the endocrine system function in humans (Chapter 21—Endocrine System). The pons and medulla oblongata generate the rhythms for regular heart rate and respiration. The cerebellum is large in humans and can be visibly separated into two hemispheres along with a vermis. There is a cerebellar cortex and a significant number of gyri and sulci in the cerebellum as well. As in other vertebrates, the cerebellum is responsible for coordinating muscle movements in response to raw signals as well as incoming sensory information.

18.6 Summary

The brain is an extremely complex structure that has changed dramatically across the span of vertebrate evolution. It is the control center of the body, monitoring all the sensory information gathered and directing all voluntary muscle movements. The changes that have occurred are the result of ever-increasing sensory information and changes to how vertebrates move in their environment. Fundamental questions of structure and function have been resolved across many vertebrates, while questions of consciousness, personality, and behavior are still unresolved in our understanding of the brain. New senses, social systems, and even more changes muddy our understanding of the brain, leading to continuing work to this day. The plastic and flexible nature of the brain reminds us that even with smaller groups of vertebrates, there is considerable variation among species.

Application Questions

1. Why might the basking shark have a lower relative brain-to-body-mass percentage than the great white or tiger shark? How do their ecology and behavior support your hypothesis?
2. How might the brain change in flightless birds like the ostrich or emu?
3. Studying the brain (and intelligence and consciousness) has been full of bias and prejudice

throughout its history, not just among different human populations, but also when comparing other vertebrates to each other and to humans. How might this have come about as we learned about the brain? How do we know now that many of those "findings" were determined incorrectly?

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19. Peripheral Nervous System

BILL RYERSON

Focus Questions—to Guide Your Reading of This Chapter

1. The cranial nerves are named for their number, structure, and function in mammals. How might this mislead you when you examine the cranial nerves in other vertebrates?
2. The nervous system interacts with many other different systems, most of them covered in other chapters in this book. How are the other systems controlled and/or monitored using the peripheral nervous system?

19.1 Introduction

When most of us are asked to think about the nervous system, our immediate response is to consider the brain. This is not the wrong response but rather a simple fact—the rest of the nervous

system does not immediately jump to mind. The brain is selfish, it only considers itself, but that is a question for metaphysics and psychology. We will instead force the brain to consider many of the other elements of the nervous system beyond itself. We consider the spinal cord a part of the **central nervous system** (**CNS**), as it shares many developmental and structural similarities to the brain, and rightfully the two are grouped together. The remainder of the nervous system is broadly categorized as the **peripheral nervous system** (**PNS**), which includes the **spinal nerves** and the **cranial nerves**. With a few notable exceptions, what these different structures all have in common is that they serve as messengers, carrying signals to/from the brain and the other parts of the body. They may carry signals from the senses to the brain or carry signals from the brain to the muscles to create a movement. In doing so, they serve as important links across the body. We can consider these elements part of the rapid-response team of the body as compared to the endocrine system (Chapter 21), which may have longer-lasting impacts. To further this interconnectedness one step forward, the endocrine and nervous systems are not independent units; action of the endocrine system impacts the nervous system and vice versa. In this chapter we will cover the general elements of the PNS, examining the structure and function over evolutionary time. We will pay especially close attention to the cranial nerves, as their story is compelling not only in their function but in how they are incredibly informative in the story of evolution as well as a key diagnostic tool in human and animal health. We will discuss small elements of nervous system physiology here as needed, but know that there are many aspects of physiology that are beyond the scope of this anatomy textbook.

19.2 Generalized Structure and Function

The spinal nerves and the cranial nerves are structurally similar in

many ways. They are nerves, with cell bodies, axons, and dendrites (mostly), and interact with other structures at synapses. They carry signals to and from the CNS via discrete pathways. The key difference between the two sets of nerves is their origin. Spinal nerves arise along the spinal cord, between pairs of vertebrae. The majority of cranial nerves connect directly with the brain itself, with either the forebrain or the brain stem, and leave through the skull. There is one exception to this rule, but you will have to contain your suspense for now. So why do we make the distinction between the cranial and spinal nerves? There are two key reasons for this separation, both of which involve the origin and path of the nerves. Cranial nerves, in addition to originating directly in the brain, remain distinct from other nerves from origin to insertion. The spinal nerves, in contrast, merge with other spinal nerves shortly after emerging from their origin on the spinal cord. In some regions of the tetrapod body, these mergers form large distinct structures known as **plexuses**. We will discuss the plexuses in more detail later, but keep this in mind as we start to explore the different nerves.

Both cranial and spinal nerves can be further subdivided based on their function. Moving forward, we will discuss the difference between where the signals originate and where they go (sensory nerves, motor nerves, and interneurons). We can also classify nerves based on how they contribute to how the body functions. For this, we can group nerves into whether they are part of the "rest-and-digest" or "fight-or-flight" systems.

The first and most direct way to break nerves down is determining in which direction the nerve is headed (Figure 19.1).

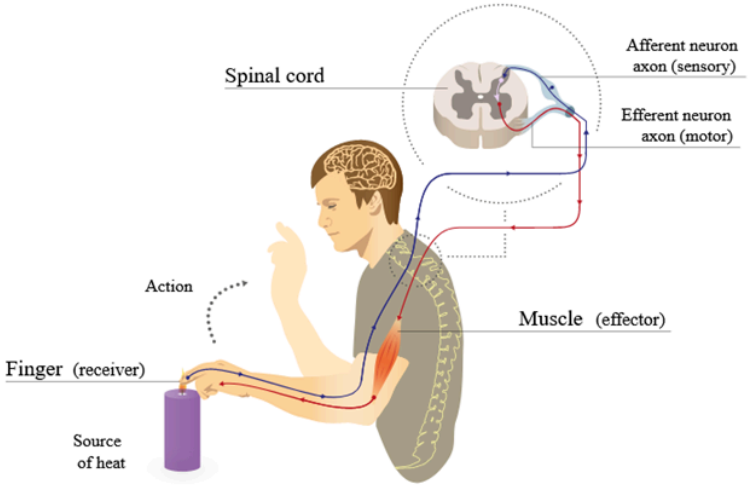

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Figure 19.1—The sensory and motor pathways for nerves in one reflex.

Axons can only carry signals in a single direction, so while we may have some difficulty determining direction in a large nerve, we know that individual axons are unidirectional. If an axon is carrying a signal toward the CNS, then we might refer to the whole cell as a **sensory** or **afferent neuron**. Signals that are carried away from the CNS do so on neurons that we can refer to as **motor** or **efferent neurons**. This is helpful more in a theoretical sense than a practical sense. This helps us visualize how the entire nervous system functions together. Information is carried from a **sensory receptor** (more on those in Chapter 20) to the CNS, where the integration and processing of signals occur. A response is sent out to an **effector**, which could be lots of different tissue types. *Sensory in, motor out*: This is the nervous system in a nutshell.

Now it's just a matter of looking at where the signals come from, how the signals arrive, what is done with that information, and how the response is carried. This is where the true complexity of the nervous system comes into play. How these all function together

is a little beyond the scope of this textbook, but it does help us separate structures out. However, you can still isolate different aspects of these subdivisions to help your understanding of the nervous system as a whole. You can see a great example of this in Figure 19.2.

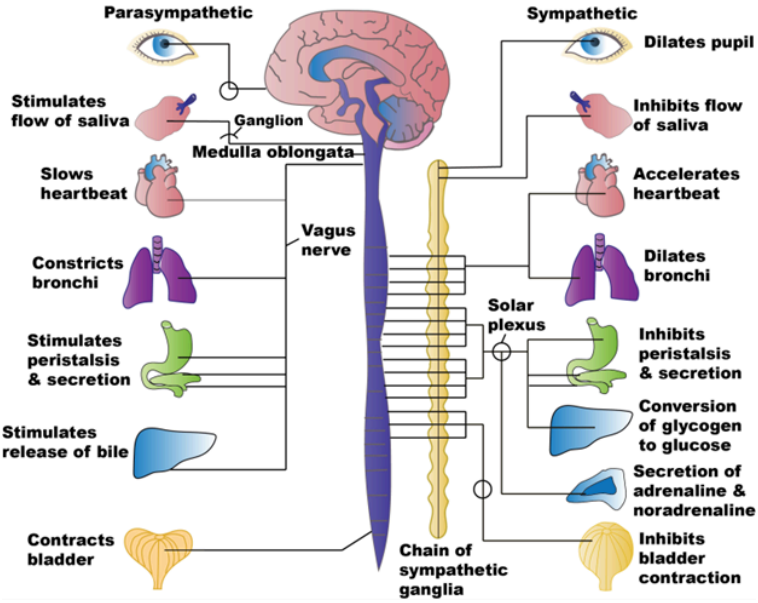

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Figure 19.2—The parasympathetic and sympathetic divisions of the autonomic system in humans.

In mammals, we can divide the **autonomic nervous system** (the components of the nervous system that regulate structures not under voluntary control) into the **parasympathetic** and **sympathetic divisions**. The parasympathetic system oversees the regulation of digestion and excretion and slows the heart rate, giving it the colloquial name of the "rest-and-digest" system. Activating the sympathetic division will increase heart rate, slow digestion, and cause the release of adrenaline, hallmarks of the

"fight-or-flight" system. Each division has different pathways (and different nerves) that carry their signals. These different nerves may eventually reach the same organs (e.g., the heart), but they have different impacts on the target organ (increasing or decreasing heart rate).

As we just discussed, individual neurons can be defined as afferent or efferent, sensory or motor, but whole nerves are a mix of both types. However, there are a few locations where the entire nerve consists of only sensory or motor nerves. One such location is right where the spinal nerves enter/leave the spinal cord. After passing through the **meningeal cuff** (an extension of the meninges associated with the spinal cord), nerves split into two pathways depending on whether they are sensory or motor (Figure 19.3).

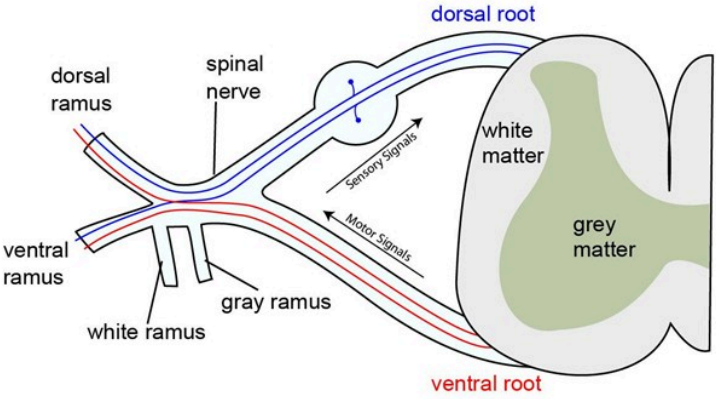

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Figure 19.3—The dorsal and ventral roots leaving the spinal cord.

These pathways form what we know as **nerve roots** and are given the same naming as the horns of the spinal cord from above: **dorsal root** and **ventral root**. This is one of the only locations where you can reliably identify a nerve as either sensory or motor in most vertebrates. The dorsal root contains only axons of sensory/afferent neurons, while the ventral root contains only axons of

motor/efferent neurons. In this way you can visualize the generalized function of the entire nervous system. Signals from sensory receptors travel through spinal nerves and move through the dorsal root. Entering the dorsal horn, the signals are processed/integrated, and a response is formulated. The response is generated in the ventral horn and leaves through the ventral root. It is then carried via spinal nerves to the target organ/muscle/gland. Note in Figure 19.3 that it seems the dorsal root and ventral root merge together right after leaving the spinal cord, and that is because they do. This condensed version of nervous system function is surprisingly similar to how reflexes work.

"What about the remaining regions of white matter in the spinal cord?!" you shout. They too can be divided into regions as they are divided by the gray matter horns. The different areas of white matter are known as **funiculi** (Latin for "string," a reference to the white nerves) or sometimes called columns: **dorsal funiculus**, **lateral funiculus**, and **ventral funiculus**. Their role is more variable across vertebrates, but there are a few generalizations we can make here. Remember that they are considered white matter as they consist of myelinated axons. Therefore, they are carrying action potentials (nerve signals) up, down, and across the spinal cord. Different regions within these funiculi are referred to as tracts, either ascending or descending. Keep this in mind when considering their function. In most groups, they make up pathways for signals coming from or to different parts of a body. There is some new terminology here, so do not hesitate to take your time and consider the wording carefully. One distinction that we have not discussed but is important here is the difference between **somatic** and **visceral**. The somatic and visceral components of the PNS represent different types of targets. To simplify the point for now, think of the somatic system as working with the skeletal muscles (voluntary) and the visceral system for all the internal organs (involuntary). Let us combine that with the sensory and motor roots/horns and note that we end up with four different ways to classify nerves in the PNS: **somatic sensory**, **somatic motor**,

visceral somatic, and **visceral motor**. And in fact, these are what the different funiculi can be divided into. The dorsal funiculus has **somatosensory tracts** (ascending to the brain), and the ventral funiculus has a large **somatomotor tract** (descending). The lateral funiculus contains two tracts: an **ascending visceral sensory** and **descending visceral motor tracts**. While this overall pattern is consistent across vertebrates, as we will discuss later, there is some variation here as well.

19.3 Development

In the previous chapter, we glossed over one element of the development of the nervous system that is very key to the development of the spinal and cranial nerves. During neurulation, small groups of cells split off from the folding neural tube. Neural crest cells are a population of mesenchymal cells that originate from the dorsolateral surface of the neural tube, between the dorsal surface of the neural tube and the epidermis just above it. Neural crest cells have multiple functions (Chapter 4), but they play a huge role in the formation of the peripheral nervous system by giving rise to all the cranial and spinal nerves. The neural crest cells will migrate all over the body and are regulated by a whole host of gene and transcription factors that will ultimately determine what they will become. Neural crest cells that migrate in the trunk region will play a large role in the development of the nerves in that region, including the sympathetic and parasympathetic pathways. Several of the **ganglia** (groups of neuron cell bodies outside of the brain) of cranial nerves are formed by these neural crest cells as well. This is still a very active area of research, with new work appearing monthly.

19.4 Evolution of the Spinal Nerves

It may come as a surprise to hear that the spinal nerves do not greatly change over evolutionary time in the same manner as many of the other structures discussed in this book. There are a few large transitions that occur in the transition from water to land and with the development of paired appendages.

Cyclostomes

There is one key element to the spinal nerves in hagfishes and lampreys that must be addressed. In the previous section, we discussed that in most vertebrates it is extremely difficult to distinguish between sensory and motor nerves, as the two groups will travel together in intermixed nerves. The emphasis there was on *most*. The hagfish and lamprey are the exception to this rule. The spinal roots containing sensory neurons and motor neurons remain separate as they become spinal nerves. These separate spinal nerves continue to their targets completely separate. This is one of the reasons we understand the nature of the dorsal and ventral roots and horns and how they translate into sensory systems and muscle activity.

Chondrichthyes and Osteichthyes

The major transition in the jawed fishes is that we now see the intermixing of the dorsal and ventral spinal roots when they emerge as spinal nerves. Coinciding with these changes are also changes to the skeletal system that impact the structure of the spinal nerves. Beginning with the cartilaginous fishes, the notochord/vertebral column is no longer a single structure that runs the length of the

body. Instead, we have discrete blocks of bones (though recall that they are made of cartilage in Chondrichthyes) known as the vertebral column. Individual blocks are called vertebrae. Between individual vertebrae, there is an intervertebral disc, resulting in a little space between the vertebrae. This, along with notches on the vertebrae themselves, creates space for the spinal nerves to leave the spinal cord. These spaces also allow for individual movement of vertebrae during locomotion that does not impact the nerves themselves.

Tetrapods—Amphibians and Reptiles

When the tetrapods emerge onto land, the factors that influence the structure and function of the spinal nerves result in some significant changes. The evolution of limbs greatly changes the structure of the spinal nerves. The limbs, with their increased range and diversity of movements, required a series of major changes to the skeletal (Chapter 10), muscular (Chapter 11), and now the nervous system.

At the beginning of our discussion, one of the major distinctions between spinal and cranial nerves is that cranial nerves don't merge with each other, while spinal nerves do. While the spinal nerves do merge closer to the spinal cord in more basal vertebrates, we see more complex patterns of merging in tetrapods. There are several areas of complex merging to be found, but we will focus on the limbs and see that the spinal nerves that innervate them will change drastically. These groups of nerves will form a network that individually we can call a **plexus** (Latin, "braid"). There are several plexuses found in tetrapods, associated with the limbs. The forelimbs are innervated by the **brachial plexus**. "Brachial" typically refers to the forelimbs, and this is no exception. Figure 19.4 illustrates the human brachial plexus.

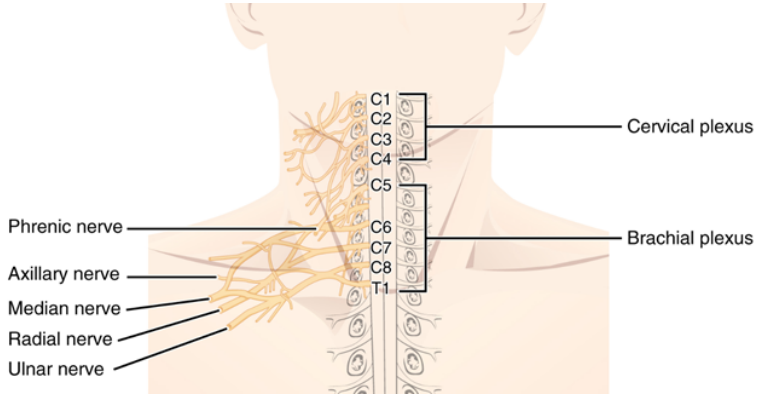

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Figure 19.4—The cervical and brachial plexus in humans.

Notice that at first glance, the plexus is a mess of connections, splits, connections, splits, and a few more connections. With a complex structure like the brachial plexus, it helps to define regions rather than individual elements until you get to the major nerves themselves. The other major plexus across tetrapods is found near the pelvis, associated with the hindlimbs. One unique element to note is that the spinal nerves are named for their location along the vertebral column. In the mammals, the vertebral column is divided into regions (Chapter 9), but outside of the mammals this is not the case. When investigating the brachial plexus of an alligator, for example, you would note it as originating from spinal nerves VII–XI.

19.5 Evolution of the Cranial Nerves

Before we begin to explore the evolution of the cranial nerves, we should take a moment to remind ourselves of what the cranial nerves are and why they are important both in the context of

evolution as well as being diagnostically important in both human and veterinary medicine. The cranial nerves function as spinal nerves do—individual neurons that carry signals to and from the brain. As was the case in the spinal nerves, they may only carry a signal in one direction, but most of the cranial nerves are intermixed, carrying both sensory and motor neurons. There are a few cranial nerves that are entirely sensory or entirely motor, and we will point these out when we encounter them. What makes them diagnostically important is how they interact with the CNS. The cranial nerves are special in that they connect directly to the brain. In a diagnostic setting, this means that disruption in their function can pinpoint damage to the brain itself without having to trace a path that includes other nerves and the spinal cord. They are also important because they are the nerves for the major external senses: vision, smell (olfaction), taste, hearing, and balance. Their evolutionary history also tells the tale of changing environments, historical functions, and how evolution tweaks existing structures through time.

As a matter of housekeeping before we dive in, we will agree to a common nomenclature when we refer to the cranial nerves. All the cranial nerves are given a name and number. The code "CN" is used in front of a Roman numeral with the name. For example, the nerve that runs from the eye to the brain is known as the optic nerve, and we will use CN II or refer to it by name. The cranial nerves number 0–XII, numbered in order from anterior to posterior (Figure 19.5).

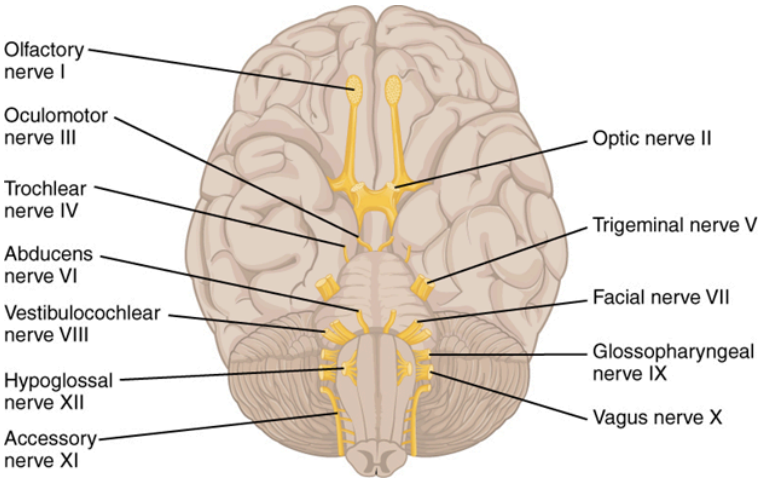

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Figure 19.5—The human cranial nerves.

We will approach this section a little differently from the other chapters in this book. Instead of our straightforward march through the major vertebrate groups, we will instead introduce each nerve and its primary function. There will be some discussion as to whether it is a primarily sensory nerve or motor nerve or contains elements of both. From there, we will work through our vertebrate groups in order to better understand how the changes in some of the cranial nerves reflect much larger changes to many of the other systems involved in the anatomy of the head and neck.

Cranial Nerve 0: The Terminal Nerve

The first cranial nerve is special in a few ways. First, it was only discovered and officially named after most of the other cranial nerves (hence it being referred to as CN 0). Second, it is only grossly visible in a few groups of fishes. However, its presence in other

vertebrates (including humans) has been known for some time. The function of the terminal nerve is still unclear. It originates in the brain near the olfactory centers and has been observed to be active in correlation with sex hormone activity. With this limited evidence, it is currently presumed to play a role in olfaction, particularly of pheromones as they relate to reproduction. It originates on the cerebrum before heading anteriorly.

Cranial Nerve I: The Olfactory Nerve

The olfactory nerve (CN I) is a great nerve to get started on the deep dive of the cranial nerves (ignoring the terminal nerve, like so many anatomists before us). As its name implies, the olfactory nerve delivers sensory information from the olfactory epithelium in the nasal passages to the brain. Unlike many of the other cranial nerves, the olfactory nerve carries only information to the CNS. In this way, we may say that the olfactory nerve is sensory only. The olfactory nerve carries information about odor, what we know as the sense of smell, to the brain. Depending on the particular vertebrate group, that may be a proper olfactory bulb or tract before diving deeper into the brain. The olfactory nerve originates on the cerebrum, associated with structures known as **olfactory bulbs**.

Cranial Nerve II: The Optic Nerve

The optic nerve (CN II) is also a purely sensory nerve, carrying visual information from the retina to the optic centers of the brain from its origin in the cerebrum. These paired nerves cross/meet each other at the optic chiasma before continuing on to the optic lobes.

Cranial Nerve III: Oculomotor Nerve

The oculomotor nerve (CN III) is the most anterior nerve to originate from the midbrain. Unlike the previous cranial nerves, the oculomotor (as the name suggests) is motor only. In many vertebrates, there are several skeletal muscles that control the movement of the eye itself, known as extraocular muscles. Most, but not all, of these muscles are controlled by the oculomotor nerve.

Cranial Nerve IV: Trochlear Nerve

The trochlear nerve (CN IV) is also a motor-only cranial nerve that also controls eye movement, originating in the midbrain. However, unlike the oculomotor nerve, the trochlear nerve controls only one muscle, a muscle that in humans we refer to as the superior oblique muscle. The superior oblique muscle has an odd course, anatomically speaking. It starts similarly to the other extraocular muscles, but its tendon passes through a cartilaginous pulley before inserting on the lateral aspect of the eye. This small pulley is known as a trochlea (Latin for "pulley"). This is where the trochlear nerve gets its name.

Cranial Nerve V: Trigeminal Nerve

The trigeminal nerve (CN V) is one of the largest cranial nerves, with a sizable and noticeable origin on the brain stem at the level of the pons. The trigeminal nerve (tri-, Latin for "three," and -geminus, Latin for "twin") is known for its three major branches that all course through the head: the **ophthalmic branch** (commonly denoted as V_1), **maxillary branch** (V_2), and **mandibular branch** (V_3). With its large branches and huge area of innervation, it should be no

surprise that the trigeminal nerve is our first mixed nerve. This means that it has both sensory and motor components. The trigeminal nerve is very important for several sensory components in the head (outside of olfaction and vision) and is the main innervator of the jaw-closing muscles. We will explore the trigeminal nerve in detail through our exploration of the evolutionary history of vertebrates, as it moves and changes frequently through time. We will also spend a lot of time with this nerve as a diagnostic tool for human health.

Cranial Nerve VI: Abducens Nerve

The abducens nerve (CN VI) is a nerve quite similar to CN IV except that it originates on the brain stem. It is a motor-only nerve, and it controls the last of the extraocular muscles we will consider here. Known as the lateral rectus muscle, it helps pull the eye laterally. This nerve follows a similar course and has the same function in all the vertebrates with the exception of the hagfish and lamprey, where it is seemingly absent.

Cranial Nerve VII: Facial Nerve

The facial nerve (CN VII) is another one of the cranial nerves that we will revisit frequently as we explore the different vertebrate groups. The facial nerve is a mixed nerve, with both sensory and motor components. Do not be fooled by the name, as it does not originate near the face (it is also in the brain stem) and its actions are also more complicated. The facial nerve only innervates muscles and sensory receptors in the face of mammals. In most of our other vertebrates, we will see that the facial nerve performs many other tasks in other regions of the head and neck. In many ways, the facial

nerve is a great example of how evolution tweaks and tinkers with existing structures. Following the track of the facial nerve, we can see quite clearly how changes in structure and function in some elements of anatomy have corresponding changes downstream. It is also a great example to see how the transition from water to land impacted not only the "major" musculoskeletal structures associated with movement and feeding but also the supporting structures. The facial nerve is also diagnostically important in humans, with many diseases and tests relying on the facial nerve.

Cranial Nerve VIII: Vestibulocochlear Nerve

The vestibulocochlear nerve (CN VIII), sometimes also referred to as the statoacoustic or auditory vestibular nerve, is a mixed nerve playing a role in hearing and balance, originating on the brain stem. These two senses are closely associated with each other in many vertebrates (see Chapter 20—Nervous System: Sensory Organs). The vestibulocochlear nerve is quickly separated into the vestibular (balance) and cochlear (hearing) divisions. Hearing and balance vary greatly in vertebrates but do exist across almost every group. While technically a mixed nerve, the motor component of CN VIII is rather minimal. In humans, the motor components are involved in a reflex involving the vestibular system and the eyes, with the vestibulocochlear nerve innervating the extraocular muscles.

Cranial Nerve IX: Glossopharyngeal Nerve

The glossopharyngeal nerve (CN IX) is much like the vestibulocochlear nerve in that its primary functions can be determined by digging into its name. Glosso- often refers to tongues, coming from a Greek origin ("tongue, word, speech"), while

the second half of the word is derived from the pharynx. There are many pharyngeal muscles, and this nerve plays a role in innervating many of those muscles. The glossopharyngeal nerve is another mixed nerve originating on the brain stem, with motor neurons for several muscles in the head and neck region (including the tongue in those vertebrates that have one). Sensory information comes from many different sources, including taste from the tongue, chemical sensing from several blood vessels, and information from the pharynx.

Cranial Nerve X: Vagus Nerve

The vagus nerve (CN X) is one of the longest nerves in the body. Elements of the vagus nerve innervate structures from the head all the way down to the abdominal organs. The vagus is also a mixed nerve; however, it is important to note some of the key differences that make the vagus unique (especially in humans). To this point, we have mostly been discussing the motor aspect of cranial nerves as controlling different skeletal muscles. However, as you will know from Chapter 11 (muscles), not all muscle in a body is skeletal. Cardiac muscle and smooth muscle still contract and still need at least some element of nervous system control (cardiac muscle will contract on its own but is regulated by the nervous system). Many of the tubular organs, with smooth muscle, are regulated by the vagus nerve. Heart rate is regulated through the vagus nerve (and the endocrine system), but there is still some controversy over the role of the vagus nerve in the force of heart muscle contraction. The vagus is also unique in that for several parts of its journey down the body, it contains both sympathetic and parasympathetic nerve fibers along its path. Along the neck and entering the chest, we refer to this conjunction as the **vagosympathetic trunk**, before the elements of the **sympathetic trunk** split off.

Cranial Nerve XI: Accessory Nerve

The accessory nerve (CN XI) is an interesting case in the world of the cranial nerves. The accessory nerve is a motor-only nerve, providing control of several muscles along the back of the neck and pectoral girdle. What makes the accessory nerve interesting is that it does not entirely originate from the brain. The accessory nerve has several rootlets that originate on the spinal cord. In mammals, these rootlets join together and travel cranially. They make a loop and reexit the skull, and this is why it was named as a cranial nerve. I hate the accessory nerve.

Cranial Nerve XII: Hypoglossal Nerve

The last official cranial nerve, the hypoglossal nerve (CN XII), originates on the medulla oblongata and immediately exits the skull. It is a motor-only nerve, and it also controls several of the muscles associated with the tongue.

Now with introductions out of the way, let us see how these nerves change through evolutionary time in response to changing structures, habitats, and functions.

Cyclostomes

While representing the earliest diverging lineage of vertebrates, it is beneficial to remind ourselves that both the hagfish and lamprey are highly specialized evolutionary lineages in their own rights. So while they are, in other body systems, used to represent the ancestral condition, they can show some specializations that may confuse the investigator. In the lamprey, all 12 pairs of cranial nerves can be located and distinguished, and the presence of a terminal nerve is

still contested. Hagfishes also seem to have all 12 nerves, but some recent work finds that CN X–XII are hypermodified in the hagfish and that CN XI and XII may actually be independent evolutions of those nerves rather than the result of shared ancestry.

Fishes

In most comparative anatomy resources, the cranial nerves of sharks serve as the de facto representative of the vertebrate ancestral condition (Figure 19.6). Fortunately for us, there are not a lot of differences between the different groups of fishes. There is some variation naturally, but much of it is beyond the scope of this book. With that in mind, we will focus on the sharks, noting key differences when they appear. Eleven pairs of cranial nerves can be found in sharks, missing CN XI, the accessory nerve. All the cranial nerves remain separate from each other until they reach their destination, unlike the hagfish and lamprey. Because they remain separate from each other, it is also easier to determine what their function is. For the first few cranial nerves, we see that the cartilaginous fishes set the trend that the other vertebrates will follow.

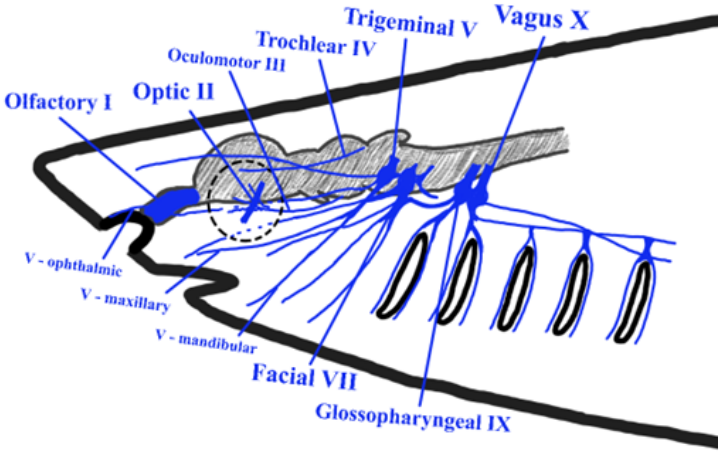

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Figure 19.6—The cranial nerves of a generalized shark. Not visible are terminal (CN 0), abducens (CN VI), and vestibulocochlear (CN VIII) nerves.

The terminal and olfactory nerves are closely associated with each other. The olfactory nerves are quite short, running to the olfactory bulbs. The terminal nerve is a much longer nerve, running from the hypothalamus section of the brain to the nares. As we stated earlier, the terminal nerve plays some role in olfaction, currently hypothesized to be involved in the detection of pheromones, but evidence is scant. The optic (CN II), oculomotor (CN III), trochlear (CN IV), and abducens (CN VI) nerves serve the same function as in other vertebrates, which we discussed earlier. The first nerve that differs from the general pattern is the trigeminal nerve (CN V). The trigeminal nerve still has four major branches in sharks, with the previously mentioned **ophthalmic branch** being separated into the **ophthalmicus profundus** and **ophthalmicus superficialis**. The mandibular branch is subdivided into superior and inferior branches. The main sensory function of the trigeminal

in sharks is to receive sensory information from the skin around the snout. However, there is an important distinction to make here. The ampullae of Lorenzini, electroreceptors in the snout of most cartilaginous fishes, are not innervated by the trigeminal nerve. The nerves that carry the signals from the electroreceptors are derived from similar nerves for the lateral line system. These nerves are technically cranial nerves, as they go directly to the brain, but they are not given official names or numbers. The trigeminal's motor nerves innervate the jaw-closing muscles: adductor mandibularis being the major jaw closer in sharks.

The facial nerve (CN VII) reveals its mammalian naming bias in the cartilaginous fishes. The facial nerve…has nothing to do with the face. It was originally thought to receive sensory information from the most anterior portions of the lateral line system, though it has been determined in several shark species that those are independent nerves that run near the facial nerve. However, the facial nerve does still play some role in the sensory system of sharks, receiving information from taste receptors scattered throughout the mouth. The motor components of the facial nerve are split between areas associated with the head and the anterior body. Within the head, the facial nerve controls several of the pharyngeal muscles, those associated with the second pharyngeal arch. It also contributes to the control of muscles and mucus membranes associated with the first gill. If you check out the muscles chapter (Chapter 11), you will see these muscles make some pretty big migrations as vertebrates moved from an aquatic environment to a terrestrial one, and the facial nerve travels along with those muscles. This will also have some implications for the role of the facial nerve in human health (Box 19.1). The statoacoustic nerve (CN VIII), as it is often referred to in the fishes, does innervate the hearing and balance sensory receptors. In addition, it also has efferent innervation of the lateral line system. This does not mean it also receives information from the lateral line system. In fact, the lateral line system is innervated by two unofficial cranial nerves, the anterior lateral line nerve and the posterior lateral line nerve.

The glossopharyngeal nerve (CN IX) represents another mammalian bias in the naming of the cranial nerves. In our fishes, CN IX plays a larger role in the first gill arch (third branchial arch). Fishes lack a true tongue, and the glossopharyngeal nerve, given its position, is more directly associated with the first few gills than the true pharynx. The last officially recognized cranial nerve in fishes is CN X, the vagus nerve. In our general introduction to the cranial nerves, we indicated that the vagus nerve serves a lot of important roles in the body, especially beyond the head. In the shark, the vagus is a very large nerve. Its impacts are widespread, from the last few gills to almost the cloaca itself. The vagus impacts the esophagus, heart, stomach, intestine, and parts of the body wall itself.

Tetrapods: A Brief Note

With the movement of the tetrapods from the water to the land, there were a few changes in the spinal nerves as limbs evolved and became the driving force for locomotion. However, these changes could be generalized across the larger groups of tetrapods. As we make our way into the cranial nerves of these same groups, we will find that this is not going to be the case. To further a theme already described in other chapters (but take a look at the skull and muscle chapters [Chapter 8 and Chapter 1] in particular), the transition from water to land caused a great deal of changes to the sensory systems and feeding apparatus of tetrapods. It should not be surprising then that the cranial nerves associated with those systems are the ones that we are going to observe the most changes in. In our remaining tour of the vertebrates, we will focus on those changes. CN I–IV and VI will largely go unmentioned, as they continue to function in the same ways as we described in the generalized section. The unnamed lateral line nerves will reduce and disappear. CN X continues to dominate control of the body wall and the visceral organs, but changes to its innervation cranially will

require some attention. The nerves not already mentioned must be considered in detail, and we will find some surprising trends among all this variation.

Nonmammalian Tetrapods

There are two seemingly innocuous changes (and one major one) that happen in the head as you move onto land that impact the skeleton, muscles, and therefore the cranial nerves. These are the evolution of the tongue and neck. The big change, the loss of the gills, is obviously going to impact a lot of these structures as well. However, many of the cranial nerves undergo little or no change as we transition to a fully terrestrial lifestyle. CN II, III, IV, VI, and VIII are quite similar in the tetrapods to their condition in the fishes. As will be discussed briefly in the sensory systems chapter (Chapter 20), there is an additional or accessory olfactory organ that appears and is particularly well developed in the reptiles (and mammals) known as the **vomeronasal organ**, or VNO for short. The olfactory nerve (CN I) serves this accessory organ, and in some groups the individual nerves that innervate the vomeronasal organ form a distinct branch. The trigeminal nerve (CN V) is more variable in the reptiles than it would seem to be in the other vertebrates. This is in large part due to the evolution of a few "new" senses and their distribution in the head. Alligators and crocodiles have unique touch receptors embedded in and around the scales of their jaws and snouts called **integumentary sense organs** (ISOs). These ISOs are innervated by the major branches of the trigeminal nerve. The same can be said for the infrared "vision" of the vipers, boas, and pythons. In these snakes, the path of the trigeminal nerve takes a detour. CN V in these snakes will have several small nerves move toward the visual processing centers of the brain, which is why we refer to this infrared reception as a type of vision. The trigeminal nerve is associated with the jaw closers, and that remains true. But it will also be part of that new structure in the mouth, the tongue.

The muscles of the tongue are not derived from the jaw closers, instead originating from farther back. However, some of the sensory components of the tongue (not taste) do send their signals to the brain along the trigeminal nerve. Conversely, the venom glands of snakes have unique muscles that control venom output during a bite. These muscles are controlled by the trigeminal nerve. Beyond the trigeminal nerve, there is more considerable variation. The facial nerve (CN VII) is highly variable, even within groups. Recall that the facial nerve in fishes innervated elements of the hyoid/pharyngeal muscles as well as the first gill arch. For our fully aquatic salamanders (like the mudpuppy), this is still the case. However, in our terrestrial amphibians and our reptiles, the changes may be pretty dramatic. It may be helpful to think of the facial nerve in the context of what is happening to the muscles in this area. As the muscles develop around the second and third visceral arch, many of them migrate cranially. The facial nerve travels with them. Part of the tongue is innervated by CN VII, and this is where the sense of taste comes in. The other brand-new element in the head is the appearance of salivary glands. The salivary glands help in the chemical digestion and transport of food along the digestive tract. They are also linked to the nervous system, which can control output and when salivation occurs. These new glands are innervated by the facial nerve.

The next really big changes occur when we reach CN IX, the glossopharyngeal nerve. In our fishes the glossopharyngeal nerve, despite its name, was confined to the gills, where it played a large role in controlling the muscles associated with the gills. Once again, this does not change in our aquatic salamanders. With the transition to land, the gills disappear. What happens to all those muscles; do they disappear as well? We know that this is not the case. Instead, the muscles migrate cranially, becoming part of the musculature of the pharynx and the majority of the tongue. The glossopharyngeal nerve travels with the muscles as they migrate and becomes one of the major nerves that control the muscles of the tongue. In our fishes, we moved on to the vagus nerve (CN X), and

that was the end. The vagus nerve in fishes we said interacted with the more caudal gills and their muscles, plus all the internal organs in the body cavity. The internal organs in our tetrapods still have a lot of vagal innervation, but it is the fate of those nerves that control the gill muscles where changes happen. There is an important shift when we get to the tetrapods (particularly the reptiles for now). With the reduction in dermal bones (Chapter 11) and the evolution of a distinct neck, the function of CN X also changed.

The neck requires muscles to provide stability and allow for movement. While homologous to the gill muscles, the innervation pattern evolves in correlation with the muscles. We get the reemergence of the accessory nerve (CN XI). The accessory nerve has an interesting developmental and evolutionary tale to tell. In fishes, the accessory nerves originate beginning at the junction of the brain stem and spinal cord and a few spinal nerve rootlets. It travels a short distance and anteriorly, where it merges with the vagus nerve. As a result, many researchers do not consider the fishes to have a true accessory nerve. With the evolution of a neck region, the origin of the accessory nerve moves more posteriorly, splitting from the vagus nerve and becoming its own distinct cranial nerve. Developmentally, the accessory nerve isn't a cranial nerve at all. It originates on the spinal cord, in roughly the sections corresponding to C1–C6. However, after it emerges, it heads cranially, enters the skull, and then promptly exits the skull again—technically a "cranial" nerve, as it leaves the cranium, but it does not originate from the brain. We will get into its specific pathway in humans, as there are some clinical implications of this movement. However, we cannot forget the vagus nerve and its role in the gill muscles. Given the original proximity of the gills to the pharynx, some of the branches of the vagus are associated with the pharyngeal muscles. This is still true, and branches also innervate the muscles of the larynx in our tetrapods. One branch of the vagus nerve, known as the recurrent laryngeal nerve, leaves the vagus in the thoracic (chest) cavity and makes its way all the way back into the neck before reaching some of those laryngeal muscles. Last, we

arrive at our discussion of the hypoglossal nerve. The hypoglossal nerve (CN XII) emerges as a new nerve not described or found in the fishes and only in tetrapods. Developmental evidence suggests that it originated as a branch of the vagus nerve, as is hypothesized for the accessory nerve. As its name implies, the hypoglossal nerves course under the tongue and also innervate several of the muscles in the tongue. These muscles are critical for moving food within the mouth and swallowing.

Humans and Other Mammals

With a few notable exceptions, we can use human cranial nerves to describe all the functions of the cranial nerves in mammals. While exploring the patterns and functions of the cranial nerves, we will also discuss their clinical relevance in humans (Figure 19.6).

The presence of a terminal nerve in mammals is still being hotly debated. While there is no doubt that some mammals communicate with pheromones, it remains unclear if the terminal remains as an independent nerve or has become a branch of the olfactory nerve (CN I). The olfactory nerve is very short in mammals, extending only a few millimeters through openings in the skull known as the **cribiform foramina**. In humans, damage to the olfactory nerve results in the loss of smell. Cranial nerves II–IV and VI (optic, oculomotor, trochlear, abducens) function in mammals as they do in other vertebrates. The optic nerve is responsible for vision. To test for damage in the optic nerve, asking someone if they are able to see is not enough. Recall from the brain chapter (Chapter 18) that the visual processing centers in humans are in the rear of the brain. How do you distinguish damage to the visual processing centers from damage to the physical structures of the eye? This is where the pupillary reflex comes in. Shining a bright light into the eyes results in contraction of the muscles of the iris, shrinking the pupil. This reflex does not require input from the visual processing center and can be used to test for damage in the optic nerve. The

other three we know control the muscles that move the eyeball within the orbit. You can test these nerves yourself. Hold your finger in front of your face and keep your head perfectly still. Now move your finger to your left, right, up, and down. Was your eye able to track your finger in each direction? What if you move it diagonally? Look at the tip of your nose? If so, congratulations, those four cranial nerves (optic, oculomotor, trochlear, and abducens) are all intact. Damage to any of those nerves results in difficulty tracking or even maintaining a straightforward gaze. The eye may "roll" in a direction even at rest with damage to the trochlear or abducens nerves.

The trigeminal nerve, as we already know, is a large and important nerve for all vertebrates. It still controls the jaw-closing muscles, as well as getting considerable sensory feedback from the many elements of the head. An excellent example of this can be found in the platypus. The platypus has electroreceptors that can detect small electric fields in water. The signals from these receptors are carried on the trigeminal nerve. In humans, damage to the trigeminal nerve can manifest itself in many different ways. Difficulty chewing, speaking, and swallowing can all be related to trigeminal nerve damage. Simultaneously, because of the amount of sensory information being carried by the trigeminal nerve, numbness in particular parts of the face and mouth points to the trigeminal nerve. If you've had a cavity filled or your wisdom teeth removed, you know this all too well. The superior and inferior alveolar nerves innervate the teeth of the upper and lower jaws, respectively. Both nerves are branches of the trigeminal nerve, coming from the maxillary and mandibular branches.

The facial nerve (CN VII) in humans and other mammals has also changed since we last visited it in the nonmammalian tetrapods. When we discussed amphibians and reptiles, the facial nerve played a large role in the tongue and hyoid apparatus. However, as mammals evolved, there were different changes to those muscles (recall that reptiles and mammals share a common ancestor from a long, long time ago). Much of the musculature,

developmentally, that originates in the pharyngeal region moves to cover the face in mammals. We call these muscles the muscles of facial expression. Smile, furrow your brow, pretend to be shocked. A lot of that movement was directed by the facial nerve. The tortuous route of the facial nerve, beginning behind the ear in humans and working its way to the face, has some diagnostically important implications, sometimes resulting in paralysis of half the face (Box 19.1). Similarly to amphibians and reptiles, the facial nerve also stimulates salivary gland activity and is the home to our sense of taste. Excessive salivation or the loss of taste may independently indicate damage to the facial nerve.

Box 19.1—The Facial Nerve, Bell's Palsy, and Ear Infections

The facial nerve serves as a good reminder that the cranial nerves are paired, with one coming off the left and one coming off the right side of the brain. A loss of one of the nerves may only impair that side of the body. This is the case for Bell's palsy, the name of the condition where one of the facial nerves is damaged, resulting in paralysis to that half of the face. The causes of Bell's palsy are myriad, but there is one that should draw our attention in the context of the evolution of vertebrates. Draw the path of the facial nerve from its origin to all those facial muscles. You notice that it passes right around the paths of the middle and inner ear. Why does this matter? In severe ear infections, the pressure buildup in the ear will compress the facial nerve, resulting in the paralysis of the facial muscles! Going from a gill muscle controller to tongue and hyoid nerve to the muscle of facial expression has twisted and

turned the facial nerve in all sorts of directions, and not always for the better.

The vestibulocochlear nerve (CN VIII) carries the information for our senses of hearing and balance. Hearing tests that include pitch, volume, and direction can all be used to determine function of the vestibulocochlear nerve. Additionally, you may test balance by standing on one foot with the other pointed forward. It is important, when testing hearing, to test both sides of the head to determine whether damage is localized to one of the nerves or to the brain itself.

The glossopharyngeal nerve (CN IX) is very important in the movement of the tongue and pharynx. It is also the key player in the gag reflex. This combination of swallowing and gagging means that while it is clearly of functional importance, it is also an easy nerve to test for function. Loss of the gag reflex may reflect damage to CN IX (if you're planning on testing this reflex, we recommend keeping a bucket nearby just in case). Beyond its motor components, the glossopharyngeal nerve serves critical sensory functions. The glossopharyngeal nerve is closely associated with the carotid arteries, and it is here that blood pressure and blood oxygen can be monitored.

The vagus nerve (CN X) is of critical importance in human health. As in other vertebrates, the vagus innervates some elements of the pharynx, larynx, and internal organs. The vagus directly interacts with the heart, lungs, and digestive tract. The vagus can impact heart rate, breathing rate, and the movement of food through the digestive system. Additionally, it receives information from blood pressure sensors and position sensors in the larynx. The recurrent laryngeal nerve is quite long in people and even longer in some mammals (Box 19.2). Diseases and disorders involving the vagus nerve are prevalent throughout the medical and veterinary

community (see 19.7 Further Reading). As the vagus plays a role in swallowing, it can be tested similarly to the glossopharyngeal nerve.

Box 19.2—Longest Nerve in the World, the Recurrent Laryngeal Nerve

The recurrent laryngeal nerve is a branch of the vagus nerve that innervates several of the muscles that are important for swallowing and producing vocal sounds. Why are we dedicating a whole box to just one nerve? The recurrent laryngeal nerve is a great example of how development and evolution tinker with existing structures, with the result not always making the most sense. In sharks and other fishes, there is no recurrent laryngeal nerve; instead, several of the branches of the vagus nerve innervate the gill muscles (Figure 19.7A). The evolution of the tetrapods changes both the musculature in this region of the body (Chapter 11) as well as the fate of the aortic arches (Chapter 15). Some of these muscles become those of the larynx. The heart and aortic arches move caudally in time, and those muscles stay close to the head. The recurrent laryngeal gets caught by the ligamentum arteriosum (the remnant of a shunt between the aorta and pulmonary trunk), wrapped around the remaining aortic arches but still needing to reach the neck, as seen in the mouse (Figure 19.7B). The most striking example of this can be seen in the giraffe (Figure 19.7C). In giraffes, the left recurrent laryngeal nerve takes a detour that is over 4.5 meters (15 feet) in length!

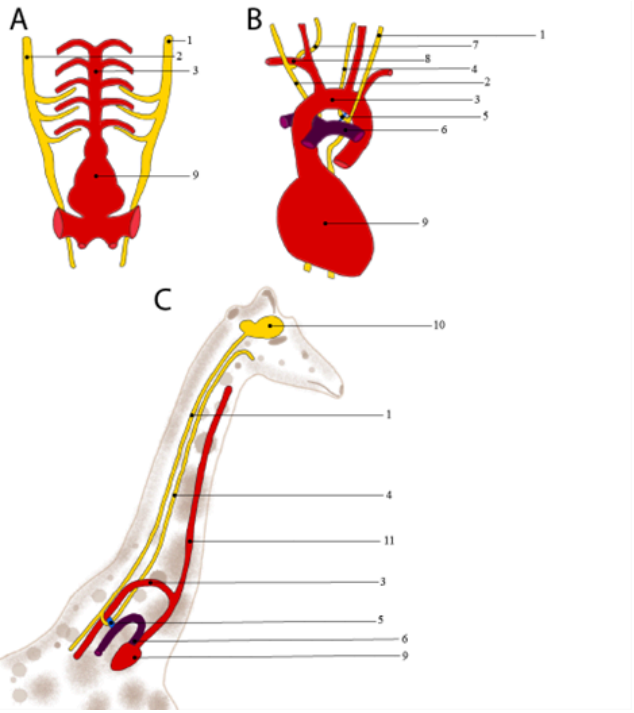

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Figure 19.7—The evolution of the recurrent laryngeal nerve from the ancestral condition in the shark (A) *to the mouse* (B) *and its extreme in the giraffe* (C)*. Labeled structures are* (1) *left vagus nerve*; (2) *right vagus nerve*; (3) *aorta*; (4) *left recurrent laryngeal nerve*; (5) *ligamentum arteriosum*; (6) *pulmonary trunk*; (7) *right recurrent laryngeal nerve*; (8) *right subclavian artery*; (9) *heart*; (10) *brain*; *and* (11) *common carotid artery.*

The accessory nerve (CN XI) is entirely motor in humans, innervating the trapezius and sternocleidomastoid muscles. The movements of these large muscles are obvious to an observer, and

any difficulty in their movement should point to damage in the nerves. Much like in reptiles, the accessory nerves actually begin in the spinal cord. Many spinal rootlets merge together before entering the skull via the foramen magnum. After entering, the accessory nerve makes an about-face, exiting the skull via the jugular foramen on its way down to the muscles.

Finally, the hypoglossal nerve, CN XII: As was the case in the reptiles, the hypoglossal nerve innervates many of the muscles of the tongue. The tongue muscles controlled by the hypoglossal nerve are of critical importance not only to swallowing but to speech as well. Speech is an incredibly complex process, involving several areas of the brain. Therefore, relying on speech as a diagnostic tool is not particularly helpful in the context of the hypoglossal nerve. Instead, a small motor test can reliably isolate the function of the hypoglossal nerve. If you can stick your tongue out, both protracting and retracting the tongue, then the function of the hypoglossal nerve is intact.

19.6 Summary

The peripheral nervous system is the link between the central nervous system and the rest of the body. It carries sensory information to the brain and outgoing effector information to muscles, glands, and organs that respond to changes. Some of these nerves, the cranial nerves, bypass the spinal cord, communicating directly with the brain and making them invaluable structures for understanding vertebrate evolution as well as clinically relevant human anatomy.

Application Questions

1. How do reflexes act as a miniature version of the nervous system itself?
2. Why does damage to one optic nerve not result in blindness in that eye?
3. How can some nerves be considered mixed?

19.7 Further Reading

1. The Developing Cranial Nerves in Zebrafish
2. Vasovagal Syncope and Laryngeal Hemiplegia in Horses
3. The Development of the Cranial Nerves

19.8 References

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20. Nervous System: Sensory Organs

BRETT AIELLO; LAUREN SIMONITIS; KATE CRISWELL; AND BILL RYERSON

Focus Questions—to Guide Your Reading of This Chapter

1. This chapter is an overview of multiple systems—a departure from other chapters, which delve heavily into one system. To keep track of them all, it may be helpful to make a table of the key components of each system. While making your table, answer the following questions: What are each system's sensory receptors? What stimuli do receptors respond to? What are the transduction pathways from sensory stimuli to neural signals for each system?
2. We explore sensory systems in both terrestrial and aquatic vertebrates. How do vertebrate sensory systems differ depending on which fluid the organism lives in—air or water? What about semiaquatic organisms?

20.1 Introduction

The ability of an animal to acquire information about the internal state of its body, the movement of its body, and the interaction of its body with the external environment is critical for survival. The acquisition and processing of sensory information help animals make decisions and responses that are appropriate for a given situation. These decisions and responses to stimuli are often highly influenced by the particular sensory information acquired by an animal. For example, sensing an internal state of hunger and the subsequent use of various sensory systems like vision and olfaction are often required to locate a food source. Sensory information on the internal state of the body and monitoring variables like blood pressure and volume, blood chemistry, and temperature allow our body to respond appropriately in order to maintain homeostasis. Thus, nearly every bodily function and behavior is triggered or modulated by the acquisition of information.

In order to acquire information from both internal and external sources, the vertebrate body relies on **sensory receptors**. A sensory receptor is responsible for the conversion of a given stimulus (often a specific type) into a neural signal that can be transmitted along a neuron to the central nervous system. The conversion of the sensory stimulus into a neural signal is called **transduction**. The specific transduction mechanism is highly dependent on the type of sensory receptor and the type of stimulus being transduced. Regardless of sensor type, transduction results in the **depolarization** (a change in the membrane potential to a more positive value) of the sensory receptor; if the stimulus is of a great enough intensity, the depolarization will reach a threshold that triggers the firing of an action potential. However, vertebrates are not aware of each piece of information being sensed at every moment. For example, we can assume that no person reading this book has ever been keenly aware of their specific blood pH level at any given moment. Differently, the feeling of pain is often a

stimulus that is hard to ignore. Thus, it is important to distinguish the difference between sensation and perception. **Sensation** is the acquisition of information, while **perception** is the conscious awareness of that sensation.

Sensory receptors can be classified according to the origin of the stimulus (inside or outside the vertebrate body), the modality of the stimulus they transduce (e.g., pain, temperature, mechanosensation, among others), or the concentration of sensory receptors used to detect a certain stimulus. Receptors that sense stimuli outside the body can be referred to as **exteroceptors**. Two examples of exteroceptors include the receptors responsible for vision and touch, as both types of stimuli (light and the interaction of the body with a physical stimulus) originate from outside the body. Receptors that sense stimuli inside the body can be referred to as **interoceptors**. An example of interoceptors includes the receptors responsible for sensing stimuli associated with internal organs (e.g., blood pressure, blood chemistry, and bladder fullness).

Stimulus modality, or the type of stimulus capable of generating a response in a given receptor, is another method for classifying sensory receptors. In other words, many types of receptors only respond to a specific type(s) of stimuli. **Thermoreceptors** respond to changes in temperature. **Mechanoreceptors** respond to the deformation of the cell or a tissue due to the application of physical force, which can include touch and pressure of the skin from an external stimulus or an internal stimulus like bladder or blood pressure. **Chemoreceptors** respond to chemicals and include receptors responsible for taste and smell as well as those responsible for sensing internal body chemistry like blood sugar level. **Nociceptors** are responsible for sensing pain and tissue damage. Finally, **radiation receptors** respond to electromagnetic radiation, which includes visible light and radiation of other wavelengths. Over evolutionary time, these receptors have diversified and undergone specializations to facilitate additional but related sensory capabilities that will be discussed throughout the chapter. It is important to note that these

classifications are not mutually exclusive, and the categorization of receptors by stimulus origin and modality can be combined to describe a receptor. For example, the receptor used to sense the pressure required for you to effectively hold a glass cup can be referred to as both a mechanosensor and an exteroceptor, while the receptor used to sense the pressure and volume of urine within the mammalian urinary bladder can be referred to as both a mechanosensor and an interoceptor.

It is also possible to classify sensory receptors based on the density of their distribution in the vertebrate body. This classification generally falls into two groups: general and specialized sensation. **General sensation** refers to senses that rely on the wide distribution of the sensory receptors across the body. General sensation can include both interoceptors and exteroceptors that could be distributed across the skin, muscles, tendons, joints, and organs. The senses involve receptors capable of mechanosensation, nociception, chemosensation, and thermosensation. In contrast, **special sensation** refers to sensory capabilities that typically rely on a more complex sensory organ. A sensory organ is composed of a high density of individual sensory receptors supported by other tissue types (as opposed to single receptors distributed across the body). A prime example of a special sensation and a sensory organ is the vertebrate eye. Indeed, many of these specialized and complex sensory organs used in special sensation are associated with the head, and interestingly, the enlargement of specialized sensory organs associated with the head is a trait that evolved incredibly early in the evolutionary history of vertebrates.

Sensory receptors are capable of coding different features of a stimulus that include stimulus location, intensity (and rate of change), and duration. The location of a given stimulus is determined by the **receptive field** of a sensory receptor, which is the specific area of the body or the relative location in the surrounding environment where a given receptor can detect a stimulus. Similarly, the neural pathway that transmits this signal to the central nervous system and the specific region of the central

nervous system that receives the signal are highly organized, allowing the body location of the stimulus to maintain its identity.

Stimulus duration, or the duration in which a stimulus is being detected by a sensor, can be encoded both by a single receptor and groups of receptors. A single sensory receptor can encode information on the duration of a stimulus in two ways, which are dependent on the type of the receptor. Receptors can respond to stimuli in different ways. **Rapidly adapting sensors** typically fire a burst of action potentials at the onset and, often, offset of a stimulus. Thus, calculating the time difference between these two bursts of action potentials reveals information on stimulus duration. Differently, **slowly adapting sensors** typically respond to a stimulus throughout the duration a stimulus is being detected, and the rate of action potential firing frequency often decreases over the duration of the stimulus. The use of both slowly and rapidly adapting fibers, which are often found working together, therefore provides two overlapping mechanisms for encoding features of stimulus duration.

Stimulus intensity, which is the magnitude of the stimulus being applied, as well as changes in stimulus intensity, can also be encoded through changes in receptor firing rate. For example, as stimulus intensity increases, the firing frequency of slowly adapting sensors can also increase over the entire stimulus duration. Similarly, as stimulus intensity increases, rapidly adapting sensors can increase both the burst duration and the frequency of action potentials over the duration of the burst, providing two additional means of encoding stimulus intensity. Relative changes in burst duration and firing frequency of rapidly adapting sensors are also known to encode information on the rate at which a stimulus intensity changes while it is being applied. In other words, rapidly adapting sensors encode how fast or slow a stimulus intensity is changing over time (e.g., flipping a light switch versus using a dimmer to slowly increase the brightness in a room).

Ultimately, it is the integration of the information sensed by various sensory receptors that allows vertebrates to perceive the

complex stimuli around the body. This is referred to as **multimodal sensory integration** and is a powerful mechanism that helps vertebrates generate the correct behavioral output in response to a variety of stimuli being experienced at the same time. Here, we will go through these different sensory modalities and types of sensory receptors.

20.2 Sense: Radiation Reception

All vertebrates are continuously exposed to **electromagnetic radiation** (**ER**). ER is a form of energy that travels in the form of waves, lying on a continuum often characterized by its wavelength or frequency (Figure 20.1). You are probably familiar with ER, as humans have been able to harness different wavelengths of ER for various purposes, like sending information in the form of radio waves, quickly heating your favorite brand of ramen noodles using microwaves, visualizing the inside of the vertebrate body using X-rays, and lighting your room at night with visible light to help you read your favorite anatomy textbook. ER is also a form of energy that naturally occurs in our universe and is constantly being emitted from solar objects like our sun. Given these various artificial and natural sources of ER and the ways they interact with natural objects in our world, it is not surprising that vertebrates (and other organisms) have evolved different ways to detect this energy to help acquire information about the world around them.

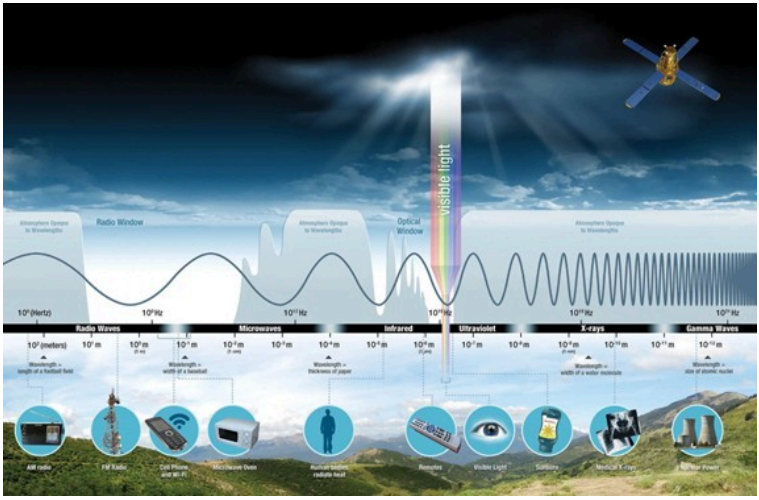

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Figure 20.1—An overview of the spectrum of electromagnetic radiation. Electromagnetic radiation consists of waves of energy that vary in wavelength. The electromagnetic spectrum is the continuum that includes all wavelengths of electromagnetic radiation. Different vertebrate species have evolved adaptations to sense different ranges of the spectrum, including the eye. However, humans (and most vertebrate species) are only capable of sensing a small portion of the spectrum called visible light.

Across vertebrates, species have evolved different sensory structures to sense different but limited ranges of the ER spectrum. While the most notable and widely utilized ER sensor is the vertebrate eye, which is capable of transducing visible light into neural signals, other sensors have evolved in select species to sense UV light and infrared radiation. In this section we will consider these different radiation receptors that have evolved and the corresponding sensory capabilities they have unlocked.

Vision

Vision is the perception of objects in the environment by detecting the visible light an object emits or reflects. Light is naturally produced in a few specialized organs that have independently evolved in various organisms like fireflies and angler fish. However, most objects in our environment cannot emit light. Instead, we rely on the detection of light that is reflected by an object in the environment. This is why, for humans and some other vertebrates that have not evolved specific adaptations, it is so difficult to see at night, as there is not much light reflecting from the objects in our environment.

The vertebrate eye is a sensory organ that has evolved to facilitate the transduction of visible light into neural signals using a set of sensors called photoreceptors. Yet due to the physical properties of light, other structures have also evolved in the vertebrate eye to help facilitate the focus of this light on the photoreceptors. Therefore, the vertebrate eye performs two primary functions: to capture and focus light and subsequently transduce that light into a neural signal that can be sent to the brain.

Box 20.1—The Eye as a Camera

The anatomy and physiology of the vertebrate eye are strikingly similar to the structure-function relationship of a photography camera. As we prepare to study the vertebrate eye, use these questions to focus your investigation. Consider the following questions regarding the structure and function of a photography camera:

1. Are there ways to regulate how much light we let into the camera? If so, how?
2. What are the consequences of letting in too much light or too little light?
3. Can we focus a camera on objects at different distances? If so, how? (If the emergence of digital cameras has made this utility obsolete in your daily life, think back to your first biology lab and consider how you focused a microscope to produce a clear and crisp image of the slide.)
4. How does light entering the camera get converted into a picture?

The Anatomy of the Vertebrate Eye

The anatomy of the eye is surprisingly conserved across vertebrates. Thus, as is often customary when studying the eye, we will begin our investigation with a mammalian (or human) perspective, as it is a good representation of the general condition found across vertebrates with few adaptations. During this initial investigation of the mammalian eye, we highlight some key differences between the mammalian condition and the condition found in other major vertebrate clades, and we will follow our investigation of the mammalian eye by examining additional differences between the mammalian condition and the condition found in other major vertebrate lineages.

The eye of all gnathostomes is approximately spherical in shape and composed of three distinct layers. These layers (in order of superficial to deep) are the superficial **fibrous layer** (sometimes

referred to as the tunica fibrosa or even incorrectly simply as the sclera) and **the uvea** (i.e., the tunica vasculosa), and the deepest layer is often referred to as the **retina** (but can also be titled the tunica interna, especially in a human anatomy course).

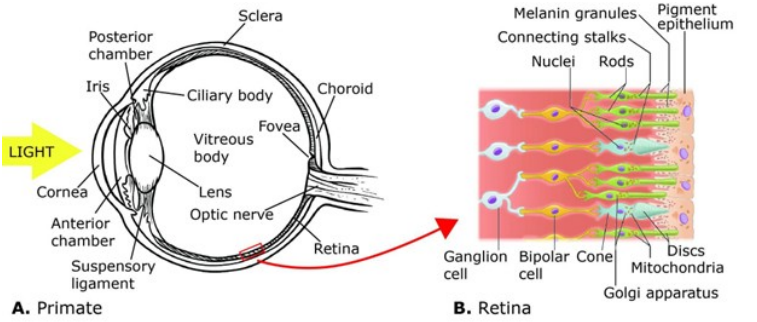

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Figure 20.2—Structure of the primate eye and retina. (A) A diagram of primate eye anatomy from a lateral perspective. The eye is composed of multiple layers and chambers. (B) The retina, the innermost layer of the eye, is composed of three cell layers. Light must first pass the ganglion and bipolar cells of the retina before reaching the photoreceptors.

The Tunica Fibrosa

The superficial fibrous layer of the eye is divided into two functionally distinct regions, the sclera and the cornea. The **cornea** is the anterior transparent portion of the fibrous tunic through which all light entering the spherical eye must first pass. In all directions surrounding the cornea, the adjacent fibrous layer is referred to as the **sclera**. The transition between the cornea and the sclera is referred to as the **limbus** and is quite distinct in most mammals because the scleral tissues in mammals possess a distinct white color. Indeed, in a human anatomy textbook, the sclera is often referred to as the white of the eye. However, it is noteworthy that the white coloration of the mammalian sclera is a derived trait;

the sclera is nearly transparent in some other extant vertebrate lineages, which is likely reminiscent of the ancestral condition.

The sclera is a tough fibrous layer composed mostly of a dense connective tissue with collagen fibers running in multiple directions. The sclera provides structure and protection, and because of the irregular arrangement of the collagen fibers, it is appropriate for resisting the internal pressures of the eye that are experienced in all directions. Indeed, the sclera is a wonderful demonstration of the structure-function relationship at the tissue level. The sclera not only resists internal forces and provides the eye with structure; it also serves as the attachment point for the extraocular eye muscles, which are responsible for moving the eyeball. The sclera is nearly devoid of cells and the metabolic needs of the sclera are so low that no direct blood supply is required. The vasculature that is found in the sclera is therefore not supplying the scleral tissue but rather passing through the superficial layer of the eye en route to the deeper uvea layer. Finally, in some vertebrates, the sclera is further reinforced by the presence of bony sheets known as **scleral ossicles**. The next time you are at a natural history museum, examine the orbits of skeletons on display (e.g., dinosaurs) and see if these scleral bones were included in the display.

The **cornea** itself is composed of three layers. The middle layer of the cornea, the substantia propria, is the thickest corneal layer and composed of multiple layers of regularly arranged collagen fibers. The substantia propria is sandwiched between epithelial tissue on both the superficial and deep surfaces. The deep epithelial layer is composed of simple squamous epithelium, while the superficial epithelium is composed of nonkeratinized stratified squamous epithelium that is actually continuous with the epithelia of the conjunctiva that lines the inner surface of the eyelids. The cornea is completely avascular, which is a critical feature of the eye, as any blood vessels traversing the corneal surface would obstruct your field of view. Although the cornea is avascular, it is densely innervated by sensory endings for pain and touch; it is these sensory endings that provide the sensory feedback that initiates the

blink reflex (and make it so difficult to withstand the puff of air that is a common component of a routine eye exam).

The Uvea (Tunica Vasculosa)

The **uvea** is the layer of the vertebrate eye just deep to the fibrous tunic. The uvea is closely associated with the sclera throughout the inside of the posterior five-sixths of the eye sphere but remains separated from the cornea anteriorly. The uvea is composed of three different regions: the choroid, the ciliary body, and the iris.

The **choroid** is the largest region of the uvea found sandwiched between the deep-laying retina and superficial sclera. The uvea is both highly vascularized and pigmented. The vascularity found in the choroid provides a source of nutrition for the surrounding ocular tissues, especially the highly metabolic retinal cells. In diurnal species, the pigmentation of the choroid can help prevent internal reflections of light and reduce the unintended entrance of light through the eye wall. However, in some nocturnal vertebrates, the choroid actually includes the **tapetum lucidum**, which is a reflective tissue that functions to reflect the minimal light entering the eye at night to further stimulate the photoreceptors of the retina. It is the tapetum lucidum that is responsible for the "eye shine" you can observe when a light is shined on the eyes of some nocturnal vertebrates—a pet cat is a great example.

The choroid continues toward the corneal surface of the eye and transitions to become the **ciliary body**, which is predominantly composed of smooth muscle called the ciliary muscle. The ciliary muscle forms a ring of smooth muscle surrounding the lens of the eye. The contraction of the ciliary muscle, in mammals, can modulate the shape of the lens to help focus on objects at different distances (see below), and it is attached to the lens by the suspensory ligament. The ciliary body is also associated with the secretion of one of the intraocular fluids filling

the internal cavity of the eye (see the subhead The Chambers and Intraocular Fluids of the Mammalian Eye).

The ciliary body also supports the **iris**, which is a thin layer of smooth muscle and pigmented epithelium. The iris is situated in the space between the cornea and the lens. The smooth muscle acts as an adjustable diaphragm that can expand and contract to regulate the amount of light entering the eye. The central opening of the iris is what is referred to as the pupil. However, the pupil is not a true structure of the eye, as it is formed from the opening of the iris. The smooth muscle of the iris is sandwiched between two pigmented layers. The corneal-facing (anterior in mammals) layer contains melanocytes, which produce the pigments that give the iris different colors. The general function of these pigmented layers is to reduce stray light from entering the eye anywhere adjacent to the pupil. The double layer is critical in reducing the entrance of stray light, as the translucent cornea is often larger in diameter than the pupil aperture of the iris.

The Retina (Tunica Interna)

The third, and deepest, layer of the eye consists of the cup-shaped **retina** and the genesis of the optic nerve (CN II). The retina is the photosensitive layer of the eye and is responsible for transduction of light into neural signals. The retina is composed of three cell layers. The most superficial retinal cell layer at the back of the eye (and just deep to the choroid) is composed of **photoreceptors**. Vertebrate photoreceptors belong to two classes: rods and cones. **Rods** are generally responsible for night vision and do not sense color, therefore producing a gray-scale image. **Cones** are generally responsible for bright light and color vision. In humans, there are three types of cones, providing trichromatic vision. Cones and the evolution of color vision will be discussed more later.

The rods and cones synapse with **bipolar cells**, which are the cells of the second retinal cell layer. Also found associated with

this cell layer are **horizontal cells**, which make horizontal connections through synapses with adjacent photoreceptors.

Bipolar cells in turn synapse with ganglion cells. **Ganglion cells** are at the front of the retina and compose the deepest retinal cell layer. The axons of the ganglion cells ultimately converge to form the optic nerve (CN II). Some ganglion cells are themselves photoreceptive but do not contribute to image formation; instead, the photoreceptive capacity of ganglion cells functions to sense light intensity that can influence the pupillary reflex as well as broad patterns of day-night cycles that can influence the circadian rhythm of the body. Also included in this layer are **amacrine cells**, which synapse to create horizontal connections between adjacent ganglion cells.

There are fewer bipolar cells than there are rods and cones, and there are fewer ganglion cells than there are bipolar cells. Thus, a single ganglion cell often synapses with multiple bipolar cells, and a single bipolar cell often synapses with more than one photoreceptor; this results in neural signal integration along this pathway from the point of transduction in the photoreceptor to the optic nerve (CN II).

It is important to note that the arrangement of the retinal cell layers means that light must first pass through the deeper, corneal-facing layers of the retina (ganglion cells and bipolar cells) before reaching the photoreceptors at the back of the eye.

The Chambers and Intraocular Fluids of the Mammalian Eye

In gnathostomes, the three layers of the eye and the connections between their associated structures result in the presence of three chambers that are each filled with a type of intraocular fluid. Two chambers are present between the lens and the cornea of the eye. The more anterior of these chambers is appropriately named the **anterior chamber** and is the space between the cornea of the tunica fibrosa and the iris of the tunica vasculosa. Posteriorly, in the space

between the iris and the lens, is the **posterior chamber**. Finally, the third chamber, known as the **vitreous (or vitreal) chamber**, is the space between the lens and cup-shaped tunica interna on the interior (deep) surface of the back of the eye.

All three chambers are filled with an intraocular fluid. The anterior and posterior chambers are both filled with a minimally viscous serous fluid secreted by epithelial cells of the tunica vasculosa layer. This watery serous fluid is often referred to as the **aqueous humor**. In comparison to the anterior and posterior chambers, the third chamber, the vitreous chamber, is filled with a relatively more viscous (jellylike) fluid called the **vitreous humor**.

Optical Components and Pathway of Light Through the Eye

As the retina, which contains the cells that transduce light into neural signals, is located on the deep surface of the posterior side of the eye, all light that hits the retina must pass through several structures. The eye structures that light penetrates en route to the retina are often referred to as the optical components of the eye and function to not only admit light but also bend (refract) and appropriately focus the light on the retina. The pathway of light through the eye occurs in the following order: the cornea, the aqueous humor of the anterior chamber, the pupil (however, this is simply a space of variable diameter determined by the contractile state of the muscles of the iris), the aqueous humor of the posterior chamber, the lens, and finally, the vitreous humor of the vitreous chamber.

It is relevant to mention this pathway, as the refraction (or bending) of light can occur to different degrees as it passes through each of these different structures or media. Ultimately, the formation of an (crisp and clear) image depends on the appropriate bending of light as it passes through the optical structures of the eye. The ability of a structure or a medium to bend light is quantified by its refractive index, and the bending or refraction of light occurs

when it passes from one structure or media to another that has a different refractive index. The larger the difference in the refractive index between two sequential structures or media, the more the light will bend. You can observe this phenomenon yourself if you place a pencil in a glass that is half full of water—the piece of the pencil outside of the water appears offset from the piece of the pencil contained within the water. Thus, you can probably already start imagining that the refraction of light must pose different challenges to animals that are fully terrestrial, fully aquatic, or in extreme cases, living at the interface of water and land.

The optical component of the eye that plays the most significant role in focusing light on the retina is strongly dependent on the habitat of the animal. In most fully terrestrial vertebrates, the cornea is responsible for the majority (~66%) of the total refraction of light entering the eye. The refractive power of the cornea is so great in terrestrial vertebrates because of the relatively large difference between the corneal refractive index and that of the air that light passes through before reaching the corneal surface. In terrestrial vertebrates, the remaining refraction is primarily accomplished by the lens, which functions to focus the light precisely on the photoreceptors of the retina. Indeed, individuals who rely on corrective lenses (i.e., glasses or contact lenses) to see clearly are using these lenses to properly focus (or bend) light so that it directly hits the retina; in humans, the cause of improper focus is usually related to differences in eye size, where an elongated eyeball causes the light to focus anterior to the retina and a flattened eyeball causes the light to focus posterior to the retina.

In fully aquatic vertebrates, the lens plays the most significant role in focusing light on the retina. The refractive index of water is nearly identical to that of the cornea and the aqueous humor filling the chambers between the cornea and lens. Thus, in fully aquatic vertebrates, it is the lens that provides the primary refractive power and is responsible for nearly 100% of the focus of light on the retina.

Accommodation and the Diversity of Accommodation Mechanisms Across Vertebrates

In order to focus on objects located at different distances within an individual's visual field, an animal usually needs to dynamically change the magnitude of light refraction in the eye. This process is known as **accommodation**. In gnathostomes, accommodation results from changes in the placement or shape of the lens, changes in the shape of the cornea, or some combination of these mechanisms. In gnathostomes, these accommodation mechanisms are generated by the contraction of muscles contained within the eye itself, while the lamprey (a jawless vertebrate) relies on extraocular eye muscles to accomplish accommodation. Interestingly, despite the generalized anatomy of the eye being well conserved across vertebrates, the mechanisms (and associated morphology) of accommodation are relatively diverse in comparison. Here, we will review this diversity in accommodation mechanism and morphology within each major vertebrate clade.

Before we continue it is important to note that not all vertebrates have a significant requirement for accommodation. The ability of the eye to focus on objects at different distances within the visual field is inversely proportional to the axial length of the eye. Therefore, vertebrates with very small eyes have little need for accommodation. In addition, vertebrates living in low light levels also have a low necessity for accommodation. Species living or behaving exclusively at low light levels usually have poor visual resolution, and that negates the need for any additional focus, as it would not significantly enhance the clarity of the image. Finally, animals that routinely transition between aquatic and terrestrial environments on a daily basis rely on accommodation to counteract the gain and loss of the refractive power of the cornea on land and underwater, respectively.

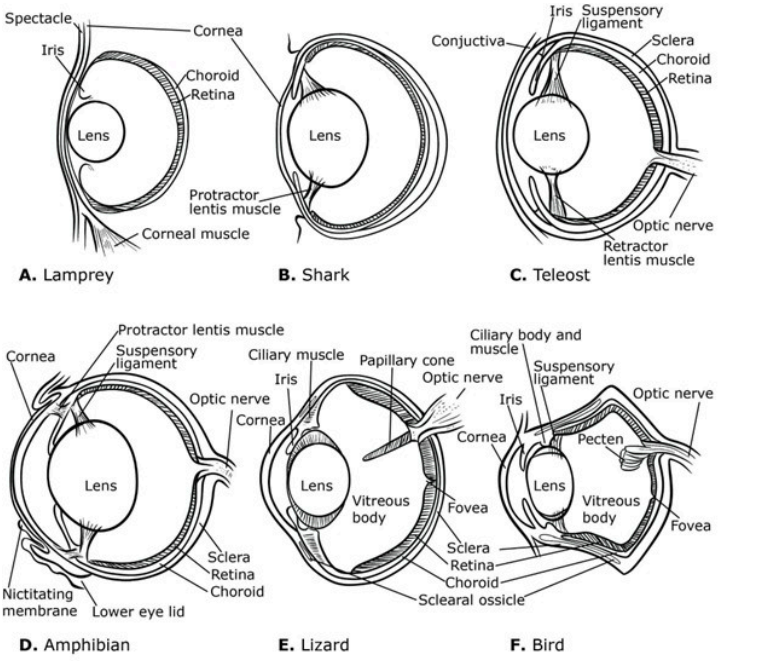

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Figure 20.3—Comparison of the eye structure across vertebrates. The vertebrate eye and its components are generally conserved across species as can be seen when comparing the eye anatomy of (A) lampreys, (B) sharks, (C) fishes, (D) amphibians, (E) lizards, and (F) birds. Despite the general conservation of the optical components of the vertebrate eye, different mechanisms and structures facilitating accommodation have evolved across vertebrates.

Lamprey

The lampreys are a lineage of vertebrates in which the mechanism of accommodation is not well studied. Thus, here, we will review what is currently known about lamprey accommodation mechanisms. Lamprey accommodation has been best studied in the genus *Petromyzon*. Interestingly, lampreys are the only currently known vertebrate lineage that relies on extraocular eye muscles to

mediate accommodation. In at least two species of *Petromyzon*, an extraocular muscle, the cornealis muscle, attaches to the superficial aspect of the cornea. The contraction of the cornealis muscle mediates the formation of lateral tension in the eyeball, causing the corneal surface to get pulled into a flatter state, and thus the front of the cornea is now positioned more posteriorly. As the corneal surface is flattened to lay more posteriorly, the corneal disposition subsequently makes contact with and pushes the lens closer to the retina (a more posterior position). The posterior movement of the lens closer to the retina allows lampreys to focus on more distant objects. In contrast, the relaxation of the cornealis muscle ultimately results in the movement of the lens to a more anterior position that is farther from the retina. Thus, just as you move the lens closer and farther from a microscope slide when focusing your microscope in general biology, some species of lamprey can move and control the position of the lens relative to the retina in order to focus on more objects at different distances.

Interestingly, it is not clear whether or not all lamprey species have the ability to undergo visual accommodation. For example, *P. marinus* does not possess the extraocular cornealis muscle. However, it has also been suggested that the simultaneous co-contraction of all six extraocular eye muscles results in the squeezing or compression of the eyeball around the equator separating the anterior and posterior halves. In this situation, the long axis of the eye between the anterior cornea and the posterior retina will elongate; the elongation of the long axis of the eye, which is the pathway light takes, will naturally lead to a mechanism of accommodation by increasing the distances between the cornea, lens, and retina. In other words, the eye goes from a spherical shape to become more of an egg-like shape with the apexes of the eggs being aligned with the anterior-posterior path of light. It will be interesting to see further research explore this hypothesis and whether this mechanism is reminiscent of the ancestral state of vertebrate accommodation methods.

Chondrichthyans and Most Teleosts

The mechanism and morphology of accommodation are generally similar in most chondrichthyans and teleost fishes. In both groups, the lens of the eye is displaced to mediate accommodation. In chondrichthyans, the lens is generally displaced anteriorly toward the cornea to focus on objects that are closer in the field of view. In teleosts, the lens is generally displaced posteriorly toward the retina to focus on objects that are farther or most distant in the field of view. The lens is generally spherical in both chondrichthyans and most teleosts. In both groups, the lens is supported dorsally by a suspensory ligament that runs between the ciliary body and the lens. Ventrally, the lens is supported and actuated by a lentis muscle. In chondrichthyans, the muscle is called the **protractor lentis muscle**, as it moves the lens anteriorly, and in teleosts, the muscle is called the **retractor lentis muscle**, as it moves the lens posteriorly. However, in some teleost species, the stimulation of the retractor lentis muscle results in oblique or vertical movements of the lens. Furthermore, an additional muscle within the choroid layer is also present in some teleost species (e.g., deep-sea fishes) that could function to modulate corneal shape and also contribute to accommodation. It is likely that accommodation is far more variable and complicated across fishes than is currently understood. Finally, while chondrichthyans do have the ability to accommodate, studies suggest that this ability is relatively limited.

Amphibians

The two primary extant clades within amphibians, Anura (frogs) and Urodela (salamanders), rely on similar mechanisms of accommodation. Similar to the chondrichthyans, both of these groups rely on the anterior/forward displacement of the lens toward the retina in order to focus on closer objects within the field of view. In Urodela, accommodation is accomplished by the

contraction of a single, ventrally located protractor lentis muscle, while the dorsal side of the lens is supported by a suspensory ligament. In Anura, anterior lens displacement is accomplished by the combined action of both a dorsal and a ventral protractor muscle; the ventral protractor muscle is prominent, however. In addition to the attachment to the lens, the lentis muscles of both Anura and Urodela also attach to the ciliary body.

Reptiles (Including Birds)

Across the group that includes turtles, birds, and other modern "reptiles," the method of accommodation relies on a change of shape to the lens, cornea, or both. The mechanisms are too diverse to cover in full detail here, but published journal articles review these methods in grave detail. Here, we will provide a few interesting highlights.

In birds, the ciliary muscle is subdivided into anterior and posterior divisions. The anterior division of the ciliary muscle attaches to both the sclera (which is thickened in birds due to the presence of scleral ossicles) and the cornea. The contraction of the anterior division of the ciliary muscle flattens the cornea and can have a significant impact on the refractive power of the cornea. Additional accommodation in birds occurs through the posterior division of the ciliary muscle, which can contract to modulate the shape of the lens. The contraction of the posterior division indirectly modulates lens shape in birds, as the shape change is mediated through the ciliary body and circumferential muscle fibers of the iris. Ultimately, contraction of the posterior division of the ciliary muscle results in the anterior portion of the lens being squeezed and distended in the anterior direction.

Lizards and Snakes

The mechanisms and morphology of accommodation have not been recently studied in lizards or snakes and warrant reevaluation in both groups. However, in lizards, all historical accounts suggest a process that is very similar to birds in that accommodation is accomplished through the modulation of both corneal and lens shape using a similar morphological mechanism to birds. In snakes, there is some evidence that the corneal shape can be modulated for accommodation. However, most historical accounts in snakes suggest the forward displacement of the lens is the primary mechanism of accommodation in most snakes. The mechanism for lens displacement in snakes is poorly understood. The interspecific differences in snake eye accommodation might correlate with interspecific differences in ecology, and the evolutionary history of the different snake clades might be a significant driver in the differences across species.

Mammals

The mammalian accommodation mechanism and morphology are best studied in primates. In primates, the primary means of accommodation is through the modulation of lens shape. Lens shape is accomplished through the contraction of the ciliary muscle. The ciliary muscle of primates is attached to the lens through the suspensory ligament. The contraction of the ciliary muscle reduces tension in these ligaments, allowing the lens to form a more spherical shape, which occurs due to the inherent elasticity of the lens. The subsequent relaxation of the ciliary muscle results in the formation of tension in the suspensory ligaments and the flattening of the lens.

It is likely that variation in the magnitude of accommodation ability and mechanism exists across mammals. For example, many mammals are nocturnal, and accommodation rarely

occurs in nocturnal animals, as it does not improve visual focus. Furthermore, many mammals possess small eyes where, again, accommodation is sometimes not needed due to the small eye size (see above). Indeed, rats and mice likely do not possess the ability to accommodate and do not seem to have the necessary morphology. Some mammals might also rely on the displacement of the lens for accommodation, as has been suggested in cats and sea otters.

The Photoreceptors of the Retina

The retina of most vertebrates contains two classes of photoreceptors: **rods** and **cones**. Rods are not able to discriminate between different colors but are very sensitive to light, enabling vision in dim and dark conditions. Cones are the photoreceptors responsible for color vision but are less sensitive than rods and function primarily in bright conditions. The retina of all vertebrate eyes (to our knowledge) contains rods, and most contain cones (with few exceptions, see below). Both rods and cones contain a class of pigment called **opsins**, which are responsible for absorbing light. Through downstream connections to a series of intracellular molecules, opsins are responsible for the transduction of light into a neural signal that ultimately travels along the optic nerve (CN II).

In cones, different subtypes of opsins exist and are directly related to differences in the color vision capacity across vertebrates. Recall that visible light ranges in wavelength, and across the spectrum of visible light, different wavelengths are directly related to different colors. The ability to sense and discriminate colors occurs because different opsin subtypes are maximally sensitive to different wavelengths of visible light (and thus different colors).

Color vision is likely to have evolved early in the history of vertebrates and since diversified significantly over evolutionary time. Across vertebrates, at least four different types of cones are known to exist and respond to different ranges of the visible light

spectrum, but the specific opsins sensitive to specific wavelengths have been lost and reevolved over evolutionary time. Further, clear and colored oil droplets, which can function to increase sensitivity to a particular wavelength, have also been incorporated into and lost from cones over evolutionary time. Thus, since the evolutionary history of color vision is complicated, we will here report major transitions and general color vision capabilities in the major vertebrate groups.

It is possible that ancient vertebrates possessed four different types of cones and were able to finely discriminate color. In support of this hypothesis, extant vertebrates belonging to ancient clades that branched early in vertebrate evolutionary history (lamprey, some chondrichthyans that have not lost color vision, and many actinopterygians) all possess four different cone types. In order of increasing wavelength, these cone types are sensitive to violet, blue, green, and orange, respectively. Many extant turtles, birds, and other reptiles maintain the presence of four cones in their retinas. Most mammals, however, only possess two cone types and have lost the cones sensitive to blue and green light. If this is hard for you to believe, given your (the reader's) ability to discriminate color, you are right to be skeptical. Humans and some other primates independently reevolved a cone type that contains an opsin allowing the perception of orange color. Thus, the capacity of color vision is quite different between humans and the majority of the pets we keep in our home (e.g., a fish or turtle, which can discriminate more colors than a human, or a cat or dog, which can distinguish fewer colors than a human).

Regardless of the number and ratio of different photoreceptors, light (and therefore images and color) is only sensed and transduced by retinal photoreceptors; it is not until those neural signals reach the brain that light is perceived. The evolution and diversification of the vertebrate brain itself are quite extensive, and thus it is difficult to succinctly review the different pathways and locations these neural signals are sent to from the retina after being carried by the optic nerve (CN II). However, in

most vertebrates, multiple synapses occur in the relay of the neural signals carrying visual information en route to the various visual processing centers. In most amniotes, the first synapse in this relay usually occurs in the thalamus, which you might recall contains nuclei relaying much of the incoming sensory information from the periphery.

Anatomical Adaptations to Dark Environments

The evolution of nocturnality and the ability to see in dark, low-light environments has evolved independently multiple times across vertebrates. In each instance, many analogous solutions have evolved. One aspect of nocturnal vision that is generally true across all nocturnal animals is its association with poor visual acuity (image clarity). This is why nocturnal species often have a reduced capacity for visual accommodation. Here, we will review a few of the most common solutions that have evolved to enable low-light vision. Note that these different adaptations for nocturnal vision are far from mutually exclusive.

One of the simplest solutions to low light is the evolution of a maximum pupil size that is large relative to the size of the eye, which results in a brighter image because proportionally more light is able to enter the eye. However, the position and size of each optical component of the eye are incredibly sensitive and tuned to others, which results in many other corresponding changes to eye and corneal shape, lens position, and relative intraocular compartment size necessary to balance the relatively larger pupil size.

A second solution that has repeatedly evolved in response to nocturnality is an increase in the ratio of rods to cones as well as an increase in the total number and density of rods within the retina. While a retina entirely composed of rods is exceedingly rare (e.g., some species of deep-sea fishes, bats, armadillos, lizards, and snakes), a significant increase in the ratio of rods to cones is quite

common. In addition, rod shape often evolves to become long and slender, which results in a higher density of rods per unit of retinal area. Ultimately, a high density of rods results in a greater degree of summation between the rods and the bipolar (and ganglion) cells, which increases sensitivity to light at the expense of visual acuity.

Interestingly, while the vertical slit pupil is regularly associated with nocturnality, the possession of a slit pupil alone does not increase light sensitivity. The slit pupil actually evolved as a mechanism needed to ensure the full and adequate closure of the pupil. The retina and photoreceptors of nocturnal animals are so sensitive to light that they must also evolve a mechanism to protect the retina from excess light (for example, when your cat decides to hang out with you during the day). A round pupil changes diameter through the contraction of circular, ring-shaped pupillary muscles, but these muscles are unable to shorten to a significant enough distance that entirely closes the pupil. However, the muscles that control pupil size in a slit-shaped pupil are arranged in a way that allows the pupil to be closed adequately to not overstimulate the highly sensitive retinal cells. Thus, the evolution of the slit pupil itself does not directly improve vision in low-light situations. Rather, the evolution of the slit pupil is an adaptation associated with nocturnality that helps protect the highly sensitive photoreceptors by allowing the pupil to fully close in comparison to a round pupil.

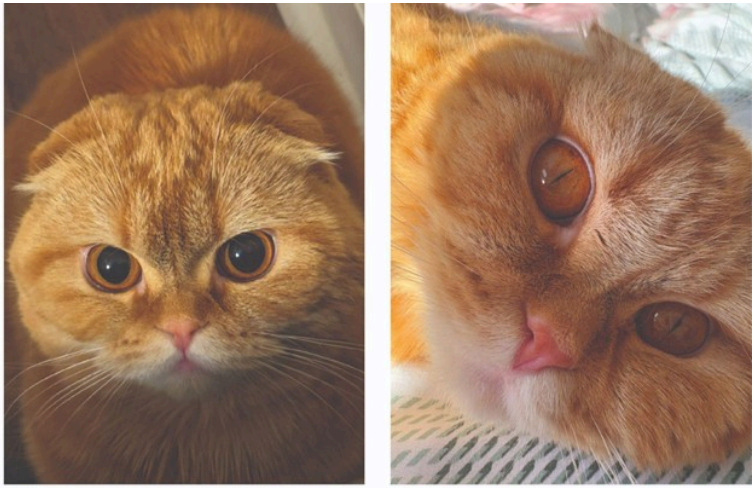

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Figure 20.4—The slit pupil is an adaptation associated with nocturnality. In species utilizing a slit pupil, the pupil shape can vary from (A) round, when fully open, to a (B) slit shape, when nearly closed. The presence of a slit pupil is demonstrated here in the house cat (Felis catus).

Finally, the repeated and independent evolution of a tapetum is another common mechanism that helps facilitate nocturnal vision. The **tapetum lucidum** is the general term we provide for a variety of reflective structures that have evolved to reflect light after it reaches the back of the eye and enhance the stimulation of the photoreceptors. It is the tapetum lucidum that is responsible for the eye shine we observe when a bright light, like the headlights of a car, is reflected from the eyes of nocturnal vertebrates. However, the tapetum lucidum has evolved independently numerous times and as such has taken multiple different forms. Most commonly, the tapetum lucidum is incorporated into the choroid layer of the eye and sits just behind the retina. Here, it has evolved in at least two ways. One way is through the evolution of a fibrous, tendinous connective tissue that

sits on the posterior surface of the choroid. The shiny, tendinous layer of tissue is then able to reflect light, which might not be too surprising if you have ever observed the shiny surface of a tendinous sheet or aponeurosis while dissecting your favorite species in a vertebrate anatomy lab. A different evolution of a choroid-based tapetum lucidum occurs by adding reflective inclusions (often guanin crystal) to the cytoplasm of epithelial cells within the choroid. Similarly, a reflective tapetum lucidum has also evolved directly within the retina through the inclusion of reflective particles inside the cytoplasm of retinal epithelial cells.

Other Types of Radiation Receptors

Pineal Gland and Eye

The pineal complex of vertebrates is another system that, at least ancestrally and in a handful of extant vertebrates, also has photoreceptive capabilities. The **pineal complex** is a group of one or more organs associated with the diencephalon of the vertebrate brain. The two most common organs of the pineal complex found across extant vertebrates are the **epiphysis** and the **parietal organ** (also known as the parietal or third eye). Ancestrally, it is most likely that the parietal organ (i.e., parietal eye) was used as a third eye that had photosensitive capabilities that were accessory to the paired eyes that also evolved early in vertebrate history. However, over the course of vertebrate history, this complex has evolved incredible variation. A pineal complex that includes a photosensitive organ (usually the parietal eye but sometimes the epiphysis) is still found in extant vertebrates including lampreys, frogs, and many reptiles. However, in other species it has been lost or transformed entirely into an endocrine organ. For example, in mammals and birds, only the epiphysis remains and is used entirely as an endocrine organ

that secretes the hormone **melatonin**, which helps regulate the internal clock and circadian rhythm of the animal. In these cases, the epiphysis is usually referred to as the pineal organ. Thus, despite no longer having photoreceptive capabilities in some clades, it still retains a connection to daily cycles of light and dark.

UV Receptors

Some vertebrate species also contain retinal photoreceptors that respond to ultraviolet (UV) radiation. There is no clear phylogenetic signal behind the distribution of retinal UV receptors, but they can be found in most major vertebrate clades other than Mammalia. The functional significance of UV reception is not clear, but in terrestrial vertebrates, it might be related to tracking prey. For example, animal urine reflects UV light, which you might have experienced when using a black light to find dog or cat urine in the carpet of your home. Similarly, one functional purpose of this might be related to predators that possess UV receptors tracking the urine of their mammalian prey.

Infrared Receptors

Receptors sensitive to infrared (IR) radiation have evolved independently at least three times across vertebrates. IR receptors have independently evolved in vampire bats (*Desmodus rotundus*), the snake families Boidae and Pythonidae, and a different clade of snakes consisting of the pit vipers (Crotalinae within the family Viperidae). In all cases, the ability to detect IR radiation aids in the detection of prey, particularly in low light levels at night. IR radiation is naturally emitted from the bodies of all living animals and therefore will also be emitted at night even when there is no visible light source able to reflect off the body. Thus, the ability to

sense the emitted IR radiation from a nearby prey item provides a significant advantage to a nocturnal species (or even a diurnal species that has poor vision) in locating its prey.

Developmental Origins of the Eye and Associated Structures

Paired eye development in vertebrates begins when the forebrain (diencephalon) **evaginates** (bulges outward) and protrudes toward the surface during the early part of neurulation (Figure 20.5). This evagination begins with a singular eye field that later splits into two paired capsules, called **optic vesicles**, on either side of the head and within the neuroectoderm. This process requires the expression of a gene called *cyclops*—if *cyclops* function is lost, a single midline eye results and the ventral forebrain fails to form. As the optic vesicles are forming, the surface ectoderm also begins to thicken into an overlying layer called the **lens placode**. The next stage of eye development consists of an invagination of the lens placode to form a pit that deepens into a lens vesicle, which will go on to differentiate into the lens. Lens fiber differentiation begins with expression of several crystallin genes, which trigger the elongation of the posterior cells of the lens vesicle and the loss of organelles like the nucleus, mitochondria, and endoplasmic reticulum. After the lens buds off from the surface ectoderm, the remaining ectodermal cells are invaded by nearby neural crest cells, and together they form the cornea.

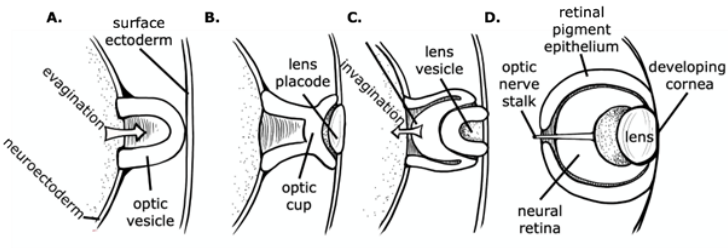

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Figure 20.5—Generalized development of the vertebrate eye begins with evagination of the forebrain to form the optic vesicle (A), continues with the formation of the optic cup and lens placode (B), proceeds with invagination of the optic cup and emergence of the lens vesicle (C), and terminates with the differentiation of the lens, sclera, and other tissue layers within the retina (D).

While the lens and cornea develop, the superficial portion of the optic vesicles extends toward the surface and invaginates to form the **optic cup**, while the deeper portion thins to form the optic stalk. The optic cup then consists of outer and inner cell layers, which at their junction form the ciliary body and iris. The outer layer then goes on to give rise to the **retinal pigment epithelium**, and the inner layer forms the photoreceptors and the neural retina (which later extends into the brain as the optic nerve [CN II]). The first type of retinal cell to form is retinal ganglion cells, then rod photoreceptors, then Müller glial cells (this is well conserved in vertebrates, with some variation). Retinal cells differentiate starting in a ventral patch near the optic stalk/future optic nerve (CN II) and then progress outward first toward the nasal capsule, then dorsally, and finally posteriorly.

Application Questions: Vision

The evolution of color vision and how it relates to hunting practices in the United States: Now that you've learned about the vertebrate eye, we should be able to start relating our understanding to real-world applications based on interspecific differences in eye anatomy.

Recall the different capacities of color vision across vertebrates, particularly within mammals.

1. How does the difference in color vision capability between humans and the majority of other mammals relate to hunting safety practices?
2. If you are unfamiliar with hunting, during deer hunting season, hunters are required to wear orange clothing to be visible and alert their presence to other nearby hunters so they aren't mistaken for a deer. Why do you think this is a wise color choice for hunters, and why does wearing bright orange allow a hunter to be easily seen by a fellow hunter while simultaneously not increasing the visibility of hunters to deer?

20.3 Sense: Chemosensation

We see it in every cartoon—a pie is left on a windowsill to cool. A tendril of smell emanates from the pie and winds its way to the nose of a dozing, mischievous character. They float along the smell tendril, following the scent to the cooling pie. They steal the pie and take a bite, sighing in satisfaction as they taste their stolen sweets. Although silly and corny, this is not too far off from the way vertebrates use chemosensation—the detection of chemicals

in the environment. They detect long-range chemicals using their olfactory system (their sense of smell), following their nose. Once they've gotten close to the source of the smell, they can use their gustatory system (their sense of taste) to detect close-range chemicals using their mouth and tongue.

Chemosensation goes beyond finding food. It allows vertebrates to avoid predator chemical cues, use pheromones to attract mates, identify conspecifics, return to their natal grounds, and care for their young. Different chemicals stimulate the olfactory system and the gustatory system. Why some chemicals stimulate one system versus the other is still unclear. Chemicals are separated out by their volatility and solubility—how well they readily vaporize and dissolve in water—and the different chemosensory systems are generally tuned to detect different chemical categories. When chemicals are detected by these systems, the chemical stimulus from the environment is transduced into a neurological signal, which can be interpreted by the animal, allowing them to make decisions based on the type of chemicals they are detecting.

Chemosensation—Olfaction

Olfaction is commonly referred to as the sense of smell. During olfaction, the nose detects chemicals and sends this information to the brain via the olfactory nerves. Most vertebrates have two types of olfactory systems: the **main olfactory system** (MOS) and the **accessory olfactory system** (AOS). Typically, the MOS, which detects airborne odorants, is what normally comes to mind when we think about the sense of smell. The AOS, also called the vomeronasal system in tetrapods, responds to water-soluble stimuli such as pheromones and is highly tied to the sense of taste. You can blame your vomeronasal system when a stuffy nose makes your food taste funny. This delineation between the MOS and AOS becomes blurry

when we think about aquatic vertebrates (like fish and amphibians), but we'll talk about that in the Aquatic Chemosensation section.

Anatomy of Vertebrate Nose

The vertebrate nose is a sensory organ that houses both the MOS and AOS. The nose has evolved to facilitate the transport of chemicals to their specific sensors called **olfactory receptor neurons** (ORNs). The anatomy of the nose is just as diverse as the animals who have them. As you learned in Chapter 14 on the respiratory system, during the evolutionary shift from water to land, vertebrates adapted to terrestrial life by developing the ability to breathe air. This is also the point in evolutionary history where olfaction and respiration are first tied. Fishes, for example, have separate olfactory and respiratory systems (more on this in the Aquatic Chemosensation section). In most tetrapods, air is brought in through the nose during respiration, allowing airborne odorants to travel into the nasal cavity along with each breath. Respiratory-tied olfaction also allows for sniffing—the active sampling of odors through intakes of air through the nose.

Airflow through the nose differs greatly across air-smelling vertebrates. Humans, for example, breathe in and out through the same cavity, causing airflow to change directions. On the other hand, air flows through dog noses in a unidirectional pathway, flowing in through the center of the nostril (or naris) and out through the outer regions of the naris, where you see the little slit on a dog's nose. In dogs, the airflow pattern helps with their keen sniffing ability—odorants are deposited in different parts of the nose based on their solubility. The ORNs in that area of the nose are sensitive to the particular class of odorants deposited there. So the airflow helps match the chemical to the right type of sensor, increasing the efficiency of the dog olfactory system.

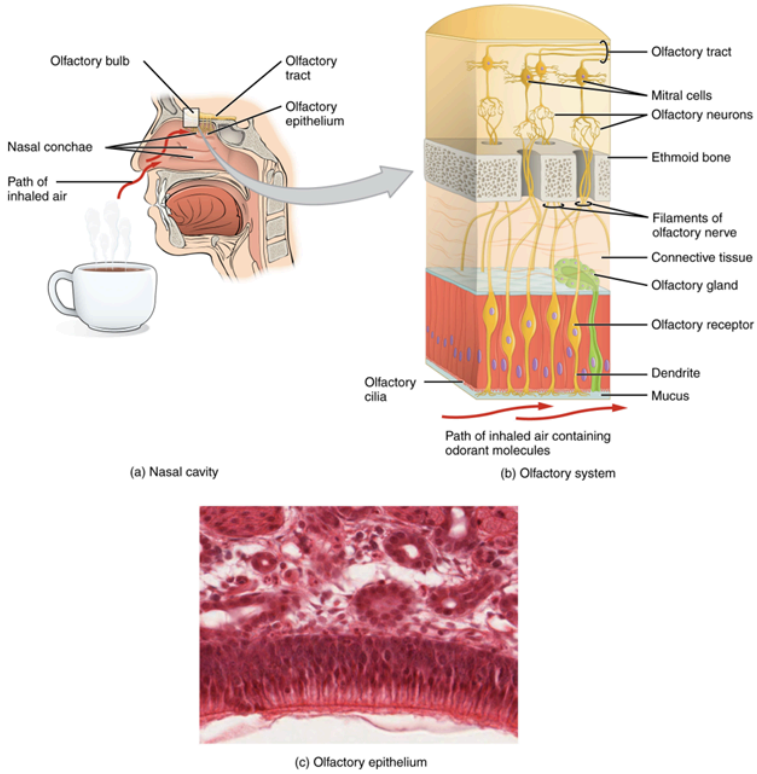

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Figure 20.6—The anatomy of the main olfactory system (MOS) for the detection of airborne chemicals. This figure portrays the olfactory morphology of a human (Homo sapiens), which is generally conserved among tetrapods. (A) MOS gross anatomy depicting the flow of air through the nares, into the nasal cavities, and across the olfactory epithelium. (B) The microstructure of the MOS. The airborne chemicals diffuse through the mucus layer (produced by the olfactory glands) and bind to the olfactory receptor neurons (ORNs). The axons of the ORNs form olfactory nerves, which synapse onto mitral cells in the olfactory bulb. Afferent axons leaving the olfactory bulb form the olfactory tract or cranial nerve I (CN I). (C) Histological section of the olfactory epithelium.

Main Olfactory System (MOS)

In tetrapods, air travels into the paired nasal cavities through the **external incurrent nares**. Tetrapods also possess internal, incurrent nares, called **choanae**, which connect the nasal cavity to the buccal cavity. The nasal cavities are partially lined with olfactory epithelium—where olfaction takes place. The rest of the nose is covered in respiratory epithelium. Nasal epithelium often covers one or more **conchae**—thin, turbinate bones on the side of the nasal cavity. These conchae increase surface area for odorant detection and help humidify the air passing through. It is hypothesized that conchae also play a role in warming the air in endotherms.

Within the olfactory epithelium there is the sensory epithelium, which houses the ORNs, and the nonsensory epithelium, without ORNs. Both tissues have supporting cells, mucus-producing cells, and basal cells. ORNs are bipolar neurons, meaning they have a dendrite on one side of the soma (cell body) and an axon on the other. The dendritic end of these ORNs extends to the olfactory epithelium, coming in direct contact with the chemical-containing air moving through the nose. ORNs are the only part of the nervous system that comes in direct contact with the external environment! The ORN dendrites terminate in cilia and/or microvilli within the sensory epithelium. The sensory epithelium is covered by a mucus layer, which is produced by Bowman's glands or olfactory glands. While runny noses may be annoying, mucus production is vital to olfaction. Chemicals in the air are caught by the mucus and are spread by diffusion to the ORNs.

The axons of ORNs project from the olfactory epithelium into the **lamina propria**, the connective tissue beneath that contains blood vessels and the Bowman's glands. These axons of the ORNs run together to form an olfactory nerve, which projects to the olfactory bulb. Here, the axons synapse onto mitral cells to form glomeruli. Afferent axons leaving the olfactory bulb form the olfactory tract or cranial nerve I (CN I), which projects to the pallium of the telencephalon.

Accessory Olfactory System (AOS)

The **vomeronasal system** is classified as a specialized accessory olfactory system based on its anatomy and the types of odorants it responds to, although the delineation between the two olfactory systems is still muddy. The **vomeronasal organ**, also sometimes called the Jacobson's organ, varies anatomically among tetrapod groups. In amphibians, the vomeronasal organ is an offshoot of the main nasal cavity. In amniotes, it is a more distinct separate organ, either connected directly to the nasal or buccal cavity or indirectly connected to both by the nasopalatine duct. The vomeronasal organ is secondarily lost in some taxa like birds.

The sensory epithelium of the vomeronasal organ is similar to olfactory sensory epithelium. One major difference is that the dendritic ends of the vomeronasal receptor neurons exclusively terminate in microvilli. The axons of these receptors terminate in the accessory olfactory bulb, which is distinct from the main olfactory bulb. The afferent axons from the accessory olfactory bulb unite with those from the main olfactory bulb to form CN I. However, the vomeronasal system's projections terminate in different areas of the brain than the main olfactory system.

The tie between nonvolatile odorants (compounds that do not easily evaporate) and the vomeronasal system is supported behaviorally. Think of a snake flicking its tongue in and out—this is a nonvolatile odorant sampling technique! Squamates transfer odorants from the environment to their vomeronasal organ on their tongue. Mammals use the **flehmen response**—which we see horses and cats do commonly. They curl back their front lips to expose their teeth, inhale with their nostrils closed, and hold the position, allowing nonvolatile chemicals to be more directly sampled by their vomeronasal organ. Elephants have an even more direct way to sample the nonvolatile pheromones of conspecifics. Elephants will dip their trunks into the urine of other elephants and transfer it directly to their vomeronasal organs. Elephants can learn a lot from urine, like the reproductive stages of potential mates. A study on

African elephants suggested that by scenting urine trails, they may be able to determine the age and maturity of the individuals who made that trail, cluing them in to who they may encounter in their environment.

Developmental Origins of the Vertebrate Olfactory System

The olfactory organs of the paired nasal epithelia in vertebrates develop from both cranial neural crest cells and ectodermal olfactory placodes located ventrally on the developing face. The olfactory placode emerges through a process of convergence and thickening of migrating epithelial cells. The placode contains a mixture of columnar cells, undifferentiated cells, and spindle-shaped cells, which serve as precursors to olfactory receptor neurons. Once established, the placode then invaginates anteriorly and dorsally and deepens into a **nasal pit**, which will give rise to the olfactory epithelium. Axons extend from bases of the sensory cells, making up the center of the nasal pit, collect into fascicles, and perforate the basement membrane to make connections with the olfactory bulb extending from the anterior part of the telencephalon. Surrounding each bundle of olfactory axons are specialized glial cells called olfactory ensheathing cells, which develop from migratory neural crest cells. At the opposite end of each receptor cell, dendrites begin to grow toward the surface of the epithelium, branch out, and sprout multiple cilia. These dendrites will then receive the odorant molecules that identify each smell.

Olfactory development across jawed vertebrates seems to be relatively conserved. However, a major difference arises in the origin of the single median nostril of cyclostomes. An additional placode that typically gives rise to the hypothalamus and anterior pituitary gland, the **adenohypophyseal placode**, forms as a separate thickening of cells in the midline of the head in gnathostomes but combines with the olfactory placode in cyclostomes to form the

nasohypophyseal placode. This placode then goes on to develop into a midline nasal sac.

Another difference in olfactory development across vertebrates concerns the vomeronasal organ, which is present in reptiles and amphibians, as well as some mammals. The vomeronasal organ forms through a secondary medial invagination of the developing nasal pit into a separate cavity within the olfactory opening. After the formation of this groove, several populations of migratory neurons colonize the vomeronasal epithelium, and the groove deepens into a well that separates from the olfactory pit completely. In many vertebrates, even those that lack a vomeronasal organ in adulthood, a temporary vomeronasal anlage forms during development and then is lost, supporting a more ancestral origin of this structure in early jawed vertebrates.

Chemosensation—Gustation

Gustation, colloquially referred to as taste, is the detection of chemicals within the oral cavity through taste buds. During gustation, taste buds on the tongue, roof of the mouth, and upper esophagus detect chemicals brought in through the drinking of liquid, licking of a substance, or the mastication of solid food. The taste buds detect these chemicals and send taste information to the brain through a suite of cranial nerves. Aquatic animals are freed from the constraint of mouth-housed taste buds. We see proliferation of extraoral taste buds not just on their heads but on their limbs (see Aquatic Chemosensation section).

The tongue is the main location for taste buds across vertebrates. Beyond being used to manipulate food (see digestion chapter, Chapter 13, for more on this), the tongue determines the palatability of a substance, a.k.a. the tastiness. The tongue can also test possible food via licking, checking if something is edible before it enters the oral cavity. It is still unknown why some chemicals

stimulate the gustatory system and not the vomeronasal system (and vice versa). Generally, the gustatory system responds to compounds that are dissolved in fluids and do not significantly become airborne.

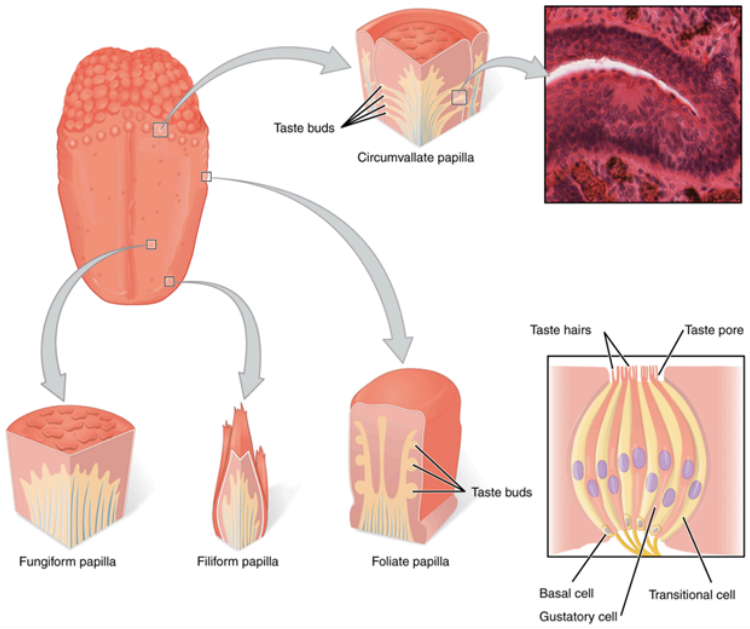

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Figure 20.7—The anatomy of human (Homo sapiens) tongue papillae. Filiform papillae cover much of the tongue and do not contain taste buds. Taste bud papillae include circumvallate, fungiform, and foliate papillae and are found in specific areas of the tongue. This figure also includes a histological section through a taste bud and a schematic showing the microstructure of a taste bud.

Taste Receptors

The functional unit of the gustatory system is the taste bud. **Taste buds** are made up of taste receptor cells, supporting cells, and basal cells. In most tetrapods, the receptors are sunken into the tongue.

Chemicals reach the receptor via a mucus-filled pit or pore. The taste bud's gustatory hairs stick out into the pore. Taste buds rapidly regenerate (every 8–12 days in humans!).

Taste buds are innervated by thin, dendritic sensory endings projecting from specific brain neurons, unlike the centrally projecting axons we see in the olfactory system. They are also not all innervated by one cranial nerve, like CN I for the olfactory system. In humans, the anterior part of our tongue is innervated by the facial nerve (CN VII) and the posterior tongue by the glossopharyngeal nerve (CN IX). The taste receptors on the roof of our mouth and beginning of our esophagus are innervated by the vagus nerve (CN X). Despite this array of innervation pathways, gustatory nerve fibers all terminate in the same nuclear complex in the medulla.

The human tongue is covered in papillae—the majority of which are not taste buds. These **filiform** papillae are small projections arranged in rows covering most of the front of the tongue. The papillae that do contain taste buds are categorized based on their shape. Humans have three different types of taste bud papillae, which are concentrated in specific areas of our tongue. **Circumvallate** taste bud papillae are larger and shaped like an upside-down V. These are concentrated in the posterior section of the tongue. **Fungiform** taste buds, as their name implies, are mushroom shaped and located in the anterior section of our tongue. Continuing with the nature-nomenclature, **foliate** taste bud papillae are leaf shaped and concentrated on the lateral portion of our tongue.

In mammals, taste bud cells have been classified into types, which are distinguished by both their morphology and cellular expression. While we have some hypotheses over which cell types respond to which types of tastes, we do not definitively know this correlation. **Type I** are glial-like cells and hypothesized to respond to salty tastes. These are the most frequent and exhibit a spindle shape with brush-like, long microvilli extending into the taste pit. **Type II** are receptor cells that express G-protein coupled receptors, which respond to bitter, sweet, and savory tastes. They are fusiform

shaped and mostly occur on the periphery of the taste bud. Type II cells also secrete ATP and ACh neurotransmitters. **Type III** cells are presynaptic cells, which sense sour taste and secrete serotonin, GABA, and norepinephrine neurotransmitters. They have unbranched apical processes, each with a single large microvillus. **Type IV** cells are basal, nonpolarized cells whose taste specificity is unknown. They are small cells at the base of the taste bud. Finally, **Type V** cells are marginal cells that are hypothesized to be taste bud stem cells that express non–taste receptor proteins.

The link between taste bud types and the types of taste they respond to is still under investigation. The majority of research in this area also primarily revolves around humans and how we identify tastes. Some functional links between taste repertoire and function are understood. Bitter taste receptors, for example, are important for identifying toxins.

Interestingly, snakes lack taste buds on their tongue. While their tongue is still a chemosensory organ, it is responsible for transport of chemicals to the vomeronasal organ, not direct detection of those chemicals. Most snakes do have taste buds; they're just in their oral cavity—namely, by the jaws. In some snakes, taste buds are elevated on papillae that also have mechanoreceptors, forming a taste/touch sensory complex that helps with the identification and manipulation of food items inside the mouth.

Developmental Origins of the Vertebrate Gustatory System

Taste buds and gustatory receptors form from sheets of epithelial cells that cover the developing tongue and differentiate first into taste placodes and then into fully formed taste buds. Unlike other sensory systems, there is good evidence that there is no neural crest contribution to taste buds in fish, amphibians, birds, or mammals. Taste bud development is broadly similar across different vertebrate groups. In mice, the taste placodes consist of a cluster of columnar

cells and begin to differentiate after sensory nerve fibers arrive and make contact with the placode cells. As the cells of the taste bud itself undergo morphogenesis, the surrounding epithelial sheets invaginate down into the mesenchyme to form the papillae that house the taste buds.

In zebrafish, taste bud primordia develop within the surface epithelium, as in mammals, where they are initially covered by layers of epithelial cells. As development proceeds, a taste pore forms between the cells, exposing the developing taste buds to the environment. However, the timing of development of gustatory receptors in zebrafish differs with location on the body. The first taste buds to differentiate are those on the lips and gill arches (3–4 days postfertilization; dpf), while taste buds inside the mouth cavity appear later (4–5 dpf), and those on the head form even later (12 dpf).

Two main models of taste bud development have been proposed: the **neural induction model** and the early specification model. The neural induction model states that innervation of each taste bud with a protrusion from either the facial, glossopharyngeal, or vagus nerves is required to induce differentiation of lingual papillae and eventually taste bud primordia. The **early specification model** suggests that patterning of taste buds is specified earlier on in development and that cell-cell interactions within the lingual epithelium, and not neural input, are the driving factors of differentiation. Despite a correlation between the appearance of sensory nerves at taste placodes, experiments in axolotls have shown that taste papillae and primordia can be induced to form without innervation. Additionally, rodent tongues cultured in a dish develop taste buds that are similar in number and size to those of control animals, suggesting that taste buds and the surrounding epithelia have their own inherent molecular signals that control their development.

Aquatic Chemosensation

Aquatic vertebrates detect chemicals in the water.

Fish

Fish are the most diverse vertebrate group, and their noses are diverse as well—far beyond the scope of what we are able to cover in this chapter. Instead, we will cover the general arrangement of the olfactory system in fish while highlighting some interesting oddballs along the way. Cyclostomes (hagfishes and lampreys) have one naris on the dorsal part of their head, which leads to one nasal chamber. The nasal chamber also connects to the pharynx via a nasopharyngeal duct. Holocephalans (a Chondrichthyan group that includes chimeras and ratfish) have a pair of forward-facing nares on the tip of their rostrum. They also have an internal nose-to-mouth connection, which allows them to irrigate their nose through buccal pumping, much like the choana we see arise later in tetrapods. Most other fish have two sets of nares on either side of their head: an incurrent and excurrent naris, which allows for unidirectional water flow through their olfactory organ. Water flows into the olfactory chamber via the incurrent naris. Within the chamber, water travels through the incurrent canal and back out of the excurrent naris through the excurrent canal. Within the olfactory chamber is the olfactory rosette, so named because of its floral-like arrangement. The "petals" of this rosette are the olfactory lamellae, sheets of olfactory tissue containing both sensory and nonsensory olfactory epithelium. Rosettes vary in size and shape. Chimeras, for example, have radially arranged rosettes where the lamellae are connected like the petals of a daisy. Sharks, in particular, have elongated rosettes with paired lamellae on either side of a connecting tube called the raphe. Shark rosettes range in lamellar count from 26 (in the Japanese angel shark) to 718 (in the

winghead shark). On the opposite end, some teleosts have as few as one or two lamellae, which are attached directly to the olfactory chamber. Of course, there are plenty of weirdos in the bunch. For example, tetraodontids, like the blackspotted puffer, have no nostrils at all. Instead, their olfactory organ is located on tentacle-like growths on their rostrum, whose inner cavities are covered by olfactory epithelium. Fish lack Bowman's glands but still rely on mucus (secreted from goblet cells) for chemical detection.

Fish also do not possess a distinct and separate vomeronasal system. However, the olfactory epithelium of teleosts is a hybrid olfactory and vomeronasal epithelium expressing both genes for olfactory and vomeronasal receptor neurons. These two neuron groups also project to different regions of the olfactory bulb. Speaking of the olfactory bulb, unlike most tetrapods, fish olfactory bulbs are not in direct contact with the telencephalon. Instead, fish olfactory bulbs are projected forward, connected to the olfactory rosettes. The olfactory bulb is connected to the telencephalon via the olfactory tracts or **peduncles**. The length of the olfactory tracts varies among species and is dependent on how far the olfactory bulb is from the telencephalon. Hammerhead sharks, for example, have long, thin olfactory tracts running from their olfactory bulbs on the distal ends of their elongated heads to their telencephalon.

Gustation-wise, fish have taste buds in their oral cavities as well as in their gills, gular region (i.e., what we would colloquially call their chin), barbels, and even fins. The abundance of extraoral taste buds highlights the issue with separating gustation from olfaction in the aquatic environment. Some fish, like the yellow bullhead, have more than 175,000 taste buds on the entire surface of their body. Typically, the facial nerve (CN VII) innervates the extraoral taste buds in fish. In some fish, like catfish, the extraoral and oral gustatory systems are two functionally separated systems—the extraoral taste buds detect chemical stimuli at a distance, while the oral taste buds are used in close stimuli detection and are used in discriminating palatable versus unpalatable foods. Adding further confusion to the delineation between olfaction and gustation in

fish—both systems respond to similar chemical types at comparable concentrations.

Box 20.2—Fish Chemosensation Is a Whole Can of Worms

Studying chemosensation in fish highlights how hard it is to separate chemosensory systems. All the chemicals they detect are dissolved in water, so separation along the lines of the volatility and solubility of the chemicals becomes muddy quickly. They have no separate vomeronasal organ but have vomeronasal receptors. They have taste buds, which are morphologically distinct from their olfactory receptors, but they detect the same types of chemicals at similar concentrations as their olfactory systems. Fish chemosensation challenges everything we laid out earlier in this section.

So what do you think? How should we separate olfaction versus gustation? Within olfaction, how do we separate the MOS and the AOS? Should we have separate definitions for aquatic versus terrestrial systems? If so, where does that leave amphibians?

Amphibians

Because of their dramatic life history change from aquatic larvae to terrestrial adults, amphibians are a fascinating subject for the evolution and development of chemosensation. While there is a large diversity in chemosensory morphology within amphibians,

here we will lay out the general trends. Larval frogs and salamanders have a specialized olfactory organ associated with a small mouth. By contrast, caecilians have relatively simple, triangular olfactory organs. The external naris is connected to the nasal sac through a nonsensory vestibule. The choana is guarded by a valve that is hypothesized to stop the backflow of water and allow for unidirectional water flow through the olfactory system. The vomeronasal organ forms as a pocket off the nasal sac. Taste buds form on papillae throughout the oral epithelium.

During metamorphosis, the chemosensory system goes through an extreme makeover. Instead of the previous unidirectional water flow, metamorphosized amphibian olfactory systems allow for bidirectional airflow. Keeping their mouth closed, they oscillate their buccal floor—bring air in and out of their excurrent nares. This new bidirectional olfactory flow eliminates the need for stopping backflow, and the choanal valve is lost. The vestibule between the external naris and the nasal sac is either reduced or completely lost along with the choanal valve. The choana normally shifts significantly in position, and the vomeronasal organ connects to the mouth. This change in positioning with a newly terrestrial lifestyle may allow for odorants to be deposited separately between the olfactory and vomeronasal organs based on their volatility. Physiological research on salamanders shows a change in the responsiveness of their olfactory epithelium with decreased sensitivity to dissolved odorants and increased sensitivity to volatile odorants.

After metamorphosis, amphibians develop the nasolacrimal duct, which connects the eyes to the nose, allowing for the reabsorption of excess **lacrimal fluid** (a.k.a. tears). Metamorphosis also cues the increased presence and function of Bowman's glands. In their new terrestrial lives, many amphibians develop the ability to close their external nares to help with lung inflation. Anurans, for example, close their nares during lung inflation via a specialized mechanism using their lower jaw and submentalis muscle.

The amphibian gustatory system also goes through a

morphological change after metamorphosis. They develop a fleshy tongue, and their taste buds are replaced by taste discs. Taste discs are made up of an epithelium similar to that of the olfactory and vomeronasal systems. Taste discs are lauded as the largest gustatory organ in all vertebrates and have a variety of different epithelial cells, chemoreceptive surface types, and neuro-epithelial systems.

Despite this shift to a more typical terrestrial olfactory system, many amphibians retain the ability to smell underwater. Frogs and salamanders can still detect pheromones in the water. In one behavioral study, blinded tiger salamanders would tap their noses and bite at bags of earthworms underwater but stopped this behavior when their noses were plugged.

Box 20.3—The Taste of Tears

Nasolacrimal duct: First seen in amphibians, the nasolacrimal duct (also known as the tear duct) is the connection between the lacrimal sac of the eye and the nasal sac. Excess fluid from the lacrimal sac is transported through the nasolacrimal duct and reabsorbed in the nose. This is why our nose runs when we cry or experience eye-irritating allergies. This is also why we sometimes can taste eye drops.

Aquatic Mammals

Mammals have the largest olfactory gene repertoire of any animal group, suggesting that olfaction is incredibly important for the majority of mammals. So what about aquatic mammals? Platypuses have very small olfactory bulbs and few olfactory turbinates in their

nasal cavities. Their closest relative, the short-beaked echidna, has large olfactory bulbs and organs and as many as seven olfactory turbinates. Platypuses do have a vomeronasal organ. For marine mammals we see similar reductions in olfactory morphology. Some, like the sirenians and most cetaceans, lack a vomeronasal organ altogether.

While underwater olfaction is assumed to be absent in mammals, there are two mammals that employ "underwater sniffing"—the star-nosed mole and water shrew. These two will exhale air bubbles onto objects or into scent trails. They then sniff back up the bubble, allowing the chemicals to travel back into the nose and olfactory system.

While marine mammals may not smell underwater, there is evidence they still use their chemosensory systems. Baleen whale blowholes act as nares. One study found a correlation between baleen nare morphology and their level of zooplanktivory. Why does this matter? Plankton release **dimethyl sulfide** (DMS), a chemical cue shown to assist in seabirds' abilities to locate prey. It's hypothesized that nares are best suited for stereo-olfaction—the ability to compare differences in chemical signal strength between two nares. Bowhead whales in particular have complex and large (for a whale) olfactory bulbs, and 51% of their olfactory gene repertoire is intact, characteristics that could be linked with their ability to find krill aggregations.

The aquatic mammal gustatory system is severely understudied, but we have some interesting findings in bottlenose dolphins. Not only can dolphins recognize different individuals based on signature whistles, but they can also use urine signals. In this study, dolphins used their gustatory system to differentiate water from urine samples. Furthermore, they differentiated between urine from familiar and unfamiliar individuals. Dolphins were able to recognize individuals by gustation and were able to integrate both the acoustic signal of the signature whistle and the gustatory signal of the urine sample when they were presented together.

The Human Connection—COVID-19 and the Chemosensory System

One of the most commonly shared side effects of COVID-19 infection is a loss of smell and/or taste. While this was commonly brushed off as insignificant, by now we hope you have realized that disruption of these chemosensory systems is more than a minor inconvenience—it's a shutdown of two of the five human senses! This is a direct, observable, and widespread neurological impact and sensory impairment. Why does this happen? The research is still not conclusive. Here's what we do know.

Lingual epithelial cells, such as taste buds, serve as a hotspot for SARS-CoV-2, the virus that causes COVID-19. SARS-Cov-2 replicates within taste buds, contributing to one of the most famous COVID-19 symptoms: ageusia (loss of taste) and dysgeusia (persistent sensation of foul tastes). On the olfactory side, experts believe that while the SARS-CoV-2 virus is not affecting the olfactory receptors or nerves directly, the evidence suggests that the virus injures the supporting cells. If these supporting cells are not able to "support" by providing nutrition and structure to the olfactory nerves, the nerves may become secondarily injured.

Additionally, early-stage COVID-19 begins with nasal congestion. Stuffy noses and sinus blockages narrow the passageways for airflow, making it more difficult for odorants to make it to the olfactory sensory epithelium. A common treatment method for congestion is nasal sprays. Nasal decongestant sprays constrict the blood vessels in nasal passages to reduce swelling. The same can be achieved by corticosteroid sprays, which reduce nasal inflammation by reducing the production of the inflammatory chemicals that cause congestion. Alternatively, saline-based sprays work by moisturizing dry nasal passages and thinning nasal mucus.

20.4 Sense: Mechanosensation

Mechanosensation is the transduction of small changes in the mechanical force applied to a tissue into a neural signal. We will broadly refer to any sensory cell capable of transducing a mechanical signal as a mechanoreceptor. Due to the generality of mechanosensation, many different senses rely on mechanoreceptors. Indeed, mechanoreceptors are involved in the sensation of stimuli, including touch, pressure, proprioception, balance, and hearing.

Somatosensation

Somatosensation is a general sense that includes multiple stimuli, including touch, pressure, pain, temperature, and proprioception. In comparison to most of the senses you will learn about in this chapter, the sensors associated with somatosensation are distributed across the body and can be associated with a variety of different organ systems. Many somatosensory sensors are found in the skin, but other locations include muscles, tendons, and direct association with organs. Consequently, somatosensation is used to sense both internal and external stimuli. For example, receptors used to sense pressure can be found in the skin of your fingers, which helps you regulate the magnitude of pressure you need to apply to a glass in order to keep it from slipping from your hand and ensuring that you don't squeeze the glass too hard so that it breaks. In contrast, a different type of pressure receptor can also be associated with arteries inside the body to monitor the pressure of blood. Thus, somatosensation is an incredibly versatile system that facilitates the sensation of many different types of stimuli from both inside and outside the vertebrate body.

Touch, Pressure, Pain, and Surface Temperature

The sensation of touch, pressure, pain, and temperature due to the interaction between the body and external stimuli results from the response of a suite of sensory receptors distributed in the skin. The external mechanical stimuli (i.e., objects and surfaces) the vertebrate body interacts with on a regular basis are incredibly complex. Simply think of an ice cube and the different variables associated with it. To do so, imagine you are blindfolded and handed an object that is relatively small, generally cube shaped; it is likely to be slippery and certain to be cold. It is likely you will be able to quickly guess the object you were handed despite being blindfolded. However, your ability to do so relied on sensing each of these different variables. Therefore, it shouldn't be surprising that the vertebrate body is outfitted with so many different types of sensors and that each of these sensors is responsible for sensing different ranges and specific stimuli associated with these external stimuli.

The different types of **sensory endings** (a term often used interchangeably with sensory receptors) responsible for somatosensation have been best studied in mammals (particularly humans). Thus, we will begin our investigation of these different types of sensory endings from a mammalian perspective (Table 20.1). To do so, we will briefly cover each sensory ending and its general function in the mammalian system. Many of these receptors might be familiar, as you might have already heard of them during your exploration of the integumentary system.

Free nerve endings associated with the skin are generally responsible for the sensation of pain and temperature. **Free nerve endings** are sensory neurons with a receptive end that lacks any kind of specialization. Free nerve endings responsible for sensing pain are often referred to as nociceptors, and sensors responsible for sensing temperature are often referred to as thermoreceptors. **Thermoreceptors** embedded in the skin are usually activated in response to changes in surface temperature relative to internal body temperature. There is a wide range of thermoreceptors that

respond to specific changes in temperature like increasing versus decreasing temperature. **Nociceptors** respond to stimuli of an intensity that surpasses a given threshold. Painful stimuli can include mechanical, thermal, or chemical. For example, stubbing your toe is a mechanical stimulus that can elicit a painful sensation, whereas eating a spicy pepper is a chemical stimulus that can elicit a painful burning sensation. In both examples, nociceptors are activated. Finally, the root of the hairs embedded in the skin of mammals is also wrapped by a free nerve ending, which provides mammals an additional sensory structure. Mammals use hair to sense a range of stimuli including wind or body movement while running or swimming through an environment and light touch associated with an animal (e.g., an insect) crawling across the body. Free nerve endings also contribute to the sensory endings associated with the whiskers of mammals (in addition to other endings like tactile discs). Free nerve endings are believed to be present in extant representative species of all major vertebrate clades. However, the specific functional capabilities of these sensors have not been evaluated in every species and therefore could vary across the phylogeny.

Table 20.1—Mechanoreceptors responsible for general somatosensation

Receptor name	Historical receptor name	Encapsulated	Receptor locations	Stimuli that activate the receptor
Free nerve endings	N/A	No	Skin, cornea, internal organs, joints, oral cavity	Mechanosensation, pain, temperature
Hair and whisker receptors	N/A	Multiple receptors are involved	Hair and whisker	Mechanosensation
Tactile discs	Merkel's discs	No	Skin, mucous membranes	Vibrations (below 20 Hz)
Bulbous corpuscle	Ruffini's corpuscle	Yes	Skin, joint capsules	Stretch
Tactile corpuscle	Meissner's corpuscle	Yes	Skin	Light touch, vibrations (below 50 Hz)
Lamellated corpuscle	Pacinian corpuscle	Yes	Skin and hypodermis	Deep pressure, higher-frequency vibrations
Muscle spindles	N/A	Yes	Tetrapod skeletal muscle	Muscle fiber length
Tendon organs	Golgi tendon organ	Often encapsulated, but structures vary across species	In tendon	Tendon stretch

Touch, pressure, and vibration are sensed by a suite of receptors that are either encapsulated or unencapsulated. These sensors are generally located in the skin, and each is tuned to sense a specific feature or range of features associated with mechanical stimuli. The specific range of stimulus features or intensity acquired by each type of sensory receptor is heavily influenced by whether the sensor is or is not encapsulated as well as the depth of the sensor in the skin of the animal. **Unencapsulated sensory receptors** are receptor endings that are not covered by any additional tissue surrounding the receptive ending of the neuron and include free

nerve endings and tactile discs. **Encapsulated sensory receptors** are receptor endings that are covered in an additional tissue (often connective) or membrane of a nearby glial cell. The encapsulation of the sensory receptor can help to either augment the responsivity of the sensor by increasing receptor sensitivity or filter the stimuli so the receptor is tuned to only respond to a very select type of stimulus or a narrow range of stimulus intensity.

Tactile discs are unencapsulated sensory receptors located in the integument of vertebrates that generally respond to light touch, help encode features of objects like shape and edge detection, and are capable of providing information about texture by being sensitive to low-frequency vibrations.

Bulbous corpuscles are slowly adapting encapsulated sensory receptors found in the deep layers of the integument as well as in joint capsules. Bulbous corpuscles respond to the stretch of the integument, movements at joints, and at least in mammals, deep pressure applied to the skin. The role of bulbous corpuscles in sensing joint movements should not be surprising given these receptors are often found in high densities within the vertebrate joint capsule. Further, the ability of these receptors to sense skin distortion and joint movements enables these receptors to also contribute to the sense of proprioception.

Tactile corpuscles are rapidly adapting encapsulated sensory receptors found in the superficial dermis of the integument. These receptors are primarily responsible for sensing light touch and some features of texture by responding to relatively low-frequency vibrations of the integument.

Lamellated corpuscles are rapidly adapting encapsulated sensory receptors in the dermis of the integument. Lamellated corpuscles respond to deep pressure and high-frequency vibrations.

The suite of sensory receptors responsible for general somatosensation is not well characterized across all vertebrates. However, it is likely that most vertebrates have at least similar mechanosensors that overlap in functional capability. For example,

actinopterygians have the ability to sense texture using the sensory receptors embedded in their fins, and the fins of Chondrichthyes have sensory receptors capable of sensing pressure. Similarly, investigations have also revealed the presence of analogous joint receptors in at least one species of all major vertebrate clades. Further investigation will be required to reveal the homology and evolutionary history of these receptors across vertebrate clades.

Proprioception

Proprioception is defined as the ability to sense the movement and position of one's own body axis and one's appendages relative to the trunk. For example, even after you fully close your eyes, many of you will be capable of touching the tip of your nose with your finger. In theme with somatosensation, proprioception itself relies on a variety of different mechanoreceptors distributed throughout the body.

Proprioceptors of the Skin

Many mechanoreceptors distributed throughout the skin are also used to sense proprioceptive stimuli. For example, in many tetrapods, the mechanoreceptors responsible for sensing skin distortion (bulbous corpuscles), especially at joints, provide complimentary proprioceptive feedback. Further, the fins of actinopterygians and chondrichthyans are also outfitted with a variety of mechanoreceptors embedded in the tissues of the skin and the fin rays, and these sensors are consistently found to encode proprioceptive information like fin ray position, bending magnitude, and bending rate. The sensory endings found in the skin and fins of actinopterygians and chondrichthyans have not been studied in enough detail to reveal their homology with the different sensory receptors that have been well described in mammalian systems.

Tendon Organs

Tendon organs are stretch receptors embedded in the tendons of skeletal muscle. These tendons respond to tendon stretch and ultimately are capable of encoding the tension or strain developed in a tendon. Thus, tendon organs are also one of the prominent vertebrate proprioceptors. The precise homology and evolutionary history of tendon organs is not clear across all vertebrates. However, analogous sensory receptors associated with muscle tendons that are capable of sensing tension of the tendon are present in at least a representative species of all major gnathostome clades.

Muscle Spindles

Muscle spindles are a proprioceptive sensory receptor that encodes details of muscle length and, therefore, changes in muscle length. You can find more details about muscle spindles within the chapter focused on muscle tissues (Chapter 11). Briefly, muscle spindles are ultimately stretch receptors found within the belly of skeletal muscle. A muscle spindle is composed of a fibrous capsule that contains a small number of muscle fibers, called intrafusal fibers, inside the capsule. Both the capsule and the intrafusal fibers are aligned in the same orientation as the rest of the muscle fibers found outside the capsule, which are referred to as extrafusal fibers. Nerve endings wrap and surround the intrafusal fibers within the capsule and respond to the stretch of the fibers, allowing muscle length to be encoded.

Muscle spindles are most likely only found in tetrapods. However, one study suggested the presence of muscle spindles in the jaw musculature of an actinopterygian fish, but this work has never been duplicated or confirmed, despite attempts. Thus, we conservatively suggest that muscle spindles are traits that first

evolved in early tetrapods sometime around the vertebrate water-to-land transition.

Finally, it is important to note that proprioceptive feedback is not unique to limbed vertebrates, and undulatory locomotion using the body axis also relies heavily on proprioceptive feedback. In these cases, mechanosensory receptors are located along the entirety of the body axis. Animals undergoing undulatory locomotion rely on the sensation of the various stimuli discussed above to encode information on myotomal muscle contraction and force, as well as the magnitude, location, and direction of body axis undulation.

Application Questions: Mechanosensation

1. How do you sense the texture of an object? First imagine you are shopping for a new shirt and hope to purchase one made with the softest material possible. In what way do you interact with the materials of different garments? You might say you touch or feel the shirt. But how do you interact with the material? Do you tap it? Do you squeeze it? Or do you sift it between your fingers or rub it against your skin?
2. Chances are you answered that you sift the material through your fingers. Or if you are discerning the difference in the roughness between different objects (e.g., sandpaper versus glass), you are likely to move your fingers across the surface of the object. Why do you do this?

The answer lies in how the integument works with sensory receptors to sense different textures and surface features of objects. The running of the integument across the surface of a textured object results in the vibration of the integument. Different textures result in vibrations of different frequencies. It is the frequency and intensity of these vibrations that are ultimately sensed by your mechanoreceptors distributed throughout the integument. Thus, the sensation of differences in texture ultimately results from differences in the vibratory frequency of the skin and the coding of these vibrations through the embedded mechanosensors.

The Neuromast Organ

The **neuromast organ** is a specialized sensory organ (composed of many individual receptors) contributing to mechanosensation in a variety of vertebrate sensory systems. The neuromast organ, or a modified version of it, is the primary sensory organ of the auditory system, vestibular system, lateral line system, and electroreception. Therefore, before the anatomy of each of these systems is examined, it is important to understand the anatomy of the neuromast organ.

The neuromast organ is a collection of mechanosensitive hair cells, supporting cells, and the afferent sensory neurons responsible for carrying the neural impulses transduced by the hair cells toward the central nervous system. A neuromast organ is also often surrounded by a cupula, which is a gelatinous membrane that embeds the underlying hair cells. In a neuromast organ, each individual hair cell is ultimately the mechanosensory receptor, and

the cupula helps tune, filter, and enhance the mechanical stimulus being transduced by the hair cells.

The primary sensory receptor of a neuromast organ is the **hair cell**. Oddly, hair cells are not neurons but actually specialized epithelial cells. These epithelial cells are called hair cells due to the presence of numerous stereocilia projecting from the apical cell surface. **Stereocilia** are nonmobile cylindrical fingerlike projections of the apical surface cell membrane and structurally are more similar to large microvilli than they are to cilia. These stereocilia are of unequal length and typically arranged in order of height along the apical cell surface, so they almost appear as a steep staircase. The edge of the cell surface with the tallest stereocilia is bordered by a single large, modified cilium called a **kinocilium**. Again, a neuromast organ is formed when multiple individual hair cells (with each cell possessing many apical surface stereocilia) are clustered together with supporting cells and surrounded by the gelatinous cupula. Thus, a neuromast organ fits the requirements of an organ, as it contains multiple tissue types working together to produce a function.

Hair cells respond to and transduce mechanical stimuli due to the physical deflection of the apical stereocilia. The bending of the stereocilia results in the opening (or closing) of mechanically gated ion channels embedded in the cell membrane, ultimately resulting in changes in cell electrical potential. Hair cells are usually highly directionally sensitive and only respond to mechanical stimuli applied in a single direction. Deflection in one direction, often along the apical surface in the direction of increasing stereocilia height, results in cell excitation, whereas deflection in the opposite direction results in cell inhibition. However, as hair cells are indeed epithelial cells, they do not have an axon directly associated with the cell. Instead, hair cells synapse (either chemically or physically) with a supporting neuron. It is the associated neuron that will ultimately carry the neural signal to the central nervous system.

Vestibular System

The vertebrate vestibular apparatus allows animals to maintain positional equilibrium, which includes the body position, orientation, and movement in three-dimensional space. Thus, the vestibular apparatus is critical for maintaining balance. All vertebrates possess a **vestibular apparatus** that includes the same basic components, although there is variation across species and clades. The common components of the vestibular apparatus include at least one **semicircular canal** and two associated vestibular chambers called the **saccule** and the **utricle**. The interconnected canal(s) and chambers are all filled with a viscous fluid known as **endolymph**. The vestibular apparatus is a bilaterally symmetrical structure found on both the right and left sides of the vertebrate head. Indeed, it is often the comparison of sensory information acquired by the right and left vestibular apparatus that allows the brain to decode the complex stimuli associated with body position, orientation, and linear and angular accelerations.

The saccule and utricle contain a sensory receptor named the **macula**, which responds to changes in orientation on the vestibular apparatus. The macula (or otolith receptor) is a modified neuromast organ where calcium carbonate protein granules called otoliths (or otoconia) are embedded in the gelatinous copula surrounding the patch of hair cells. The macula of the utricle and saccule detect changes in orientation of the head because the otoliths are subjected to gravitational forces and therefore bend in response to movements of the head. For example, as the head is tilted, the otoliths cause the otolith-occupied cupula to bend and ultimately stimulate the hair cells. The macula is also capable of sensing changes in linear acceleration due to the inertia of the otoliths suspended in the cupula of the macula. Imagine riding a roller coaster (or even driving a car); as you accelerate, the mass of your body is deflected in a given direction. The same concept applies to the macula within the utricle and saccule, where linear

accelerations result in the displacement of the cupula of the macula that stimulates the hair cells.

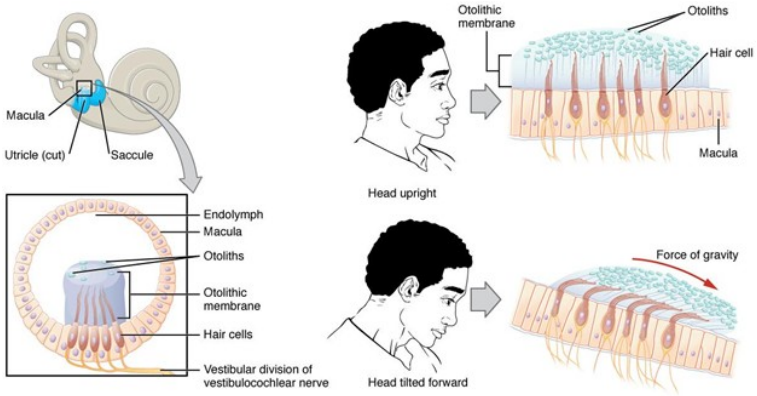

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Figure 20.8—Vestibular apparatus anatomy and the encoding of linear acceleration by the macula. (Top left) The vertebrate vestibular apparatus is composed of the utricle, saccule, and up to three semicircular canals generally arranged in orthogonal angles with respect to each other. This figure portrays the vestibular apparatus of a human (Homo sapiens), which is generally consistent with the mammalian condition. Also note the tight relationship and direct connection between the vestibular apparatus and the associated cochlea (coiled, snail shell organ) that is typically associated with hearing. Note the cochlea is typically only coiled like this in mammals. (Bottom left) A diagram of the anatomy of the macula, which is the specialized sensor contained within the vertebrate vestibular apparatus. (Right) A summary of how the macula responds to changes in the orientation of the human head.

The semicircular canal(s) of the vestibular apparatus senses changes in angular acceleration. A semicircular canal is a semicircular duct filled with endolymph that contains a dilated sac at its end called an ampulla. Enlarged neuromasts, called cristae, are found within the ampulla of the semicircular canal and are the sensory receptors responsible for sensing changes in angular acceleration. Due to the viscosity, and therefore interior properties, of the endolymph contained in the semicircular canals, accelerations of the vestibular apparatus result in the movement of the circulating endolymph lagging behind the physical movement of

the vestibular apparatus. The delayed movement of the endolymph contained within the semicircular canal ultimately results in the deflection of the cristae contained within the ampulla, thereby stimulating the hair cells. The magnitude of the acceleration will result in differences in the magnitude and timing of endolymph movement, thereby providing a means to encode angular acceleration. All extant gnathostomes have three semicircular canals (on each side of the head) that are generally oriented to capture acceleration around each of the three body axes. Thus, extant gnathostomes can generally sense acceleration associated with body pitch, yaw, and roll. However, species of lamprey are only known to have two semicircular canals, and species of hagfish are only known to have a single semicircular canal, thereby limiting the different rotational angles lampreys and hagfishes are capable of sensing.

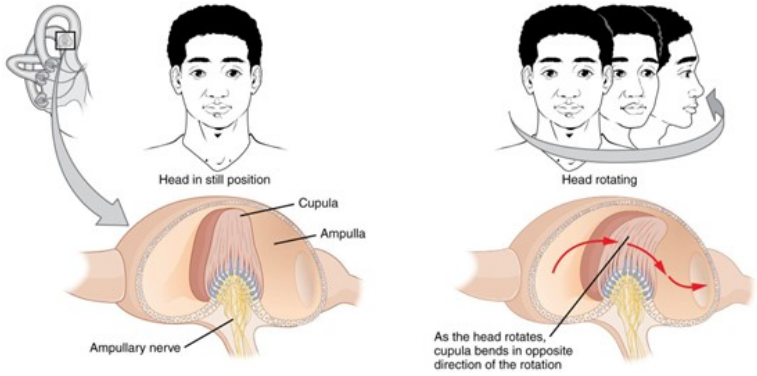

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Figure 20.9—The anatomy and function of the semicircular canal(s) for the encoding of rotational accelerations. This figure portrays the vestibular apparatus of a human (Homo sapiens), which is generally consistent with the gnathostome semicircular canal condition. (Left) A summary of the state of an individualized sensory organ while the head is stationary. (Right) A summary of the movement of the sensory organ in response to human head rotation (roll). A comparison of the two illustrations demonstrates the movement of the sensory organ due to the movement of the fluid through the semicircular canal.

Vestibular Development

Vertebrate vestibular and auditory systems begin their development as one structure with the emergence of the otic placode. The otic placode is a thickened region of ectoderm that sits adjacent to rhombomeres four through six in the embryonic hindbrain. As development proceeds, the otic placode invaginates to form an otic pit, which then closes to form a vesicle that is lined by pseudostratified epithelial cells. While the otic pit is closing, ventromedial cells detach from their epithelial layer and extend toward the neural tube to form the vestibulocochlear ganglion, which later differentiates into separate vestibular and cochlear ganglia. The cells of the otic vesicle then proliferate and differentiate to form an otocyst, which includes primordia of the semicircular canals, cochlea (in mammals), and endolymphatic duct. Genes expressed in the nearby hindbrain influence the patterning and morphogenesis of different cell types in the ear.

The development of the semicircular canals varies slightly across vertebrates. In amniotes, the canal primordia emerge as anterior, posterior, and lateral bulging epithelial plates. The plates then fuse and the supporting tissue surrounding the canals is resorbed, leaving the canals themselves behind. In fishes and frogs, rather than forming as fusion plates, the semicircular canals begin as cylindrical projections of the epithelium that extend into the lumen of the otic vesicle. The projections meet corresponding bulges on the opposite sides of the vesicle and fuse to form pillars of tissue that will differentiate into the semicircular canals.

Auditory System

The auditory system is present in most major vertebrate lineages and enables the ability to sense sound, which humans often refer to as hearing. The auditory system is inherently connected to the vestibular system, and in some species, a clear separation of

function of certain structures is not fully distinct. Common to both systems, mechanosensitive neuromast organs are used to transduce the stimulus. The portion of the vestibular apparatus that is typically utilized for sound transduction is known broadly as the **lagena**, which can mostly simply and inclusively be considered a fluid-filled chamber. This chamber can range in size and shape from that of a small sphere resembling the saccule and utricle of the vestibular apparatus (e.g., many fishes) to that of a lengthened and coiled tube, which is found in most mammals and sometimes referred to as the cochlea. Across all vertebrates that possess a specialized or shared (with the vestibular apparatus) organ for sound transduction, the components of the vestibular apparatus and the lagena are together referred to as the inner ear and usually embedded within the skeletal component of the skull. Similar to the chambers of the vestibular apparatus, the primary chamber(s) responsible for sound transduction is also filled with endolymph. In addition, in most vertebrate lineages, other fluid-filled canals are also contained within the inner ear and contain perilymph and can be broadly referred to as the perilymphatic canals (also referred to as the scala vestibuli and the scala tympani in amniotes).

Interestingly, one of the most variable components of the vertebrate auditory system is how sound is transmitted from the external environment to the component of the inner ear responsible for sound transduction. The routes for sound transmission can include other chambers often referred to as the middle and external ear.

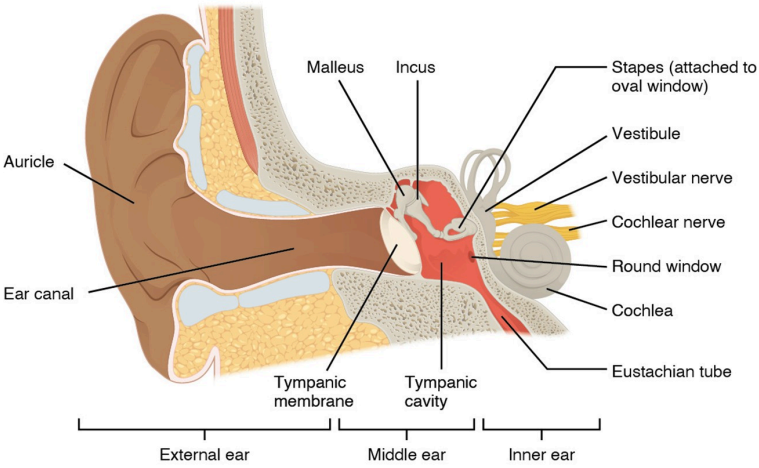

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Figure 20.10—The anatomy of the mammalian auditory system. This figure portrays the auditory system of a human (Homo sapiens), which is generally consistent with the mammalian condition. In addition, the relationship between the cochlea and the rest of the vestibular apparatus is generally consistent across all vertebrates. All vertebrates possess an inner ear that is equipped with a chamber responsible for the transduction of auditory stimuli. All tetrapods, with few exceptions, also possess a middle ear, which is a hollow chamber contained within the skeleton that houses at least one skeletal element (usually referred to as the columella or stapes) that helps propagate sound from the outside world to the inner ear. Finally, mammals (and to some degree birds) have also evolved an external canal that leads between the surface of the head and the middle ear. The transition between the external and middle ear is usually denoted by the presence of the tympanic membrane.

In this section, we will begin our study of the vertebrate auditory system by considering the anatomy of the inner ear and then add on the additional structures used for sound transmission that evolved in each major vertebrate lineage.

Actinopterygii

The auditory system of actinopterygian fishes generally lacks any

structure associated with the vestibular apparatus that is specialized for auditory transduction. Studies find that the saccule of the vestibular apparatus is the primary auditory chamber used for sound wave transduction in most fishes. However, in other species, the lagena as well as the utricle have also been identified as contributing to auditory transduction. Regardless of the specific chamber, transduction relies on the deformation of the mechanosensitive macula (modified neuromast organs) for sound transduction. The sound waves propagated to the chamber result in the vibration of the macula at the frequency of the propagating sound waves. The mechanical stimulation of the macula from sound waves is therefore distinct from the unidirectional bending that occurs during equilibrium sensation. Thus, the macula can contribute to both vestibular and auditory sensation.

Actinopterygian fishes do not possess any specialized structures that can be associated with the middle or external ear. Instead, in the vast majority of fish species, because the bodies of vertebrates are primarily composed of water, the sound waves received by fishes are easily propagated from the surrounding water and through the body to stimulate the macula. Other mechanisms might also contribute to sound transmission in fishes. For example, in some species, the air-filled swim bladder includes extensions that directly contact the chambers of the inner ear. In other species, a chain of three to four tiny ossicles helps propagate sound waves from the swim bladder to the saccule for transduction. The role of the swim bladder in propagating sound waves could help enhance sound detection by increasing the sensitivity of the system.

Amphibia

The auditory system of amphibians is best studied in frogs (Anura) and will be the basis of this section. Additional notes will be included for hearing in the other two main amphibian lineages, salamanders (Urodela) and caecilians (Apoda).

Two distinct auditory receptors are found in the amphibian inner ear. The amphibian **papilla** is an auditory receptor unique to amphibians and generally common across all species of amphibians. The amphibian papilla is located in a small recess in the medial portion of the saccule. Here, a modified neuromast organ is suspended from the roof of this recess. Thus, it is the motion of the fluid moving across the suspended neuromast organ that results in the mechanical deformation of the hair cells.

The other auditory receptor found in the amphibian inner ear is the basilar papilla. The **basilar papilla** is not present in every amphibian species and it is possible that it independently and convergently evolved in each major amphibian lineage. In Anura the basilar papilla is also found within the saccule, but it is found within the lagena of Urodela and the utriculus of Apoda. The basilar papilla is also stationary and deforms in response to fluid movement.

The movement of the inner ear fluid occurs in response to the reception and propagation of external sound waves, but these sound waves need amplification in order to reach and then subsequently stimulate the fluid contained within the inner ear. This amplification is necessary because the fluid contained within the inner ear is of higher viscosity than the air of the surrounding terrestrial environment. The energy of the sound waves needs to be amplified in order to produce movement of the fluid of the inner ear. Thus, terrestrial vertebrates evolved additional structures to concentrate, amplify, and deliver sound waves traveling through the air to the inner ear. Tetrapods initially solve this problem by evolving the middle ear cavity. Later, in mammals and birds, the external ear also evolves to further aid in this endeavor.

The structures contained within the middle ear cavity aid in the amplification of sound from the external environment. The middle ear consists of a **tympanum** (also referred to as the eardrum), which is set in vibration by the reception of external sound waves. These sound waves are then transmitted from the tympanum to the inner ear by way of one or more middle ear **ossicles** (or bones). One middle ear ossicle common to most

amniotes is the **columella** (or stapes). The stapes is homologous to the hyomandibula, which is a bone that first evolved in early gnathostomes and is involved in jaw suspension. In species that possess more than one middle ear ossicle, the columella is always the bone that is closest to the inner ear and the bone that is therefore responsible for the final propagation of sound waves to the middle ear. The vibrating columella is capable of initiating the movement of the fluid of the inner ear through the oval window (fenestra ovalis). The surface of the columella that initiates the movement of the inner fluid is often expanded to fit snugly into the oval window and secured via a ligament. Finally, other structures of the middle ear cavity include the eustachian tube, which is a tube that forms continuity between the cavity of the middle ear and the pharynx. Indeed, it is the presence of the eustachian tube that allows you (and other tetrapods) the ability to equalize pressure within the middle ear cavity, which is a process you might be familiar with if you have ever flown on an airplane, participated in scuba diving, or even changed elevation in a vehicle.

In amphibians, there are at least two mechanisms of sound propagation from the external environment to the inner ear. One occurs through the tympanum, which in many amphibians is directly on the outside surface of the head. The vibration of the tympanum then sets in motion the bones traversing the middle ear cavity. In amphibians, there are usually two in-series middle ear ossicles, the tympanum-facing **extracolumella** and oval window–facing **columella**. The tympanum–columella route is the primary mechanism of propagating high-frequency airborne sounds, which are primarily transduced by the basilar papilla auditory receptor. However, seismic waves, or sounds initiating from the vibration of the ground, can be delivered to the inner ear through a different mechanism. Interestingly, in amphibians, the oval window is also partly covered by a third bone, the operculum, which is not in series with the columella. A small muscle, the opercularis, is found traversing the space between the operculum and the pectoral girdle. Thus, ground-based sounds and vibrations

can be transferred to the inner ear through the series consisting of the ground-facing forelimb bones, pectoral girdle, opercularis muscle, and operculum. The operculum will then transfer vibrations directly to the fluid of the inner ear. The operculum route is the primary mechanism for propagating low-frequency ground-based sounds, which are primarily transduced by the amphibian papilla sound receptor.

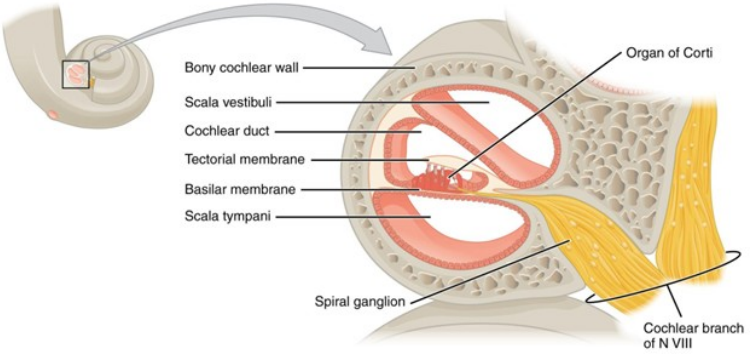

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Figure 20.11—Cross-sectional anatomy of the human (H. sapiens) cochlea. A cross section of the human cochlea reveals the internal anatomy, which contains three-tubular spaces: the scala vestibuli, scala tympani, and the cochlear duct (scala media). The cochlear duct also houses the auditory organ (referred to here as the Organ of Corti). Although this diagram is a cross section from a mammalian cochlea, which is coiled, the internal structure of this cross section is generally representative of the condition found across amniotes regardless of the specific shape and size of the cochlea in a given species.

Amniotes

The inner ear and its connection to the middle ear cavity are generally similar across most lineages of amniotes. The middle ear cavity of amniotes is similar to that of amphibians; however, the structures associated with the middle ear do diversify across amniotes. Thus, we will introduce the general structure of the inner ear here and in the following subsections highlight key differences

of the middle and inner ear that exist between major vertebrate lineages. The inner ear of amniotes is again categorized by a series of tubes within the skeleton. The skeletal tube that is typically associated with hearing is generally referred to as the **cochlea** (or **lagena**) in amniotes and is an extension from the chambers of the vestibular apparatus. Contained within the cochlea are three fluid-filled tubes separated by membranes. Two tubes found within the cochlea in amniotes are the **scala vestibuli** and the **scala tympani**, which both contain **perilymph** and reside adjacent to the skeleton. Sandwiched between the scala vestibuli and the scala tympani is the cochlear duct (or **scala media**), which is filled with endolymph. A **vestibular membrane** (formerly Reissner's membrane) separates the scala media from the scala vestibuli, and the basilar membrane separates the scala media from the scala tympani. It is the basilar membrane that supports the auditory receptor, which we generally refer to as the auditory organ (formerly the Organ of Corti and still referred to as the spiral organ in some human anatomy textbooks). However, we make note of additional naming conventions specific to each vertebrate lineage below. The **auditory organ** is a series of neuromast organs along the length of the basilar membrane that transduces sound waves and are connected to the brain via the auditory nerve (CN VIII). One interesting difference in comparison to nonamniote vertebrates is that in amniotes, fluid movement through the cochlea results in the movement of the basilar membrane (and the subsequent stimulation of the supported neuromast organs).

The movement of the basilar membrane occurs in response to the movement of the inner ear fluids. The sound waves approaching the ear will ultimately generate the movement of the tympanum, which through a series of middle ear ossicles (ending in the columella) transmit these sound waves to the inner ear fluids through the oval window. The **oval window** is a window in the region of the scala vestibuli. The perilymphatic fluid within the inner ear is not compressible, and thus, the movement of the fluid through the scala vestibuli results in the subsequent downward

movement of the following structures (in order): the vestibular membrane, endolymph contained within the cochlear duct, the basilar membrane, and finally, the perilymphatic fluid within the scala tympani. After the sound wave is removed from the tympanic membrane, the exact same series of events will occur in reverse. Thus, the basilar membrane will oscillate up and down at the frequency of the propagating sound waves. Because the perilymphatic fluid is not compressible, a second small region of the tube must also be mobile or elastic in order to yield to the moving perilymphatic fluid, or else the basilar membrane would not oscillate. In most amniotes, the yielding structure is the membrane covering the round window. The round window sits between the scala tympani and the middle ear cavity. The round window is covered by a thin, elastic membrane and is free to oscillate with the fluid because the middle ear cavity is hollow and filled with air. Finally, in most amniotes the hair cells of the neuromast organs along the basilar membrane (i.e., the acoustic organ) are covered by the tectorial membrane, which is firm and does not oscillate in response to inner ear fluid movement. Thus, the vibration of the basilar membrane results in the deflection of hair cells against the stationary tectorial membrane. Typically, different regions of the basilar membrane respond differently to different frequencies of sound waves resulting in the ability to discriminate between sound waves of different frequencies (tones). However, across vertebrates, several different mechanisms have evolved in order to discriminate between tones.

Nonavian Reptiles

The inner ear of most species of reptile follows the general condition of other amniotes. Here, we classify reptiles for the purpose of this section as any species of amniote that does not belong to either Aves or Mammalia. In most reptiles, the bones (or middle ear ossicles) traversing the middle ear cavity responsible

for sound wave propagation between the tympanum and the oval window of the inner ear are the extracolumella and the columella (stapes). These bones are homologous and arranged in the same order as those referred to previously in the amphibian section. The inner ear of most species is consistent with the general amniotic condition. The lagena is slightly expanded in most reptilians. The reptilian acoustic organ, which likely evolved in early reptiles, is often referred to as the auditory papilla within reptiles. The auditory papilla is anchored directly to the surface of the basilar membrane found bordering the scala media (or cochlear duct). The apical surface of individual hair cells is roofed with the tectorial membrane.

Finally, the movement of fluid within the inner ear is variable across species of reptiles, as not every species has evolved a round window. Instead, in some reptilian lineages (e.g., turtles and snakes) displacement of the incompressible fluids of the inner ear are able to be mobilized due to the evolution of an open circuit where fluid flow from the scala tympani is pushed into the scala vestibuli. Thus, the same volume of fluid within the scala vestibuli displaced by the movement of the columella on the oval window is returned to the starting point from the scala tympani.

Aves

The auditory system of birds is generally similar across Aves. In birds, the primary chamber of the inner ear associated with hearing is the lagena (i.e., cochlea). In birds, the cochlea is of significantly greater length in comparison to the lineages we consider reptiles above. The cochlea of birds is also slightly curved, and this curvature is most pronounced in owls. The auditory organ is distributed along the now lengthened basilar membrane traversing through the cochlear duct. Again, the apical surface of individual hair cells is roofed with the tectorial membrane.

The middle ear cavity of birds is similar to that described

in reptiles and amphibians. The two middle ear ossicles responsible for propagating sound waves between the tympanum and the oval window are again the extracolumella and the columella (stapes), which are arranged in the same order.

Birds also evolved a true external ear. In birds, the external ear, characterized as a short canal, runs from the tympanic membrane toward the external surface of the head. This canal, referred to as the **external auditory meatus** (but also known as the external auditory canal, external acoustic meatus/canal), is analogous to convergent evolution of the similar structure in mammals. Birds have not evolved a true outer ear, which in mammals is a skin-covered, cartilaginous structure referred to as the **pinna** or the **auricle** (usually in human anatomy). Instead, in birds, the facial feathers surrounding the opening of the external auditory meatus are sometimes raised to provide similar functionality to the mammalian pinna (discussed below).

Mammals

The mammalian inner ear is characterized by a highly elongated and (to different degrees) coiled cochlea. In mammals, this elongated and coiled chamber is almost exclusively referred to as the **cochlea**. In monotremes, the cochlea is only slightly coiled, and the degree of cochlear coiling ranges from one to four total coils across therian mammals. The inside of the cochlea follows the same condition described above in the introduction to hearing in amniotes. However, the number (and rows) of hair cells is significantly greater than in other vertebrate lineages, thereby increasing sensitivity and the ability to discriminate different tones. Sadly, for the humans reading this chapter, the range of tones that mammals are capable of transducing is limited in humans in comparison to many other mammalian species (especially bats and dolphins).

Mammals also evolved modification of the middle ear that contributes to their auditory sensitivity. While other tetrapods

utilize the two-structure chain composed of the extracolumella and the columella to propagate sound waves from the tympanum to the oval window of the inner ear, mammals utilize three middle ear ossicles. In mammals (in order from the tympanum to the oval window), these three middle ear ossicles are the malleus, incus, and stapes. The stapes is indeed homologous to the columella of other amniotes. The malleus and incus are actually homologous to two bones that constitute the bones of the jaw joint in other vertebrates, the articular and the quadrate. Over evolutionary times, these two bones were freed from use at the jaw joint and migrated toward the middle ear cavity in mammals.

Finally, mammals also rely on an external auditory meatus that runs from the tympanum toward the external surface of the head. In addition, most mammals (all but monotremes) possess the pinna (or auricle), which is the visually noticeable external feature that humans often consider "the ear." The pinna, which has different degrees of mobility across mammals (compare the mobility of the pinna of your pet cat to that of your own pinna in humans), functions to help direct sound into the external auditory meatus, improving the ability of an animal to localize the source of the sound.

Ear Development

As described above, development of the auditory system begins with the emergence of the otic placode. The placode formation stage is similar across all vertebrate groups, but in zebrafish no otic cup or pit forms; rather, the placode thickens into a solid sphere of cells. These cells then become polarized, with their nuclei moving to more superficial positions within the cells, and an elongated hollow cavity appears in the center of the sphere to form the lumen of the otic vesicle. This lumen then expands to quickly enlarge the otic vesicle, and the cells sink into the lumen to form the different chambers of the ear.

The ears of zebrafish, like most other fishes, also include **otoliths**, which develop in an interesting way. Within the otic vesicle, small seeding particles are bound by the kinocilia of early forming tether hair cells. Other cilia within the vesicle beat to move additional seeding particles around to prevent them from clumping up prematurely. When these particles contact those already bound to the tether, they aggregate, and the otoliths increase in size.

Lateral Line System

The lateral line is an aquatic mechanosensory system that allows fish and amphibians to "feel the flow" around them. Picture a bait ball of schooling silvery fish. A swordfish dives through and a hole opens up in the shimmering sphere. This interaction is mediated by the lateral line system: The baitfish school closely together without crashing into each other by utilizing their lateral line. The swordfish senses the vibrations of the bait ball in the water using its lateral line. The baitfish dodge out of the way in time thanks to their lateral line feeling the water pressure wave created by the plunging swordfish.

The sensor of the lateral line system is the neuromast organ (discussed above). Each hair cell has a single kinocilium as well as several stereocilia. As water passes over the neuromasts, their cupulas are deflected, and the hair cells embedded in the cupula are deflected with it. Because neuromasts are morphologically asymmetrical, the way that the stereocilia are deflected impacts relays directional information to the rest of the animal. In other words, the directional response of the hair cell is determined by the bending of the stereocilia toward the kinocilium, resulting in depolarization of the cell. If the stereocilia bend away from the kinocilium, it causes hyperpolarization of the cell.

Neuromasts are distributed both on the skin (**superficial neuromasts**) and in fluid-filled canals (**canal neuromasts**). These two types of neuromasts have slightly different functions.

Superficial neuromasts respond to direct fluid flows. Canal neuromasts respond to water flows indirectly, instead sensing the pressure difference between the pores of the canals they sit in. Simply, superficial neuromasts are velocimeters and canal neuromasts are accelerometers. Canal neuromasts are oriented in the direction of their canal. However, superficial neuromasts can be oriented in lots of directions, but mostly they are oriented parallel or perpendicular to axes of their neighboring canals.

While the lateral line is most well known in fish, most amphibian larvae and some adult amphibians possess a lateral line system. In amphibians, neuromasts are mostly superficial, but some more basal amphibians have recessed neuromasts in canals or grooves, like the canal neuromasts seen in fish. Studies on the effects of metamorphosis on the lateral line have shown that larval neuromasts are very similar to those found in adults. They change slightly in shape (adult neuromasts are longer and more slender), which is attributed to the thickening of the epidermis during metamorphosis.

The Acousticlateralis System: The Historical Overlap Between Sound and Touch

The lateral line was originally lumped in with the acoustic system as the "acousticlateralis" system. This is because (1) they share a similar developmental pathway via dorsolateral placodes, (2) they both project to similar parts of the hindbrain, and (3) they use the same functional unit: the hair cell. Both systems respond to low frequencies, but the lateral line responds to lower frequencies of 0–200 Hz. Although they are both responding to mechanosensory stimuli, we now know they're two separate systems thanks to physiological and morphological studies. That's not to say that they're not related. Some fish have portions of their lateral line adapted to sensing sound pressure. This is mediated by their close association with compressible gas cavities. If you're interested in a

thorough history of the lateral line and personal histories of leaders in the field, check out Coombs and Bleckmann, 2014; Bleckmann, 2023; and Webb, 2023.

Lateral Line Development

The lateral line system is widely distributed along the head and body of fishes and amphibians and consists of mechanosensory neuromasts and their afferent neurons. Anteriorly, the lateral line develops from anterodorsal and anteroventral lateral line placodes, through proliferation and differentiation of a combination of migratory embryonic cells and stationary progenitors. The posterior lateral line develops from separate lateral line placodes located just posterior to the otic vesicle and gives rise to a set of sensory primordial cells. This cluster of migratory cells deposits neuromasts at intervals as it moves along the body and induces overlying epidermal cells to form a ringlike pore around each neuromast. Posteriorly, the posterior lateral line placode generates a line of seven or eight neuromasts during embryonic development, while three more lateral lines form during larval development.

Electroreception

Electroreception, or the ability to sense electrical stimuli, can be separated into passive and active. **Passive electroreception** is when organisms detect electric fields already present in the environment, from both abiotic and biotic sources. Alternatively, **active electroreception** is when an organism generates and senses its own electrical fields or those generated by other actively electroreceptive organisms.

Passive Electroreception

Passively electroreceptive fish are able to sense both abiotic and biotic electric fields. **Abiotic** electric fields are things like the electric fields produced by the Earth's magnetic field or even those produced by strong water currents due to water being a polar molecule. **Biotic** electric fields are produced by other animals, such as the electrical signal of contracting muscles or the ion imbalance between an animal's internal and external environment. This is how hammerhead sharks, for example, can sense a stingray buried in the sand.

Electrosensory receptors, or **ampullary receptors**, are named for their shape. Elasmobranchs are probably the most famous passively electroreceptive animals, but 16% of fish are passively electroreceptive. Elasmobranchs have the famous ampullae of Lorenzini, first described by Lorenzini in 1678. Ampullary receptors are modified neuromasts and share many of the morphological characteristics discussed already in this section. Pores in the skin connect to an ampullary organ via jelly-filled canals, which are lined with tightly packed cells providing a high resistance barrier and stopping electrical leakage. The jelly found in the ampullae of Lorenzini is actually the most conductive naturally occurring material on Earth. The ampullary organs at the end of the canals are made up of electrically excitable sensory cells, which are "always on," a.k.a. tonically active. These cells are constantly producing neurotransmitters, and their membrane potential differences change based on external electrical stimuli. Fish don't have all the electric fun—some amphibians have passive electrosensory systems as well that are similar in morphology, function, development, and evolutionary origins.

Some aquatic mammals are also passively electroreceptive. Platypuses use their bills to sense electrical fields via modified mucus or serous glands. Their close cousins, the short-beaked and western long-beaked echidnas, also have electroreceptors despite being fully terrestrial. They use their electrosensory abilities when

they snuffle around with their snouts underwater. Guiana dolphins are also electroreceptive. Their electroreceptive sensors are suspected to be their vibrissal crypts—the empty pores formally filled by vibrissae (whiskers) during neoteny. These crypts are similar morphologically to the ampullary organs seen in platypuses and fish. While other dolphins have similar crypts, electrical sensitivity has yet to be shown in other species.

Active Electroreception

Actively electroreceptive animals can generate their own electric fields and sense both their own and that of conspecifics. This cool sensory modality is most famous in electric fish. Strongly electric fish use powerful electric discharges to stun prey, while weakly electric fish mostly use their generated electrical fields for intraspecific communication. The electric organ of these fish is made of modified muscle cells called **electrocytes**. They have a smooth side, which is innervated, and a rough side, which is not, causing an unequal voltage distribution. Much like a battery, they have a positive end and a negative end, maintained by an Na^+/K^+ ion pump, keeping Na^+ excluded from the cell and keeping K^+ inside. When the nerve is stimulated, Na^+ rushes out much faster than K^+ rushes in, causing a sharp increase in electrical potential difference. These cells are stacked positive end to negative end (once again, think of batteries in a remote) to generate larger voltages, forming an electrical field around their body.

Weakly electric fish, like knife fish or elephant fish, generate weak electric discharges. They can create pulses of electric fields that extend around their body via variation in the arrangement of these electrocytes. They can modulate this field based on habitat, behavior, navigational need, or motivational state. Socially, they can use these fields to defend territories or advertise to mates. They sense these fields via tuberous receptors, which can sense fields up to 1 kHz. Like ampullary organs, they are named for

their shape. Each tuberous receptor sits in a tuber-shaped capsule in the epidermis. At the base of the capsule sit the electrosensitive sensory cells, which are different from those in ampullary organs. These sensory cells are covered in microvilli, which are hypothesized to act as a high-pass filter for the system.

Electroreceptor Development—Ampullary Organs

Most of our current knowledge of the development of electrosensory ampullary organs in vertebrates comes from axolotls, nonteleost actinopterygians, and chondrichthyans, with little data coming from teleost fishes, since those that are known to have electroreceptors are not commonly used as developmental models. Cell lineage tracing studies have shown in all three groups mentioned previously that ampullary organs, like neuromasts, develop from the lateral line placodes but are confined to just the head. Numerous small lateral line placodes covering the head first elongate into ridges, and then ampullary organs form deep within the epidermis and then open to the surface on the lateral sides of the ridges.

20.5 Summary

You have learned how the different vertebrate sensory systems are made up of specialized structures that facilitate perception and enable animals to interact with their environment. Each of the discussed sensory systems has receptors that respond to stimuli in the environment—converting physical stimuli into neural signals, specialized anatomy to help the stimuli reach their intended receptors, and a network of neural pathways to carry the sensory neural outputs to the brain.

It's important to remember that these specialized systems

do not work in isolation—animals integrate sensory information to navigate their world. It's easy to forget this, especially when we think of animals who are lauded as having a particularly good sense (a shark's sense of smell or a bat's ability to hear, for example.) While in these cases other senses may be reduced, it's vital to think about the whole suite of sensory systems, not just the "famous" one.

The way sensory systems are integrated can also change over time. Sensory adaptation, for example, occurs when the sensitivity to a constant stimulus decreases over time. Think about entering a bakery: When you walk in, you're initially overwhelmed with the smell of cookies and pastries, but the longer you wait in line to purchase your sweets, you will become "nose blind" to the smell, no longer noticing the aromas. Sensory systems can also experience cross-modal interactions where one sensory modality impacts another. A famous example of this is the McGurk effect, which impacts how we hear speech. When we watch someone speak, our visual cues (the speaker's lip movements) impact our auditory perception.

Studying sensory systems across vertebrate groups gives great insight into the pressures that have driven vertebrate evolution and radiation. Sensory systems evolved as vertebrates survived by adapting to diverse ecological niches. Studying sensory system anatomy gives us insights into not only how these systems work but how they have evolved. The exploration of vertebrate sensory anatomy deepens our understanding of basic biological functions, the evolution of these systems, and the complexity of sensory system integration. We hope that after this quick tour through vertebrate sensory systems, you have learned to appreciate the intricacies that underlie the sensory experiences that allow us to interact with the world.

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21. Endocrine System

JENNIFER L. HOUTZ

Focus Questions—to Guide Your Reading of This Chapter

1. How is the endocrine system different from the nervous system?
2. How do endocrine gland structure and complexity change through the evolution of vertebrates?
3. What are the main hormones secreted by each major endocrine gland? What are each hormone's main functions, and how do they differ across vertebrate taxa?
4. What endocrine glands and hormones are part of the following hormonal axes: (*a*) hypothalamic-pituitary-thyroid (HPT) axis, (*b*) hypothalamic-pituitary-adrenal (HPA) axis, and (*c*) hypothalamic-pituitary-gonadal (HPG) axis?

21.1 Introduction

There are two major control systems in the body: the nervous

system (covered in detail in Chapters 19 and 20) and the endocrine system. These two systems frequently work together to coordinate organ activity and maintain **homeostasis** or a stable internal physiological state.

The endocrine system is composed of a series of **endocrine glands** that are spread throughout the body (Figure 21.1). Unlike **exocrine glands** (e.g., sweat glands, sebaceous glands; see Chapter 6), which deposit their products on the body's surface or into organs through ducts and act only in the immediate vicinity of the gland, endocrine glands produce chemical messengers known as **hormones**, which can affect the function of body parts far removed from the gland's location. Such far-reaching effects are possible because endocrine glands deposit hormones into circulating body fluids (typically the bloodstream but also occasionally the lymph or cerebrospinal fluid). The hormones are then transported throughout the body to act on their target tissues. Target tissues must express **receptors** specific to that hormone for it to affect the function of the target.

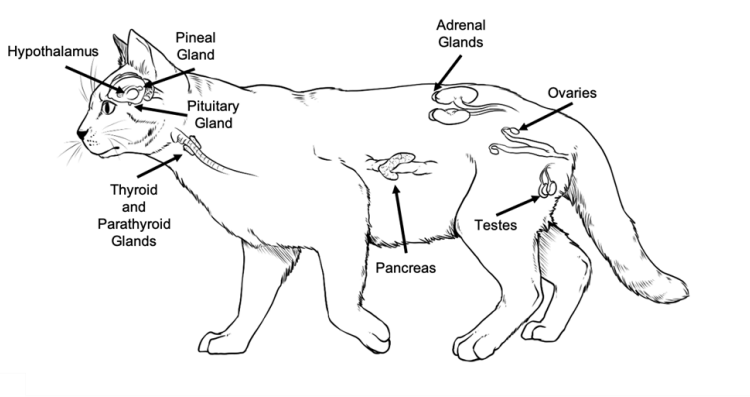

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Figure 21.1—Overview of the major endocrine glands evolutionarily conserved across vertebrates.

We know at this point that form and function go hand in

hand, so it should come as no surprise that endocrine glands and exocrine glands have some basic structural differences related to their different functions. The primary structural difference between these two gland types is the presence or absence of a duct through which products are released. Exocrine glands contain ducts that may be simple (i.e., a singular duct) or compound (i.e., a branched duct), and their products are released into these ducts for transport to the epithelial surface they act on (Figure 21.2). Again, because the products of exocrine glands are released at a specific site, they can only act locally and within the general vicinity of the gland itself. Endocrine glands, in contrast, lack ducts entirely. Instead, these glands tend to be highly vascularized, so their products can diffuse directly into the capillary beds and then be transported throughout the body via the bloodstream (Figure 21.2). Because endocrine products can be transported throughout the body via the bloodstream, these glands can act globally (i.e., impact areas of the body beyond the immediate vicinity of the gland).

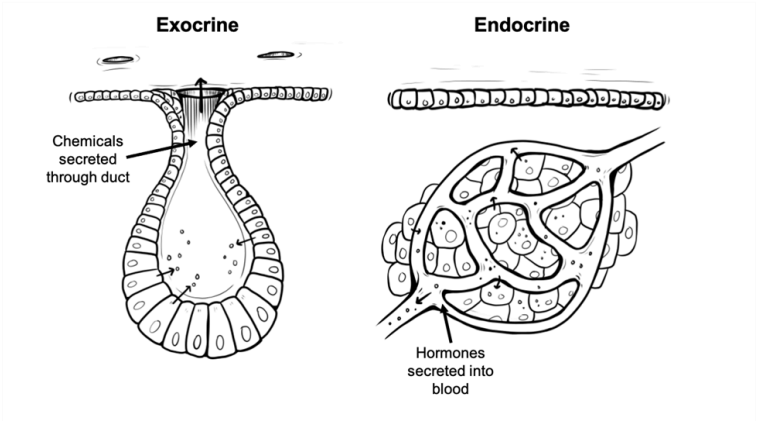

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Figure 21.2—Structural differences between exocrine and endocrine glands.

The effects of hormones on target tissues can be categorized as activational or organizational depending on the stage

of development or age of the organism at the time of hormone exposure. **Activational effects** of hormones are temporary and reversible changes in the organism's physiology and/or behavior. These activational effects occur when hormones secreted at certain levels or times of the year in the bloodstream act on target tissues to regulate their function. For example, increased testosterone secretion in adult testes-bearing songbirds during the breeding season can lead to increased aggression, territory defense, and courtship behavior; however, testosterone levels decrease in the nonbreeding season and these behaviors are not expressed as intensely or frequently. **Organizational effects** of hormones occur during critical periods of development (i.e., prenatal or early postnatal stages) and permanently shape anatomical, physiological, and/or behavioral traits. For example, exposure to certain sex steroid hormones (e.g., androgens and estrogens) during fetal development can permanently influence the sexual differentiation of the brain and reproductive organs (e.g., gonads). Thus, it is important to consider the age and stage of development of the vertebrate in question at the time of hormone exposure when discussing the functions of hormones.

Hormones can be classified as **protein hormones** (water-soluble; Figure 21.3A) or **steroid hormones** (lipid-soluble; Figure 21.3B). The chemical properties associated with each class of hormone determine how they are produced and stored in endocrine cells in addition to their mode of action (i.e., which types of receptors they bind to on target cells). Protein hormones are made of amino acids that form hydrophilic (i.e., attracted to or soluble in water) polypeptides. Depending on their size (i.e., number of amino acids), these hydrophilic hormones can be categorized as amines, peptides, or proteins. Glycoproteins are another class of hydrophilic hormone, which are proteins with oligosaccharide chains (glycans) bonded covalently to the amino acids. Steroid hormones are derived from cholesterol, creating hydrophobic (i.e., repelled by water or insoluble in water) molecules. Protein hormones are synthesized in the endoplasmic reticulum of endocrine cells where they undergo

posttranslational modifications and are stored in secretory vesicles (i.e., membrane-bound organelles that carry proteins, peptides, or neurotransmitters to the exterior of a cell). Because they can be stored in advance (i.e., require active transport out of cell membranes), protein hormones generally elicit rapid responses by binding to membrane-bound receptors on the outside of the target cell. Due to their lipophilic (i.e., attracted to or soluble in fats and oils) structure, steroid hormones can easily diffuse out of the cell membrane; therefore, the effects of steroid hormones are slower and more prolonged, as they cannot be stored in advance. Steroid hormones typically bind to intracellular receptors on the nucleus, but some do bind extracellular membrane-bound receptors. Last, protein hormones circulate through the bloodstream in their unbound form because they can dissolve in the blood, whereas steroid hormones require carrier proteins for transport in the bloodstream.

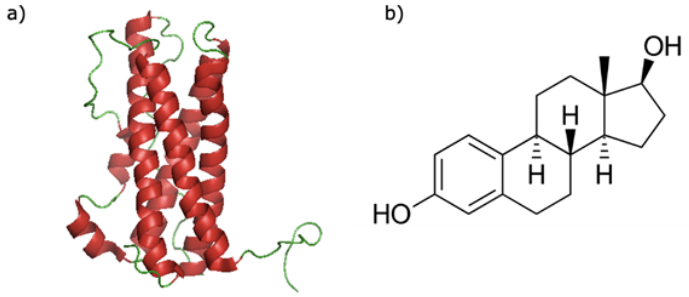

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Figure 21.3—Protein versus steroid hormones including (a) prolactin (protein hormone) and (b) estradiol (steroid hormone).

How does the body naturally keep hormone levels within a specified range? Positive and negative feedback are two mechanisms by which the endocrine system promotes or inhibits hormone secretion, respectively. In **negative feedback**, increasing

levels of a hormone initiate physiological responses that eventually inhibit the secretion of that hormone. Once circulating hormones rise to a certain threshold, the binding of the hormone to receptors on various endocrine glands signals to those glands to shut off production of the target hormone or other hormones in a hormonal axis. For example, the adrenal glands secrete glucocorticoid hormones in response to stressful stimuli (Figure 21.4). Once glucocorticoid levels pass a threshold concentration in the blood, glucocorticoids bind to receptors on the adrenal gland to shut off their own production but also bind to receptors on the pituitary gland and hypothalamus that each secrete other hormones that stimulate glucocorticoid secretion from the adrenal glands. We will cover this topic in more detail when we discuss the hypothalamic-pituitary-adrenal (HPA) axis. In **positive feedback**, increasing levels of a hormone continue to promote secretion of that hormone. For example, as labor progresses in humans, oxytocin is released from the pituitary gland in response to uterine contractions. Oxytocin stimulates further contractions, leading to more oxytocin release. This positive feedback loop continues until childbirth is completed.

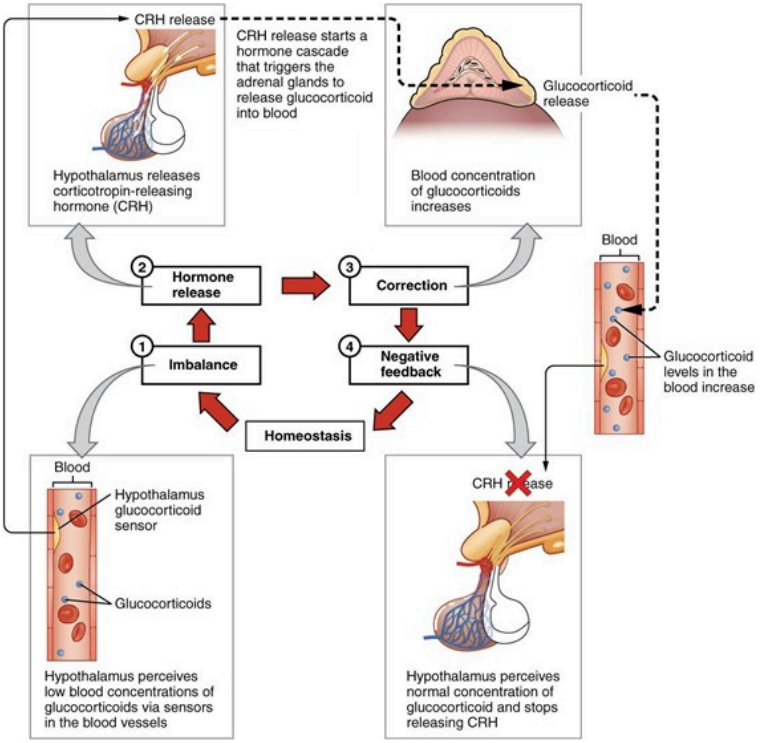

Figure 21.4—Negative feedback loop for glucocorticoid hormones.

In this chapter, we will survey the major endocrine glands of the vertebrate body, looking at their structures, development, and evolution. Because these glands are tightly integrated with other systems, we will link back to other chapters in this textbook for details related to function.

21.2 Hypothalamus and Pituitary Gland

Structure and Function

The **hypothalamus** and **pituitary gland** are considered the masters of the endocrine system because they coordinate numerous homeostatic processes throughout vertebrates (Figure 21.5).

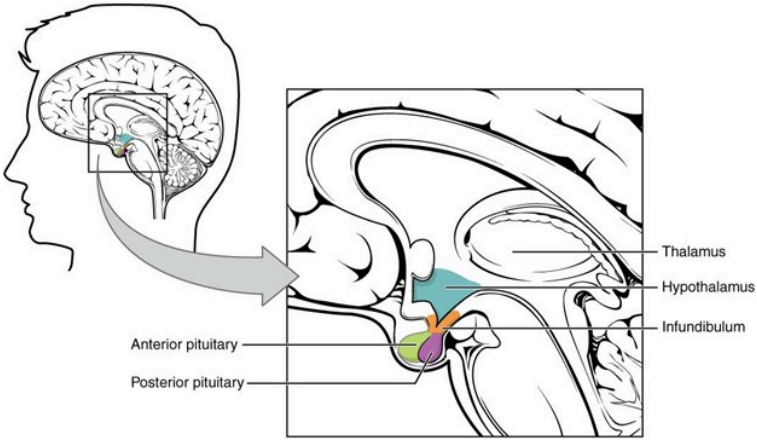

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Figure 21.5—Location of the hypothalamus and pituitary gland in a human.

Together they form **hypothalamus-pituitary (HP) axes** that control the functions of other peripheral endocrine glands such as the thyroid gland (**hypothalamic-pituitary-thyroid [HPT] axis**), adrenal gland (**hypothalamic-pituitary-adrenal [HPA] axis**), and gonads (**hypothalamic-pituitary-gonadal [HPG] axis**). **Neurosecretory neurons** (i.e., neurons that secrete neurohormones into the blood) in the hypothalamus secrete **hypothalamic releasing** or **inhibiting hormones**, which regulate secretion of **tropic hormones** (i.e., hormones that have other endocrine glands as their targets) from the pituitary gland. These hypothalamic hormones travel to the pituitary via two routes: (1) release into the **median eminence** at the base of the hypothalamus, which contains a portal capillary system that vascularizes the anterior pituitary; or (2) direct axon innervation into the posterior pituitary. The tropic hormones

are released from the pituitary into the bloodstream where they travel to peripheral endocrine glands (Figure 21.6). The peripheral endocrine glands will release their hormones into the blood to target other endocrine or nonendocrine tissues with corresponding target receptors.

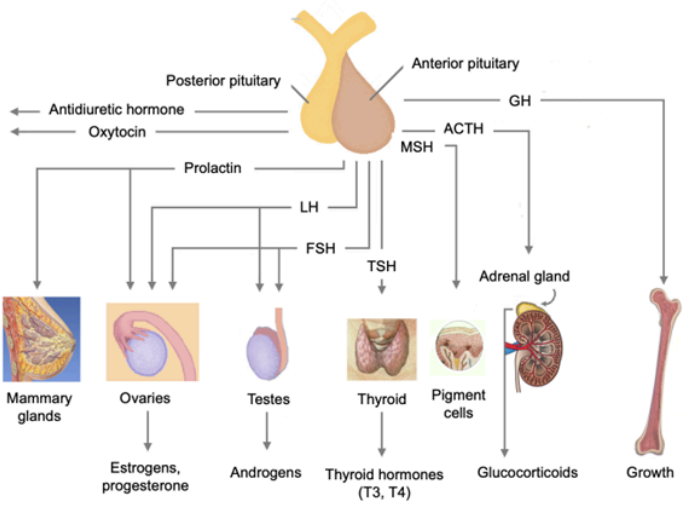

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Figure 21.6—Tropic hormones from the pituitary gland and their target peripheral endocrine glands. Abbreviations for hormones are provided in the text.

Hormones from other endocrine glands can induce positive or negative feedback at the level of the pituitary and/or hypothalamus. However, the class of the hormone (i.e., protein vs. steroid) determines whether it can reach receptors within the hypothalamus, as it is protected by the **blood-brain barrier**. The blood-brain barrier is a selectively permeable membrane that regulates the passage of ions and molecules from the blood into the brain, maintaining ionic homeostasis and protecting the brain from neurotoxins found in the blood. The blood-brain barrier is formed by microvascular endothelial cells lining the cerebral capillaries that

penetrate the brain and spinal cord of most mammals and other organisms with a well-developed central nervous system. Endothelial cell tight junctions limit the passage of hydrophilic molecules across the blood-brain barrier. Hydrophilic molecules including protein hormones can only pass through the barrier via carrier- or receptor-mediated active transport. In contrast, lipophilic molecules such as steroid hormones can passively diffuse across the membrane barrier. The pituitary gland is not protected by the blood-brain barrier, allowing protein tropic hormones to freely circulate to the rest of the body through the bloodstream.

Hypothalamus

The hypothalamus evolved before the vertebrate brain (see Lemaire et al., 2021). The structure of the hypothalamus is highly conserved across vertebrates due to its integral function in regulating fundamental aspects of physiological homeostasis. It is located at the most ventral position in the forebrain (see Chapter 19). The mammalian hypothalamus contains bilateral neurosecretory nuclei (i.e., groupings of neurons based on proximity and shared function) such as the preoptic nucleus (PON) or paraventricular nucleus (PVN), among others. The axons of the hypothalamic neurosecretory cells extend directly into the posterior pituitary or into the median eminence where neurohormones travel to the anterior pituitary. These neurohormones are categorized as releasing hormones or inhibiting hormones depending on whether they stimulate or prevent tropic hormone release from the pituitary. All hypothalamic releasing hormones are protein hormones ranging in size (i.e., number of amino acids). These hormones are synthesized in the cell bodies of the hypothalamus and stored in secretory vesicles. Each hypothalamic regulatory hormone is named after the first tropic hormone it was discovered to control in

addition to whether its primary action is stimulatory or inhibitory. We outline the functions of each one here.

1. **Corticotropin-releasing hormone (CRH)** is a peptide hormone that stimulates the synthesis and the secretion of adrenocorticotropic hormone (ACTH) by corticotroph cells of the anterior pituitary gland. It is the first hormone in the HPA axis, which coordinates the vertebrate hormonal stress response.
2. **Thyrotropin-releasing hormone (TRH)** is a peptide hormone that stimulates the synthesis and secretion of thyrotropin (thyroid-stimulating hormone) by thyrotroph cells of the anterior pituitary gland. It is the first hormone in the HPT axis, which regulates internal body temperature and metabolism.
3. **Gonadotropin-releasing hormone (GnRH)** is a peptide hormone that stimulates the synthesis and secretion of the two gonadotropin hormones including Luteinizing Hormone (LH) and follicle-stimulating hormone (FSH) by gonadotroph cells of the anterior pituitary. It is the first hormone in the HPG axis, which coordinates reproduction and expression of primary and secondary sex characteristics.
4. **Gonadotropin-inhibitory hormone (GnIH)** is a peptide hormone that suppresses gonadotroph function, preventing the release of LH and FSH from the anterior pituitary.
5. **Growth hormone–releasing hormone (GHRH)** or somatocrinin is a peptide hormone that stimulates synthesis and release of growth hormone (GH, somatotropin) by somatotroph cells of the anterior pituitary.
6. **Growth hormone–inhibiting hormone (GHIH)** or somatostatin is a peptide hormone that inhibits a variety of physiological functions in the gastrointestinal tract, such as gastrointestinal motility and gastric acid production. It also inhibits the secretion of pancreatic and intestinal hormones such as insulin and glucagon.

Pituitary Gland

The pituitary gland is also called the **hypophysis** due to its location below the hypothalamus where it is attached to the brain via a stalk. In adult mammals, it is located ventral to the brain and posterior to the optic chiasm. The term *pituitary* is derived from the Latin word for phlegm (pituita) due to its assumed function of channeling mucus from the brain into the nasal cavity. The pituitary is divided into two lobes including the **adenohypophysis** (anterior pituitary; Figure 21.7) and **neurohypophysis** (posterior pituitary; Figure 21.7). The anterior pituitary or adenohypophysis is made of different endocrine cell types (e.g., somatotrophs, lactotrophs, gonadotrophs, thyrotrophs, corticotrophs) that synthesize and secrete a wide array of tropic hormones (see below). The anterior pituitary further divides into three regions: pars distalis, pars intermedia, and pars tuberalis. The *pars distalis* comprises the majority of the anterior pituitary across vertebrates. The *pars tuberalis* is located anterior to the pars distalis but is only found in tetrapods. The *pars intermedia* lies between the anterior pituitary and posterior pituitary, physically connecting them. The posterior pituitary or neurohypophysis consists of neurosecretory neurons that project down from the hypothalamus. These neurosecretory neurons release neurohormones into capillaries within the median eminence. The posterior pituitary further divides into two regions: the pars nervosa and median eminence. The pars nervosa has a separate blood supply from the body than that of the anterior pituitary.

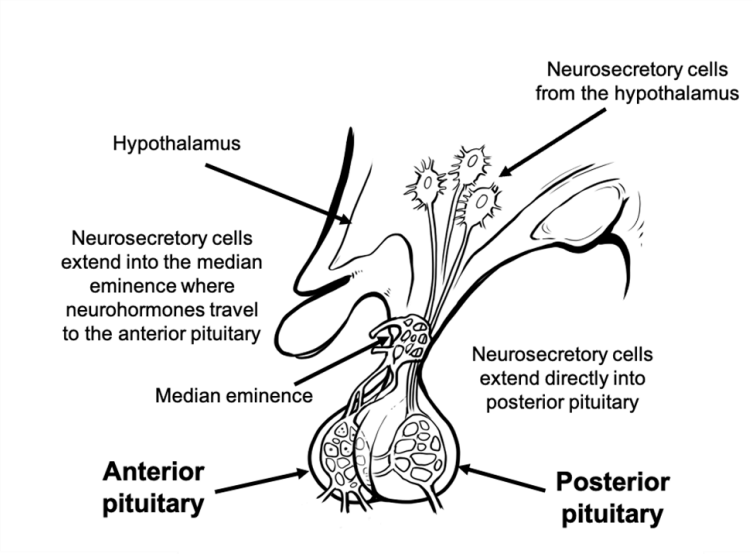

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Figure 21.7—The two lobes of the pituitary including the anterior pituitary (adenohypophysis) and posterior pituitary (neurohypophysis).

Anterior Pituitary (Adenohypophysis) Tropic Hormones

1. **Thyroid-stimulating hormone (TSH)** or thyrotropin is a glycoprotein hormone synthesized and secreted by thyrotrophs of the anterior pituitary. It is the secondary hormone in the HPT axis that is released in response to thyrotropin-releasing hormone (TRH). TSH stimulates follicular cells within the thyroid gland to release thyroid hormones.
2. **Adrenocorticotropic hormone (ACTH)** is a peptide hormone synthesized and secreted by corticotrophs of the anterior pituitary. It is the secondary hormone in the HPA axis that is released in response to corticotropin-releasing hormone

(CRH). ACTH stimulates the adrenal cortex to release glucocorticoid and androgen hormones.

3. **Luteinizing hormone (LH)** is a glycoprotein hormone that is synthesized and co-secreted along with follicle-stimulating hormone (FSH) by gonadotroph cells of the anterior pituitary. It is one of the secondary hormones in the HPG axis, triggered by gonadotropin-releasing hormone (GnRH). LH stimulates the Leydig cells of the testes to produce testosterone. LH triggers the secretion of steroid hormones from the ovaries and release of progesterone after ovulation from the corpus luteum (i.e., a temporary mass of cells that forms during the mammalian menstrual cycle inside an ovary). Additionally, LH helps regulate the length and order of the menstrual cycle by playing roles in both ovulation and implantation of an egg in the uterus (see Box 21.3).
4. **Follicle-stimulating hormone (FSH)** is a glycoprotein hormone that is synthesized and co-secreted along with LH by gonadotroph cells of the anterior pituitary. It is one of the secondary hormones in the HPG axis, triggered by GnRH. FSH promotes follicle development in ovaries and spermatogenesis in testes.
5. **Prolactin (PRL)** is a polypeptide hormone that is synthesized and secreted by lactotroph cells in the anterior pituitary. PRL promotes milk production and the development of mammary glands within breast tissues in mammals. It can stimulate crop milk production (i.e., nutrient-rich substance secreted from the lining of the crop) in some bird species including pigeons (Figure 21.8). PRL also promotes parental care behavior in some vertebrates.



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Figure 21.8—An adult common wood pigeon (Columba palumbus) feeds its offspring.

6. **Growth hormone (GH)** is a protein hormone synthesized and secreted by acidophilic and somatotroph cells of the anterior pituitary. GH promotes cartilage and bone growth in addition to increased production of insulin-like growth factor-1.
7. **Melanocyte-stimulating hormone (MSH)** is a protein hormone that is synthesized and secreted by melanocytes or melanophores of the anterior pituitary. MSH promotes the development of pigmentation in skin, hair, and feathers by depositing the pigment melanin. It has also been shown to suppress appetite.

Posterior Pituitary (Neurohypophysis) Tropic Hormones

1. **Oxytocin (OXY)** is a nonapeptide hormone that is synthesized by neurosecretory cells in the hypothalamus and then stored and released by the posterior pituitary. It promotes uterine

contractions via positive feedback during birth in mammals and promotes social behavior (e.g., pair bonding between mates) in certain animals.

2. **Antidiuretic hormone (ADH)** is also known as arginine vasopressin (AVP) or vasopressin. AVP is a nonapeptide hormone that is synthesized by neurosecretory cells in the hypothalamus and then stored and released by the posterior pituitary. AVP regulates blood volume and osmolarity of the body by promoting water reabsorption by the kidneys.

Development

The hypothalamus arises from the ventral portion of the diencephalon. The pituitary develops from two sources including a ventral outgrowth of the diencephalon called the infundibulum and an invagination off the anterior roof of the stomodeum (i.e., invagination of the ectoderm that turns into the mouth) called Rathke's pouch. The infundibulum maintains a connection to the brain forming the posterior pituitary, whereas the connection between the stomodeum and Rathke's pouch is severed forming the anterior pituitary. The secretory cells of the anterior pituitary and neurosecretory cells of the hypothalamus arise from the neuroectoderm (neural ridge). The embryonic origins of the pituitary gland are the same across all vertebrates with the exception of hagfishes. The anterior pituitary of the hagfish originates from endoderm rather than stomodeal ectoderm.

Evolution

The evolution of the vertebrate brain is covered in detail in Chapter 18, but we will highlight differences in the neurovascular connection between the hypothalamus and pituitary. Releasing hormones from

the hypothalamus exert control over tropic hormone release from the pituitary via vascular connection through the median eminence. The neurovascular connection between the hypothalamus and pituitary is found across all vertebrates with a few exceptions. Hagfishes do not have vascular or neural connections between the brain and anterior pituitary. However, a primitive neurohemal area has been described in the Pacific hagfish similar to a median eminence. In chondrostean fishes, the median eminence is separated from the pars distalis by connective tissue so that no neurons penetrate the pars distalis but a portal system is present. Teleost fishes also do not possess vascular connections between the hypothalamus and anterior pituitary but the axons of the hypothalamus directly feed into the pituitary.

The organization and location of the pituitary gland differs across vertebrates (Figure 21.9).

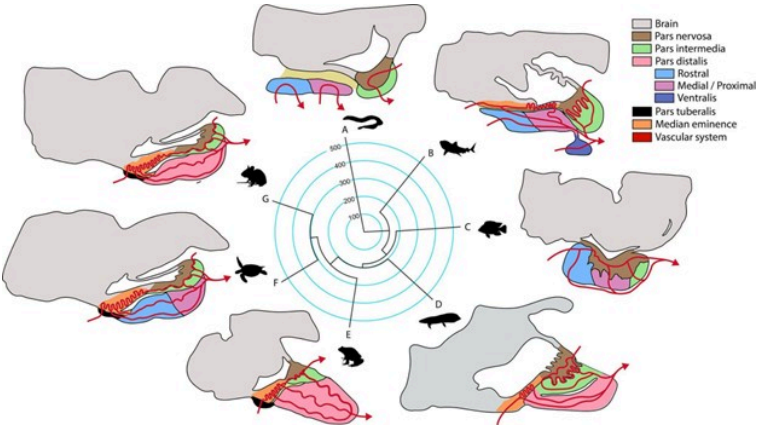

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Figure 21.9—Anatomical pituitary organization across vertebrates. From Santiago-Andres, Golan, and Fiordelisi (2021).

The hagfish anterior pituitary arises from a different embryonic source than other vertebrates. The hagfish anterior pituitary consists of patches of cells in the connective tissue with

no differentiated regions. The hagfish posterior pituitary consists of the pars nervosa in the shape of a long sac that extends from the diencephalon with no median eminence. In lamprey, the anterior pituitary consists of a pars intermedia and a pars distalis that is divided into rostral and proximal subregions. The lamprey posterior pituitary does not have a median eminence. It extends from the ventral region of the brain and is in contact with the anterior pituitary. The anterior pituitary of gnathostome fish contains a pars distalis and pars intermedia, and the posterior pituitary is made of a pars nervosa and median eminence.

In the elasmobranch anterior pituitary, there is a projection from the pars distalis called the ventral lobe or pars ventralis. Though the function is unknown, it is thought to be homologous to the pars tuberalis of tetrapods. The elasmobranch pars distalis is subdivided into rostral and proximal regions like lampreys. There is a vascular portal system between the pars distalis of the anterior pituitary and the median eminence of the posterior pituitary. Above the elasmobranch posterior pituitary, there is a structure derived from the hypothalamus called the saccus vasculosus, which senses seasonal changes in day length.

The pars tuberalis first appears in amphibians and persists through the majority of later vertebrates. In most tetrapods, the anterior pituitary consists of a pars distalis, pars tuberalis, and pars intermedia, whereas the posterior pituitary consists of the pars nervosa and median eminence. The amphibian pars distalis has no distinct regions and no known functions. In reptiles, the pars distalis has cephalic and caudal lobes. The reptilian pars tuberalis is absent in snakes. In birds, there is no pars intermedia in the anterior pituitary but the remaining structure is similar to the typical tetrapod pituitary. The avian pars distalis has cephalic and caudal lobes like reptiles. Though size and structure vary, the mammalian pituitary follows the typical pattern of the anterior pituitary consisting of a pars distalis, pars tuberalis, and pars intermedia, and the posterior pituitary consisting of the pars nervosa and median eminence.

21.3 Pineal Gland

Structure and Function

The **pineal gland** or **epiphysis** is an unpaired gland located behind the third ventricle in the brain midline (Figure 21.10). The term *pineal* comes from the Latin word *pinea* due to the pine cone shape of the gland. The pineal gland and the adjacent **parapineal organ** make up the **epiphysial complex** in mammals. The pineal gland is not protected by the blood-brain barrier. The main hormone synthesized, stored, and secreted by the pineal gland is **melatonin** (protein hormone). In the 1900s, the pineal gland was originally thought to only affect pigmentation. Pineal extracts caused lightening of the skin in amphibian larvae by concentrating melatonin levels within melanophores. We know now that pineal gland secretions of melatonin also coordinate circadian rhythms (i.e., a pattern of an organism that follows a 24-hour period) in most higher vertebrates. The parapineal organ is an adjacent structure that may convey photosensory information to the pineal gland in lower vertebrates.

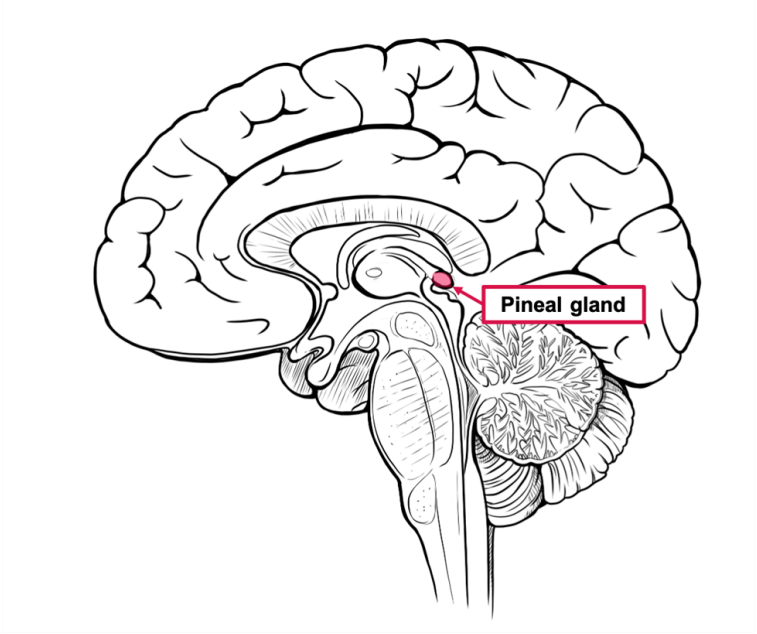

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Figure 21.10—Location of human pineal gland.

In mammals, melatonin regulates endogenous rhythms including the suppression of thyroid function and reproductive activity. Circulating melatonin levels are higher at night than during the day. The retina of the eyes detects changes in photoperiod (i.e., day length) and entrains the **suprachiasmatic nucleus** (**SCN**) of the hypothalamus to the timing of light/dark cycles. Changes in photoperiod detected by the retina sends neural signals to the SCN via the **retinohypothalamic pathway** that controls melatonin synthesis and release from pinealocytes (Figure 21.11). Light suppresses melatonin production by reducing sympathetic input signals to the pineal gland. In photoperiodic species (i.e., seasonally breeding species that coordinate reproductive cycles based on photoperiod), melatonin suppresses reproductive hormones such as

luteinizing hormone (LH) during the nonbreeding season (i.e., short day/photoperiod). As days become longer (i.e., longer photoperiod), light suppresses melatonin production, leading to an increase in LH during the breeding season. During gestation in mammals, maternal melatonin provides the fetus with information on photoperiod until the fetal pineal gland is formed. The pineal gland also impacts melanophore expression in the skin of lower vertebrates such as fish and amphibians.

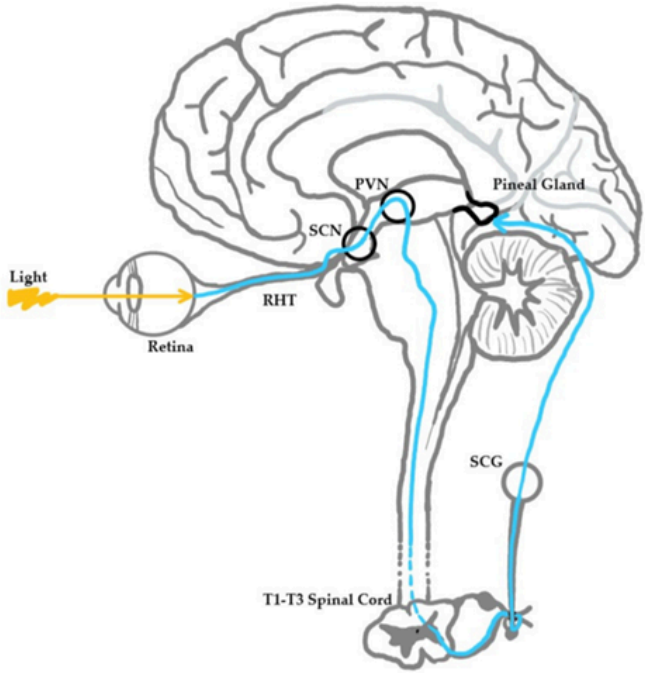

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Figure 21.11—Neuroanatomical pathway of light stimulus to the pineal gland depicting the retinohypothalamic tract (RHT) to suprachiasmatic nucleus (SCN) to paraventricular nucleus (PVN) to the brain stem to the spinal cord to the superior cervical ganglion (SCG) to the pineal gland. From Vasey, McBride, and Penta (2021).

Development

The pineal gland is derived from the roof of the diencephalon of the brain called the epithalamus. During development, evaginations of the epithalamus differentiate into multiple secretory structures including the epiphyseal complex (pineal and parapineal organs), paraphysis, and dorsal sac. The paraphysis develops from the telencephalon as the anterior most evagination of the epiphysial complex. The paraphysis may function like the choroid plexus, a branching network of cells found in each ventricle of the brain that secretes cerebrospinal fluid. The dorsal sac develops as an evagination anterior to the epiphyseal complex and posterior to the paraphysis. The dorsal sac contributes to the formation of the choroid plexus in most vertebrates. It is hypothesized that the organs of the epiphyseal complex arose in primitive fishes as a pair of diverticula and then continued to differentiate into the structures we see today.

Evolution

Through vertebrate evolution, there has been a shift from the pineal gland acting as its own photoreceptor to a heavy reliance on the SCN-pineal pathway to coordinate photoperiod detection and melatonin secretion. Almost all vertebrates possess a pineal gland and a parapineal organ that compose the epiphyseal complex. In cyclostomes, the epiphyseal complex consists of a pineal organ and a parapineal gland that are both potentially photosensory. In Chondrichthyes, the pineal organ is photosensitive but we do not know how functionally important it is. There is no documented existence of a parapineal organ in chondrichthyans.

In teleost fishes, the epiphyseal complex consists of a pineal organ but a reduced parapineal organ is only found in some

species. There are no generalized patterns of melatonin secretion across teleost fish species with peaks occurring regardless of photoperiod. Melatonin has been shown to influence melanophore function in several teleost species. Melatonin is also produced in the retinas of teleost fishes where increased melatonin levels could cause the concentration of pigment in retinal melanophores to increase light sensitivity of retinal cells. Some teleost species also exhibit neural connections to the SCN similar to the mammalian retinohypothalamic pathway. The strength of phototaxis (i.e., an organism alters its movement in response to light stimuli) expressed by a teleost species depends on the opaqueness and thickness of the skull covering their pineal spot.

In amphibians, the epiphyseal complex contains a pineal organ and parapineal organ. There is no documented retinohypothalamic pathway in amphibians; though melatonin expresses a typical diurnal rhythm. Some anuran species have a parapineal end vesicle known as the frontal organ or stirnorgan. The parapineal organ penetrates the skull and possesses photosensory cells (Figure 21.12). It is referred to as the **parietal eye** (i.e., due to the opening in the parietal bones of the skull) or the "third eye" (i.e., due to its ability to transmit photosensory information to the pineal organ). The pineal organ has been thought to evolve in a single direction by losing photosensory capacity and augmenting secretory function in the transitions from amphibians and reptiles to birds and mammals. New evidence reveals that a "fourth eye" reevolved from the pineal organ at least once within reptiles, specifically in an extinct monitor lizard (*Saniwa ensidens*) in which the pineal and parapineal penetrated the skull simultaneously.



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Figure 21.12—Parietal eye of an anole (Anolis carolinensis).

The arrangement of the epiphyseal complex is variable across reptiles. Crocodylians do not possess a pineal or parapineal organ but do have blood levels of melatonin from the retina. Similar to amphibians, lizards have a parapineal organ that penetrates the skull (i.e., parietal eye) that can communicate with the photoreceptive pineal organ. Turtles and snakes only have the glandular portion of the pineal organ, suggesting that they may coordinate photoperiod and melatonin secretion via the retinohypothalamic pathway.

In birds, the pineal organ only possesses the glandular basal portion making it the pineal gland. The pineal gland does not possess photoreceptive cells, and there is no parapineal organ in birds. The pineal gland is innervated with sympathetic fibers, suggesting a retinohypothalamic pathway as in mammals as described above. Mammals possess the typical epithalamus

structure with a pineal gland and parapineal organ, relying on the retinohypothalamic pathway to coordinate melatonin secretion.

21.4 Thyroid and Parathyroid Glands

Structure and Function

The **thyroid gland** or glandula thyroidea is named after the Greek word for shield due to its shield-shaped appearance at the base of the neck in humans. The thyroid gland is responsible for regulating metabolism, growth, body temperature, and reproduction. Across all vertebrates, the thyroid gland secretes two main hormones: **thyroxine** (**T4**) and **triiodothyronine** (**T3**). Both hormones are protein hormones composed of large quantities of iodine. T3 and T4 are the end products of the HPT axis (Figure 21.13). The hypothalamus releases TRH, which travels to the anterior pituitary, which then secretes TSH. TSH stimulates the release of T3 and T4 from the thyroid gland. T4 is considered inactive but makes up about 90% of circulating thyroid hormone levels. T3 is the bioactive form, which makes up the other 10% of circulating thyroid hormone. T4 is converted to T3 by deiodinases (i.e., enzymes involved in the activation or deactivation of thyroid hormones) in tissues with high blood flow such as the liver and kidneys. Both thyroid hormones (T3 is more effective than T4) can initiate negative feedback on TRH at the level of the hypothalamus and on the thyrotropes of the anterior pituitary, decreasing TSH secretion.

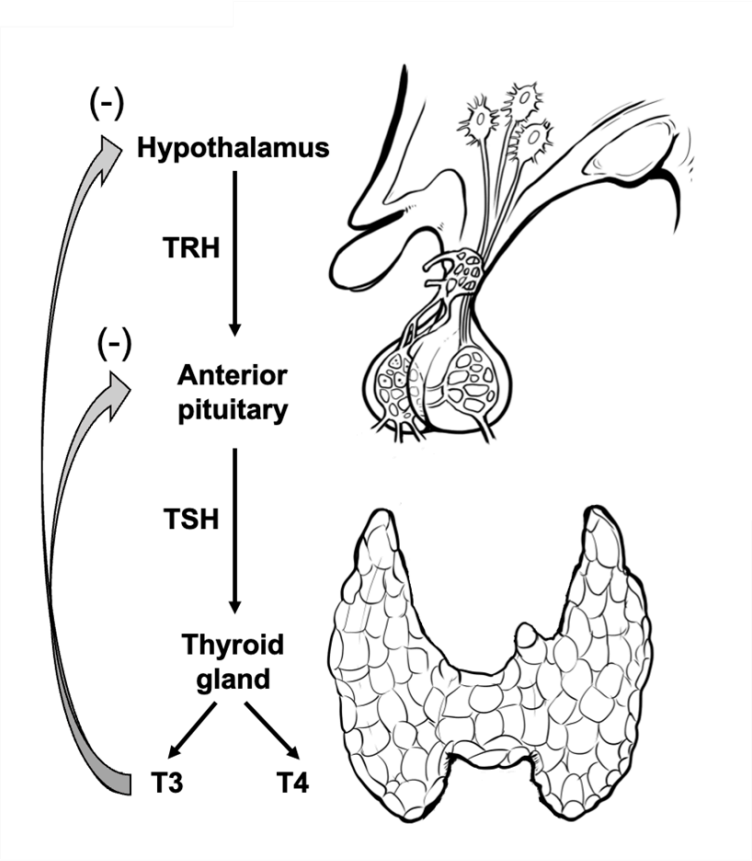

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Figure 21.13—Hypothalamic-pituitary-thyroid (HPT) axis. Gray arrows denote negative feedback. TRH = thyrotropin-releasing hormone; TSH = thyroid-stimulating hormone; T3 = triiodothyronine; T4 = thyroxine.

The parathyroid glands are positioned posterior to the thyroid gland in tetrapods and humans, which normally possess four parathyroid glands (Figure 21.14). Within the thyroid gland, there are follicles made of follicular cells that synthesize and secrete thyroid hormones. **Follicular cells** also secrete a glycoprotein-rich colloid called **thyroglobulin** that stores the secreted thyroid

hormones in the space between the follicular cells (inside the thyroid follicles). In between these thyroid follicles or within the wall of the thyroid follicles, there are **parafollicular cells** or **C cells** that produce **calcitonin**, a peptide hormone that regulates calcium levels in blood. Calcitonin is hypocalcemic, meaning it lowers levels of calcium in the blood. When calcium levels are too high, the parathyroid gland secretes **parathyroid hormone** (**PTH**). PTH is hypercalcemic, meaning it increases blood calcium levels. Calcitonin and PTH work antagonistically to each other via a negative feedback loop to maintain calcium homeostasis in the blood (Box 21.1).

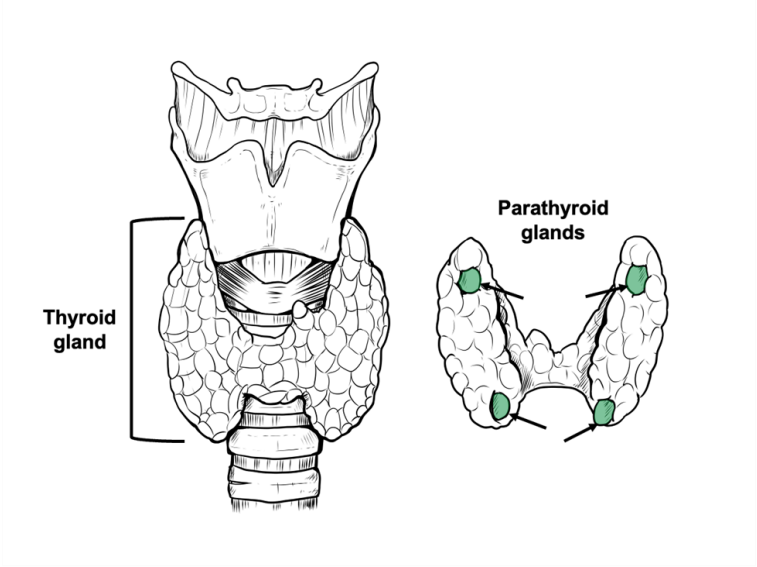

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Figure 21.14—Location of the human thyroid and parathyroid glands.

Box 21.1—Calcium Ion Homeostasis

PTH and calcitonin, primarily secreted by the parathyroid glands and thyroid gland, respectively, are key players in calcium ion regulation. PTH raises blood calcium levels by stimulating bone resorption, which releases calcium into the bloodstream. PTH also enhances calcium reabsorption in the kidneys, which reduces urinary calcium excretion. Calcitonin acts antagonistically to PTH to decrease calcium levels in the blood. Calcitonin inhibits bone resorption and promotes calcium deposition in bones.

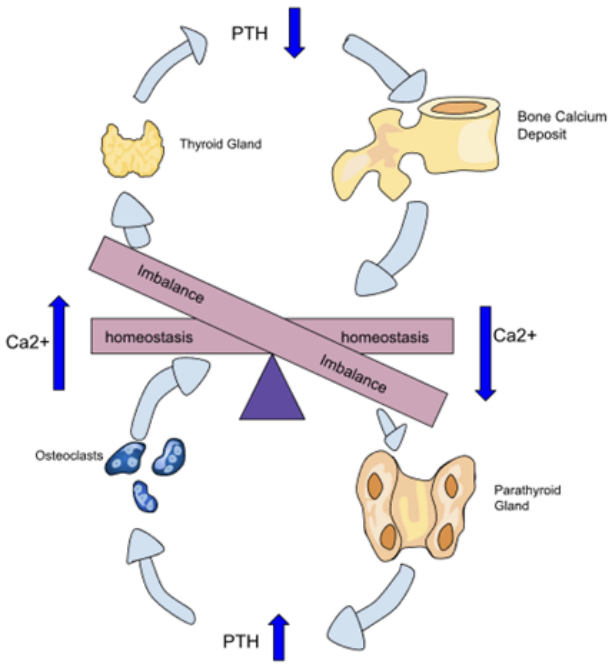

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Figure 21.15—The regulation of blood calcium levels.

Thyroid hormones regulate metabolism and can help generate body heat by increased oxidation of glucose. Basal metabolic rate is linked to thyroid hormone concentrations in birds and mammals. Thyroid hormones also promote growth through a permissive effect on growth hormone (GH) release from the anterior pituitary. Finally, thyroid hormones can also promote reproduction through a permissive effect on gonadotropins (e.g., LH and FSH) from the pituitary that stimulate gonad growth and sex steroid

hormone release. Thyroid hormones are also involved in metamorphosis of lower vertebrates such as fishes and amphibians. Thyroid hormones also regulate proximodistal patterning in fin rays of many teleost fish species.

Development

Across vertebrates, the thyroid gland initially develops as an outgrowth from the floor of the embryonic pharynx. The gland will eventually separate from the pharynx, but the mature gland looks different across vertebrates. The thyroid gland is derived from a ventral bud in the floor of the embryonic pharynx or endoderm between the first and second pharyngeal pouches. There is an initial differentiation into cellular cords, which separate into cell clusters and form thyroid follicles. Unlike the endodermal origin of thyroid gland follicular cells, the thyroid parafollicular cells have a neural crest origin and then migrate into the embryonic glands. Parafollicular cells (C cells) that occur adjacent to or between the follicles arise from the ultimobranchial body, another pharyngeal derivative. The ultimobranchial body originates from neural crest embryonic primordia from the pharyngeal pouches of embryos. The parathyroid gland originates from the ventral edges of the embryonic pharyngeal pouches, but like parafollicular cells, the secretory cells of the parathyroid gland are also of neural crest origin and migrate to the gland later in embryonic development.

Evolution

In adult cyclostomes, the thyroid consists of scattered follicles, and T3 and T4 are stored intracellularly. Cyclostomes also do not possess pituitary thyrotropes or TSH. In most teleost fishes, the

thyroid gland consists of clumps of follicles that are spread across the pharyngeal region, and the follicles contain colloid that stores the hormones extracellularly. In other types of fish and tetrapods, the thyroid gland is a discrete single- or double-lobed gland located at the base of the throat anterior to the heart. Though not present in cyclostomes, the ultimobranchial bodies are located in the neck as paired cell masses in fishes, amphibians, reptiles, and birds during development. In nonplacental mammals, the ultimobranchial body fuses with the thyroid gland. Derived from the ultimobranchial bodies, the parafollicular cells (C cells) are interspersed among the thyroid follicles.

The location of the mature parathyroid gland varies across vertebrates. The parathyroid is completely absent in fishes and does not appear in amphibians until after the gills are lost during metamorphosis. After maturation, the parathyroid gland(s) are either located on the thyroid or interspersed throughout the neck veins in amphibians, reptiles, and birds. In mammals, the glands are either within the thyroid gland or adjacent to it. The parathyroid gland is made of **chief cells**, which secrete PTH. In some mammalian species including humans, there are also oxyphil cells present in the parathyroid gland. We do not know the function of these cells, but they only appear at the onset of sexual maturity, whereas chief cells are present at birth.

21.5 Pancreatic Islets

Structure and Function

The **pancreas** is made of exocrine and endocrine cells that play essential roles in digestion and metabolism (Figure 21.16). The exocrine component includes **acini**, which are made of **acinar cells** that secrete digestive enzymes into ducts. These enzymes include

trypsin and chymotrypsin to digest proteins, amylase for the digestion of carbohydrates, and lipase to break down fats. However, we will focus on the endocrine component of the pancreas in this chapter.

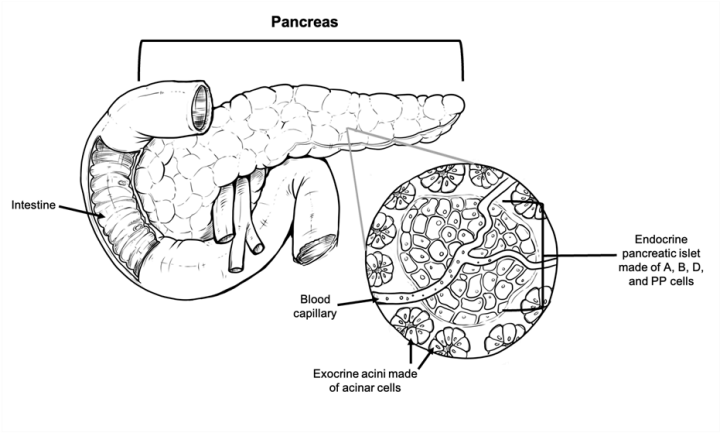

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Figure 21.16—Location of the human pancreas depicting endocrine pancreatic islets versus exocrine acini.

Pancreatic islets (otherwise known as **islets of Langerhans**) are masses of different endocrine cell types that secrete hormones with varying metabolic functions. Within the islets, **A cells** produce **glucagon**, a hormone that promotes the conversion of glycogen to glucose in the liver. This hyperglycemic protein hormone serves to increase blood glucose levels. Within the islets, **B cells** secrete **insulin**, which serves to decrease blood glucose levels in an antagonist role to the function of glucagon. This hypoglycemic protein hormone triggers intracellular absorption of glucose from the bloodstream. Insulin and glucagon regulate blood glucose levels via a negative feedback loop (Figure 21.17).

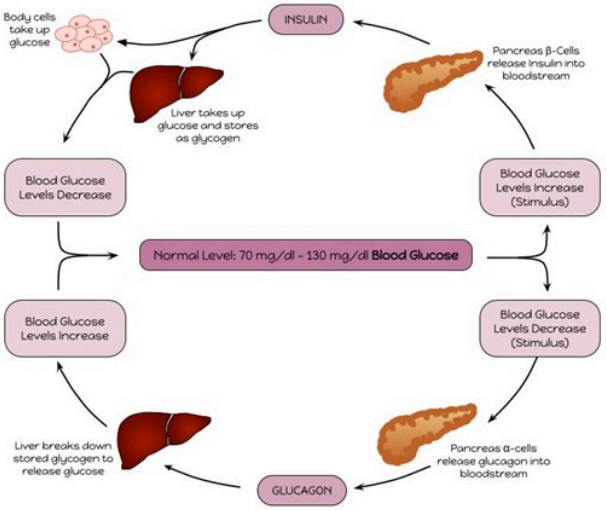

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Figure 21.17—Hormonal control of blood glucose levels by insulin and glucagon in humans.

D cells secrete **somatostatin** or **growth hormone–inhibiting hormone**, which inhibits the secretion of insulin and glucagon in certain contexts. The final cell type, **PP cells**, secrete **pancreatic polypeptide**, which promotes the release of hydrochloric acid into the stomach immediately following a meal.

Development

Across vertebrates, the pancreas arises from a diverticulum that grows from the endodermal lining of the embryonic gut through surrounding mesenchyme. A dorsal bud from the intestine fuses with one or two ventral buds to form the pancreas. The exocrine tissue forms small ductules that merge into larger ducts that

eventually become a large duct connecting the exocrine part of the pancreas to the small intestine. The pancreatic islets develop as small buds from the ductules; however, the true embryonic origin of the endocrine cells remains unclear. There are hypotheses that they may originate from mesoderm, neuroectodermal neural crest cells, or gut endoderm.

Evolution

In cyclostomes, acini and endocrine islets form separate groups of tissue but are close to each other. In hagfishes, the islets occur at the base of the common bile duct. In lampreys, islets occur within the mucosal wall of the intestine and the liver. Cyclostomes have B and D cells but lack A and PP cells. In chondrichthyans, the islets are located around the ducts of the exocrine portion of the pancreas. Chondrichthyans have A, B, D, and PP cells. In most bony fishes, islets are clumped together in **Brockmann bodies** that occur along the gallbladder, bile ducts, liver, and surface of the intestines (Figure 21.18). In a few bony fish species, islet tissue occurs in the exocrine portion of the pancreas. These Brockmann bodies contain B, A, and D cells but PP cells occur only in pyloric Brockmann bodies. In most tetrapods, the islets are distributed into clumps within a discrete, extraintestinal gland. In many birds and in the toad *Bufo*, islets form lobes embedded in the exocrine component of the pancreas and contain all four cell types. In mammals, the pancreatic islets consist of small masses of endocrine cells among the acinar tissue. In addition to A, B, D, and PP cells, another cell type called amphophils are found in the islets of mammals, sharks, teleost fishes, amphibians, and reptiles with no known function.

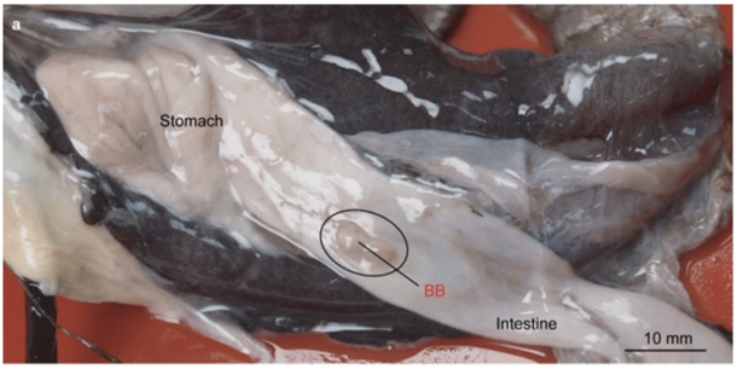

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Figure 21.18—Brockmann body (BB) of a freshly dissected Pacific jellynose fish (Ateleopus japonicus). Modified from Chanet et al. (2023).

21.6 Adrenal Glands

Structure and Function

Adrenal glands are named for their location superior to the kidneys in humans (ad-renal). Each adrenal gland consists of an inner **adrenal medulla** consisting of **chromaffin cells** and an outer **adrenal cortex** made of steroidogenic **adrenocortical cells** (Figure 21.19). The adrenal medulla coordinates the fight-or-flight response by secreting catecholamine neurotransmitters (e.g., epinephrine and norepinephrine) within seconds of a stressful stimulus. Minutes later, a region within the adrenal cortex will secrete **glucocorticoid hormones** (steroids) that coordinate physiological and behavioral responses to stressors. At low levels, glucocorticoids mediate basic metabolic processes, but when elevated in response to a stressor, glucocorticoids cause shifts in energy usage to counteract acute and chronic stressors. Another region of the adrenal cortex secretes

mineralocorticoids when stimulated by the **renin-angiotensin system** that regulates ions (potassium K^+ and sodium Na^+) and water balance in the body (Box 21.2). In addition to chromaffin cells and adrenocortical cells, a novel cell type was recently discovered in the adrenal gland of the monogamous oldfield mouse (*Peromyscus polionotus*) that expresses an enzyme that converts progesterone into 20α-hydroxyprogesterone and induces monogamous-typical parental behavior.

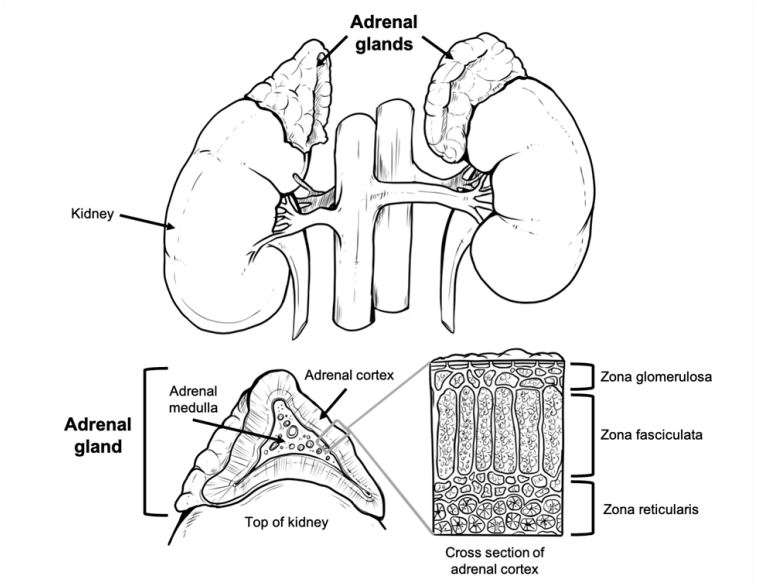

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Figure 21.19—Location of the human adrenal gland depicting zonation of the adrenal cortex.

In mammals, there are distinct zones of the adrenal cortex that each secrete different hormones. The outermost **zona glomerulosa** releases mineralocorticoids to regulate water balance in the kidneys. This zone contains receptors for angiotensin II (described below as part of the renin-angiotensin system), which triggers the endocrine glands to release aldosterone, the primary

mammalian mineralocorticoid hormone. The middle region **zona fasciculata** is responsible for secreting glucocorticoids, which mediate the hormonal stress response. This zone is involved in the **HPA axis** (Figure 21.20). A stressful stimulus is perceived by the hypothalamus, which secretes CRH to the anterior pituitary. The pituitary releases ACTH, which attaches to receptors in the zona fasciculata that trigger the release of glucocorticoids (e.g., cortisol or corticosterone). Glucocorticoids can initiate negative feedback on the HPA axis at the level of the hypothalamus and anterior pituitary, suppressing CRH and ACTH, respectively. The innermost **zona reticularis** secretes androgens and other glucocorticoids. This zone contains receptors for ACTH, LH, and human chorionic gonadotropin (hCG), which trigger the release of DHEA, DHEA-S, and androstenedione.

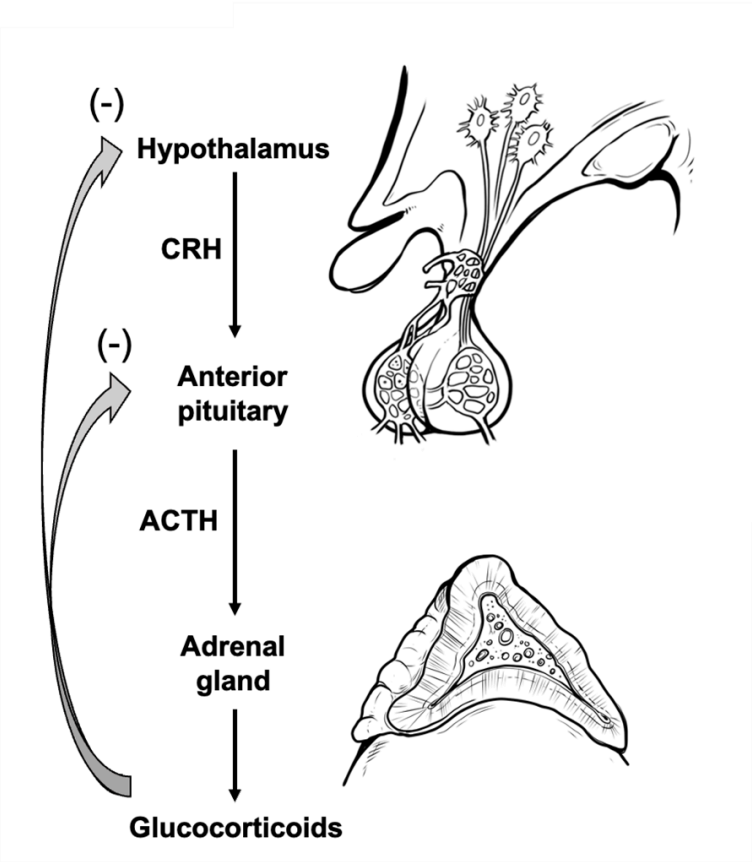

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Figure 21.20—Hypothalamic-pituitary-adrenal (HPA) axis. Gray arrows denote negative feedback. CRH = corticotropin-releasing hormone; ACTH = adrenocorticotropic hormone.

The adrenal medulla does not possess the zonation patterns found in the cortex of mammals. The medulla has direct communication with the nervous system via synapses of preganglionic sympathetic neurons. Any signals from these neurons trigger the release of catecholamine neurotransmitters **epinephrine (adrenaline)** and **norepinephrine (noradrenaline)**.

Known as the "fight-or-flight" response, catecholamines are released as the first wave of the stress response during a perceived challenge. The adrenal cortex and medulla are supplied with blood directly from the connective tissue capsule, but the medulla receives a secondary blood source from the cortical sinuses.

In addition to coordinating the vertebrate stress response, the adrenal glands are also essential in regulating sodium and potassium ion balance in the body but also controlling extracellular fluid volume. When blood pressure is low, the kidneys release an enzyme called **renin** that transforms the protein **angiotensinogen** into **angiotensin I** in the bloodstream. The inactive angiotensin I is converted to a more active form called **angiotensin II** by the angiotensin-converting enzyme. Angiotensin II causes the walls of arterioles to constrict, which raises blood pressure. Angiotensin II also triggers the release of aldosterone from the adrenal cortex, which stimulates the kidneys to retain sodium and excrete potassium. Increased sodium concentrations retain water, increasing blood pressure and volume.

Box 21.2—Blood Pressure Regulation

The renin-angiotensin-aldosterone system is the central player in blood pressure regulation in vertebrates. Renin is an enzyme released by the kidneys in response to low blood pressure or decreased sodium levels. Renin converts angiotensinogen to angiotensin II, which constricts blood vessels to elevate blood pressure. Angiotensin II also stimulates the release of aldosterone from the cortex of the adrenal gland. Aldosterone promotes sodium reabsorption in the kidneys, which leads to water retention and blood pressure elevation. Another hormone involved in blood

pressure regulation is **atrial natriuretic peptide** (**ANP**). ANP works antagonistically to the renin-angiotensin-aldosterone system. ANP is released by the atria of the heart in response to increased blood volume and pressure. ANP acts to lower blood pressure by promoting vasodilation, increasing urinary sodium excretion, and inhibiting renin and aldosterone secretion.

Development

The adrenal gland is composed of two tissue types that each develop from a different source. Interrenal or adrenocortical tissue is derived from splanchnic mesoderm adjacent to the urogenital ridge. This tissue is responsible for secreting mineralocorticoids and glucocorticoids. Chromaffin tissue arises from neural crest cells and is responsible for releasing catecholamines. In some mammal species (e.g., human and nonhuman primates), the adrenal cortex is dominated by the fetal zone prior to birth. The fetal zone produces steroid hormones that serve as precursors of the estrogens secreted by the placenta. At birth, the fetal zone will stop functioning and decline in size throughout postnatal development.

Evolution

Across vertebrates, the adrenocortical or interrenal tissue and chromaffin tissue occur in different arrangements. In adult cyclostomes and teleost fishes, the two tissue types are separated.

In cyclostomes, clusters of chromaffin cells are not in contact with the adrenocortical tissue, which is found in clumps above the pronephric funnels in the kidney and dispersed along the posterior cardinal veins above the pronephros. There is no evidence that the adrenocortical cells in lampreys can produce cortisol or corticosterone but there is circulating 11-deoxycortisol and 11-deoxycorticosterone in sea lampreys. In elasmobranchs, chromaffin tissue remains separate as cell clusters along the border of the kidneys. Some elasmobranch species have one large unpaired adrenal gland that is entirely adrenocortical tissue located posterior to the kidneys. Elasmobranchs secrete a unique corticosteroid called 1a-hydroxycorticosterone in addition to 11-deoxycorticosterone.

As stated in Chapter 16, bony fish kidneys vary in their function and structure across species, so we will consider actinopterygian kidneys for most of this section. In some teleost fishes, adrenocortical tissue is found inside the pronephros in clusters or in a strip around the posterior cardinal veins. Teleost adrenocortical tissue is located in the most anterior portion of the kidney known as the "head kidney" that has lost its renal function (Figures 16.11 and 21.20). Adrenocortical tissue of some species without a head kidney are not associated with kidney elements and surround the posterior cardinal veins. Chromaffin tissue occurs in clumps near the adrenocortical tissue in teleosts. The main glucocorticoid in teleost fishes is cortisol; however, the teleost HPA differs from that of other vertebrates. The hypothalamic neurosecretory cells directly innervate the pars distalis of the pituitary. Amphibian adrenocortical cells are extrarenal and vary in their location. In anurans, adrenocortical tissue occurs in clumps loosely organized into a pair of interrenal glands on the ventral side of the kidneys. Anuran chromaffin cells occur near adrenocortical cells in clumps. The main hormones secreted by the amphibian adrenal gland are aldosterone and corticosterone, though cortisol is found in amphibian species.

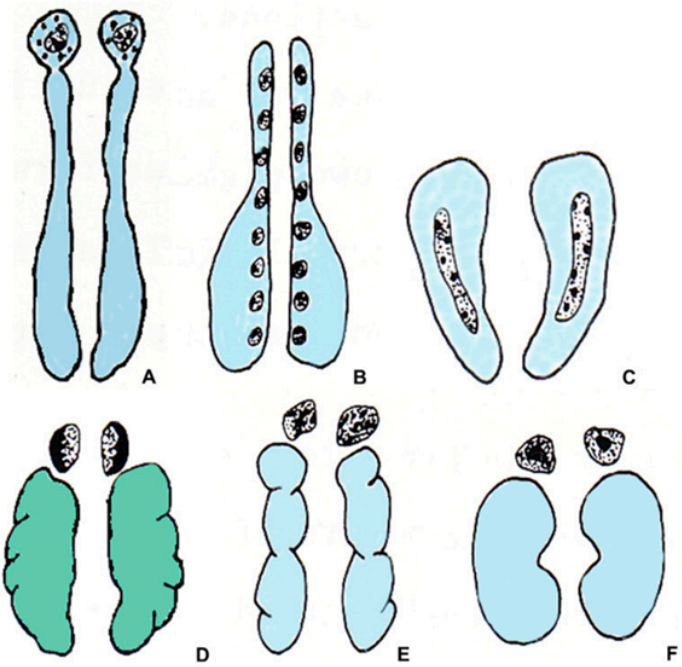

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Figure 21.21—Different arrangements of steroidogenic tissue (gray), chromaffin tissue (black), kidneys (light blue), and gonads (green) across vertebrates including (A) teleost, (B) urodele amphibian, (C) anuran amphibian, (D) lacertilian reptile, (E) bird, and (F) mammal. From Capaldo (2023).

In reptiles, the arrangement and positioning of the adrenal glands differs by group. Crocodylians and most snakes have paired suprarenal adrenal glands with cords of chromaffin cells within the adrenocortical cells. In lizards and some snakes, the adrenocortical cells are partially encapsulated by chromaffin cells similar to the mammalian medulla. The reptilian adrenal gland evolved the first independent vascular supply and venous drainage in vertebrates. Reptiles produce aldosterone and corticosterone. Similar to crocodylians and most snakes, the two tissue types of the avian adrenal gland occur together forming distinct glands on or near

the kidneys. Birds also secrete aldosterone and corticosterone as the main hormones from their adrenal glands. In mammals, adrenocortical tissue forms the adrenal cortex, and chromaffin tissue forms the medulla. As described above, the mammalian adrenal cortex exhibits distinct zonation including zona glomerulosa, zona fasciculata, and zona reticularis. There is also zonation of the cortex in anurans, reptiles, and birds, but it is less distinct.

21.7 Gonads

Structure and Function

In addition to gamete production (see Chapter 17—Reproduction), **gonads** (i.e., testes and ovaries) are responsible for production of **sex steroid hormones** (e.g., androgens and estrogens) that coordinate onset of sexual maturity in juveniles and reproductive processes in adult vertebrates. In **testes**, **Leydig** or **interstitial cells** produce **androgens** and are located between the seminiferous tubules (i.e., tubes within the testes that produce, maintain, and store sperm; Figure 21.22). Testes also consist of **Sertoli cells** that are responsible for spermatogenesis or sperm development (covered in Chapter 17). In ovaries, **ovarian follicles** consist of the oocyte surrounded by a layer of **granulosa cells** and then a layer of **theca cells** (Figure 21.22). The theca cells produce androgens in response to LH as well as progesterone in the preovulatory follicles. The granulosa cells produce estrogen in response to FSH. Granulosa cells in the preovulatory follicle also respond to LH and produce progesterone. The **corpus luteum** (i.e., temporary mass of cells that forms in the ovary after ovulation) also produces progesterone. Egg development (covered in detail in Chapter 17—Reproduction) is regulated by gonadotropins released from the anterior pituitary.

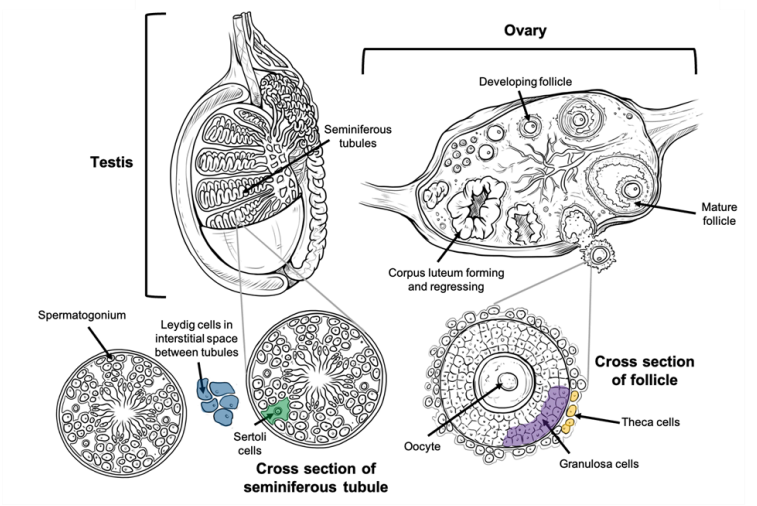

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Figure 21.22—Cross section of seminiferous tubules inside a human testis depicting Leydig cells (blue) and Sertoli cells (green) and a cross section of a follicle inside a human ovary depicting theca cells (yellow) and granulosa cells (purple).

Sex steroids also induce the expression of **secondary sex characteristics** (i.e., phenotypic traits that emerge at sexual maturity) in vertebrates such as changes in body size/mass, ornamentations, hair or feather colors, and behaviors that are used to either attract mates or compete with others for access to mates (Figure 21.23). This can result in sexual dimorphism or phenotypic distinctions between sexes within a species.

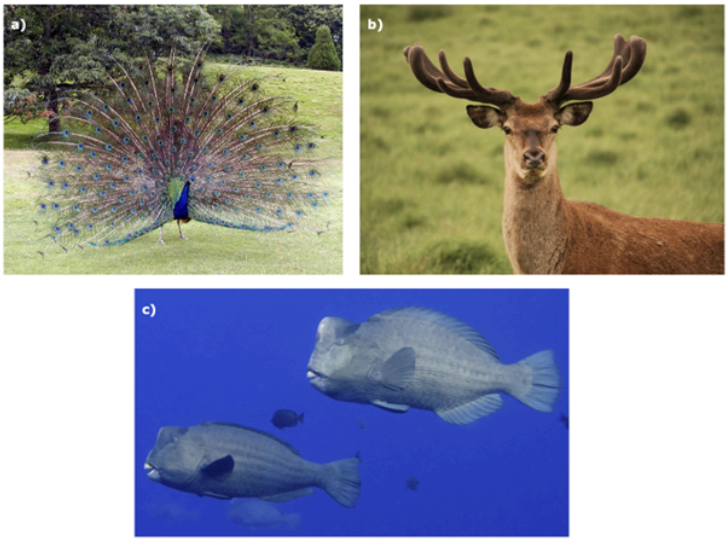

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Figure 21.23—Examples of secondary sex characteristics including (a) feathers of a testes-bearing Indian peafowl (Pavo cristatus), (b) antlers of a testes-bearing red deer (Cervus elaphus), and (c) sexual dimorphism in green humphead parrotfish (Bolbometopon muricatum).

The **hypothalamic-pituitary-gonadal axis** (**HPG**) has been identified in all vertebrates (though the specific hormones involved may differ; Figure 21.24). The hypothalamus releases GnRH, which travels to the anterior pituitary via the portal system. The anterior pituitary releases gonadotropins including FSH and LH. Most vertebrates have two separate gonadotropins similar to mammalian FSH and LH. Teleost fishes, amphibians, birds, and most reptiles have separate FSH- and LH-like hormones. Squamate reptiles are an exception that only require an FSH-like hormone for reproduction. LH controls spermiation (i.e., mature sperm are released from the Sertoli cells into the lumen of the seminiferous tubules) in testes and ovulation in ovaries. FSH controls spermatogenesis or sperm development in testes (see Figure 17.7) and follicular development in ovaries (Box 21.3).

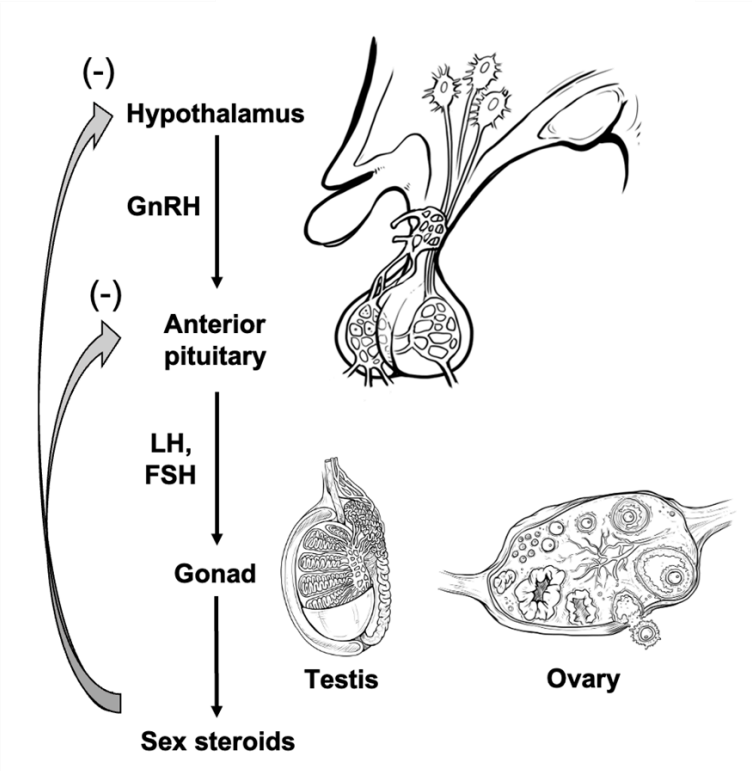

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Figure 21.24—Hypothalamic–pituitary-gonadal axis (HPG axis). Gray arrows denote negative feedback. GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone; FSH = follicle-stimulating hormone.

The gonadotropins travel to the gonads (either testes or ovaries), which triggers the release of sex steroids (e.g., androgens and estrogens depending on the type of gonad). The main circulating estrogen produced from ovaries is **estradiol**, whereas testes produce different androgens including **testosterone**, 5a-dihydrotestosterone (DHT), and 11-ketotestosterone (11-KT). *Relative levels of androgens to estrogens are not always characteristic or predictable across sexes as both testes and ovaries produce*

androgens and estrogens. For example, testosterone is converted to estradiol by the enzyme **aromatase**. Once circulating levels reach a certain threshold, gonadal steroids initiate negative feedback at the level of the hypothalamus and pituitary.

Box 21.3—Reproductive Hormones: Hormonal Contraception

The most common types of hormonal contraception (birth control) include pills, patches, injections, and hormonal intrauterine devices (IUDs). These methods typically contain combinations of synthetic steroid hormones including estrogen and progestin. These hormones work together to prevent pregnancy by inhibiting ovulation (i.e., the release of an egg from the ovary) during the fertile period.

Using the human menstrual cycle as an example, FSH stimulates the growth of follicles during the follicular phase. As the follicle matures, estrogen production begins to thicken the endometrium (i.e., the lining of the uterus). Increased estrogen production triggers a surge of LH, which initiates ovulation. After releasing the ovum, the ruptured follicle is transformed into the corpus luteum (i.e., a temporary mass of cells that produces progesterone) during the luteal phase. Progesterone helps maintain the uterine lining in preparation for implantation of a fertilized egg. If there is no fertilization of the ovum by sperm, the corpus luteum deteriorates and production of progesterone stops, causing the uterine lining to shed (i.e., menstruation). The continued delivery of estrogen and

progestin maintains the suppression of FSH and LH via negative feedback.

The synthetic estrogen and progestin found in birth control harness the power of negative feedback of the HPG axis. The estrogen suppresses production of FSH in the pituitary gland, preventing follicle maturation. Constant low levels of progestin that bind to receptors on the hypothalamus and pituitary prevent the LH surge that would otherwise trigger ovulation. The constant level of progestin also makes the lining of the uterus inhospitable to implantation of an embryo.

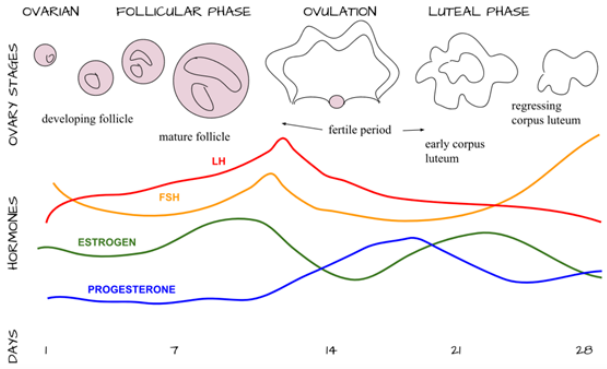

Figure 21.25—The cycling of reproductive hormones and their relationship to follicle development.

Development

Here, we discuss the development of the testes and ovaries. Development of gametes (i.e., oogenesis and spermatogenesis) and reproductive accessory organs such as ducts are covered in Chapter 17—Urogenital System: Reproduction. In cyclostomes, there is fusion of paired primordia early in development. The gonad arises from the embryonic cortex and subsequently differentiates into a testis or an ovary. In hagfishes, there is a single ovary because one primordium fails to develop. In lampreys, the indifferent gonad will undergo one of two pathways to form a testis or ovary. To form a testis, the posterior portion develops into testicular tissue, but the anterior portion degrades. To form an ovary, the anterior gonad develops into the ovary and the posterior portion degenerates. In elasmobranchs, the ovary is covered by germinal epithelium and may contain a cavity derived from large lymph spaces within the stroma. In teleost fishes, the gonad develops from a cortical primordium.

In most amphibians, the gonads develop from a bipotential gonad that differentiates into a testis or ovary early in development. However, there are a few species that undergo semidifferentiated gonadal development during which the gonads first develop as ovaries regardless of genetic sex followed by further differentiation into testes. Bufonid toads have rudimentary ovaries called Bidder's organs that develop from cortical remnants of embryonic genital ridge prior to gonadal differentiation. Amphibian ovaries are derived from the embryonic cortex and covered by germinal epithelium.

Reptilian gonads also start as bipotential and then differentiate into testes or ovaries. Granulosa cells are derived from germinal epithelium. In birds, the gonads develop from a pair of undifferentiated primordia associated with the embryonic nephrotome. Primordial germ cells migrate through the blood to the gonads where they develop into germinal epithelium. To form an avian ovary, the cortical tissue of the bipotential gonad

differentiates while the medullary tissue is suppressed. To form an avian testis, the medullary tissue of the bipotential gonad differentiates while the cortical tissue is suppressed. As outlined below, most avian species only develop the left ovary while the right regresses whereby more germ cells migrate to the left ovary during development. Both testes usually develop but the left is usually larger than the right in adults.

In mammals, the gonads begin as paired primordia from the mesoderm as a genital ridge on either side of the midline in close association with the transitory mesonephric kidney. Each primordium consists of an outer cortex derived from peritoneum and an inner medulla that differentiates into primary sex cords. To form a mammalian testis, the primary sex cords differentiate into seminiferous cords and the cortex regresses. Germ cells migrate from the yolk sac endoderm to the medulla where Sertoli and interstitial or Leydig cells form. Interstitial cells arise from the medulla surrounding the primary sex cords. To form a mammalian ovary, the primary sex cords degenerate, and secondary sex cords arise from the cortical tissue. Germ cells migrate from the yolk sac endoderm to the cortex where oogonia develop (eventually become primary oocytes) surrounded by follicular cells.

Evolution

Amniote testes exhibit a tubular pattern of seminiferous elements interspersed with clumps of interstitial cells. In vertebrates that are not amniotes, the testes exhibit a cystic organization. The testes of elasmobranchs and cyclostomes have isolated cellular cysts. The testes of bony fishes and amphibians consist of lobes or lobules composed of large cellular cysts. In anamniotes, Leydig cells are present between testicular cysts or in the periphery of or adjacent to the testis. In amniotes, Leydig cells develop between the tubules in the interstitial region.

In lampreys, there is either a single ovary or a single lobular testis with cystic organization. Interstitial cell masses are located between the lobules in the testis. Lamprey gonads secrete 15a-hydroxylated steroids that are not secreted by other vertebrates. In hagfishes, there is also a single gonad that differentiates into an ovary or testis when hagfishes reach 20 cm in length. Hagfish testes do not appear to have interstitial cells, but they secrete testosterone. Egg-producing Atlantic hagfish (*Myxine glutinosa*) also possess a functional corpus luteum (plural: corpora lutea) that can produce progesterone.

Elasmobranchs have paired testes with Sertoli cells that secrete androgens and estrogens. Leydig cells are present between testicular cysts in a few species but are not a significant source of androgens. Testosterone, 11-KT, and DHT have been documented in some elasmobranch species. Testes also produce estradiol and progesterone. Elasmobranch ovaries are hollow and contain follicles similar to mammals where most estrogen production occurs in mature follicles with well-developed granulosa. Granulosa cells are the major source of testosterone and estrogens in atretic follicles and corpora lutea. Ovaries of viviparous sharks (see Chapter 17 for definition of viviparous) also secrete relaxin (i.e., a similar hormone called raylaxin has been found in rays and skates), which prevents premature uterine contractions in sharks. Elasmobranchs are the only other vertebrate group besides mammals that produces relaxin.

Teleost fishes can be **gonochoristic** (i.e., individuals have only ovaries or testes) or **ambisexual** (i.e., simultaneous or sequential hermaphrodites). Simultaneous hermaphroditic fishes contain both functional ovaries and testes within the same individual, but sequential hermaphrodites start with one functional gonad type and then shift to the other type later in life. The testes of teleost fish (cystic organization) produce 11-KT and testosterone from Leydig cells. The ovary of most teleost fishes is hollow, but a few species have solid ovaries. There are masses of follicles embedded in stroma and the ovarian cavity is lined with epithelial

tissue. Theca cells produce androgens (testosterone), and granulosa cells produce estrogens.

In amphibians, adults typically only possess ovaries or testes but there are cases of juvenile hermaphroditism in certain species. Fat bodies lie adjacent to the gonads of amphibians and can secrete steroid hormones. The testes of anurans and urodeles secrete both testosterone and DHT in response to LH (urodeles also produce 11-KT). The testes of urodeles are of cystic organization consisting of one or more lobes. The testes of anurans are also of cystic organization with Leydig cells that resemble those of mammals. The ovaries of anurans and urodeles are hollow, sac-like structures. Granulosa cells are the major producer of estrogens, but theca cells also produce them. Amphibian ovaries produce progesterone, estradiol, estrone, testosterone, androstenedione and DHT.

In reptiles, the testes consist of convoluted seminiferous tubules each surrounded by the connective sheath tunica propria. Leydig cells and Sertoli cells are both steroidogenic. Reptilian ovaries are paired hollow structures with little stromal tissue. Granulosa cells in most species are the major source of follicular estrogen, but skinks produce estrogen from the theca cells instead. Crocodylians and turtles possess LH and FSH, but squamate reptiles only have one FSH-like hormone.

In birds, GnRH stimulates gonadotropin release from the anterior pituitary, whereas GnRH inhibits their secretion. Testes are paired masses of convoluted seminiferous tubules lined with germinal epithelium surrounded by connective tissue. There are steroidogenic interstitial cells between the seminiferous tubules as in reptiles and mammals. LH stimulates Leydig cells to secrete androgens, whereas FSH stimulates spermatogenesis and local androgen release from Sertoli cells. There are two ovaries during development but typically the left ovary will reach maturity while the right regresses, which is interpreted as an adaptation for flight (i.e., lighter body mass). The avian ovary consists of follicles containing oocytes surrounded by a layer of granulosa cells and

then another layer of theca cells, both of which are steroidogenic. FSH stimulates follicular development, whereas both LH and FSH stimulate estrogen synthesis.

Photoperiod (i.e., day length) tightly regulates gonad size and function in birds. Melatonin secreted from the pineal gland suppresses sex steroid release from the gonads during short days. As spring approaches and day length increases, an increase in LH and FSH release from the adenohypophysis leads to increased synthesis of spermatogenesis and androgen secretion of interstitial cells. Gonads will regress in size during the nonbreeding season and/or migration. Prolactin can inhibit gonadotropins from the pituitary but stimulates the formation of a brood patch (i.e., a patch of featherless, highly vascularized skin that forms in incubating adult birds to provide direct skin to egg contact) and secretion of crop milk in some species.

In mammals, the testes and ovaries are paired. Testes are composed of Sertoli cells (i.e., sperm production) and Leydig cells (i.e., androgen production) between the seminiferous tubules. Ovaries contain follicles that consist of follicular cells as depicted in Figure 21.22. Mammalian sex steroid release follows the typical HPG axis pattern described in Figure 21.24 whereby GnRH from the hypothalamus stimulates the release of LH and FSH from the anterior pituitary. FSH controls gametogenesis and estrogen synthesis, whereas LH controls androgen synthesis and gamete release. The role of reproductive hormones in mammalian menstruation are described in Box 21.3. The brains of egg-producing mammals contain a surge center that controls the LH surge in response to increased estrogen. The other notable endocrine organ of eutherian mammals is the placenta, which secretes estrogens and progesterone in addition to various polypeptide hormones (e.g., chorionic gonadotropins) during pregnancy. Finally, lactogenesis (i.e., milk production) in the mammary gland is controlled by prolactin release from the anterior pituitary, whereas lactation (i.e., milk ejection) is triggered by oxytocin release from the posterior pituitary.

21.8 Summary

This chapter highlights the myriad functions of vertebrate hormones due to their ability to travel long distances in the bloodstream to target tissues throughout the body. Hormones can be classified as either proteins or steroids and exert their effects on target tissues by binding to corresponding receptors, which can initiate positive or negative feedback. Across vertebrates, the hypothalamus and pituitary (hypothalamic-pituitary axes) control the function of other endocrine glands. The hypothalamus synthesizes and secretes releasing or inhibiting hormones, whereas the pituitary releases hormones that travel to other endocrine and nonendocrine target tissues. Hypothalamic neurohormones and pituitary tropic hormones are the first and second steps in many hormonal axes including the HPT, HPA, and HPG axes. Endocrine glands differ in developmental pathways, location, shape, and function across vertebrates. The photosensitive pineal gland secretes melatonin, which coordinates endogenous rhythms in higher vertebrates and pigmentation in lower vertebrates. The thyroid gland regulates metabolism and growth in addition to working with the parathyroid gland to regulate blood calcium levels. Pancreatic islets regulate blood glucose levels via a negative feedback loop with insulin and glucagon. The adrenal glands secrete glucocorticoids, which regulate the physiological and behavioral responses to stressors, in addition to secreting mineralocorticoids, which help regulate ion and water balance in the kidneys. Last, the gonads (i.e., testes and ovaries) secrete androgens and estrogens that regulate reproductive timing and activity across vertebrates. Throughout this chapter, we have highlighted the fact that hormones control nearly every function in the vertebrate body. The feedback between form and function has led to the evolution of diverse endocrine glands that influence the physiology, behavior, and fitness of nearly all vertebrates.

Application Questions

1. How can knowledge of comparative vertebrate endocrinology inform veterinary medicine?
2. What are the potential implications for human health based on insights from comparative vertebrate endocrinology studies?
3. How does the class of hormone (protein vs. steroid) affect its mode and speed of action at target cells?
4. What is positive versus negative feedback in terms of endocrine function?
5. What are the structural and functional differences between endocrine glands vs. exocrine glands?
6. What is an activational versus organizational effect of a hormone?

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Bill Ryerson is a Senior Lecturer in Anatomy and Foundation Course Leader at Cornell University's College of Veterinary Medicine. His research focuses on the functional morphology, behavior, and biomechanics of feeding in vertebrates, particularly reptiles. He also participates in scholarly work in veterinary education, particularly in the teaching of anatomy and physiology to students interested in human and animal medicine. He has taught Comparative Anatomy and Human Anatomy and Physiology at the undergraduate and

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Update History

April 2026: Accessibility updates: alt text, contrast, tables

June 2026: Accessibility updates: headings, underlines