

A new plant species from Idaho, Sacajawea's bitterroot (*Lewisia sacajaweanana*) was formally described in 2006. It is named in honor of Sacajawea, the Native American woman who guided Lewis and Clark in their exploration of the Pacific Northwest in the early 1800s.

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The twin goals of systematics are reconstruction of evolutionary history and classification of species

Systematics provides essential information for all of the biological sciences

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Courtesy of U.S. Forest Service, Boise National Forest (Kathryn M. Beall photo)



# 23 Systematic Biology: Phylogeny and Classification

## WHY IT MATTERS

Mention the word “malaria,” and people envision the tropics: explorers wander through the jungle in pith helmets and sleep under mosquito netting; clouds of insects hover nearby, ready to infect them with *Plasmodium*, the parasite that causes this disease. You may be surprised to learn, however, that less than 100 years ago, malaria was also a serious threat in the southeastern United States and much of western Europe.

Scientists puzzled over the cause of malaria for thousands of years. Hippocrates, a Greek physician who worked in the fifth century B.C., knew that people who lived near malodorous marshes often suffered from fevers and swollen spleens. Indeed, the name malaria is derived from the Latin for “bad air.” By 1900, scientists had established that mosquitoes, *Plasmodium*'s intermediate hosts, transmit the parasite to humans. Mosquitoes breed in standing water, and anyone living nearby is likely to suffer their bites.

Until the 1920s, scientists thought that the mosquito species *Anopheles maculipennis* carried malaria in Europe. But some areas with huge mosquito populations had little human malaria, whereas other areas had relatively few mosquitoes and a high incidence of the disease.

Then, a French researcher reported variation in the mosquitoes, and Dutch scientists identified two forms of the “species,” only one of which seemed to carry malaria. The breakthrough came in 1924, when a retired public health inspector in Italy discovered that individual mosquitoes—all thought to be the same species—produced eggs with one of six distinctive surface patterns (**Figure 23.1**).

Further research revealed that the name *Anopheles maculipennis* had been applied to six separate mosquito species. Although the adults of these species are very similar, their eggs are clearly different. The species are reproductively isolated from each other, and they differ ecologically: some breed in brackish coastal marshes, others in freshwater inland marshes, and still others in slow-moving streams. Only some of these species have a preference for human blood, and researchers eventually determined that only three of them routinely transmit malaria to humans.

These discoveries explained why the geographical distributions of mosquitoes and malaria did not always match. And government agencies could finally fight malaria by eradicating the disease-carrying species. Health workers drained marshes to prevent mosquitoes from breeding. They applied insecticides to kill mosquito larvae or introduced *Gambusia*, the mosquito

fish, which eats them. These targeted control programs were very successful.

The eradication of malaria in Europe owes a debt to **systematics**, the branch of biology that studies the diversity of life and its evolutionary relationships. Systematic biologists—systematists for short—identify, describe, name, and classify organisms, organizing their observations within a framework that reflects evolutionary relationships. In this chapter we first describe the goals of systematics and the traditional classification scheme that has been used for more than 200 years. Next we consider some of the evidence that systematists use and how that evidence must be interpreted to infer evolutionary relationships. Finally, we consider the analytical methods that contemporary systematists embrace.

## 23.1 Systematic Biology: An Overview

By organizing information about the biological world, systematics facilitates research in all fields of biology.

### The Twin Goals of Systematics Are Reconstruction of Evolutionary History and Classification of Species

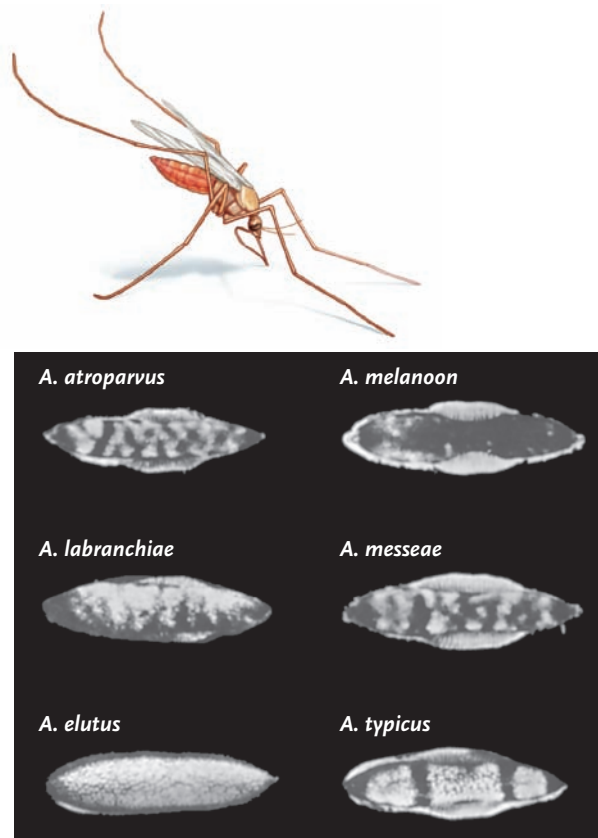
The science of systematics has two major goals. One is to reconstruct the **phylogeny**, the evolutionary history, of a group of organisms. Phylogenies are illustrated in **phylogenetic trees**, formal hypotheses that identify likely relationships among species. Like all hypotheses, they are revised as scientists gather new data.

The second goal of systematics is **taxonomy**, the identification and naming of species and their placement in a classification. A **classification** is an arrangement of organisms into hierarchical groups that reflect their relatedness. Most systematists want classifications to mirror phylogenetic history and, thus, the pattern of branching evolution.

### Systematics Provides Essential Information for All of the Biological Sciences

Systematics is sometimes maligned as “stamp collecting” by those who think that systematists just collect, describe, and maintain specimens. In fact, systematists study the patterns of phenotypic and genetic variation discussed in Chapters 20 and 21. Thus, their work enhances our understanding of microevolution, speciation, adaptive radiation, and extinction. While studying these phenomena, systematists also prepare guidebooks to biodiversity.

The ability to identify species is also crucial for controlling agricultural pests and agents of disease, such as malaria-carrying mosquitoes. Systematics also helps us to identify endangered species, manage wild-



**Figure 23.1** Eggs of European mosquitoes. Differences in surface patterns on the eggs of *Anopheles* mosquitoes in Europe helped researchers identify six separate species. The adults of all six species look remarkably alike. An adult *Anopheles atroparvus* is illustrated.

life effectively, and choose wild plants and animals for selective breeding and genetic engineering projects.

Data collected and organized by systematists also allows biologists to select appropriate organisms for their work. Most biological experiments are first conducted with individuals of a single species, because each species is a closed genetic system that may respond uniquely to experimental conditions. If a researcher inadvertently used two species, and these species responded differently, the mixed results probably wouldn't make much sense.

Finally, accurate phylogenetic trees are essential components of the comparative method, which biologists use to analyze evolutionary processes. Without a good phylogenetic hypothesis, we could not distinguish similarities inherited from a common ancestor from those that evolved independently in response to similar environments. For example, if biologists did not know the ancestry of sharks, penguins, and porpoises, they could not determine that their similarities were produced by convergent evolution (see Figure 22.9).

## STUDY BREAK

1. What is the difference between a phylogenetic tree and a classification?
2. How does work in systematics allow biologists to select appropriate organisms for research?

## 23.2 The Linnaean System of Taxonomy

The practice of naming and classifying organisms originated with the Swedish naturalist Carl von Linné (1707–1778), better known by his Latinized name, Carolus Linnaeus. A professor at the University of Uppsala, Linnaeus sent ill-prepared students around the world to gather specimens, losing perhaps a third of his followers to the rigors of their expeditions. Although not a commendable student adviser, Linnaeus developed the basic system of naming and classifying organisms still in use today.

### Linnaeus Developed the System of Binomial Nomenclature

Linnaeus invented the system of **binomial nomenclature**, in which species are assigned a Latinized two-part name, or **binomial**. The first part identifies a group of species with similar morphology, called a **genus** (plural, *genera*). The second part is the **specific epithet**, or species name.

A combination of the generic name and the specific epithet provides a unique name for every species. For example, *Ursus maritimus* is the polar bear and

*Ursus arctos* is the brown bear. By convention, the first letter of a generic name is always capitalized; the specific epithet is never capitalized; and the entire binomial is italicized. In addition, the specific epithet is never used without the full or abbreviated generic name preceding it because the same specific epithet is often given to species in different genera. For instance, *Ursus americanus* is the American black bear, *Homarus americanus* is the Atlantic lobster, and *Bufo americanus* is the American toad. If you were to order just “*americanus*” for dinner, you might be dismayed when your plate arrived—unless you have an adventurous palate!

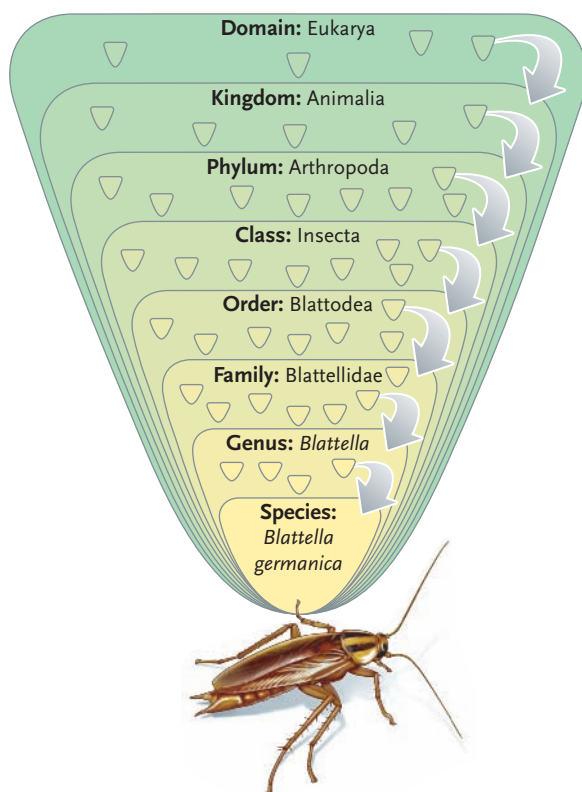
Nonscientists often use different common names to identify a species. For example, *Bothrops asper*, a poisonous snake native to Central and South America, is called *barba amarilla* (meaning “yellow beard”) in some places and *cola blanca* (meaning “white tail”) in others; biologists have recorded about 50 local names for this species. Adding to the confusion, the same common name is sometimes used for several different species. Binomials, however, allow people everywhere to discuss organisms unambiguously.

Many binomials are descriptive of the organism or its habitat. *Asparagus horridus*, for example, is a spiny plant. Other species, such as the South American bird *Rhea darwinii*, are named for notable biologists. The naming of newly discovered species follows a formal process of publishing a description of the species in a scientific journal. International commissions meet periodically to settle disputes about scientific names.

### The Taxonomic Hierarchy Organizes Huge Amounts of Systematic Data

Linnaeus described and named thousands of species on the basis of their similarities and differences. Keeping track of so many species was no easy task, so he devised a **taxonomic hierarchy** for arranging organisms into ever more inclusive categories (**Figure 23.2**). A **family** is a group of genera that closely resemble one another. Similar families are grouped into **orders**, similar orders into **classes**, similar classes into **phyla** (singular, *phylum*), and similar phyla into **kingdoms**. Finally, all life on Earth is classified into three **domains**, described in Section 1.3. The organisms included within any category of the taxonomic hierarchy compose a **taxon** (plural, *taxa*). Woodpeckers, for example, are a taxon (Picidae) at the family level, and pine trees are a taxon (*Pinus*) at the genus level.

Linnaeus did not believe in evolution. His goals were to illuminate the details of God's creation and to devise a practical way for naturalists to keep track of their discoveries. Nevertheless, the taxonomic hierarchy he defined was easily applied to Darwin's concept of branching evolution, which is itself a hierarchical phenomenon. As we discussed in the preceding two chapters, ancestral species give rise to descendant species through repeated branching of a lineage. Organ-



**Figure 23.2**

**The Linnaean hierarchy of classification.** The classification of a common household pest, the German cockroach (*Blattella germanica*), illustrates the nested hierarchy that Linnaeus developed. The German cockroach is one of many closely related species classified together in the genus *Blattella*, which is in turn one of nine genera in the family Blattellidae. Six distinctive cockroach families compose the order Blattodea, one of about 30 orders grouped into the class Insecta. The phylum Arthropoda contains about a dozen classes of animals, including insects, horseshoe crabs, spiders, crabs, and centipedes. Arthropoda is one of approximately 30 phyla, each representing a major lineage and body plan, within the kingdom Animalia. The classification of animal diversity is described in detail in Chapters 29 and 30.

isms in the same genus generally share a fairly recent common ancestor, whereas those assigned only to the same class or phylum share a common ancestor from the more distant past.

### STUDY BREAK

1. How does the system of binomial nomenclature minimize ambiguity in the naming and identification of species?
2. Which taxonomic category is immediately above family? Which is immediately below it?

## 23.3 Organismal Traits as Systematic Characters

Systematists compare organisms and then group species that share certain characteristics. Linnaeus focused on external anatomy. For example, he defined

birds as a class of oviparous (egg-laying) animals with feathered bodies, two wings, two feet, and a bony beak. No other animals possess all these characteristics, which distinguish birds from “quadrupeds” (his term for mammals), “amphibians” (among which he included reptiles), fishes, insects, and “worms.”

For roughly 200 years, systematists building on Linnaeus’ work relied on a variety of organismal traits to analyze evolutionary relationships and classify organisms: chromosomal anatomy; details of physiological functioning; the morphology of subcellular structures, cells, organ systems, and whole organisms; and patterns of behavior. Today, systematists often focus on the molecular sequences of nucleic acids and proteins (see Section 23.6). Here we consider two commonly studied organismal characteristics: *morphological traits* and *behavioral traits*.

### Morphological Characters Provide Abundant Clues to Evolutionary Relationships

Morphological differences often reflect genetic differences between organisms (see Section 20.1), and they are easy to measure in preserved or living specimens. Moreover, morphological characteristics are often clearly preserved in the fossil record, allowing the comparison of living species with their extinct relatives.

Useful morphological traits vary from group to group. In flowering plants, the details of flower anatomy often reveal common ancestry. Among vertebrates, the presence or absence of scales, feathers, and fur as well as the structure of the skull help scientists to reconstruct the evolutionary history of major groups. Sometimes systematists use obscure characters of unknown function. But differences in the number of scales on the back of a lizard or in the curvature of a vein in the wing of a bee may be good indicators of the genetic differentiation that accompanied or followed speciation—even if we do not know *why* these differences evolved.

Sometimes we rely on characteristics found only in the earliest stages of an organism’s life cycle to provide evidence of evolutionary relationships. As described in Chapter 30, analyses of the embryos of vertebrates reveal that they are rather closely related to sea cucumbers, sea stars, and sea urchins and even more closely related to a group of nearly shapeless marine invertebrates called sea squirts or tunicates.

### Behavioral Characters Offer Additional Data When Species Are Not Morphologically Distinct

Sometimes external morphology cannot be used to differentiate species. For example, two species of treefrog (*Hyla versicolor* and *Hyla chrysoscelis*) commonly occur together in forests of the central and eastern United

States. Both species have bumpy skin and adhesive pads on their toes that enable them to climb vegetation. They also have gray backs, white bellies, yellowish-orange coloration on their thighs, and large white spots below their eyes. The frogs are so similar that even experts cannot easily tell them apart.

How do we know that these frogs represent two species? During the breeding season, males of each species use a distinctive mating call to attract females (**Figure 23.3**). The difference in calls is a prezygotic reproductive isolating mechanism that prevents females from mating with males of a different species (see Section 21.2). Prezygotic isolating mechanisms are excellent systematic characters because they are often the traits that animals themselves use to recognize members of their own species. The two frog species also differ in chromosome number—*Hyla chrysoscelis* is diploid and *Hyla versicolor* is tetraploid—which is a postzygotic isolating mechanism.

### STUDY BREAK

1. Why are morphological traits often helpful in tracing the evolutionary relationships within a group of organisms?
2. Why are prezygotic isolating mechanisms useful characters for systematic studies of animals?

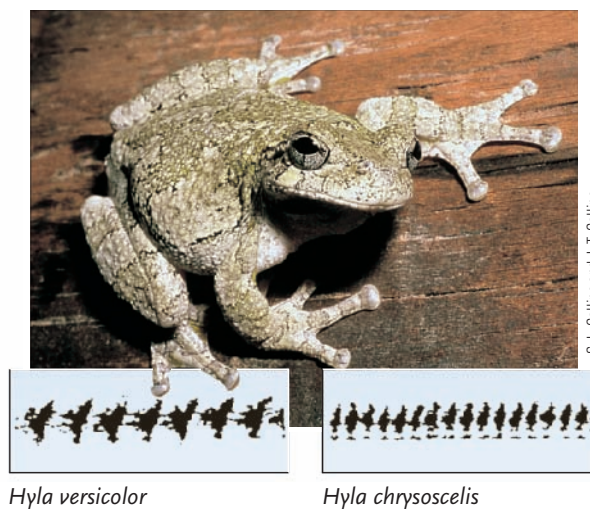
## 23.4 Evaluating Systematic Characters

With a wealth of traits available for analysis, systematists use several guidelines to select characters for study. In this section we examine the most important of these principles.

### Characters Must Be Independent Markers of Underlying Genetic Similarity and Differentiation

Ideally, systematists would create phylogenetic hypotheses and classifications by analyzing the genetic changes that caused speciation and differentiation. But in many cases they have had to rely on phenotypic traits as indicators of genetic similarity or divergence. Thus, systematists study traits in which phenotypic variation reflects genetic differences; they exclude differences caused by environmental variation (see Section 20.1).

Characters must also be genetically *independent*, reflecting different parts of the organisms' genomes. This precaution is necessary because different organismal characters can have the same genetic basis—and we want to use each genetic variation only once in an analysis. For example, tropical *Anolis* lizards climb



**Figure 23.3**

Look-alike frog species. The frogs *Hyla versicolor* and *Hyla chrysoscelis* are so similar in appearance that one photo can depict both species. Male mating calls, visualized in sound spectrograms for the two species, are very different. The spectrograms, which depict call frequency on the vertical axis and time on the horizontal axis, show that *H. chrysoscelis* has a faster trill rate.

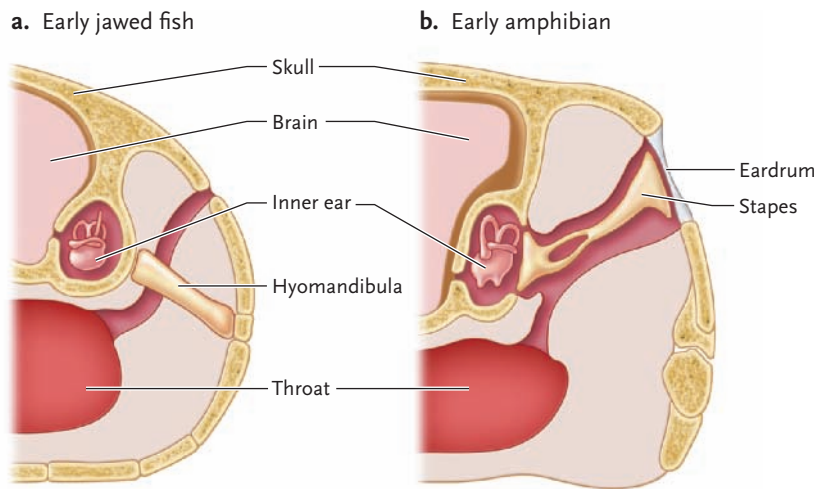
(Sound spectrograms from *The Amphibians and Reptiles of Missouri*, by T. R. Johnson © 1987 by the Conservation Commission of the State of Missouri. Reprinted by permission.)

trees using small adhesive pads on the underside of their toes. The number of pads varies from species to species, and researchers have used the number of pads on the fourth toe of the left hind foot as a systematic character. They do not also use the number of pads on the fourth toe of the *right* hind foot as a separate character, because the same genes almost certainly control the number of pads on the toes of both feet.

### Only Homologous Characters Provide Data about Evolutionary Relationships

A basic premise of systematic analyses is that phenotypic similarities between organisms reflect their underlying genetic similarities. As you may recall from Figure 19.3, species that are morphologically similar have often inherited the genetic basis of their resemblance from a common ancestor. Similarities that result from shared ancestry, such as the four limbs of all tetrapod vertebrates, are called **homologies** (or homologous characters). *Systematic analyses rely on the comparison of homologous characters as indicators of common ancestry and genetic relatedness.*

Even though homologous structures were inherited from a common ancestor, they may differ greatly among species, especially if their function has changed. For example, the stapes, a bone in the middle ear of tetrapod vertebrates, evolved from—and is therefore homologous to—the hyomandibula, a bone that supported the jaw joint of early fishes. The ancestral function of the bone is retained in some modern fishes, but its structure, position, and function are different in tetrapods (**Figure 23.4**).



**Figure 23.4**  
Homologous bones, different structure and function. The hyomandibula, which braced the jaw joint against the skull in early jawed fishes (a), is homologous to the stapes, which transmits sound to the inner ear in the four-legged vertebrates, exemplified here by an early amphibian (b). Both diagrams show a cross section through the head just behind the jaw joint.

As you know from the discussion of convergent evolution in Section 22.2, organisms that are not closely related sometimes bear a striking resemblance to one another, especially when they live in similar environments. Phenotypic similarities that evolved independently in different lineages are called **homoplasies** (or homoplasious characters). Some biologists use the terms *analogies* or *analogous characters* for homoplasious characters that serve a similar function in different species. *Systematists exclude homoplasies from their analyses, because homoplasies provide no information about shared ancestry or genetic relatedness.*

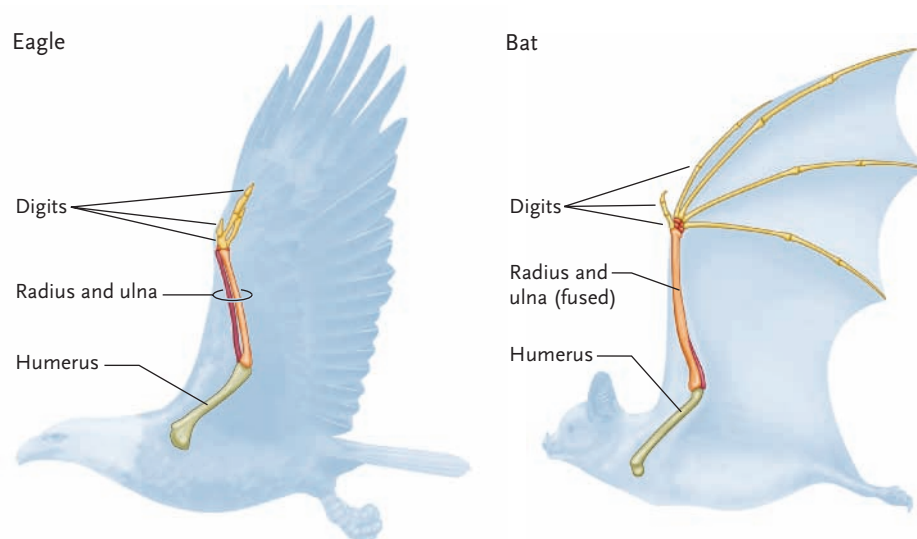
If homoplasies are similar and homologies are sometimes different, how can we tell them apart? First, homologous structures are similar in anatomical detail

and in their relationship to surrounding structures. For example, the bones within the wings of birds and bats are homologous (Figure 23.5). Both wings include the same basic structural elements with similar spatial relationships to each other and to the bones that attach the wing to the rest of the skeleton. However, the large flat surfaces of their wings are homoplasious, the products of convergent evolution. The bird's wing is made of feathers, whereas the bat's wing is formed of skin.

Second, homologous characters emerge from comparable embryonic structures and grow in similar ways during development. Systematists have put great stock in embryological indications of homology on the assumption that evolution has conserved the pattern of embryonic development in related organisms. Indeed, recent discoveries in evolutionary developmental biology (described in Section 22.6 and explored further in Chapters 29 and 30) have revealed that the genetic controls of developmental pathways are very similar across a wide variety of organisms.

### Systematists Focus Attention on Derived Versions of Characters

In all evolutionary lineages, some characteristics evolve slowly and others evolve rapidly, a phenomenon called **mosaic evolution**. Because mosaic evolution is pervasive, every species displays a mixture of **ancestral characters** (old forms of traits) and **derived characters** (new forms of traits). Derived characters provide the most useful information about evolutionary relationships because once a derived character becomes established, it is usually present in all of that species' descendants. Thus, unless they are lost or replaced by newer characters over evolutionary time, derived characters serve as markers for entire evolutionary lineages.



**Figure 23.5**  
Assessing homology. The wing skeletons of birds and bats are homologous structures with the same basic elements. However, the flat wing surfaces are homoplasious structures.

a. Caddis fly



b. Orange palm dart butterfly



c. Monarch butterfly



**Figure 23.6**

**Outgroup comparison.** Most adult insects, like the (a) caddis fly (family Limnephilidae) and the (b) orange palm dart butterfly (*Cephrenes auglades*), have six walking legs. This comparison of butterflies with other insects suggests that the four walking legs of the (c) monarch butterfly (*Danaus plexippus*) represents the derived character state.

Systematists score characters as either ancestral or derived only when comparing them among organisms. Thus, any particular character is derived *only in relation to* what occurs in other organisms—either an older version of the same character or, in the case of an entirely new trait, the absence of it altogether. For example, most species of animals lack a vertebral column and the other components of an internal skeleton. However, one animal lineage—the vertebrates, including fishes, amphibians, reptiles, birds, and mammals—has those structures. Thus, when systematists compare vertebrates to all of the animals that lack a vertebral column, they score the absence of a vertebral column as the ancestral condition and the presence of a vertebral column as derived.

How can systematists distinguish between ancestral and derived characters? In other words, how can they determine the direction in which a character has evolved? The fossil record, if it is detailed enough, can provide unambiguous information. For example, biologists are confident that the presence of a vertebral column is a derived character because fossils of the earliest animals lack that structure.

For some traits, researchers use embryological evidence. Derived characters often appear later during embryonic development as modifications of an ancestral developmental plan. Recall, for example, that the early embryos of mammals first develop fishlike features in their circulatory systems (as shown in Figure 19.13) and only later develop the characteristic adult morphology. This developmental sequence suggests that the two-chambered linear hearts of fishes are ancestral, and that the four-chambered, double-loop hearts of mammals are derived.

Systematists frequently use a technique called **outgroup comparison** to identify ancestral and derived characters by comparing the group under study to more distantly related species that are not otherwise included in the analysis. Most modern butterflies, for example, have six walking legs, but species in two families have four walking legs and two small, nonwalking

legs. Which is the ancestral character state, and which is derived? Outgroup comparison with other insects, most of which have six walking legs as adults, suggests that six walking legs is ancestral and four is derived (Figure 23.6).

### STUDY BREAK

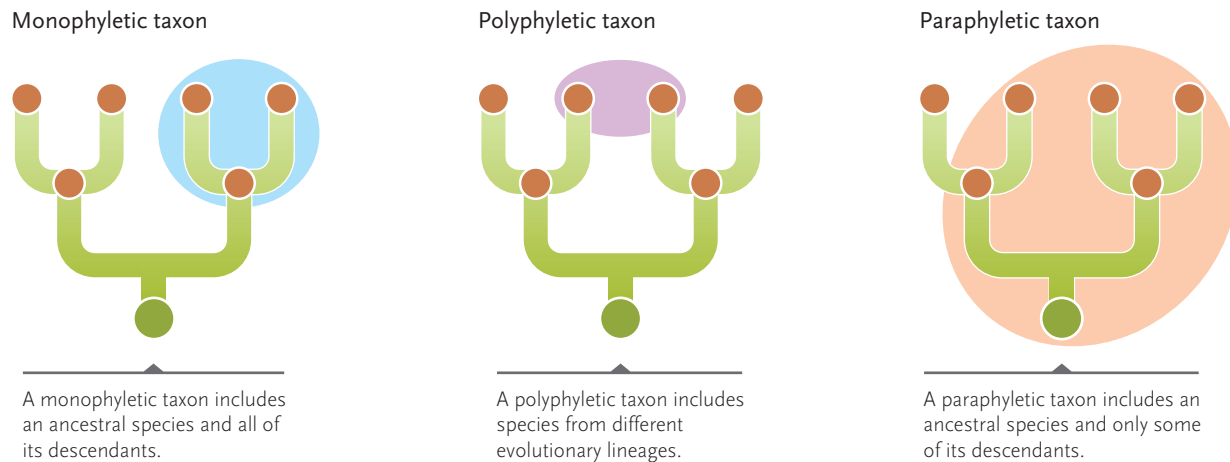
1. Why do systematists use homologous characters in their phylogenetic analyses?
2. What is outgroup comparison?

## 23.5 Phylogenetic Inference and Classification

After exploring two guiding principles of research in systematics, we describe how systematists use their analyses of organismal characters to reconstruct phylogenetic histories and create classifications.

### Many Systematic Studies Rely on the Principles of Monophyly and Parsimony

Phylogenetic trees portray the evolutionary diversification of lineages as a hierarchy that reflects the branching pattern of evolution. Each branch represents the descendants of a single ancestral species. When converting the phylogenetic tree into a classification, systematists use the **principle of monophyly**; that is, they try to define **monophyletic taxa**, each of which contains a single ancestral species and all of its descendants (Figure 23.7). By contrast, **polyphyletic taxa**—which systematists never intentionally define—would include species from separate evolutionary lineages. A taxon that included convergent species, such as sharks, penguins, and dolphins, would be polyphyletic. **Paraphyletic taxa** each contain an ancestor and some, but not all, of



**Figure 23.7**

**Defining taxa in a classification.** Systematists can create different classifications from the same phylogenetic tree by identifying different groups of species as a single taxon (shaded).

its descendants. For example, the traditional taxon *Reptilia* is paraphyletic, as described in the next section. These distinctions are crucial when making classifications.

Many systematists also strive to create *parsimonious* phylogenetic hypotheses, which means that they include the fewest possible evolutionary changes to account for the diversity within a lineage. According to the **principle of parsimony**, any particular evolutionary change is an unlikely event; therefore it is extremely unlikely that the same change evolved twice in one lineage. For example, phylogenetic trees place all birds on a single branch, implying that feathered wings evolved once in their common ancestor. This hypothesis is more parsimonious than one proposing that feathered wings evolved independently in two or more vertebrate lineages.

### Traditional Evolutionary Systematics Was Based on Linnaeus' Methods

For a century after Darwin published *On the Origin of Species*, most systematists followed Linnaeus' practice of using phenotypic similarities and differences to infer evolutionary relationships. This approach, called **traditional evolutionary systematics**, groups together species that share both ancestral and derived characters. For example, mammals are defined by their internal skeleton, vertebral column, and four limbs—all ancestral characters among the tetrapod vertebrates—as well as hair, mammary glands, and a four-chambered heart—all of which are derived characters.

The classifications produced by traditional systematics reflect both evolutionary branching and morphological divergence (**Figure 23.8a**). For example, among the tetrapod vertebrates, the amphibian and mammalian lineages each diverged early, followed shortly thereafter by the turtle lineage. The remaining organisms then diverged into two groups: lepidosaurs gave rise to lizards and snakes, and archosaurs gave rise to crocodilians, dinosaurs, and birds. Thus, although

crocodilians outwardly resemble lizards, they share a more recent common ancestor with birds. Birds differ from crocodilians because birds experienced substantial morphological change when they emerged as a distinct group.

Even though the phylogenetic tree shows six living groups, the traditional classification recognizes only four classes of tetrapod vertebrates: Amphibia, Mammalia, *Reptilia*, and Aves (birds). These groups are given equal ranking because each represents a distinctive body plan and way of life. The class *Reptilia*, however, is clearly a paraphyletic taxon: it includes *some* of the descendants of the common ancestor labeled A in **Figure 23.8a**, namely turtles, lizards, snakes, and crocodilians; but it omits birds, and thus does not include *all* descendants.

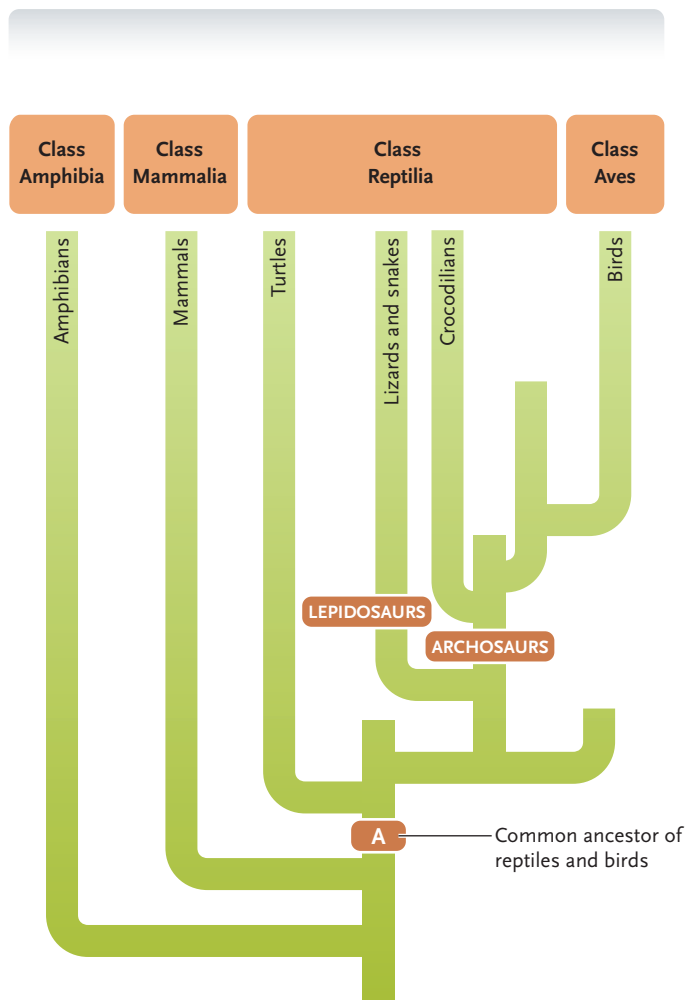
Traditional evolutionary systematists justify this definition of the *Reptilia* because it includes morphologically similar animals with close evolutionary relationships. Crocodilians are classified with lizards, snakes, and turtles because they share a distant common ancestry and are covered with dry, scaly skin. Traditional systematists also argue that the key innovations initiating the adaptive radiation of birds—wings, feathers, high metabolic rates, and flight—represent such extreme divergence from the ancestral morphology that birds merit recognition as a separate class.

### Cladistics Uses Shared Derived Characters to Trace Evolutionary History

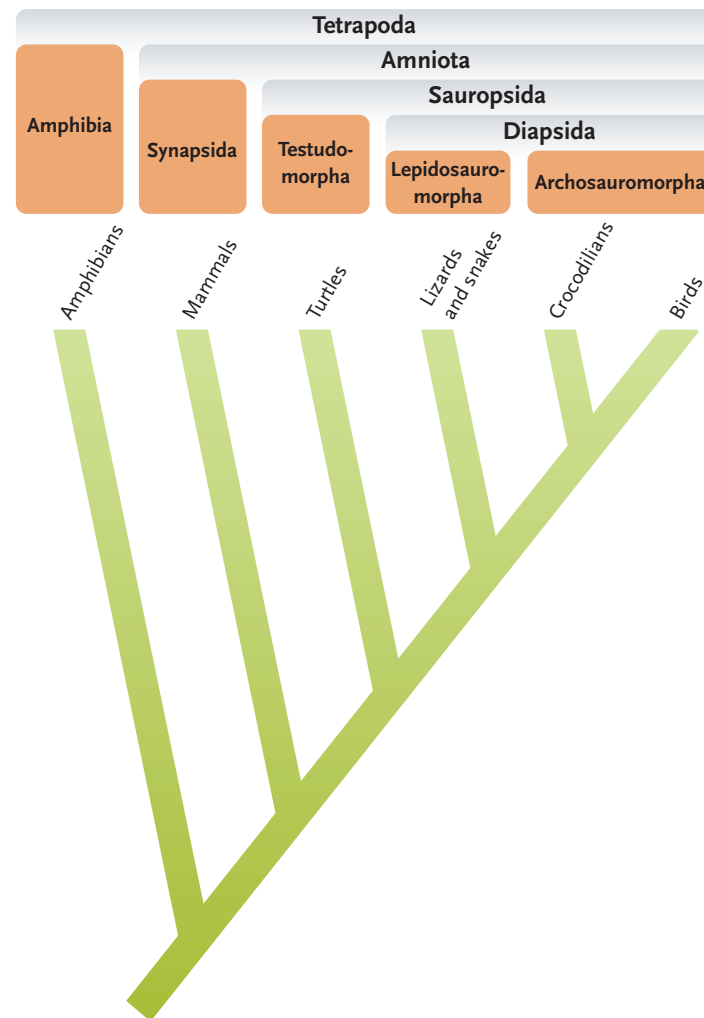
In the 1950s and 1960s, some researchers criticized classifications that were based on two distinct phenomena, branching evolution and morphological divergence, as inherently unclear. After all, how can we tell *why* two groups are classified in the same higher taxon? They may have shared a recent common ancestor, as did lizards and snakes. Alternatively, they may have retained similar ancestral characteristics after being separated on different branches of a phylogenetic tree, as is the case for lizards and crocodilians.



a. Traditional phylogenetic tree with classification



b. Cladogram with classification



To avoid such confusion, many systematists quickly followed the philosophical and analytical lead of Willi Hennig, a German entomologist, who published an influential book, *Phylogenetic Systematics*, in 1966. Hennig and his followers argued that classifications should be based solely on evolutionary relationships. **Cladistics**, as this approach is known, produces phylogenetic hypotheses and classifications that reflect only the branching pattern of evolution; it ignores morphological divergence altogether.

Cladists group together only species that *share derived characters*. For example, cladists argue that mammals form a monophyletic lineage—a **clade**—because they possess a unique set of derived characters: hair, mammary glands, reduction of bones in the lower jaw, and a four-chambered heart. The ancestral characters found in mammals—internal skeleton, vertebral column, and four legs—do not distinguish them from other tetrapod vertebrates, so these traits are excluded from the analysis.

The phylogenetic trees produced by cladists, called **cladograms**, thus illustrate the hypothesized sequence

of evolutionary branchings, with a hypothetical ancestor at each branching point (**Figure 23.8b**). They portray strictly monophyletic groups and are usually constructed using the principle of parsimony. Once a researcher identifies derived, homologous characters, constructing a cladogram is straightforward (**Figure 23.9**).

The classifications produced by cladistic analysis often differ radically from those of traditional evolutionary systematics (compare the two parts of **Figure 23.8**). Pairs of higher taxa are defined directly from the two-way branching pattern of the cladogram. Thus, the clade Tetrapoda (the traditional amphibians, reptiles, birds, and mammals) is divided into two taxa, the Amphibia (tetrapods that do not have an amnion, as discussed in Section 30.3) and the Amniota (tetrapods that have an amnion). The Amniota is subdivided into two taxa on the basis of skull morphology and other characteristics: Synapsida (mammals) and Sauropsida (turtles, lizards, snakes, crocodilians, and birds). The Sauropsida is further divided into the Testudomorpha (turtles) and the Diapsida (lizards and snakes, crocodilians, and birds). Finally, the Diapsida is subdivided

**Figure 23.8**

Phylogenetic trees and classifications for tetrapod vertebrates. **(a)** Traditional and **(b)** cladistic approaches produce different phylogenetic trees and classifications. Classifications are presented above the trees.

## Figure 23.9 Research Method

### Constructing a Cladogram

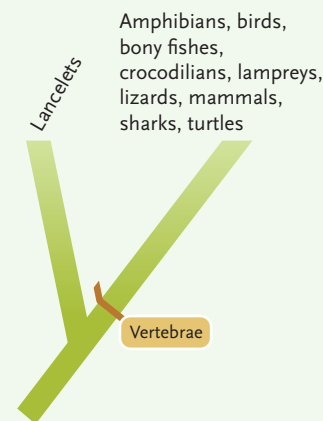
#### PROTOCOL:

- Select the organisms to study.** To demonstrate the method, we develop a cladogram for the nine groups of living vertebrates: lampreys, sharks (and their relatives), bony fishes (and their relatives), amphibians (frogs and salamanders), turtles, lizards (including snakes), crocodilians (including alligators), birds, and mammals. We also include marine animals called lancelets (phylum Chordata, subphylum Cephalochordata), which are closely related to vertebrates (see Chapter 30). Lancelets are the outgroup in our analysis.
- Choose the characters on which the cladogram will be based.** Our simplified example is based on the presence or absence of 10 characters: (1) vertebral column, (2) jaws, (3) swim bladder or lungs, (4) paired limbs (with one bone connecting each limb to the body), (5) extraembryonic membranes (such as the amnion), (6) mammary glands, (7) dry, scaly skin somewhere on the body, (8) two openings on each side near the back of the skull, (9) one opening on each side of the skull in front of the eye, and (10) feathers.
- Score the characters as either ancestral or derived in each group.** As the outgroup, lancelets possess the ancestral character; any deviation from the lancelet pattern is derived. Because lancelets lack all of the characters in our analysis, the presence of each character is the derived condition. We tabulate data on the distribution of ancestral (–) and derived (+) characters, listing lancelets first and the other organisms in alphabetical order.

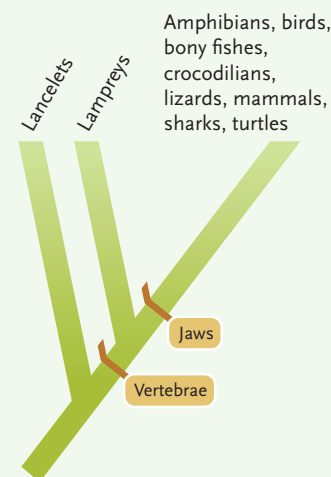
	Vertebrae	Jaws	Swim bladder or lungs	Paired limbs	Extraembryonic membranes	Mammary glands	Dry, scaly skin	Two openings at back of skull	One opening in front of eye	Feathers
Lancelets	–	–	–	–	–	–	–	–	–	–
Amphibians	+	+	+	+	–	–	–	–	–	–
Birds	+	+	+	+	+	–	+	+	+	+
Bony fishes	+	+	+	–	–	–	–	–	–	–
Crocodilians	+	+	+	+	+	–	+	+	+	–
Lampreys	+	–	+	+	–	–	–	–	–	–
Lizards	+	+	+	+	+	–	+	+	–	–
Mammals	+	+	+	+	+	+	–	–	–	–
Sharks	+	+	–	–	–	–	–	–	–	–
Turtles	+	+	+	+	+	–	+	–	–	–

**PURPOSE:** Systematists construct cladograms to visualize hypothesized evolutionary relationships by grouping together organisms that share derived characters. The cladogram also illustrates where derived characters first evolved.

- Construct the cladogram from information in the table, grouping organisms that share derived characters.** All groups except lancelets have vertebrae. Thus, we group organisms that share this derived character on the right-hand branch, identifying them as a monophyletic lineage. Lancelets are on their own branch to the left, indicating that they lack vertebrae.



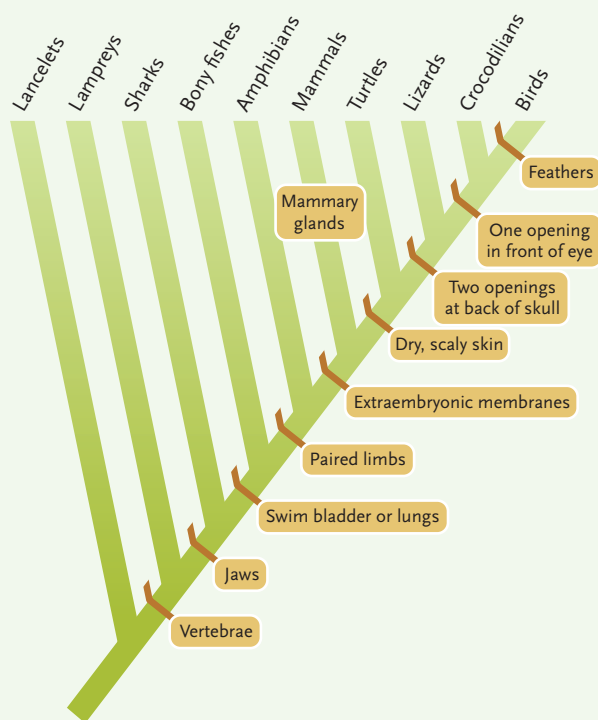
All of the remaining organisms except lampreys have jaws. (Lancelets also lack jaws, but we have already separated them out, and do not consider them further.) Place all groups with jaws, a derived character, on the right-hand branch. Lampreys are separated out to the left, because they lack jaws. Again, the branch on the right represents a monophyletic lineage.



into two more recently evolved taxa—the Lepidosauromorpha (lizards and snakes) and the Archosauromorpha (crocodilians and birds). Thus, a strictly cladistic classification exactly parallels the pattern of branching evolution that produced the organisms included in the classification. These parallels are the essence and strength of the cladistic method.

Most biologists now use the cladistic approach because of its evolutionary focus, clear goals, and precise methods. In fact, some systematists advocate abandoning the Linnaean hierarchy for classifying and naming organisms. They propose using a strictly cladistic system, called **PhyloCode**, that identifies and names clades instead of pigeonholing or-

5. Construct the rest of the cladogram using the same step-by-step procedure to separate the remaining groups. In our completed cladogram, seven groups share a swim bladder or lungs; six share paired limbs; and five have extraembryonic membranes during development. Some groups are distinguished by the unique presence of a derived character, such as feathers in birds.



**INTERPRETING THE RESULTS:** Although cladograms provide information about evolutionary relationships, the common ancestors represented by the branch points are often hypothetical. You can tell from the cladogram, however, that birds are more closely related to lizards than they are to turtles. Follow the branches of the cladogram from birds and lizards back to their intersection, or node. Next, trace the branches of birds and turtles to their node. You can see that the bird–turtle node is closer to the bottom of the cladogram than the bird–lizard node. Nodes that are closer to the bottom of the cladogram indicate a more distant common ancestry than those closer to the top.

organisms into the familiar taxonomic levels embraced by more traditional systematists. Although traditional evolutionary systematics has guided many people’s understanding of biological diversity, we use a cladistic approach to describe evolutionary lineages and taxa in the Biodiversity unit that follows this chapter.

## STUDY BREAK

1. How does a monophyletic taxon differ from a polyphyletic taxon?
2. Why is the traditionally defined group Reptilia a paraphyletic taxon?
3. What characteristics are used to group organisms in a cladistic analysis?

## 23.6 Molecular Phylogenetics

Most systematists now conduct phylogenetic analyses using molecular characters, such as the nucleotide base sequences of DNA and RNA or the amino acid sequences of the proteins for which they code. Because DNA is inherited, shared changes in molecular sequences—insertions, deletions, or substitutions—provide clues to the evolutionary relationships of organisms. Technological advances have automated many of the necessary laboratory techniques, and analytical software makes it easy to compare new data to information filed in data banks accessible over the Internet.

### Molecular Characters Have Both Advantages and Disadvantages over Organismal Characters

Molecular sequences have certain practical advantages over organismal characters. First, they provide abundant data: every amino acid in a protein and every base in a nucleic acid can serve as a separate, independent character for analysis. Moreover, because many genes have been conserved by evolution, molecular sequences can be compared between distantly related organisms that share no organismal characteristics. Molecular characters can also be used to study closely related species with only minor morphological differences. Finally, many proteins and nucleic acids are not directly affected by the developmental or environmental factors that cause nongenetic morphological variations such as those described in Section 20.1.

Molecular characters have certain drawbacks, however. For example, only four alternative character states (the four nucleotide bases) exist at each position in a DNA or RNA sequence and only 20 alternative character states (the 20 amino acids) at each position in a protein. (You may want to review Sections 14.2 and 15.1 on the structure of these molecules.) And if two species have the same nucleotide base substitution at a given position in a DNA segment, their similarity may well have evolved independently. As a result, systematists often find it difficult to verify that molecular similarities were inherited from a common ancestor.

For organismal characters, biologists can establish that similarities are homologous by analyzing the characters’ embryonic development or details of their func-



## INSIGHTS FROM THE MOLECULAR REVOLUTION

### Whales with Cow Cousins?

More than 50 million years ago, whales evolved from terrestrial mammals into streamlined creatures, spectacularly adapted to life in the sea. But which mammals were their ancestors? Using morphological comparisons of living and fossil species, evolutionists had hypothesized that modern cetaceans—whales, dolphins, and porpoises—evolved from wolflike mammals called mesonychians. However, recent work by molecular biologists suggests that cetaceans are part of a lineage that includes an ungulate ancestor of cows and hippopotamuses.

Several molecular studies support this surprising conclusion. Mitsuru Shimamura and his colleagues at the Tokyo Institute of Technology and other Japanese institutions examined the distribution of transposable elements (TEs) in whales and ungulates. TEs are sequences that move to new locations in DNA (see Section 17.3). The TEs that the researchers studied in whales move by making RNA copies of themselves; the RNA copies then act as templates for making DNA copies, which are inserted in new locations. The mechanism leaves the original copy still in place in the DNA.

The TEs studied by the Shimamura team are called SINEs (for Short *IN*ter-

persed Elements). These elements, which occur only in mammals, are particularly useful for evolutionary studies because the pattern by which they duplicate and move to new locations is unique in each evolutionary lineage. If SINEs occur at the same sites in the nuclear DNA of several species, those species are likely to be members of the same lineage.

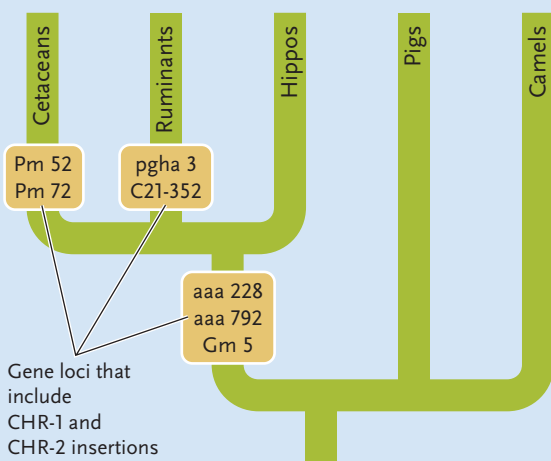
To begin their work, Shimamura and his coworkers isolated two types of SINEs from whales, which they designated CHR-1 and CHR-2. They found that the DNA of these SINEs could pair with sequences in the nuclear DNA of hippos, cows, and other ruminants, but not with sequences of pigs and camels. This result showed that the CHR-1 and CHR-2 SINEs are present in whales, cows, and hippos but not in pigs and camels.

The researchers then used similar techniques to work out the locations of the SINEs in the DNA, with particular focus on SINEs that may have inserted into known protein-encoding genes. SINEs can insert into genes without serious damage if they do so in introns, the surplus segments that are transcribed but spliced out of the messenger RNA copy of the gene (see Section 15.3). To find genes containing the SINEs, the researchers added probes—labeled DNA sequences that could pair with CHR-1 and CHR-2—to DNA preparations containing all the genes of the species under study. They also searched through electronic databanks of known gene sequences of the species, looking for genes with introns containing either of the two SINEs.

The probes and computer searches produced seven “hits” among protein-encoding genes. Three CHR-1 insertions were found at the same locations in genes of cetaceans, ruminants, and hippos, but were absent from these locations in camels and pigs. The results indicate that the SINEs inserted at these locations in a

common ancestor of cetaceans, ruminants, and hippos after camels and pigs had split off as a separate group (see **figure**). Additionally, some other SINEs evidently inserted later, after an evolutionary split had separated the ruminants and cetaceans. Two CHR-1 insertions were found in ruminants but not in cetaceans, hippos, camels, or pigs; two CHR-2 insertions were found only in cetaceans. These data enabled the investigators to construct the phylogenetic tree shown in the figure; the gene loci within which they found CHR-1 and CHR-2 insertions are labeled on the branches of the tree. Molecular studies testing the distribution of other DNA sequences, including mitochondrial DNA, support the close relationships between whales and cows suggested by the Shimamura experiments.

Some evolutionists contested the conclusions from molecular studies because they considered the database too limited and because morphological studies supported other hypotheses. Pigs, ruminants, camels, and hippos share a mobile heel joint that is different from the nonmobile joint in all other mammals. With their greatly reduced hind limbs, modern whales have no heel joint; but a land-living fossil believed to be an ancestor of whales has a nonmobile heel joint. Further, the teeth of pigs, ruminants, camels, and hippos are different from those of cetaceans. These morphological characters support a traditional classification in which ruminants, pigs, camels, and hippos form one lineage, and cetaceans a separate one. However, in 2001, Philip D. Gingerich of the University of Michigan and his colleagues in Pakistan reported the discovery of two ancient whale fossils, both of which had mobile heel joints. These new findings provide strong evidence in support of the conclusion that whales are closely related to ruminants and hippos.



tion. But molecular characters have no embryonic development, and biologists still do not understand the functional significance of most molecular differences. Despite these disadvantages, molecular characters represent the genome directly, and researchers use them with great success in phylogenetic analyses. *Insights from the Molecular Revolution* describes an example using sequences called transposable elements.

### Variations in the Rates at Which Molecules Evolve Govern the Molecules Chosen for Phylogenetic Analyses

Although molecular phylogenetics is based on the observation that many molecules have been conserved by evolution, different adaptive changes and neutral mutations accumulate in separate lineages from the moment they first diverge. Mutations in some types of DNA appear to arise at a relatively constant rate. Thus, differences in the DNA sequences of two species can serve as a **molecular clock**, indexing their time of divergence. Large differences imply divergence in the distant past, whereas small differences suggest a more recent common ancestor.

Because mosaic evolution exists at the molecular level, different molecules exhibit individual rates of change, and every molecule is an independent clock, ticking at its own rate. Researchers study different molecules to track evolutionary divergences that occurred over different time scales. For example, mitochondrial DNA (mtDNA) evolves relatively quickly; it is useful for dating evolutionary divergences that occurred within the last few million years. Studies of mtDNA have illuminated aspects of the evolutionary history of humans, as described in Section 30.13. By contrast, chloroplast DNA (cpDNA) and genes that encode ribosomal RNA evolve much more slowly, providing information about divergences that date back hundreds of millions of years.

To synchronize molecular clocks, some researchers study DNA sequences that are not parts of protein-encoding genes. Because they don't affect protein structure, mutations in these sequences are probably not often eliminated by natural selection. Thus, the sequence differences between species in noncoding regions probably result from mutation alone and therefore reflect the ticking of the molecular clock more directly. Some researchers also calibrate molecular clocks to the fossil record, so that actual times of divergence can be predicted from molecular data with a fair degree of certainty.

### The Analysis of Molecular Characters Requires Specialized Approaches

Molecular phylogenetics relies on the same basic logic that underlies analyses based on organismal characters: species that diverged recently from a common

ancestor should share many similarities in their molecular sequences, whereas more distantly related species should exhibit fewer similarities. Nevertheless, the practice of molecular phylogenetics is based on a set of distinctive methods.

**Determining the Molecular Sequence.** After selecting a protein molecule or appropriate segment of a nucleic acid for analysis, systematists determine the exact sequence of amino acids (in the case of proteins) or nucleotide bases (in the case of DNA or RNA) that compose the molecule.

Amino acid sequencing allows systematists to compare the primary structure of protein molecules directly. As you may recall from Chapter 15, the amino acid sequence of a protein is determined by the sequence of nucleotide bases in the gene encoding that protein. When two species exhibit similar amino acid sequences for the same protein, systematists infer their genetic similarity and evolutionary relationship. For example, researchers have used sequence data from the protein cytochrome *c* to construct a phylogenetic tree for organisms as different as slime molds, vascular plants, and humans (**Figure 23.10**).

Most systematic studies are now based, at least in part, on DNA sequencing data, which provide a detailed view of the genetic material that evolutionary processes change. The polymerase chain reaction (PCR) makes it easy for researchers to produce numerous copies of specific segments of DNA for comparison (see Section 18.1). This technique allows scientists to sequence minute quantities of DNA taken from dried or preserved specimens in museums and even from some fossils.

**Aligning Molecular Sequences.** Before comparing molecular sequences from different organisms, systematists must ensure that the homologous sequences being compared are properly “aligned.” In other words, they must be certain that they are comparing nucleotide bases or amino acids at exactly the same positions in the nucleic acid or protein molecule. This crucial step is necessary because mutations often change the length of a DNA sequence and the relative locations of specific positions through the insertion or deletion of base pairs (see Section 15.4). Such mutations make sequence comparisons more difficult; but, by determining where such insertions or deletions have occurred, systematists can match up the positions of—in other words, *align*—the nucleotides for comparison. Although alignments can be done “by eye” in many cases, most systematists use computer programs to accomplish this task. **Figure 23.11** provides a simplified example of this step in the process.

**Constructing Phylogenetic Trees.** Once the molecules are aligned, a systematist can compare the nucleotide base or amino acid sequences to determine whether

## Figure 23.10 Observational Research

### Using Amino Acid Sequences to Construct a Phylogenetic Tree

**HYPOTHESIS:** Because the amino acid sequences of proteins change over evolutionary time, sequence differences between organisms should reflect their evolutionary relationships.

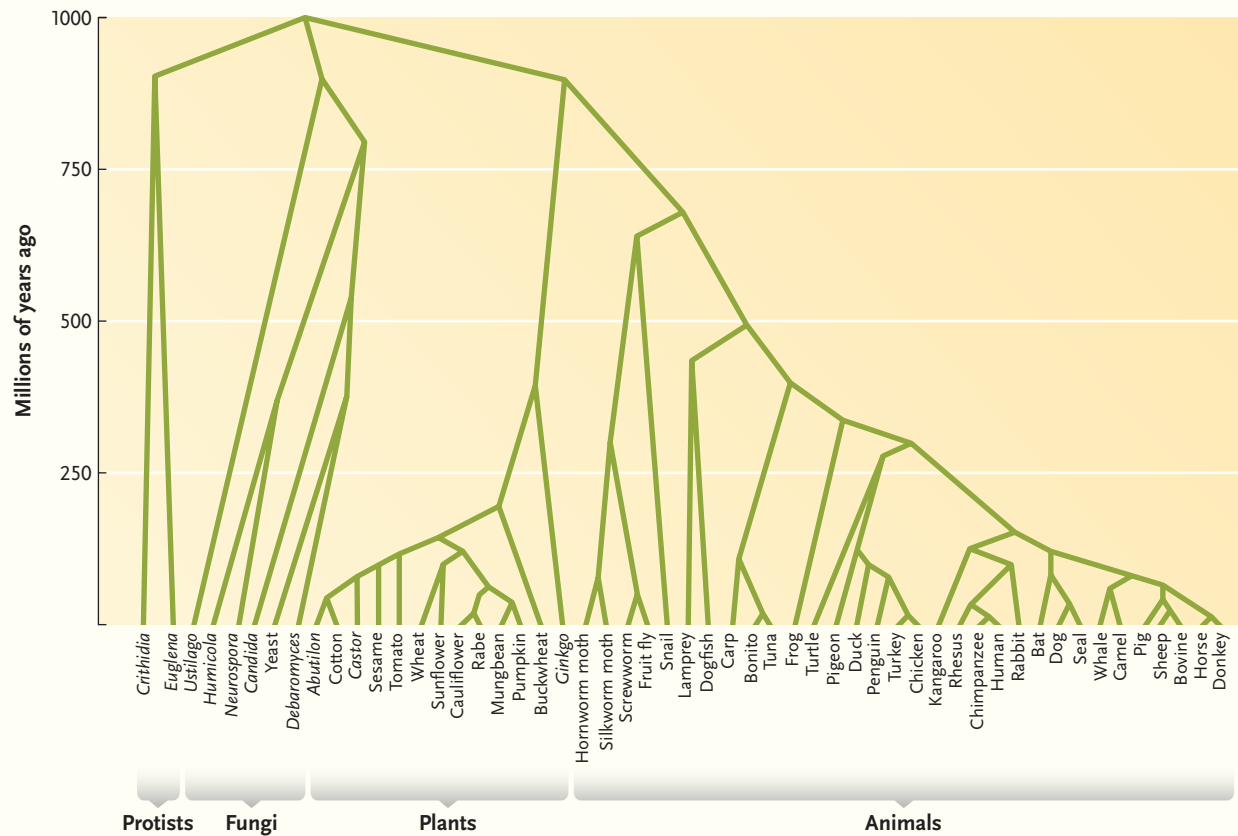
**PREDICTION:** Closely related species will exhibit similar amino acid sequences, whereas more distantly related species will exhibit greater differences in their amino acid sequences.

**METHOD:** Researchers determined the amino acid sequence of cytochrome *c*, a protein in the electron transport system that has been conserved by evolution, using samples from a wide variety of eukaryotic species classified in four kingdoms. They compared the data derived from the different species and used the sequences to construct a phylogenetic tree.

**RESULTS:** The amino acid sequence of cytochrome *c* is surprisingly similar in distantly related organisms that diverged from a common ancestor hundreds of millions of years ago. Gold shading marks the amino acids that are identical in the sequences for yeast (top row), wheat (middle row), and human (bottom row). Abbreviations for the amino acids listed below are derived from those in Figure 3.15.



The phylogenetic tree based on similarities and differences in cytochrome *c* sequences is remarkably consistent with trees based on organismal characters. The vertical axis gives the approximate time of each evolutionary branching, estimated from the amino acid sequence data.



**CONCLUSION:** Amino acid sequence data can be used to construct phylogenetic trees for species that share essentially no organismal characteristics.

mutations or other processes have produced evolutionary changes in the sequences. The similarities and differences can then be used to reconstruct the phylogenetic tree. Every phylogenetic tree is a hypothesis about evolutionary relationships, and different assumptions can yield multiple alternative trees for any data set. Indeed, systematists have developed several approaches for comparing molecular sequences and constructing trees.

For DNA sequences, the simplest approach is to count the number of similarities and differences between every pair of organisms being compared. Systematists use such data to estimate the *genetic distances* between species and to construct a phylogenetic tree by grouping together those organisms that exhibit the smallest genetic distances. However, this approach reconstructs phylogenies with both ancestral and derived characters, the same criticism that was leveled against traditional evolutionary systematics.

An alternative approach for converting molecular sequence data into a phylogenetic tree follows a cladistic method, using the principle of parsimony, which requires the identification of ancestral and derived character states. In other words, systematists must determine, for each position in the sequence, which nucleotide base is ancestral and which is derived. As is the case for organismal characters, the analysis of homologous sequences in a designated outgroup can provide that information. Under the parsimony approach, a computer program then tests all possible phylogenetic trees and identifies the one that accounts for the diversity of organisms in the group with the fewest evolutionary changes in molecular sequences.

In recent years, researchers have faulted the parsimony approach because identical changes in nucleotides often arise independently. To avoid this problem, systematists have begun using a series of sophisticated statistical techniques collectively called *maximum likelihood methods*. This approach reconstructs phylogenetic history from molecular sequence data by making assumptions about variations in the rate at which different segments of DNA evolve. These statistical models can take into account variations in the rates of evolution between genes or between species as well as changes in evolutionary rates over time. Maximum likelihood programs construct numerous alternative phylogenetic trees and estimate how likely it is that each tree represents the true evolutionary history. Systematists then accept the phylogenetic tree that is most likely to be true—until more data are available.

### Molecular Phylogenetics Has Clarified Many Evolutionary Relationships

As you will see in the next unit, molecular phylogenetics has enabled systematists to resolve some long-standing disputes about evolutionary relationships.

## Figure 23.11 Research Method

### Aligning DNA Sequences

**PURPOSE:** The insertion or deletion of base pairs often changes the length of a DNA sequence and the relative locations of specific positions along its length. Systematists must therefore “align” the sequences that they are comparing. This procedure ensures that the nucleotide bases being compared are at exactly the same positions in the nucleic acid molecules. By determining where insertions or deletions have occurred, systematists can match up the positions of—in other words, *align*—the nucleotides for comparison. In this hypothetical example, imagine that the DNA segments were obtained from three different species. A comparable procedure is used to align the amino acid sequences of proteins.

**PROTOCOL:**

1. Before alignment, three DNA segments differ in length and exhibit nucleotide differences in many positions.

Segment A AATTGACCTTCTAAGTGTAAT  
 Segment B AATTGAGCCTTCTAAGTCTAAT  
 Segment C AATTGATTCTAAGTGTAAT

2. The computer program detects similar sequences in parts of the three segments.

Segment A AATTGACCTTCTAAGTGTAAT  
 Segment B AATTGAGCCTTCTAAGTCTAAT  
 Segment C AATTGATTCTAAGTGTAAT

3. The three segments are aligned under the hypotheses that segment B included a one-nucleotide insertion and segment C had experienced a two-nucleotide deletion.

One-nucleotide insertion  
 Segment A AATTGA CCTTCTAAGTGTAAT  
 Segment B AATTGAGCCTTCTAAGTCTAAT  
 Segment C AATTGA TTCTAAGTGTAAT  
 Two-nucleotide deletion

**INTERPRETING THE RESULTS:** After alignment, the sequences can be compared at every position. In addition to the one-nucleotide insertion in segment B and the two-nucleotide deletion in segment C, the comparison reveals one nucleotide substitution in segment B.

Insertion Substitution of C for G  
 Segment A AATTGA CCTTCTAAGTGTAAT  
 Segment B AATTGAGCCTTCTAAGTCTAAT  
 Segment C AATTGA TTCTAAGTGTAAT  
 Deletion

As one example, analyses of morphological data had produced conflicting hypotheses about the origin and relationships of flowering plants. In 1999, four teams of researchers, analyzing different parts of flowering plant genomes, independently identified

**Figure 23.12**

The ancestral flowering plant. DNA sequencing studies identified *Amborella trichopoda* as a living representative of the earliest group of flowering plants.

*Amborella* branch



Thomas J. Lemieux, University of Colorado

*Amborella* flower

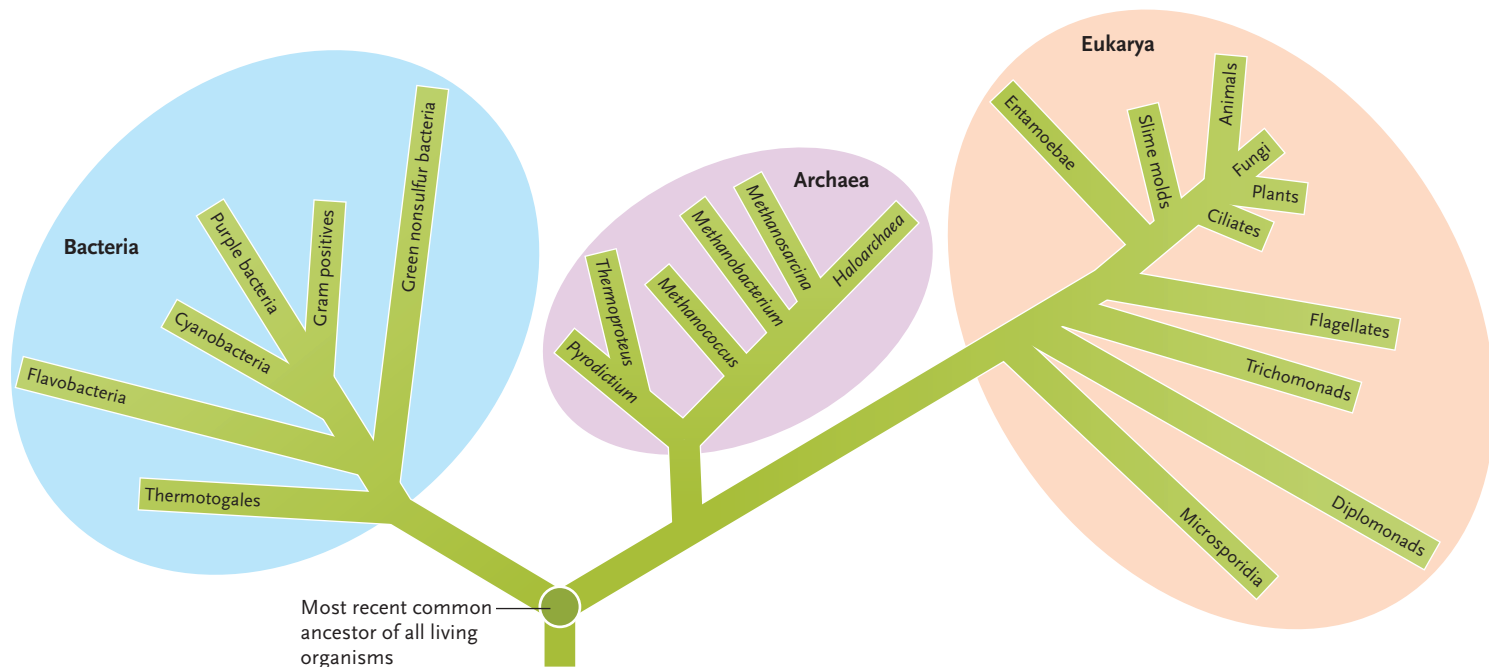


Sandra Floyd, University of Colorado

*Amborella trichopoda*, a bush native to the South Pacific island of New Caledonia, as a living representative of the most ancient group of flowering plants yet discovered (Figure 23.12). The first team to publish their results, Sarah Mathews and Michael Donoghue of Harvard University, studied phytochrome genes (*PHYA* and *PHYC*) that had duplicated early in the evolutionary history of this group. Other researchers, who studied chloroplast, mitochondrial, and ribosomal sequences, obtained similar results, providing strong support for this phylogenetic hypothesis.

On a very grand scale, molecular phylogenetics has revolutionized our view of the entire tree of life. The first efforts to create a phylogenetic tree for all forms of life were based on morphological analyses. However, these analyses did not resolve branches of the tree containing prokaryotes, which lack significant structural variability, or the relationships of those branches to eukaryotes.

In the 1960s and early 1970s, biologists organized living systems into five kingdoms. All prokaryotes were grouped into the kingdom Monera. The eukaryotic organisms were grouped into four kingdoms: Fungi, Plantae, Animalia, and Protista. The Protista was always recognized as a polyphyletic “grab bag” of unicellular or acellular eukaryotic organisms. Unfortunately, phylogenetic analyses based on morphology were unable to sort these organisms into distinct evolutionary lineages.



**Figure 23.13**

Three domains: the tree of life. Carl R. Woese's analysis of rRNA sequences suggests that all living organisms can be classified into one of three domains: Bacteria, Archaea, and Eukarya.



In the 1970s, biologists realized that molecular phylogenetics provides an alternative approach. They simply needed to identify and analyze molecules that have been conserved by evolution over billions of years. Carl R. Woese, a microbiologist at the University of Illinois at Urbana-Champaign, identified the small subunit of ribosomal RNA as a suitable molecule for analysis. Ribosomes, the structures that translate messenger RNA molecules into proteins (see Section 15.1), are remarkably similar in all forms of life. They are apparently so essential to cellular

processes that the genes specifying ribosomal structure exhibit similarities in their nucleotide sequences in organisms from bacteria to humans. Thus, it is possible to sequence these genes and align them for analysis.

The phylogenetic tree based on rRNA sequences divides living organisms into three primary lineages called domains: Bacteria, Archaea, and Eukarya (**Figure 23.13**). According to this hypothesis, two domains, Bacteria and Archaea, consist of prokaryotic organisms, and one, Eukarya, consists of eukaryotes. Bacteria in-

## UNANSWERED QUESTIONS

### Should we abandon the traditional Linnaean hierarchy in favor of a more evolutionary classification?

Diligent Kindly Professors Cannot Often Fail Good Students—or some equally silly mnemonic device for remembering the Linnaean taxonomic hierarchy—is all that many students recall about systematics. Even if they remember the underlying rank names—is *G* for “group” or “genus”?—they often forget that Linnaeus conceived his system of classification more than a century before Darwin articulated his theory of evolution, which revolutionized our understanding of biological diversity. In the roughly 150 years since Darwin published *On the Origin of Species*, systematists have sought not only to categorize life’s diversity but, more importantly, to understand its origins. The broad relevance of studies in systematics has become increasingly clear as biologists have discovered that systematic principles are as important to tracing the emergence and spread of avian flu as they are to distinguishing a duck from a dove.

As we approach the sesquicentennial of Darwin’s theory, its impact becomes increasingly revolutionary. Perhaps the most striking recent example is a call for the complete abandonment of the Linnaean taxonomic hierarchy. Although biologists thought they had reconciled the perspectives of Darwin and Linnaeus, a growing minority of systematists now believe that any effort to catalog and categorize life’s diversity must be explicitly phylogenetic and free of the arbitrary ranks that Linnaeus invented. This movement, which has been codified in the PhyloCode initiative, is fueled largely by newly available molecular data, vastly improved phylogenetic methodologies, and increasingly fast computers. These advances offer the potential to reconstruct accurate and fully resolved phylogenetic trees at a scale never before possible. For the first time, biologists see real progress in accurately reconstructing the entire tree of life. Although we are still far from achieving this goal, every day millions of new, phylogenetically informative DNA fragments are being sequenced and analyzed by thousands of computers running around the clock.

Although PhyloCode’s synthesis of taxonomy and evolutionary systematics may be long overdue, this attempted coup is not without controversy. For example, some systematists contend that such a radical revision of our taxonomic system will introduce confusion and in-

stability in the naming of species. Even the revolution’s adherents recognize that we still face many challenging limitations to the synthesis between taxonomic practice and Darwinian principles. Nowhere is this more evident than in the definition of species.

During Linnaeus’ time, species were viewed as immutable natural types created by God. Darwin, however, formulated his theory on the principle that species change over time. Although the truth of this basic hypothesis is no longer a subject of debate, its practical implications for delimiting species boundaries and understanding how new species form are among the most exciting areas of study in modern systematics. Most practicing systematists view species as real (that is, biologically meaningful) categories, but the criteria for recognizing species vary dramatically among systematists working on different types of organisms (plants versus animals, or organisms that reproduce asexually versus those that reproduce sexually). Biologists are now beginning to use new molecular tools to address the challenge of understanding the origin of new species. Using these tools and sophisticated genetic experiments, evolutionary biologists are beginning to probe the precise genetic basis of species. Over the past decade a small number of “speciation genes” have been identified; more such discoveries are sure to follow in the coming years. Although many of these studies have been restricted to model research organisms, such as fruit flies, the new tools offered by the fields of genomics and bioinformatics offer the potential to address similar questions in an increasingly broad array of organisms.

Simply put, the systematics of today is not that of your grandparents. Given the enormous challenge involved in categorizing and understanding the origin and evolutionary relationships of millions of species, many additional changes are on the horizon. For the next generation of systematists, however, a better mnemonic to remember may be “Keep Probing Charles’ *Origin* For Good Systematics.”



Rich Glor conducts research on the evolution of *Anolis* lizards at the University of Rochester. To learn more about his research, go to <http://www.lacertilia.com>.

cludes well-known microorganisms, and Archaea includes microorganisms that live in physiologically harsh environments, such as hot springs or very salty habitats. Eukarya includes the familiar animals, plants, and fungi, as well as the many lineages formerly included among the Protista. The next unit of this book is devoted to detailed analyses of the biology and evolutionary relationships between and within these three domains.

## STUDY BREAK

1. What are three advantages of using molecular characters in phylogenetic analyses?
2. How can molecular sequence data be used as a molecular clock?
3. Why was a phylogenetic analysis of prokaryotes based on molecular sequence data more successful than the analysis based on morphological data?

## Review

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### 23.1 Systematic Biology: An Overview

- Systematic biology has two goals: the reconstruction of evolutionary history and the naming and classification of organisms. Phylogenetic trees and classifications are hypotheses about the relationships of organisms.
- By providing a guide to biological diversity, systematics allows biologists to identify species for research, for the control of harmful organisms, and for conservation (Figure 23.1).

### 23.2 The Linnaean System of Taxonomy

- Linnaeus invented a system of binomial nomenclature in which each species receives a unique two-part name.
- Species are organized into a taxonomic hierarchy (Figure 23.2), which reflects the pattern of branching evolution. Species classified in the same genus or family have a more recent common ancestor than species classified only in the same class or phylum.

**Animation: Classification systems**

### 23.3 Organismal Traits as Systematic Characters

- Systematists have always studied organismal characters, such as morphology, chromosome structure and number, physiology, and behavior.
- Morphological traits often allow the reconstruction of a group's phylogeny, that is, its evolutionary history.
- Behavioral characters are useful for understanding the relationships of animals that are not morphologically different (Figure 23.3).

**Animation: Evolutionary tree for plants**

### 23.4 Evaluating Systematic Characters

- Systematists study characters that are genetically independent, reflecting different parts of the organisms' genomes.
- Most systematists use homologous characters that reflect genetic similarities and differences among species (Figures 23.4 and 23.5).
- Because characters evolve at different rates, systematists select traits that evolved at a rate consistent with the timing of branching evolution.

- Systematists base their analyses on derived versions of homologous traits (Figure 23.6).

### 23.5 Phylogenetic Inference and Classification

- Phylogenetic trees and classifications include only monophyletic taxa, each of which contains a single ancestral species and all of its descendants (Figure 23.7). Many systematists create parsimonious phylogenies, which include the fewest possible evolutionary changes to account for the diversity within a lineage.
- Traditional evolutionary systematics emphasizes branching evolution and morphological divergence. Using both ancestral and derived characters, this approach sometimes creates classifications with paraphyletic taxa, which include an ancestor and some, but not all, of its descendants (Figure 23.8a).
- Cladistics emphasizes only evolutionary branching to define monophyletic taxa (Figure 23.8b). Cladists create phylogenetic hypotheses and classifications by grouping organisms that share derived characters (Figure 23.9).

**Animation: Constructing a cladogram**

**Animation: Interpreting a cladogram**

**Animation: Current evolutionary tree**

### 23.6 Molecular Phylogenetics

- Contemporary systematists use the structure of proteins and nucleic acids in their analyses. Molecular characters provide abundant data and can be compared among many morphologically distinct forms of life, but because molecular similarities in different species may have evolved independently, systematists cannot always verify that they were inherited from a common ancestor.
- Molecular characters may act as molecular clocks, providing data that allows researchers to determine the times when lineages first diverged (Figure 23.10).
- The use of molecular characters in phylogenetic studies requires the sequencing and alignment of molecules (Figure 23.11). Several methods, including genetic distances, parsimony, and maximum likelihood, have been proposed for the construction of phylogenetic trees.
- Molecular phylogenetics has clarified relationships among the flowering plants (Figure 23.12) and provided insights into the evolutionary relationships of all organisms (Figure 23.13).

**Animation: Cytochrome *c* comparison**

## Questions

### Self-Test Questions

- The evolutionary history of a group of organisms is called its:
  - classification.
  - taxonomy.
  - phylogeny.
  - domain.
  - outgroup.
- In the Linnaean hierarchy, the organisms classified within the same taxonomic category are called:
  - a phylum.
  - a taxon.
  - a genus.
  - a binomial.
  - an epithet.
- When systematists study morphological or behavioral traits to reconstruct the evolutionary history of a group of animals, they assume that:
  - similarities and differences in phenotypic characters reflect underlying genetic similarities and differences.
  - the animals use exactly the same traits to identify appropriate mates.
  - differences in these traits caused speciation in the past.
  - the adaptive value of these traits can be explained.
  - variations in these traits are produced by environmental effects during development.
- Which statement best describes the concept of mosaic evolution?
  - Some phenotypic variation is caused by environmental factors.
  - Homologous characters are those that different organisms inherit from a common ancestor.
  - Different organismal traits may reflect the same part of an organism's genome.
  - Some characters evolve more quickly than others.
  - The fossil record provides clues about the ancestral versions of characters.
- Which of the following pairs of structures are homoplasious?
  - the wing skeleton of a bird and the wing skeleton of a bat
  - the wing of a bird and the wing of a fly
  - the eye of a fish and the eye of a human
  - the bones in the foot of a duck and the bones in the foot of a chicken
  - the adhesive toe pads on the right hind foot of an *Anolis* lizard and those on the left hind foot
- Which of the following does *not* help systematists determine which version of a morphological character is ancestral and which is derived?
  - outgroup comparison
  - patterns of embryonic development
  - studies of the fossil record
  - studies of the character in more related species
  - dating of the character by molecular clocks
- In a cladistic analysis, a systematist groups together organisms that share:
  - derived homologous traits.
  - derived homoplasious traits.
  - ancestral homologous traits.
  - ancestral homoplasious traits.
  - all of the above.
- A monophyletic taxon is one that contains:
  - an ancestor and all of its descendants.
  - an ancestor and some of its descendants.
  - organisms from different evolutionary lineages.
  - an ancestor and those descendants that still resemble it.
  - organisms that resemble each other because they live in similar environments.

- Which of the following is *not* an advantage of using molecular characters in a systematic analysis?
  - Molecular characters provide abundant data.
  - Systematists can compare molecules among species that are morphologically very similar.
  - Systematists can compare molecules among species that share few morphological characters.
  - Amino acid sequences in proteins are generally not influenced by environmental factors.
  - Systematists can easily determine whether base substitutions in the DNA of two species are homologous.
- To construct a cladogram by applying the principles of parsimony to molecular sequence data, one would:
  - start by making assumptions about variations in the rates at which different DNA segments evolve.
  - group together organisms that share the largest number of ancestral sequences.
  - group together organisms that share derived sequences, matching the groups to those defined by morphological characters.
  - group together organisms that share derived sequences, minimizing the number of hypothesized evolutionary changes.
  - identify derived sequences by studying the embryology of the organisms.

### Questions for Discussion

- Systematists use both amino acid sequences and DNA sequences to determine evolutionary relationships. Think about the genetic code (Section 15.1), and explain why phylogenetic hypotheses based on DNA sequences may be more accurate than those based on amino acid sequences.
- Traditional evolutionary systematists identify the Reptilia as one class of vertebrates, even though we know that this taxon is paraphyletic. Describe the advantages and disadvantages of defining paraphyletic taxa in a classification.
- The following table provides information about the distribution of ancestral and derived states for six systematic characters (labeled 1 through 6) in five species (labeled A through E). A "d" means that the species has the derived form of the character, and an "a" means that it has the ancestral form. Construct a cladogram for the five species using the principle of parsimony; in other words, assume that each derived character evolved only once in this group of organisms. Mark the branches of the cladogram to show where each character changed from the ancestral to the derived state.

Species	Character					
	1	2	3	4	5	6
A	a	a	a	a	a	a
B	d	a	a	a	a	d
C	d	d	d	a	a	a
D	d	d	d	a	d	a
E	d	d	a	d	a	a

- Imagine that you are a systematist studying a group of little-known flowering plants. You discover that the phylogenetic tree based on flower morphology differs dramatically from the phylogenetic tree based on DNA sequences. How would you try to resolve the discrepancy? Which tree would you believe is more accurate?

5. Create an imaginary phylogenetic tree for an ancestral species and its 10 descendants. Circle a monophyletic group, a polyphyletic group, and a paraphyletic group on the tree. Explain why the groups you identify match the definitions of the three types of groups.

### Experimental Analysis

Imagine that you are trying to determine the evolutionary relationships among six groups of animals that look very much alike because they have few measurable morphological characters. What data would you collect to reconstruct their phylogenetic history?

### Evolution Link

How do the two models of macroevolution (gradualist versus punctuated equilibrium) relate to the philosophies of phylogenetic inference espoused by traditional evolutionary systematists and cladists? You may want to review material in Section 22.3 before answering this question.