CHAPTER 2

Methods and Principles of Biological Systematics

Biological systematics (or taxonomy) is the theory and practice of grouping individuals into species, arranging those species into larger groups, and giving those groups names, thus producing a classification. Classifications are used to organize information about plants, and keys can be constructed to identify them.

There are many ways in which one might construct a classification. For example, plants could be classified on the basis of their medicinal properties (as they are in some systems of herbal medicine) or on the basis of their preferred habitat (as they may be in some ecological classifications). A phylogeny-based classification, such as that followed in this book, attempts to arrange organisms into groups on the basis of their evolutionary relationships. There are two main steps in producing such a classification. The first is determining the **phylogeny**, or evolutionary history. The second is basing the classification on this history. These two steps can be, and often are, separated, such that every new theory of relationships does not lead automatically to a new classification. This chapter will outline how one goes about determining the history of a group, and then will discuss briefly how one might construct a classification, given that history.

What Is a Phylogeny?

As described in Chapter 1, evolution is not simply descent with modification, but also involves the process of separation of lineages. Imagine for a moment a population of organisms that all look similar to each other. By some process, the population divides into two populations, and these two populations go on to evolve independently. In other words, two **lineages** (ancestor–descendant sequences of populations) are established. We know this has happened because the members of the two new populations acquire, by the process of mutation, new characteristics in their genes, and possibly changes in their overall form, making the members of one population look more similar to each other than to members of the other population or to the ancestral population. These characteristics are the evidence for evolution.

For example, a set of plants will produce offspring that are genetically related to their parents, as indicated by the lines in Figure 2.1. The offspring will produce more offspring, so that we can view the population over several generations, with genetic connections indicated by lines.

If the population divides into two separate populations, each will have its own set of genetic connections, and eventually will acquire distinctive characteristics. For example, the population on the right could develop red flowers, whereas the stems of the population on the left could become woody. Red flowers and woodiness are evidence that each of the two populations constitutes a single lineage. The same process can repeat, and each of the new populations can divide (Figure 2.2). Again, we know this has happened because of a new set of characteristics acquired by the newly formed populations. Some of the woody plants have fleshy fruits, and another group has a spiny seed coat. Meanwhile, some of the red-flowered plants now have only four stamens, and another set of red-flowered plants have hairy leaves.

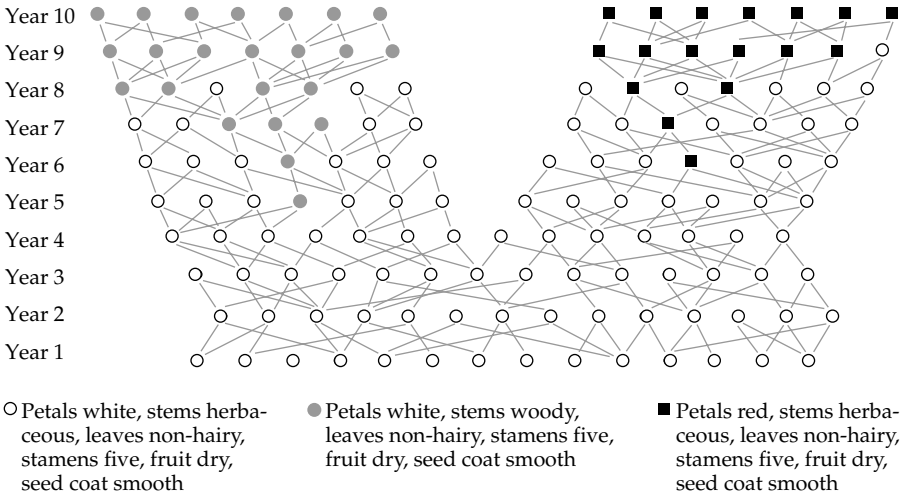


Figure 2.1 The evolution of two hypothetical species. Each circle represents a plant. A mutation in the lineage on the left causes a change to woody stems, which is then transmitted to descendant plants. Woody-stemmed plants gradually replace all the herbaceous ones in the population. A similar mutation in the lineage on the right leads to a group with red petals.

The characteristics of plants, such as flower color or stem structure, are generally referred to as **characters**. Each character can have different values, or **character states**. In this case, the character "flower color" has two states, white and red. The character "stem structure" also has two states, woody and herbaceous, and so forth. All else being equal, plants with the same state are more likely to be related than those with different states.

The critical point in this example, however, is that characteristics such as red petals and woody stems are new, and they are **derived** relative to the ancestral population. Only such new characters tell us that a new lineage has been established; retaining the old characteristic

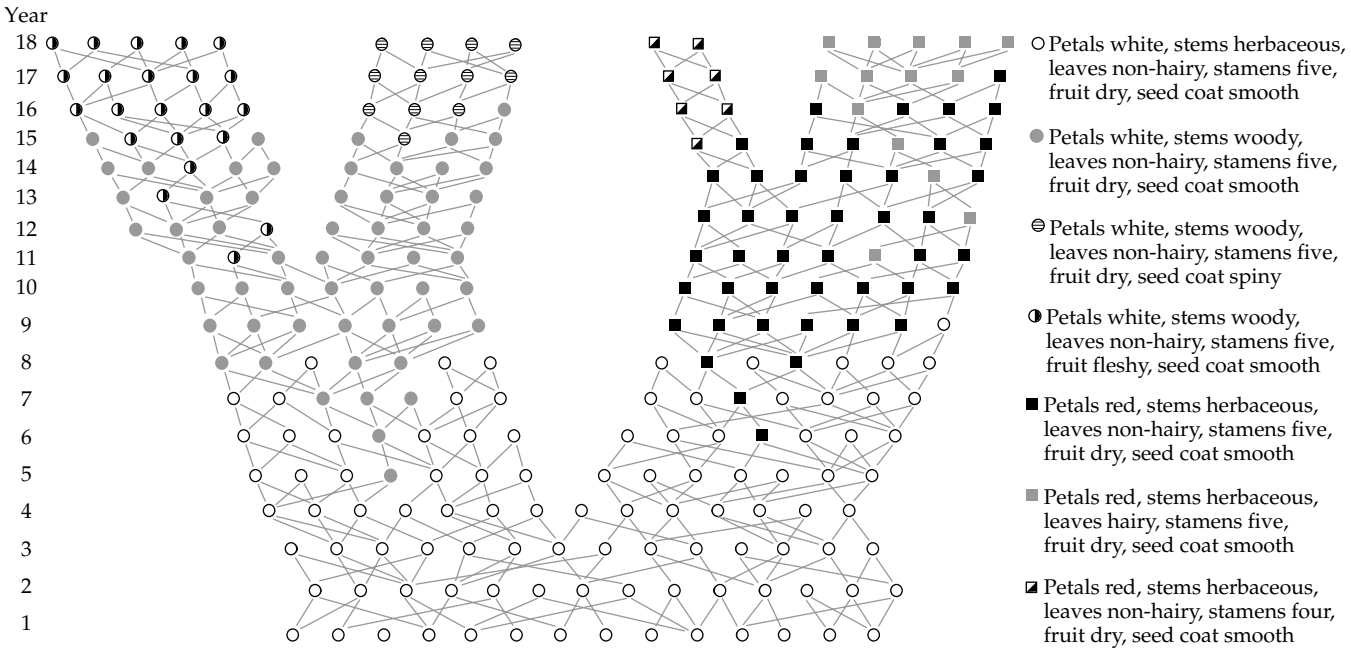


Figure 2.2 The same hypothetical set of plants as in Figure 2.1 after eight years and two more speciation events.

(white flowers, herbaceous stems, non-hairy leaves, five stamens, dry fruit, smooth seed coat) does not tell us anything about what has happened.

A character state that is derived at one point in time will become ancestral later. In Figure 2.2, woody stems are derived relative to the original population, but are ancestral relative to the groups with fleshy fruits or spiny seed coats.

A group composed of an ancestor and all of its descendants is known as a **monophyletic group** (*mono*, single; *phylum*, lineage). We can recognize it because of the shared derived characters of the group (**synapomorphies**). These are character states that have arisen in the ancestor of the group and are present in all of its members (albeit sometimes in modified form). This concept was first formalized by Hennig (1966) and Wagner (1980).

The diagrams of Figures 2.1 and 2.2 are cumbersome to draw, but can be summarized as a branching tree (Figure 2.3A). It is also inconvenient to repeat the ancestral character states retained in every group, so systematists commonly note only the characters that have changed, with tick marks placed on the appropriate branches to indicate the relative order in which the character states originated (Figure 2.3B).

The shared derived characters in Figure 2.3B can be arranged in a hierarchy from more inclusive (e.g. stems woody or petals red) to less inclusive (e.g., leaves hairy, seed coat spiny). These then lead to the obvious conclusion that the plants themselves can be arranged in a hierarchical classification that is a reflection of their evolutionary history. The plants could be divided into two groups, one with herbaceous stems and red petals, the other with woody stems and white petals. Each of these groups can also be divided into two groups. Thus the classification can be derived directly from the phylogeny.

Note that the hierarchy is not changed by the order in which the branch tips are drawn. The shape, or **topology**, of the tree is determined only by the connections between the branches. We can tell the evolutionary "story" by starting at any point in the tree and working up or down. This means that the terms "higher" and "lower" are not really meaningful, but simply reflect how we have chosen to draw the evolutionary tree. From this point of view, a plant systematics course could as well begin by covering the Asteraceae, which some textbooks consider an "advanced" family, and then working out to other members of the asterid clade, instead of starting with the so-called "primitive" families, such as Magnoliaceae and Nymphaeaceae. The latter simply share a set of characters thought to be ancestral, but these are combined with a large set of derived characters as well.

Determining Evolutionary History

CHARACTERS, CHARACTER STATES, AND NETWORKS

In the example in Figures 2.1, 2.2, and 2.3, we have described evolution as though we were there watching it happen. This is rarely possible, of course, and so part of the challenge of systematics is to determine what went on in the past. The relatives of an extant species must be

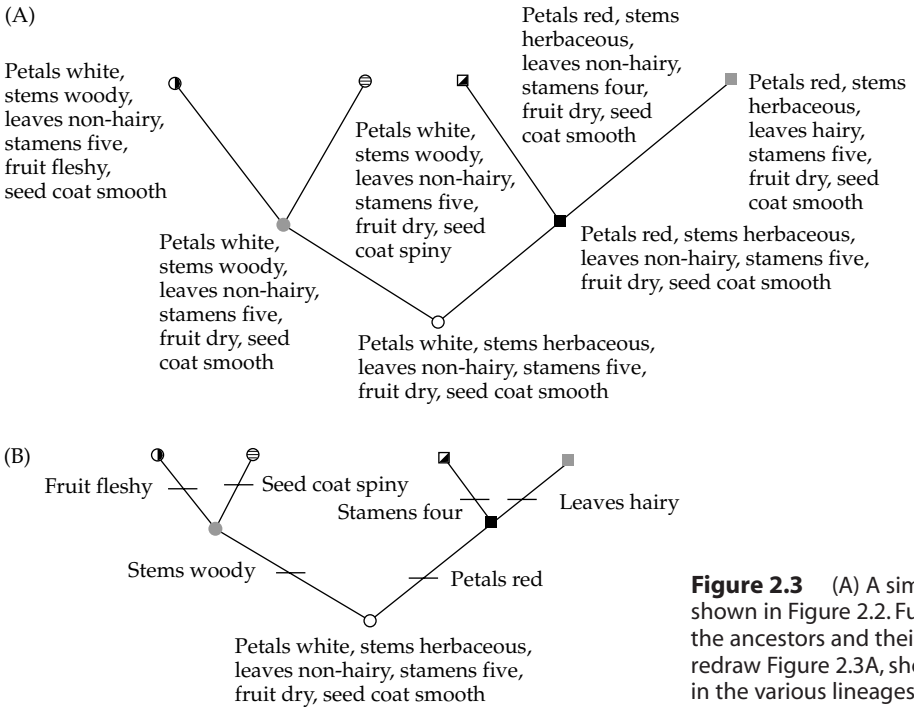


Figure 2.3 (A) A simple way to redraw the pattern of change shown in Figure 2.2. Full descriptions are provided for each of the ancestors and their descendants. (B) A simpler way to redraw Figure 2.3A, showing only the mutations that occurred in the various lineages.

determined by examining that species closely for characteristics that are believed to be heritable. These can be any aspect of the plant that can be passed down genetically through evolutionary time and still be recognizable. For example, petal color, inflorescence structure, and plant habit are all known to be under genetic control. Although they often show considerable phenotypic variation, they are generally stably inherited from one generation to the next and thus would provide good taxonomic characters. Many examples of such heritable characters are described in Chapter 4. Characters of DNA and RNA can also be used, and are described in Chapter 5.

It may be harder than you think to determine which structures in one plant can be compared to structures in another plant. Two structures may be similar because they are in a similar position in the different organisms, or because they are similar in their cellular and histological structure, or because they are linked by intermediate forms (either intermediates at different developmental stages of the same organism or intermediates in different organisms). These are **Remane's criteria** of what he called homology, but what we are calling similarity. The assessment of similarity is the basis of all of comparative biology and of systematics in particular.

Systematics entails the precise observation of organisms. Without accurate comparative morphology, classification of any sort is impossible. Without careful description of characters and their states, phylogeny reconstruction and the description of history are meaningless.

Determining similarity is the first step in determining **homology**, or identity by descent. Be aware, however, that the word *homology* has many different meanings, and it is impossible to summarize the literature here. Many phylogenetic systematists argue that homology can only be determined by constructing an evolutionary tree, a viewpoint that will be followed in this text. When reading the literature, it is worth checking what particular authors mean when they use the term.

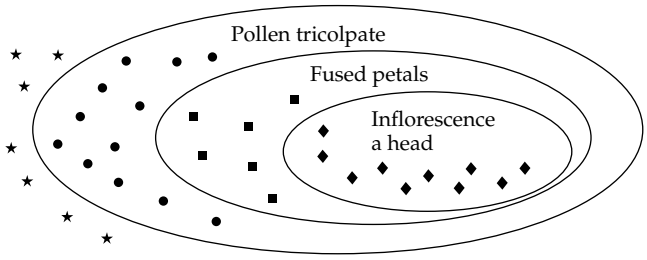
From observing plants, groups that share particular characteristics can be identified. For example, a large group of plant species has pollen with three grooves, or germination furrows, called colpi; the pollen is thus described as tricolpate. Within this large group is a smaller group that has fused petals, and within the fused-petal group is a still smaller group with flowers arranged in a head. These nested groups can be diagrammed as a set of ovals (a **Venn diagram**) as in Figure 2.4A, with the individual shapes representing plant species.

The same information can be drawn as a **network** (Figure 2.4B). In this case, number of colpi in pollen, fusion of petals, and type of inflorescence are shown as vertical lines. All shapes (species) to the left of the pollen line have fewer than three colpi, whereas everything to the right of the line has tricolpate pollen, as indicated by the numeral 3. Likewise, the line for petals indicates a shift between free petals and fused petals, and the inflorescence line a shift between flowers clustered in a head versus flowers borne separately.

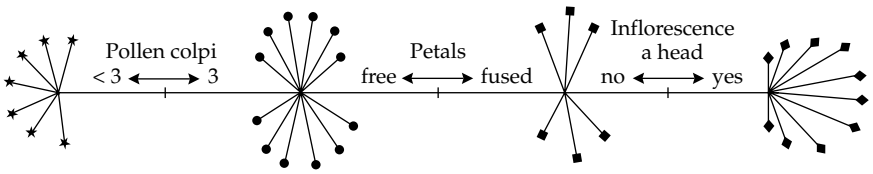
All the plants indicated by the same shape are drawn as though they arose at the same point in time. This is because, for the purposes of this simplified example, we have not provided any information on their order of origin.

We can determine the length of the network, which is the number of changes. For example, proceeding from right to left, there is one change each in inflorescences,

(A)



(B)



(C)

	Pollen grooves	Petals	Inflorescence in heads
Star plants	< 3	free	no
Circle plants	3	free	no
Square plants	3	fused	no
Diamond plants	3	fused	yes

Figure 2.4 (A) Venn diagram of a set of plants. A large group has tricolpate pollen. Of those plants, a smaller group has fused petals, and of the plants with tricolpate pollen and fused petals, a subset has flowers arranged in a head. (B) The pattern of Figure 2.4A redrawn as an unrooted network. (C) The pattern of Figures 2.4A and B redrawn as a matrix.

petals, and pollen grooves, so the network can be described as having a length of three.

The same information can also be presented as a **matrix** (Figure 2.4C). This time the rows correspond to plants, and the columns correspond to characters of the plants. The character states are then used to fill in the matrix. These are, or are hypothesized to be, genetic changes that potentially distinguish the groups of plants in the matrix. Thus the three changes in the network of Figure 2.4B represent three changes in character states or genes of plants.

In the example, we have implied that the determination of the character states is perfectly obvious. This is often not the case, however, particularly with morphological characters. The variation among similar structures must be described by dividing the character into character states. This is a hypothesis of underlying genetic control, although it is rarely framed this way. For example, if two species differ in the color of their flowers, we may score the character petal color as having two states, red and blue. By scoring it this way, we are hypothesizing that there are underlying genes that switched, over evolutionary time, to produce red flowers from a blue-flowered ancestor, or blue flowers from a red-flowered ancestor.

In this case, we know that there are genes (for example, components of the anthocyanin pathway) that do in fact control flower color, and thus the inference of two states controlled by a genetic switch is probably a reasonable guess. In most cases, however, we have no idea of the genetic control of the structural characters observed. In making hypotheses about the nature of the underlying switches, then, often the only recourse is to be sure that the character states really are distinct. For quantitative characters such as leaf length or corolla tube width, this means drawing a graph to be sure that the species we are studying have measurements that do not overlap. For many characters, the measurements do overlap, such that any guess as to the underlying switches, and therefore division into character states, is unsupported by any evidence. In these cases, the characters should be omitted from the phylogenetic analysis (unless the overlap is caused by only a few plants, in which case the character could be scored as polymorphic and retained in the analysis). Even though such characters probably reflect genetic changes over evolutionary time, with our current knowledge it is difficult to extract from them information on the underlying changes, although methods of dealing with plants with variable characters have been developed.

Variability and overlap in morphological characters are, of course, good reasons why many systematists have turned to molecular data in constructing phylogenies. The recognition of molecular character states (i.e., nucleotides) is often easier and more precise, although even this can be difficult if gene sequences are hard to align, or if restriction fragments are similar in size (see Chapter 5).

EVOLUTIONARY TREES AND ROOTING

Figure 2.4 shows three different ways of recording and organizing observations about plants. Even though the network looks somewhat like a time line, it is not. It could be read from left to right, right to left, or perhaps from the middle outward. To turn it into an **evolutionary tree**, we must determine which changes are relatively more recent and which occurred further in the past. In other words, the tree must be **rooted**, which causes all character changes to be **polarized**, or given direction. (Some workers distinguish between an evolutionary tree, a phylogeny, and a branching diagram or cladogram, but in this text the terms are used interchangeably.)

If you imagine that the network is a piece of string, you can keep the connections exactly the same, even when you pull down a root in many different places. The network from Figure 2.4B is redrawn in Figure 2.5, but rooted in three different places. Notice that the length of each tree (or **cladogram**) is the same as the length of the original network—three—and that all the connections are the same, but that the order of events differs considerably. For example, in the rooting shown in Figure 2.5A, the ancestral plants had pollen with fewer that three colpi, petals not fused, and flowers not in heads, whereas in Figure 2.5B, we would conclude that the ancestral plants had exactly the opposite. In Figure 2.5C, the tree is rooted in such a way that the ancestor had tricolpate pollen. The pollen later changed to having fewer than three colpi in one lineage, whereas the other lineage kept the pollen character state of three colpi and later acquired fused petals and flowers in heads.

As is obvious from inspection of Figure 2.5, the rooting of the tree is critical for interpreting how plants evolved. Different roots suggest different patterns of changes (character polarizations). There has been much discussion among systematists of how the position of the root should be determined. One frequent suggestion is that one should use fossils. But just because a plant has been fossilized does not mean that its lineage originated earlier than plants now living; we know only that it died out earlier. In determining evolutionary history we are interested in determining when lineages—**taxa** or taxonomic groups—diverged from one another (when taxa originated). When taxa die out is interesting to know, but it is not relevant to determining origins.

In general, evolutionary trees are rooted using a relative of the group under study: an **outgroup**. When selecting an outgroup, one must assume only that the ingroup members (members of the group under study) are more closely related to each other than to the outgroup; in other words, the outgroup must have separated from the ingroup lineage before the ingroup diversified. Often several outgroups are used. If an outgroup is added to the network, the point at which it attaches is determined as the root of the tree.

In the case of the plants in Figures 2.4 and 2.5, the plants shown are all flowering plants (angiosperms),

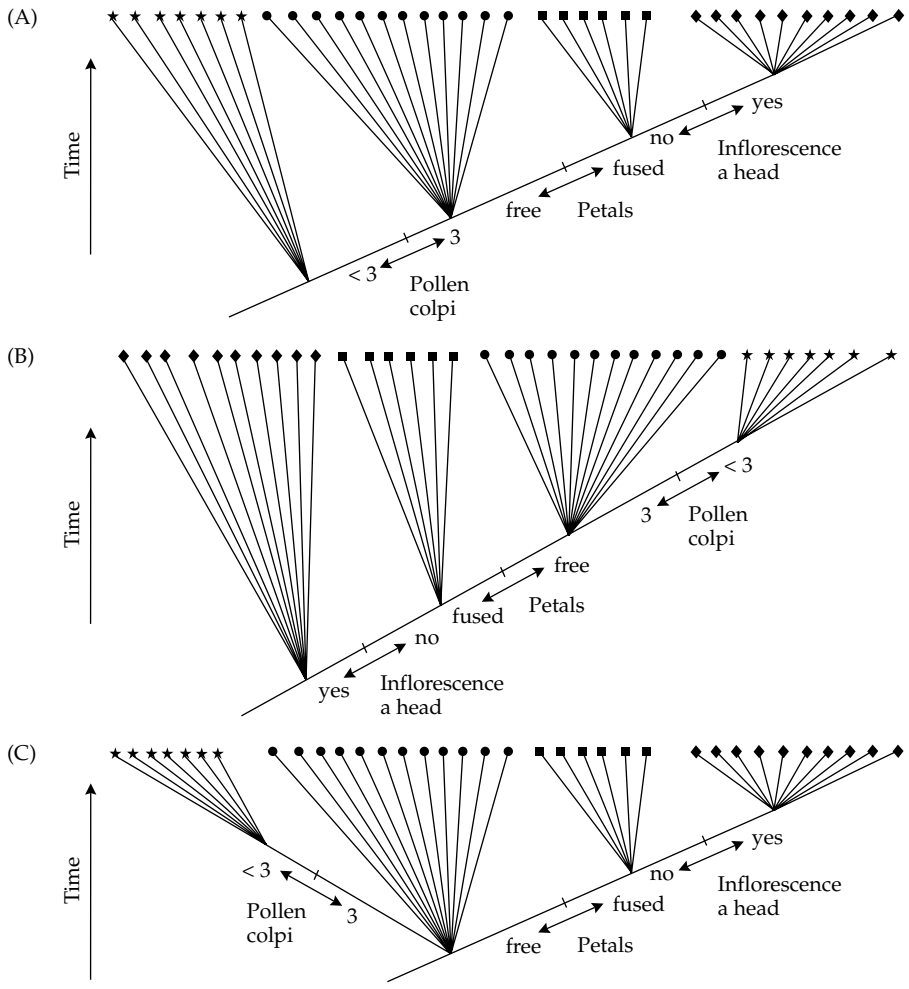


Figure 2.5 (A) One possible rooting of the network in Figure 2.4B. Note that the number of evolutionary steps (character state changes) is the same as the unrooted network. (B) A second possible rooting of the same network. (C) A third possible rooting of the same network.

and their closest living relatives are either the conifers, cycads, gnetophytes, or ginkgos (see Chapter 7). In Figure 2.6A, a conifer is added to the matrix from Figure 2.4C. (We could have used all "gymnosperms" as outgroups, but have chosen only one for simplicity of the example.) Because conifers do not have petals or flowers, two of the characters must be scored as not applicable, but we do know that conifer pollen does not have three colpi. With this information, the conifer can be added to the network as an outgroup, as in Figure 2.6B. Because it attaches among the star species, the tree can be rooted and redrawn as in Figure 2.6C. This corresponds to the rooted tree in Figure 2.5A and strengthens the hypothesis that Figure 2.5A accurately reflects evolutionary history.

Note that the tree can be drawn in different ways and still reflect the same evolutionary history. Comparing Figures 2.7A and B with Figure 2.6C shows that the branches of the tree can be "rotated" around any one of the branch points (**nodes**) and not affect the inferred order of events.

With a rooted tree (and only with a rooted tree), we can determine which groups are monophyletic. Therefore, in the example laid out in Figure 2.6C, the diamond plants are monophyletic (i.e., they form a **clade**). In fact, the flowering plants with fused petals and flowers arranged in a head are the family Asteraceae, which are known to form a monophyletic group. Thus having flowers in heads is a synapomorphy for (is a shared derived character for, indicates the monophyly of) the Asteraceae, having fused petals is a shared derived character (synapomorphy) uniting the square species with the diamond species, and having tricolpate pollen indicates the monophyly of the circle plus square plus diamond species.

Notice how important rooting is for determining monophyly. If Figure 2.5B were the correct rooting of the flowering plant phylogeny, then fused petals and flowers in heads would be ancestral character states (usually called **symplesiomorphies**) rather than derived (**synapomorphies**). In this case, the species indicated by diamonds and squares would not share any *derived* character. Notice also that a group including the diamond plus square plants in Figure 2.5B does not include *all* the descendants of their common ancestor—some of those descendants went on to become the circle and the star plants. Therefore, if Figure 2.5B were correct, then the diamond plus square species would not be a monophyletic group. Such a group is called **paraphyletic**; a

(A)

	Pollen grooves	Petals	Inflorescence in heads
Star plants	< 3	free	no
Circle plants	3	free	no
Square plants	3	fused	no
Diamond plants	3	fused	yes
Conifer	< 3	not applicable	not applicable

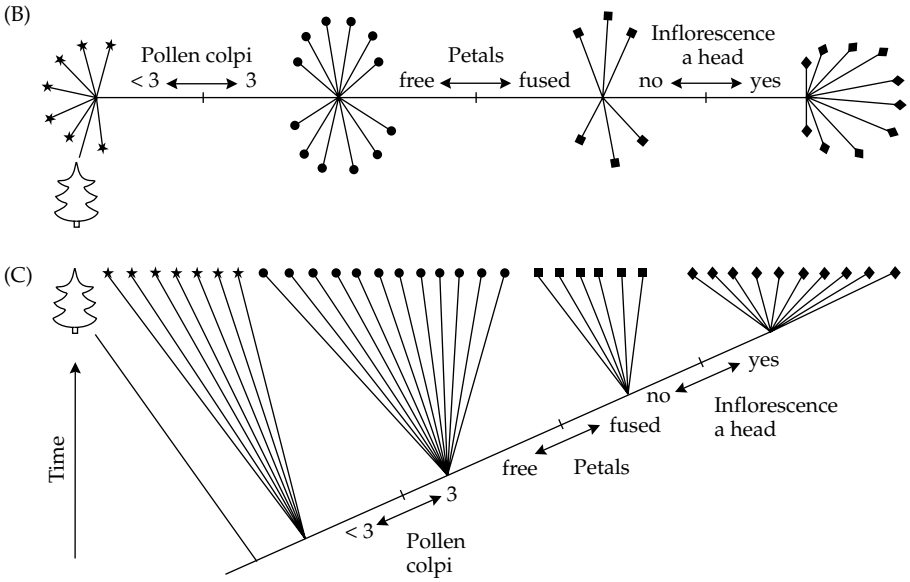


Figure 2.6 (A) The matrix from Figure 2.4C, but with character states added for a conifer. (B) The unrooted network from Figure 2.4B, but with the conifer attached according to the character states in Figure 2.6A. (C) The network of Figure 2.6B rooted with the conifer. Note that the evolutionary history is now the same as in Figure 2.5A.

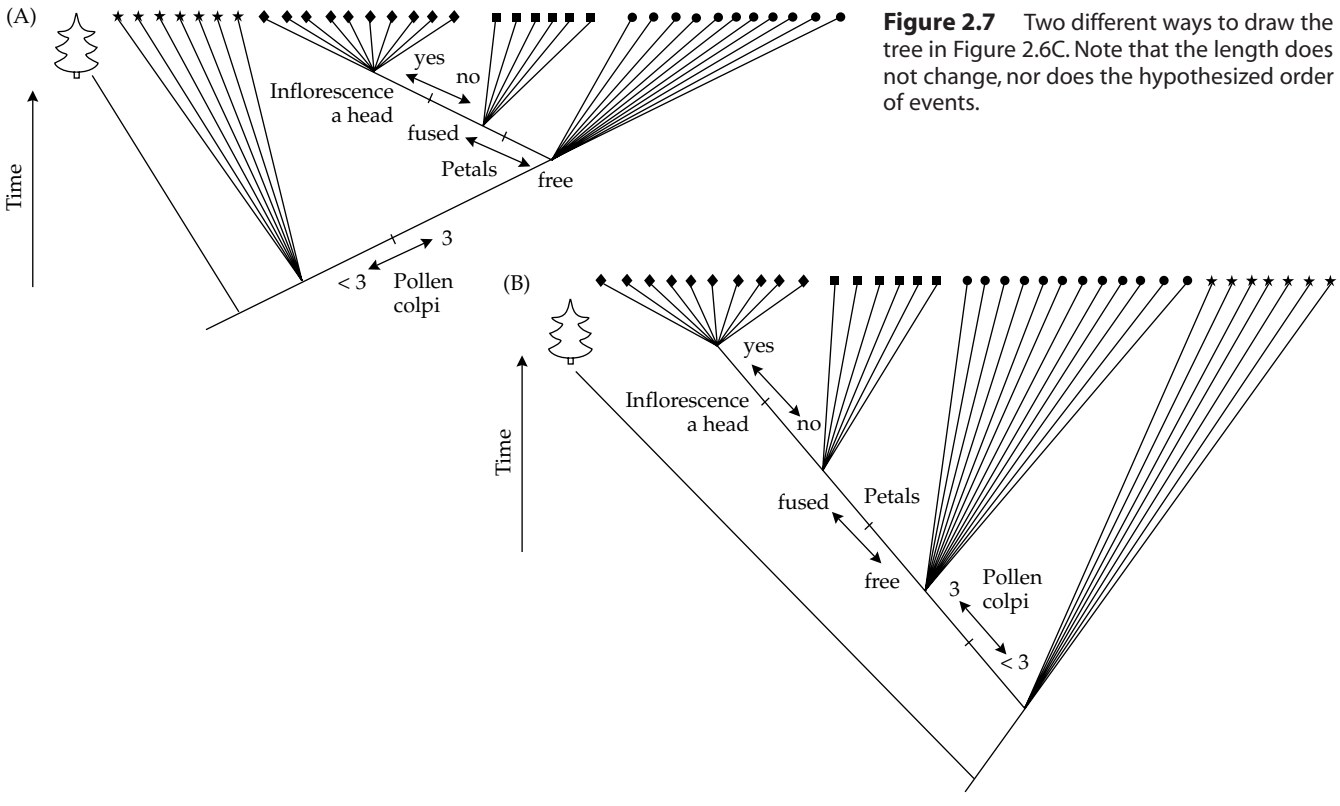


Figure 2.7 Two different ways to draw the tree in Figure 2.6C. Note that the length does not change, nor does the hypothesized order of events.

paraphyletic group includes a common ancestor and some but not all of its descendants.

Some taxonomic groups are cladistically unresolved; they cannot be determined to be either positively paraphyletic or positively monophyletic, and are referred to as **metaphyletic**. The way we have drawn the circle plants in Figure 2.5, for instance, indicates that we do not know whether they have a synapomorphy or not, and thus they form a metaphyletic group.

As mentioned earlier, a character state that is derived (synapomorphic) at one point in time will become ancestral later. In this example, tricolpate pollen is a shared derived character of a large group of flowering plants. It is a synapomorphy and indicates monophyly of the group sometimes called the eudicots. For the group with fused petals, however, tricolpate pollen is an ancestral, or **plesiomorphic** character. It is something they all inherited from their common ancestor and thus does not indicate relationship. Plesiomorphic similarities cannot show genealogical relationships in the group being studied because they evolved earlier than any of the taxa being compared, and merely have been retained in various lineages (taxa).

It is sometimes possible to determine monophyly of a group by observing that the characters do not occur in any other organism. For example, all members of the grass family (Poaceae) have an embryo that is unlike the embryo of any other flowering plant. We can thus hypothesize that the grass embryo is uniquely derived in (is a synapomorphy for) the family and indicates that the family is monophyletic. This is the same as saying that any reasonable rooting of the phylogenetic tree will lead to the same conclusion.

It is often possible to find evidence that a group is monophyletic even without a large computer-assisted phylogenetic analysis. Indeed, most cladistic analyses were done by hand until the mid-1980s. Characters are divided into character states, as with any cladistic analysis. The character state in the outgroup (or outgroups) is then assumed to be ancestral (Stevens 1980; Watrous and Wheeler 1981; Maddison et al. 1984). In other words, the character is polarized, or given direction. The shared derived, or synapomorphic, state can then be used as evidence of monophyly, and cladograms can be constructed on the basis of shared derived character states. This kind of thinking is often useful in providing a first guess as to whether taxonomic groups might be monophyletic and thus named appropriately.

CHOOSING TREES

As can be seen from the preceding sections, determining the evolutionary history of a group of organisms is conceptually quite simple. First, characters are observed and divided into character states. Second, using the character states, a Venn diagram (Figure 2.4A), character × taxon matrix (Figure 2.4C), and branching network (Figure 2.4B) can be constructed. Third, using an outgroup, the network can be rooted to produce an evolutionary tree, cladogram, or phylogeny.

Two phenomena, however, make it much harder in practice to determine evolutionary history: **parallelism** and **reversal**, which sometimes are referred to together as **homoplasy**. Parallelism is the appearance of similar character states in unrelated organisms. (Many authors make a distinction between parallelism and convergence, but for this discussion we will treat them as though they are the same.) A reversal occurs when a derived character state changes back to the ancestral state. To provide a clear example, divide the group that we have called "star plants" into black star plants, gray star plants, and white star plants. Let us assume that the gray star and white star plants have only one cotyledon, whereas all the rest of the organisms have more than one (including the conifer). Let us further assume that the white star plants have fused petals. Add the character cotyledon number to the matrix in Figure 2.6A to give the matrix in Figure 2.8A, which gives the same information as the network in Figure 2.8B.

Now we see that, according to this network, there have been *two* changes in petal fusion. Counting the number of changes on this network (its length), we find five: one each in pollen colpi, flowers in heads, and cotyledon number, and two in petal fusion.

A group based on fused petals would be considered **polyphyletic**. Polyphyletic groups have two or more ancestral sources in which the parallel similarities evolved. (Although we distinguish here between paraphyletic and polyphyletic, many systematists have observed that the difference is slight, and simply call any para- or polyphyletic group non-monophyletic.) Petal fusion in this case is nonhomologous since it fails the ultimate test of homology— congruence with other characters in a phylogenetic analysis.

Why not draw the network in such a way that petal fusion arose only once? Such a network is shown in Figure 2.8C. Now we have one change in petal fusion, but that requires two changes in cotyledon number, and also two changes in number of pollen colpi, making the network six steps long.

Each of the networks (Figures 2.8B and C) can be converted to a phylogeny by rooting at the conifer, but they make different suggestions about how plants have evolved. In one case, cotyledon number and number of pollen colpi have been stable over evolutionary time, whereas petal fusion has appeared twice, independently. In the other case, we postulate that cotyledon number and number of pollen colpi have changed twice over evolutionary time, while petal fusion has evolved only once. By drawing either of these networks, we are making a hypothesis about how evolution has happened—about which genetic changes have occurred, at what frequency, and in which order.

As you can see, the hypotheses differ. How do we determine which is correct? There is no way to be certain.

(A)

	Pollen grooves	Petals	Inflorescence in heads	Cotyledon number
Black star plants	< 3	free	no	2
Gray star plants	< 3	free	no	1
White star plants	< 3	fused	no	1
Circle plants	3	free	no	2
Square plants	3	fused	no	2
Diamond plants	3	fused	yes	2
Conifer	< 3	not applicable	not applicable	> 2

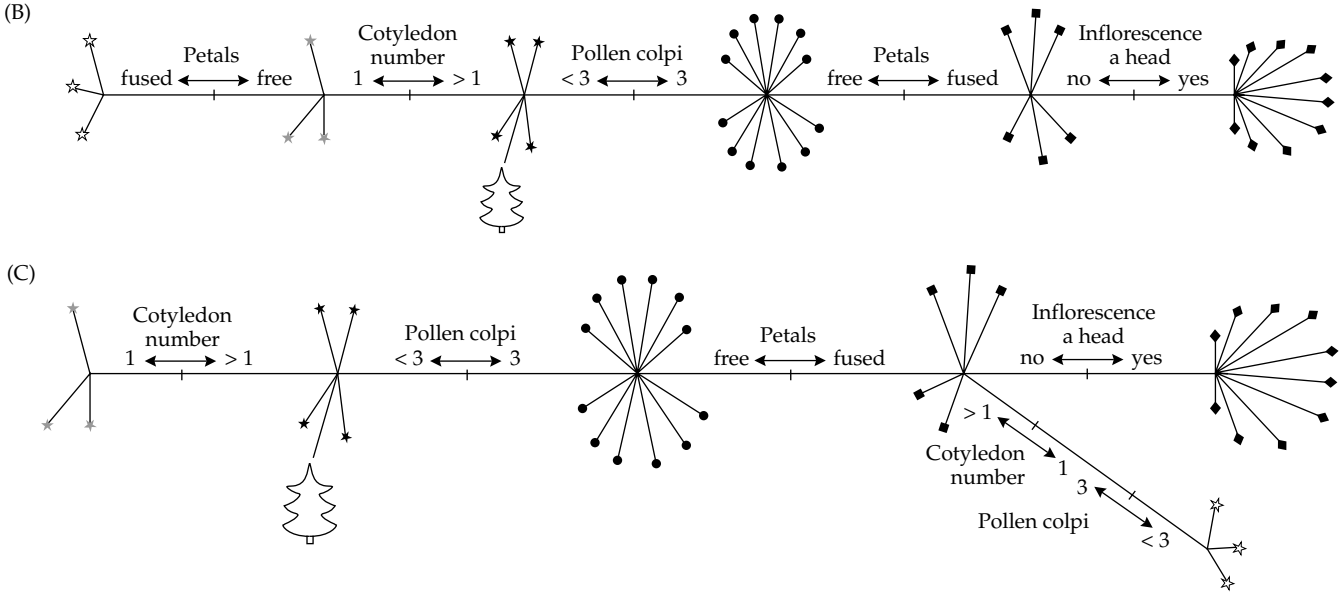


Figure 2.8 (A) A plant by character matrix. (B) Unrooted network based on the matrix in Figure 2.8A. Note that petal fusion appears to change twice. Network length is 5. (C) Another possible unrooted network based on the matrix in Figure 2.8A. Unlike the network in Figure 2.8B, petal fusion changes only once, but cotyledon number and pollen colpi change twice. Network length is 6.

No one was there to watch the evolution of these plants. We can, however, make an educated guess, and some guesses seem more likely than others to be correct. One way to proceed is to ask, "What is the simplest explanation of the observations?" This rule, used throughout science, is known as **Ockham's razor**: Do not generate a hypothesis any more complex than is demanded by the data. Applying this principle of simplicity, or **parsimony**, leads us to prefer the shorter network. The fact that it is shorter does not make it correct, but it is the simplest explanation of the data.

There are other ways to construct and to choose among evolutionary networks and trees. We have presented a simple step-counting (parsimony) method here because it is the most widely used, the most easily applicable to morphological changes, and possibly also the most intuitive method. Parsimony works well when evolutionary rates are not so fast that chance similarities (due to the evolution of identical derived characters independently in two or more lineages) overwhelm characters shared by the common ancestor.

Other methods use other optimality criteria. Instead of choosing the tree with the fewest evolutionary changes, one could convert the character matrix to a measure of similarity or dissimilarity among the plants, and then build a network that minimizes the dissimilarity; this is known as the **minimum distance method**. Alternatively, one could develop theories about the probability of change from one character state to another and then use those probabilities to calculate the likelihood that a given branching diagram would lead to the particular set of data observed. The tree with the highest likelihood is preferred—the **maximum likelihood method** (Felsenstein 1981; Hillis et al. 1993; Huelsenbeck 1995; Swofford et al. 1996). Maximum likelihood methods are particularly suited to molecular data (see Chapter 5), for which it is easier to model the probability of genetic changes (mutations). For a more comprehensive description of methods of phylogeny reconstruction, see Swofford et al. (1996).

In the examples we have presented, in which there are few characters and little homoplasy, it is easy to construct the shortest network to link the organisms. In most real

cases, however, there are many possible networks, and it is not immediately obvious which one is the shortest. Fortunately, computer algorithms have been devised that compare trees and calculate their lengths. Some of the most widely used of these are *PHYLIP* (Felsenstein 1989), *hennig86* (Farris 1989), *NONA* (Goloboff 1993), and *PAUP* 3.1.1/*PAUP** 4.0 (Swofford 1993). These programs either evaluate data over all possible trees (an exhaustive search), or make reasonable guesses as to the topology of the shortest trees (branch-and-bound searches or heuristic searches). In analyses of numerous taxa, only heuristic algorithms can be used. These algorithms may not succeed in finding the shortest tree or trees because of the large number of possible dichotomous trees. For example, the possible interrelationships of three taxa can be expressed by only three rooted trees, [A(B,C)], [B(A,C)], and [C(A,B)]. But the number of potential trees expands rapidly given larger numbers of taxa; for example, four taxa yield 15 trees, five yield 105 trees, six yield 945 trees, and ten yield 34,459,425 trees!

SUMMARIZING EVOLUTIONARY TREES

Often parsimony analyses will find multiple trees, all with the same length but with different linkages among the taxa. Sometimes, too, different methods of analysis will find trees showing different topologies and therefore different histories for the same taxa. In addition, studies using different kinds of characters (e.g., gene sequences, morphology) may find still other trees. Rather than choosing among the trees in these cases, often systematists simply want to see what groups are found in all the shortest trees, or by all methods of analysis, or among different kinds of character matrices. The information in common in these trees can be summarized by the use of a **consensus tree**.

Strict consensus trees contain only those monophyletic groups that are common to all trees. For example, analyses of different sets of data have produced different results regarding the early evolution of the angiosperms. A study of morphological characters and 18S rRNA sequences led to the evolutionary tree shown in Figure 2.9A (Doyle et al. 1994; we have omitted some taxa for the purposes of this example). A study of *rbc*L sequences led to the tree in Figure 2.9B (Albert et al. 1994). For descriptions of 18S rRNA and *rbcL* data, see Chapter 5. The trees both show *Gnetum* as sister to *Welwitschia*, and those two as sister to *Ephedra*. (These three genera together make up the gnetophytes; see Chapter 7.) Both trees also suggest that the Calycanthaceae and Laurales are closely related (see Chapter 8). The strict consensus of the two cladograms (Figure 2.9C) also shows the Gnetophytes and the Calycanthaceae/Laurales clade.

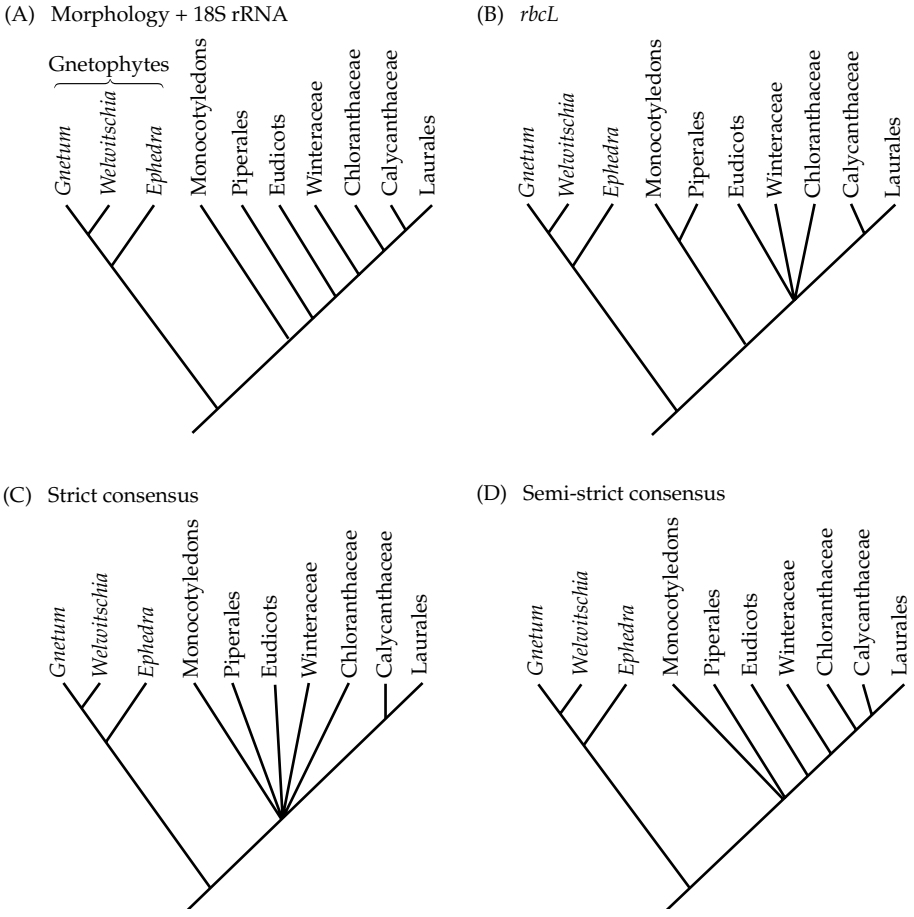


Figure 2.9 (A) Phylogeny of angiosperms based on data from morphology and 18S rRNA sequences (Based on Doyle et al. 1994). (B) Phylogeny of angiosperms based on data from *rbcL* sequences. (C) Strict consensus of trees in A and B. (D) Semi-strict consensus of trees in A and B. (Modified from Albert et al. 1994.)

There are differences between the two evolutionary hypotheses, however. The *rbcL* tree suggests that the Piperales are sister to the monocots, but the morphology/rRNA tree tells us that the Piperales arose after the monocot lineage diverged, such that the Piperales are more closely related to the rest of the dicots. In Figure 2.9B, the eudicots, Winteraceae, and Chloranthaceae appear as though they arose at the same time. This means that *rbcL* data cannot tell us whether they did arise together, or one after the other, nor can we determine the order. Having multiple lineages arising at the same apparent point in the diagram is usually an expression of ambiguity. The difference in the position of the Piperales combined with the ambiguity in the *rbcL* tree leads us to conclude that we really do not know which early angiosperm lineages appeared first. This is reflected in the strict consensus tree by drawing all those lineages as though they arose at the same time.

When many trees are being compared, it is sometimes interesting to know whether a clade appears in most of the trees, even if it doesn't occur in all of them. A **majority-rule consensus tree** can show all groups that appear in 50% or more of the trees. If a particular clade is present in the majority of the most-parsimonious trees, then this clade will be represented on the majority-rule tree (along with an indication as to the percentage of most-parsimonious trees showing that clade). The majority-rule consensus tree will be inconsistent with some of the original trees, and thus provides only a partial summary of the phylogenetic analyses.

A **semi-strict**, or **combinable component**, consensus tree is often useful, particularly when comparing phylogenies with slightly different terminal taxa, or from different sources of characters. It is common, for example, to construct trees from two different sets of characters (e.g., a gene sequence and morphology) and to find that both sets of characters indicate monophyly of a particular group of species. Only one set of characters, however, may resolve relationships among the species. The semi-strict consensus then indicates all relationships supported by one tree or both trees and not contradicted by either. For example, although the *rbcL* tree (Figure 2.9B) does not give us information on the order in which eudicots, Winteraceae, and Chloranthaceae originated, the tree in Figure 2.9A does. The two trees are not really conflicting; the morphology/rRNA tree just provides more precise information. The semi-strict consensus thus follows the morphology/rRNA arrangement of those three groups (Figure 2.9D).

THE PROBABILITY OF EVOLUTIONARY CHANGE IN CHARACTERS

In trying to infer the evolutionary history of a group, we depend on an implicit or explicit model of the evolutionary process. The more accurately the model reflects the underlying process, the more accurately we will be able to estimate the evolutionary history. For nucleotides in a DNA sequence, the starting point is usually a model in which mutation is assumed to be random, although this assumption is often modified to reflect hypothesized mechanisms of molecular evolution. The model is much more difficult for morphological characters, because we usually have no idea how many genes are involved, nor do we know what kinds of changes in the genes lead to different character states. Nonetheless, certain assumptions must be made if one is to proceed at all. (And, we note, there are *no* methods that are entirely free of assumptions!) The major assumptions have to do with the likelihood of particular changes of character states, and the likelihood of reversals and parallelisms.

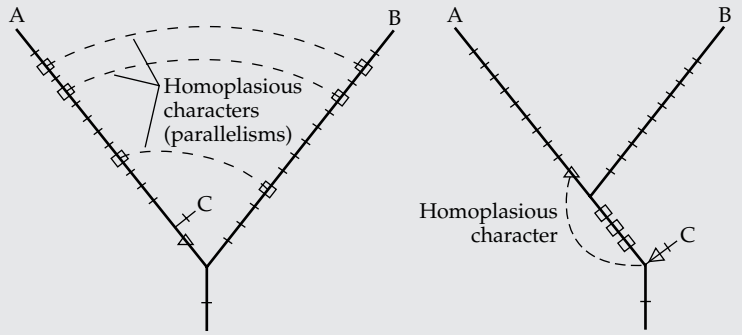
Ordering character states The characters in Figure 2.8A have only two states. Such two-state characters are interpreted as representing a single genetic switch— "on" producing one state (e.g., pollen is tricolpate), "off" resulting in the other state (e.g., pollen is one-grooved, or monosulcate). Over evolutionary time, of course, such characteristics can continue to change. For example, tricolpate pollen is modified in some Caryophyllales so that it is spherical, with many pores evenly spaced around it (looking rather like a golf ball); this pollen is pantoporate. If we were to include the character "pollen grooves" in a matrix, it would now have three states—monosulcate, tricolpate, and pantoporate. This is now a **multistate character**, in contrast to the two-state or **binary** characters discussed previously. Multistate characters create a dilemma: how many genetic switches are involved?

It is possible that monosulcate pollen changed to tricolpate, which then changed to pantoporate pollen, and this actually matches what we think happened in evolutionary time (Figure 2.11A). (Recall that the outgroup does not have tricolpate pollen.) This implies two genetic switches. It also implies that they must have occurred in order—pantoporate pollen could arise only after tricolpate pollen. If we accept this series of events, the multistate character is considered to be **ordered**. If we decide to allow for reversals of character states—that is, consider the possibility that pantoporate pollen might switch back to tricolpate and tricolpate to monosulcate pollen—the character is still ordered. It requires two evolutionary (genetic) steps to go from monosulcate to pantoporate pollen, or two to go from pantoporate to monosulcate pollen. A phylogenetic analysis in which all characters are treated as ordered is sometimes referred to in the literature as **Wagner parsimony**.

If we didn't know anything about the plants involved, we might consider the possibility that monosulcate pollen might have changed to tricolpate pollen, and, in an independent event, monosulcate pollen might have changed to pantoporate pollen (Figure 2.11B). This would suggest that there is a genetic switch from monosulcate to tricolpate pollen and there is also a switch that allows change from monosulcate to pantoporate pollen, but a change from tricolpate to pantoporate pollen is impossi-

BOX 2A *Long Branch Attraction*

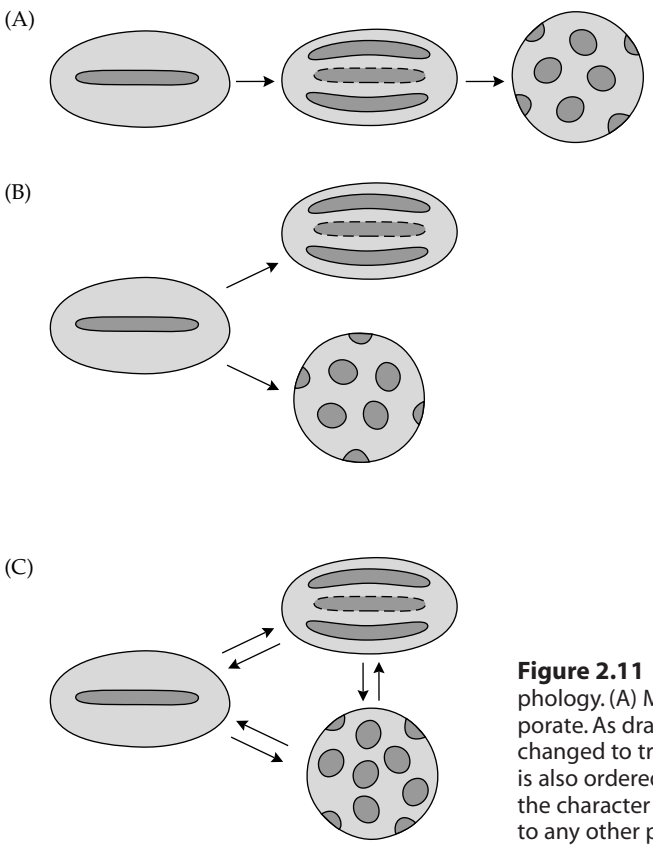
If there are great differences in the rates of character evolution between lineages such that some lineages are evolving very rapidly, and if the pattern of variation is sufficiently constrained (i.e., only a limited number of character states exist), then unusually long branches tend to be connected to each other whether or not they are actually closely related (Figure 2.10; Felsenstein 1978). This occurs because the numerous random changes, some of which occur in parallel in the two lineages, outnumber the information that shows common ancestry. The problem cannot be circumvented by acquiring more characters; these would merely add to the number of parallelisms linking the two rapidly evolving lineages. This situation, often called **long branch attraction** or the "Felsenstein zone," can affect all methods of tree construction. With the correct model of evolution, however, maximum likelihood methods will not have this problem (although determining the correct model may be difficult). The situation basically is a sampling problem, and may be alleviated by including taxa that are related to those terminating the long branches.



(A) True phylogeny: 28 steps

(B) Tree generated by parsimony analysis: 26 steps

Figure 2.10 Long branch attraction, a situation in which strongly unequal evolutionary rates cause parsimony to fail. (A) True phylogeny. Dotted lines show character states that have arisen in parallel in the lineages leading to A and B. (B) Phylogeny as reconstructed by parsimony. The number of parallelisms shared by A and B is greater than the number of characters linking A and C, so A and B appear to be sister taxa, with parallelisms (in the true phylogeny) treated as shared derived characters of A and B.



ble. The character in this case is still ordered, but in a different way from Figure 2.11A. If reversals are possible, then it requires two steps to get from tricolpate to pantoporate and two from pantoporate to tricolpate pollen.

With morphological characters and character states, we are usually unsure of which switches are possible, so it is common to treat multistate characters as unordered (Figure 2.11C); this is sometimes called **Fitch parsimony**. In the case of an unordered character, we postulate only one switch between any two states. DNA sequence characters are multistate characters with four states (adenine, thymine, guanine, cytosine). To treat these as ordered would be nonsensical; adenine does not need to change to cytosine before changing to guanine. DNA characters are therefore always treated as unordered and fully reversible.

Reversals, parallelisms, and character weighting In the network in Figure 2.8B, we hypothesized that petal fusion arose twice, independently. To make the slightly longer network in Figure 2.8C, we had to let cotyle-

Figure 2.11 Three alternative hypotheses about the evolution of pollen morphology. (A) Monosulcate changed to tricolpate, which then changed to pantoporate. As drawn, the character is ordered and irreversible. (B) Monosulcate changed to tricolpate and independently changed to pantoporate. The character is also ordered and irreversible. If the arrows were drawn as double headed, then the character would be interpreted as reversible. (C) Any pollen type can change to any other pollen type. The character is unordered and reversible.

don number change from one to more than one and back to one again—that is, to reverse. In comparing the trees in Figures 2.8B and 2.8C, therefore, we are comparing the hypotheses that (1) mutations in the genes leading to petal fusion have happened more than once versus (2) mutations in the genes controlling cotyledon number have happened and then their effects have been reversed. In deciding that the network in Figure 2.8B was shorter than the one in Figure 2.8C, we counted all the steps equally, whether they were parallelisms, reversals, or unique origins.

This may or may not be reasonable. Dollo's law, for example, suggests that for very complex characters, parallel origin is highly unlikely, whereas reversal may be quite easy (Mayr and Ashlock 1991). The assumption is that many genes must change to create a morphological structure, but only one of those genes needs to be modified to lose it. Dollo's law can be built into the process of choosing a tree by making gains of structures count for more than losses; the process is then known as **Dollo parsimony**. (To define the terms "gain" and "loss," of course, requires a rooted tree; hence Dollo parsimony cannot be applied to an unrooted network.)

Certain characters are sometimes **weighted** in cladistic analyses. This reflects the assumption that certain characters should be harder to modify in evolutionary time than others. One might hypothesize, for example, that leaf anatomy is less likely to change than leaf hairiness (pubescence), and therefore a change in a leaf anatomical character could be counted as equivalent to two changes in pubescence for the purposes of counting steps in the tree. Such weighting decisions can easily become subjective or arbitrary, and risk biasing the outcome of the study toward finding particular groupings. (For example, the investigator might theorize, "My favorite species group has interesting leaf anatomy; therefore I think that leaf anatomy is phylogenetically important; therefore I will give it extra weight in the phylogenetic analysis." In this case, it is no surprise when the favorite species group is shown to be monophyletic.)

Because of the possibility of bias, systematists generally attempt to base weighting decisions on an objective criterion. One approach is to do a preliminary phylogenetic analysis with all characters assigned equal weights. The results of this analysis will identify which characters have the least homoplasy on the shortest tree(s); these characters with less homoplasy can then be given more weight in subsequent analyses, a process known as **successive weighting**.

Another approach is to base weights on knowledge of the underlying genetic basis of characters. For example, transversions (purine → pyrimidine or pyrimidine → purine changes) are weighted over transitions (purine → purine or pyrimidine → pyrimidine changes) because transitions are known to occur more frequently and be easier to reverse. Restriction site gains may be weighted over site losses because there are fewer ways to gain a restriction site than to lose one (see Chapter 5). And complex characters (presumably controlled by many genes) may be weighted over simple characters (presumably controlled by fewer genes), again because the latter are thought to be more labile over evolutionary time.

The most common approach, used in most preliminary analyses, is to consider all characters of equal weight. Although this sometimes is described as "unweighted," in fact it assumes that all characters are equally likely to change and weights them accordingly.

Underlying all discussion of weights is the assumption that characters of organisms evolve independently. This assumption requires that change in one character does not increase the probability of change in another character. As with the previous assumption, this one may be violated frequently. For example, a change in flower color may well lead to a shift in pollinators, which would then increase the probability that corolla shape would change. Violating this assumption obviously affects character weighting, in that the likelihood of change of two characters is not the same.

DO WE BELIEVE THE EVOLUTIONARY TREE?

An evolutionary tree is simply a model or hypothesis, a best guess about the history of a group of plants. It follows that some guesses might be better, or at least more convincing, than others. Much of the current literature on phylogeny reconstruction involves ways of determining how much credence we should give to a particular evolutionary tree. Use of an optimality criterion is one way to evaluate the evolutionary tree; of all possible descriptions of history, we prefer the one that requires the fewest steps, or gives the maximum likelihood, or the minimum distance. It is usually possible to evaluate trees more precisely, however. For the purposes of this discussion, we will continue to focus on phylogenies generated according to maximum parsimony (i.e., the fewest evolutionary steps).

Measuring support for the whole tree: Assessing homoplasy Parsimony analyses minimize the number of characters that change in parallel or reverse. If there are many such homoplasious characters, then it is possible that the phylogenetic tree is an artifact of the characters we have chosen, and a slight change in characters will lead to a different tree. The simplest, and most common, measure of homoplasy is the **consistency index** (CI), which equals the minimum amount of possible evolutionary change (the number of genetic switches) divided by the actual tree length (the number of actual genetic changes on the tree). In the network of Figure 2.4B, each of the three characters represents a single genetic switch, and each one changes only once, so the consistency index is 3/3 = 1.0. In the network in Figure 2.8B, there are four binary (one-switch) characters, but one of them (petal fusion) changes twice on the tree, so that the consis-

tency index is 4/5 = 0.80. Consistency indices may also be calculated for individual characters and equal the minimum number of possible changes (one, for a binary character) divided by the actual number of changes on the tree. For example, the CI of petal fusion (Figure 2.8B) is 1/2 = 0.50. For a given matrix (set of characters and taxa), the shortest network or tree will also have the highest consistency index. Lower consistency indices indicate many characters that contradict the evolutionary tree.

Comparing consistency indices across data sets is hazardous because the CI has some undesirable properties. For one thing, a character that changes once in only one taxon will have a consistency index of 1.0, but it in fact says nothing about relationships. Such a uniquely derived character is sometimes called an **autapomorphy**. For example, if one of the black star plants in Figure 2.8B had hairy leaves while all other plants studied had hairless ones, leaf hairiness would not be of any help in indicating the relationship of the hairy-leaved plant. The character is **uninformative**. Because the uninformative character changes only once, however, it has a CI of 1.0. If we added many uninformative characters into the analysis, the overall CI would be inflated accordingly and would give a misleading impression that many characters supported the tree. Uninformative characters, therefore, are often omitted before calculating the consistency index.

The consistency index is also sensitive to the number of taxa in an analysis (Sanderson and Donoghue 1989): analyses with many taxa tend to have lower CIs than analyses with fewer taxa. This occurs with both molecular and morphological data, and with analyses of species, genera, or families.

Other measures are used to describe how characters vary over the tree. One of these, the **retention index** (RI), is designed to circumvent another limitation of the CI (Forey et al. 1992; Wiley et al. 1991). The CI is designed to vary between near 0 (a character that changes many times on the tree) and 1.0 (a character that changes only once). But consider the plants in Figure 2.8 once more. For those described by the matrix in Figure 2.8A, only two groups—the white star plants and the gray star plants—have a single cotyledon. If the single-cotyledon plants form a clade, as in Figure 2.8B, then the CI for cotyledon number is 1.0. If they are unrelated, as in Figure 2.8C, then the CI is 0.5 (1/2), which is the lowest possible value on the tree. Thus, instead of varying between 0 and 1, the CI in this case varies between 0.5 and 1.0. The RI corrects for this narrower range of the CI by comparing the actual number of changes in the character to the maximum possible number of changes.

The RI is computed by calculating the maximum possible tree length, which is the length that would occur if the derived character state originated independently in every taxon in which it appears (i.e., if all taxa with the derived character state were unrelated). The minimum tree length and actual tree length are computed the same way they are for calculating the CI. The RI then equals the maximum length minus the actual length, divided by the maximum length minus the minimum length, or $(Max - L)/(Max - Min)$. In Figure 2.8B, then, the RI is $(9 - 5)/(9 - 4) = 4/5 = 0.80$.

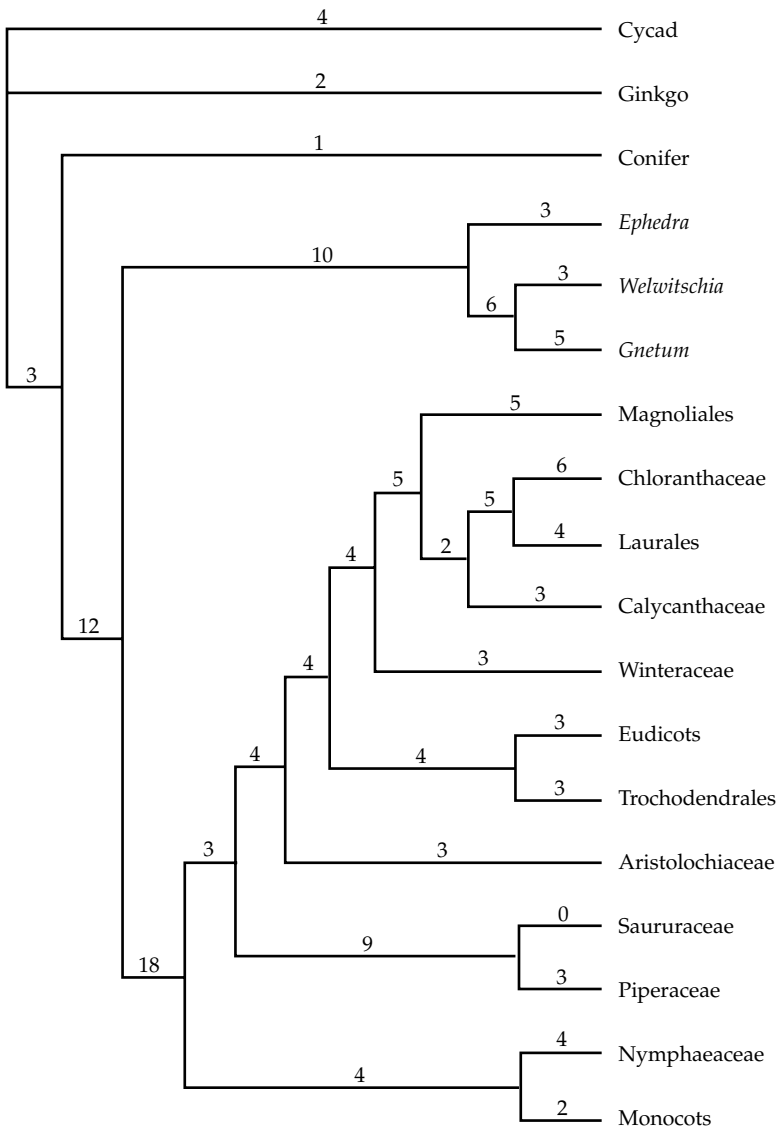


Figure 2.12 Phylogeny of the angiosperms, based on data from Doyle et al. (1994). Numbers above branches indicate the number of characters changing along that branch.

Measuring support for parts of trees With parsimony methods, the shortest available tree is preferred over one that is longer. It is possible, however, that some parts of the tree are more reliable than others. This will occur if reversals and parallelisms (or simple misinterpretation of characters) affect some groups of plants more than others, or if there were very few evolutionary changes in the history of a particular group. One simple way to evaluate this is to note the number of genetic changes that occur on the branch leading to a particular group, along with the consistency indices of the characters. For example, one of the morphological trees produced by Doyle et al. (1994) found 18 changes on the branch leading to the angiosperms (Figure 2.12), and of these 11 were in characters that had a CI of 1.0. In other words, over half of the genetic changes that occurred during the origin of the angiosperms produced novel characteristics, found nowhere else. Groups like the angiosperms that share numerous characters that do not change elsewhere on the cladogram are more believable than a group that shares only a few highly homoplasious characters.

Another way to assess how well the data support the tree is to determine whether a group of interest occurs in other trees that are almost equally short. Suppose, in other words, we ask if there are other ways to analyze the homoplasious characters that lead to trees that are one, two, or three steps longer.

For example, in the tree shown in Figure 2.12, the shortest trees indicate that the earliest diverging lineages in the angiosperms were the monocots and the water lilies (Nymphaeaceae; see Chapter 8). This implies that the character of herbaceous stems is gained once and then lost, whereas reducing the number of ovules per carpel to one occurs only once, and oil cells are gained once and lost once (Figure 2.13A). On the other hand,

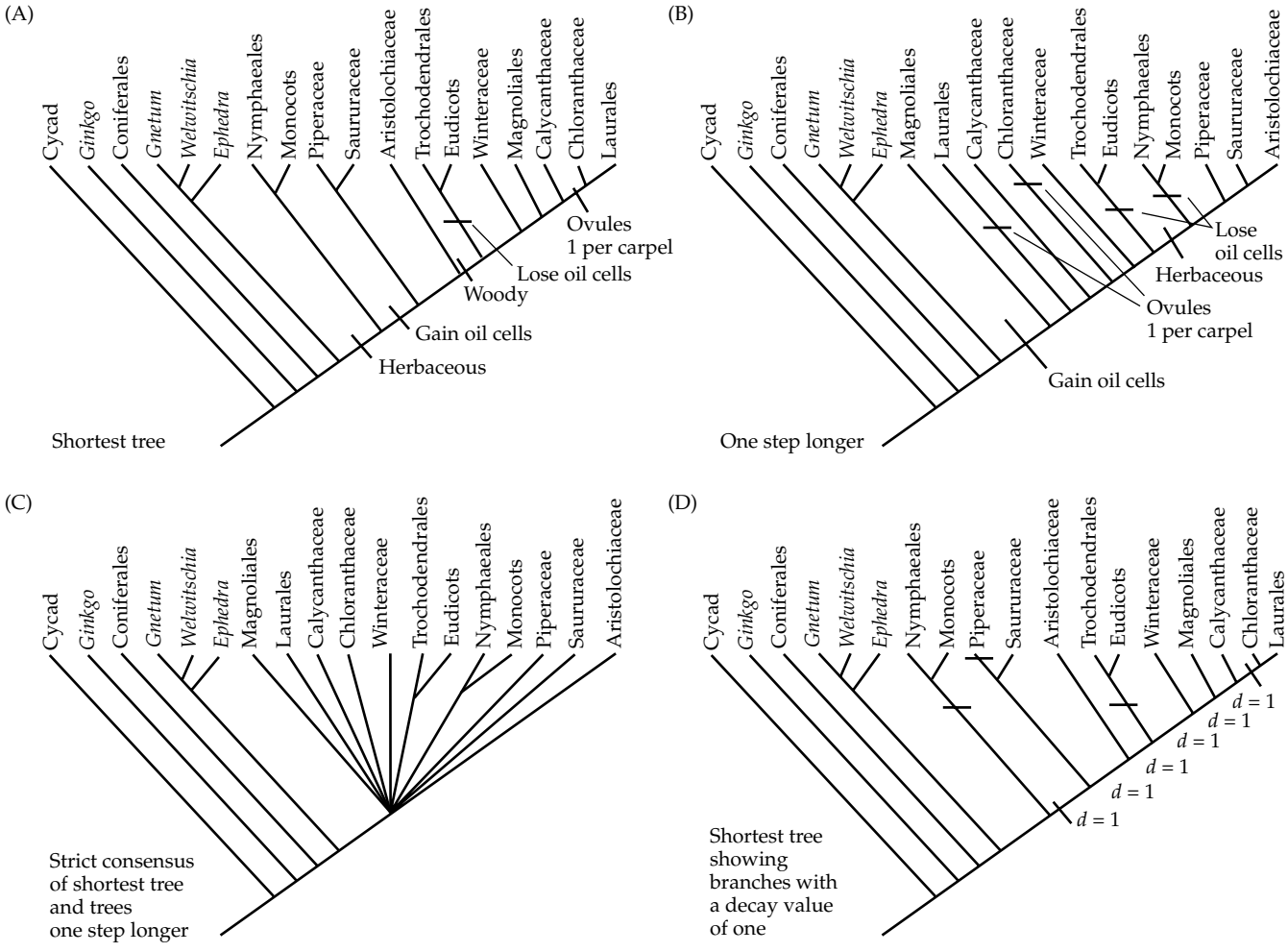


Figure 2.13 (A) The same tree as in Figure 2.12, indicating patterns of change in presence/absence of oil cells, ovule number per carpel, and plant habit. (B) An alternative tree, only one step longer than the tree in Figure 2.13A, showing patterns of change in the same characters. Note that herbaceousness now is hypothesized to have evolved only once, but loss of oil cells and reduction of ovule number occur twice. (C) Strict consensus of the shortest trees and trees one step longer (Figures 2.13A and B). (D) The same tree as Figures 2.12 and 2.13A, showing branches with a decay value of one. (Data from Doyle et al. 1994.)

trees one step longer, in which the earliest angiosperm lineages led to the magnolias, suggest that herbaceous stems evolved once, but reduction in ovule number occurred twice, and there were three changes in oil cells (gained once and lost twice or vice versa) (Figure 2.13B). Thus, by looking at trees one step longer, some characters are hypothesized to be less homoplasious, but some to be more so. If we now take the strict consensus of all the trees, including the shortest ones and those one step longer, all the early angiosperm lineages are drawn as though they radiate from a single point, indicating uncertainty about the order in which they evolved (Figure 2.13C). In other words, many of the branches that are evident in the shortest trees do not appear in trees one step longer. Thus all those branches are not drawn in the strict consensus; they "collapse." This can be indicated by placing a one next to each of the collapsing branches of the shortest tree (Figure 2.13D). The number is the **decay index**, sometimes called Bremer support, which represents how many extra steps are required to find trees that do not contain a particular group. It provides a relative measure of how much the homoplasy in the data affects support for a particular group.

The decay index is not statistical, which, depending on one's point of view, is either a virtue or a drawback. Because history, and therefore the phylogeny, happened only once and cannot be repeated, it is impossible to replicate the evolutionary experiment. It is certainly possible, however, to test whether character data are different from random, although there are many possible ways to randomize systematic data. Many tests have been devised that use some sort of randomization technique. Probably the most widely used is bootstrap analysis.

Bootstrap analysis randomizes characters with respect to taxa. As an example, begin with the matrix in Figure 2.8A and randomize the columns while leaving the rows in place. Choose a column at random to become the first column of the new matrix. Then choose another column from the original matrix to become the second column, and so on until a new matrix is created with the same number of columns as the original. Because one returns to the original matrix each time to choose a new column, some characters may be represented several times in each new matrix, while others are omitted. This is usually described as random sampling with replacement. Thus, Figure 2.14 shows the matrix in Figure 2.8A sampled with replacement; note that the first character (pollen colpi) has been selected twice, whereas the third character (inflorescence a head) was missed by the random selection process. Multiple such randomized matrices are constructed, and the most-parsimonious tree(s) found for each new matrix. This leads to a set of at least 100 trees, which can be summarized by a consensus tree (see pages 18–19). In the bootstrap consensus tree, a clade with a bootstrap value of, say, 95% was present in 95% of the cladograms generated in the bootstrap analyses.

An example of a cladistic analysis giving both bootstrap and decay values (along with branch lengths) is represented in Figure 2.15. We see that bootstrap and decay values are high for the genus *Lyonia*, indicating that the data support monophyly of the genus, whereas the linkage of *Agarista* and *Pieris* is supported by only 51% of the bootstrap trees, and in trees only one step longer the two genera are not sisters, indicated by the notation *d* (decay) = 1.

Another excellent way to gain confidence in the groupings present in a tree is to compare phylogenies that have been based on different sets of characters. For example, phylogenies based on morphology, chloroplast DNA nucleotide sequences (cpDNA), and nuclear DNA nucleotide sequences could be (and often are) compared. If these phylogenies show similar groups, then we can be more confident that they reflect the true order of events. For example, the monophyly of such families as the Poaceae, Onagraceae, Ericaceae, Asteraceae, and Orchidaceae has been supported by phylogenetic analysis of many kinds of data, including information from morphology, chloroplast gene sequences, and nuclear gene sequences.

Comparing trees is often particularly intriguing when the data come from different genes; a more extensive discussion of this is in Chapter 5. It is also common to combine morphological and DNA characters in a single phylogenetic analysis, which often leads to more strongly supported phylogenies than either sort of data can produce alone.

	Pollen colpi	Petals	Pollen colpi	Cotyledon number
Black star plants	< 3	free	< 3	2
Gray star plants	< 3	free	< 3	1
White star plants	< 3	fused	< 3	1
Circle plants	3	free	3	2
Square plants	3	fused	3	2
Diamond plants	3	fused	3	2
Conifer	< 3	not applicable	< 3	> 2

Figure 2.14 The matrix from Figure 2.8A sampled with replacement, as it would be for the first step of a bootstrap analysis. Note that in the sampling process, the character "pollen colpi" has been sampled twice, whereas the character "inflorescence a head" has been omitted.

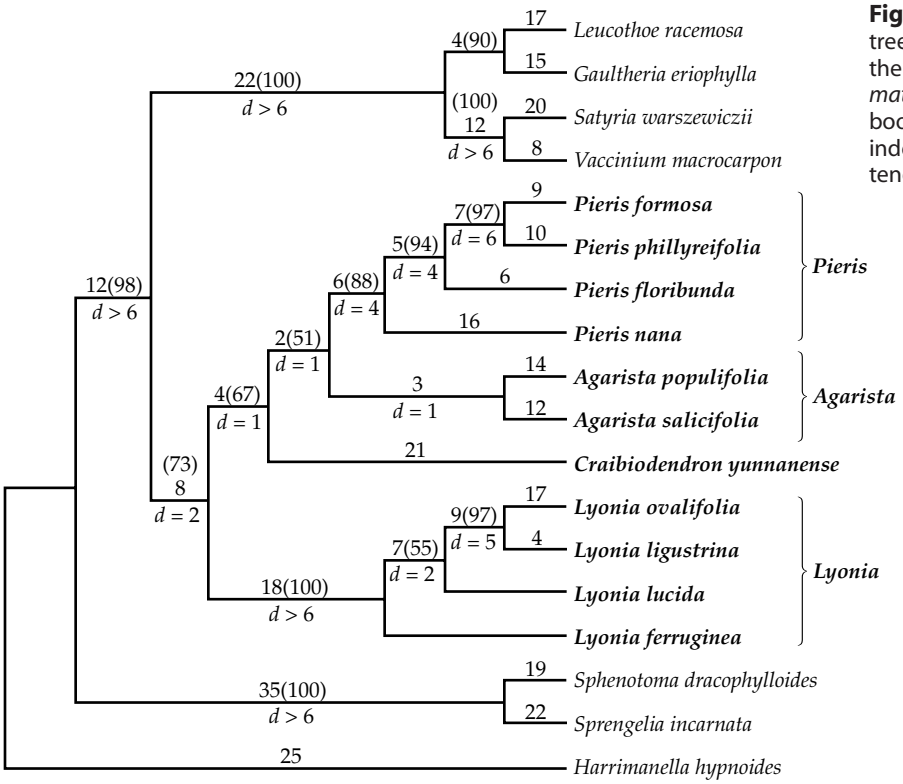


Figure 2.15 The single most parsimonious tree found in branch-and-bound analysis of the *Lyonia* group (taxa in boldface type) using *matK* data. Branch lengths appear above lines; bootstrap values are in parentheses; decay index (*d*) is below lines. Length = 425, consistency index 0.60. (From Kron and Judd 1997.)

Describing Evolution: Mapping Characters on Trees

Once created, phylogenies may be used as the basis of classification. This is one major goal of systematics and is described in detail in the next section. Phylogenies can also be used to describe the evolutionary process and to develop hypotheses about adaptation, morphological and physiological change, or biogeography, among many other uses. If a phylogeny is to be used to describe history, however, it requires careful attention to the characters and character states used in the description. In what follows we will focus on morphological characters, but many of the points apply to any sort of characters.

Consider a group of plants for which the tree is known; a good example is the Ericaceae, for which much information is available (Figure 2.16). Assume for the purposes of this discussion that this tree is an accurate

BOX 2B *Phylogenetic Analysis Assumes That Evolution Can Be Diagrammed as a Branching Tree*

Phylogenetic studies assume that after two lineages diverge from each other, they never exchange genetic information again. This assumption may in fact be violated frequently. If hybridization is common, a plant may share the derived characters of two unrelated parent plants, and the history will look more like a piece of macramé than like a tree. Phylogenetic analysis will always produce a treelike diagram, whether appropriate or not. Phylogenetic methods presuppose divergent evolution and cannot give the correct phylogeny for hybrids, which have reticulating evolutionary histories.

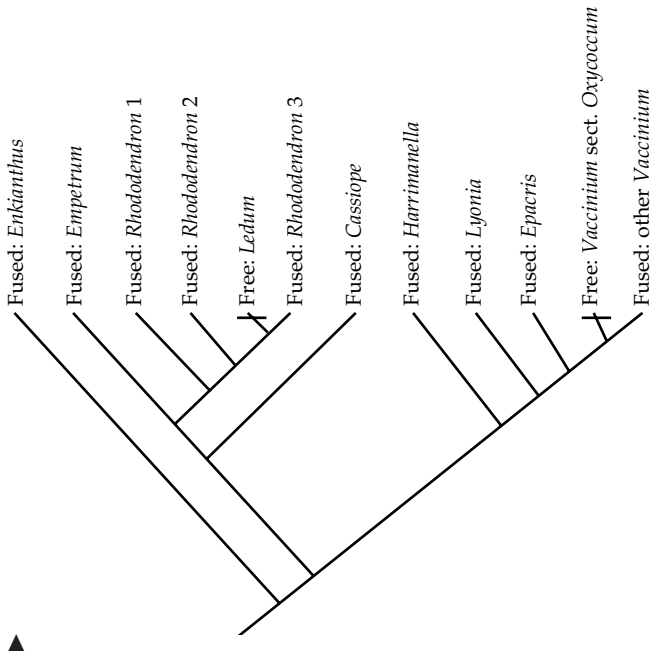
Interspecific hybridization is known to be common in plants, and the proper treatment of hybrids in cladistic analyses has been much discussed (Bremer and Wanntorp 1979; Bremer 1983; Wagner 1980, 1983; Funk 1985; Kellogg 1989; Kellogg et al. 1996). Most systematists have suggested that hybrids be identified and removed from analyses because their inclusion could lead to increased homoplasy, an increased number of most-parsimonious trees, and a distortion of the patterns of relationships among nonhybrid taxa. However, recent studies by McDade (1990, 1992, 1997) indicate that hybrids are unlikely to create problems in phylogenetic analysis unless they are between distantly related parental species. When hybrids are recognized and their ancestry determined (see Chapter 6), they can be manually inserted into the cladogram, which then indicates not only cladogenetic events (brought about through speciation) but also reticulating histories (developed through interspecific hybridization).

reflection of history, and that each of the terminal genera really is monophyletic, which has been demonstrated by studying multiple species of each. Then consider a study that is concerned with the gain or loss of fused petals, which are intimately connected with the evolution of pollination systems. This is the kind of study that systematists frequently engage in, because the details of character evolution lead to hypotheses about how natural selection has worked. Also, when constructing classifications, one frequently wants to know what morphological characters can be attributed to and distinguish a particular monophyletic group.

In Figure 2.16, we show the observed character states for the genera. It seems trivially obvious from looking at the distribution of characters and character states that free petals must have evolved in the lineage leading to *Ledum* (Labrador tea) and again in the lineage leading to *Vaccinum* sect. *Oxycoccum* (cranberries). Phrasing this another way, the ancestor of *Vaccinium* sect. *Oxycoccum* and all other vacciniums (blueberries) had fused petals, as did the ancestor of *Ledum* plus *Rhododendron* sect. 3.

Examine this "obvious" conclusion more closely. If we were studying only species of *Vaccinium*, we would have no way of knowing whether fused petals were ancestral or derived (Figure 2.17A). There must have been one genetic change, but it could as easily have happened in the lineage leading to the cranberries (sect. *Oxycoccum*) as in the lineage leading to the blueberries. It is only by reference to the outgroup *Epacris* that we can determine when petal fusion was lost. Because *Epacris* has fused petals, free petals must have originated within *Vaccinium*; it is simplest (most parsimonious) to assume just one genetic change, from fused to free (Figure 2.17B). This is the same as saying that the ancestor of blueberries plus cranberries had fused petals. If we were to postulate that the ancestor had free petals, then we would need two changes to fused petals—one in *Epacris* and one in the blueberries. The same argument applies in the case of *Rhododendron* and *Ledum*.

Now suppose that we were studying only species of *Vaccinium*, but this time, instead of using *Epacris* or other Ericaceae as outgroups, we used only *Ledum*. This could easily happen if material of the other genera were hard to obtain, or if they were extinct and we didn't even know they had existed. Now we would conclude that the ancestor of all vacciniums had free petals, and that in response to some unknown selective pressure there was a change to fused petals (Figure 2.18A). *This is exactly the opposite conclusion from the one reached above*, and the only difference is the genera included in the analysis.



▲ **Figure 2.16** Phylogeny of a portion of the Ericaceae, based on data summarized in Stevens (1998). The genus Rhododendron is paraphyletic and is represented by three separate lineages, numbered one to three. Two changes to free petals are hypothesized.

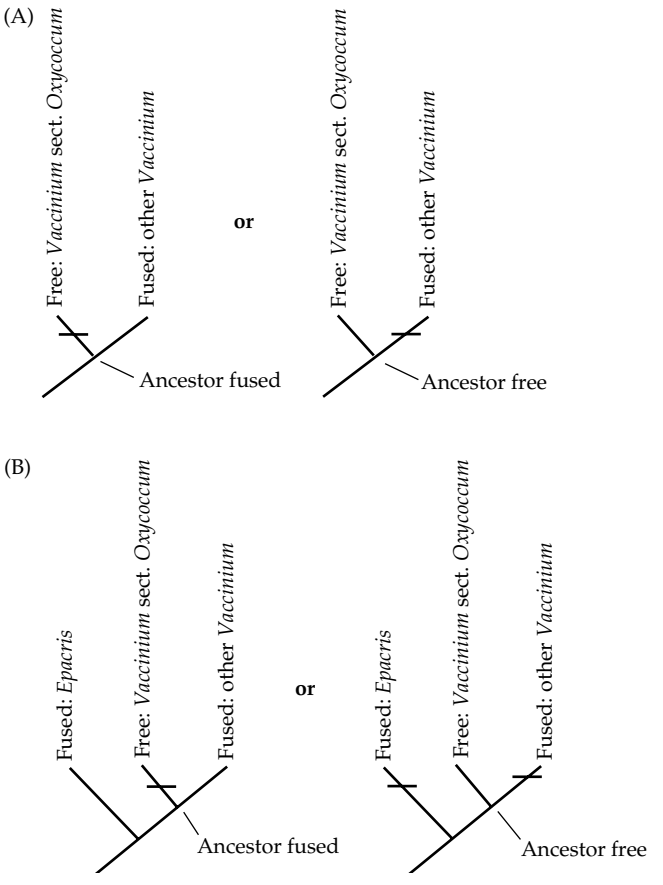


Figure 2.17 (A) Two taxa differ in character states. It is impossible to determine from this information alone what the character state of the ancestor was because either assumption will involve one change in one descendant lineage. (B) The addition of an outgroup determines the character state of the ancestor. In this case, it is simpler (requires fewer steps) to assume that the ancestor had fused petals. ►

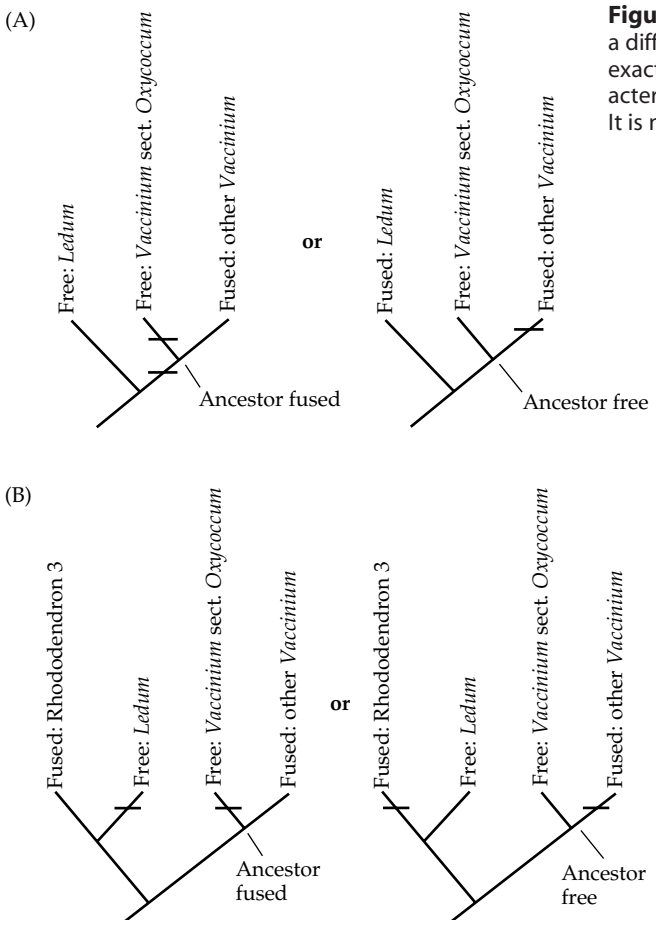


Figure 2.18 (A) Analysis of character state change in *Vaccinium* using a different outgroup. Note that the inference of the ancestral state is exactly the opposite of that reached using *Epacris*. (B) Analysis of character state change in *Vaccinium* using two outgroups that differ in state. It is now impossible to determine the character state of the ancestor.

One might try to improve the situation by using additional outgroups. For example, consider the same study of *Vaccinium*, but now use both *Ledum* and *Rhododendron* as outgroups. In this case, the direction of change is completely ambiguous (Figure 2.18B). It is as simple to postulate that the ancestor of the group had fused petals and there were two changes to free as it is to postulate that the ancestor had free petals and there were two changes to fused. These two choices are known as **equally parsimonious reconstructions**. It is safe to say that for many characters on many trees, there are multiple equally parsimonious reconstructions. In other words, there are multiple equally good hypotheses about the direction and timing of character state change. If you return to the example in Figure 2.13, you should be able to find equally parsimonious reconstructions that differ from the ones shown.

Ambiguity can also come from including taxa for which the character state is not known. Suppose, for example, two new taxa are discovered such that, on the basis of other characters, one is clearly sister to *Vaccinium* sect. *Oxycoccum*, and the other sister to the rest of *Vaccinium* (Figure 2.19). In addition, suppose that it is unclear whether the petals are fused or free. (This is more common that you might think; it can occur when the original description is vague and/or illustrations are unclear, or when the original plant is known only from fruiting material.) This now means that we do not know what the ancestral state was for *Vaccinium*, so that we cannot make any hypothesis about direction of evolutionary change. It also means that we cannot be sure that fused petals is a synapomorphy for the genus.

Various algorithms have been developed to assign character state changes to particular portions of trees (see Chapter 5 of Maddison and Maddison 1992 for a lucid and comprehensive discussion of these). Depending on the algorithm used, the character changes can be biased in favor of parallelisms (the so-called "delayed transformation," or DELTRAN algorithm) or in favor of reversals ("accelerated transformation," or ACCTRAN). The results can have implications, sometimes major, for hypotheses about the evolutionary process, and may also affect how organisms are described in a classification.

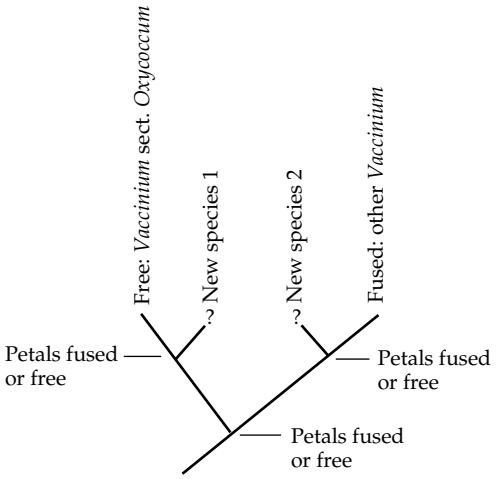


Figure 2.19 Addition of species for which the character state is unknown can prevent any inference about the ancestral state.

Constructing a Classification

The theory of classification is a topic with which systematists have been wrestling for centuries, leading to a broad and frequently contentious literature (see Chapter 3). The principles of phylogenetic classification outlined here are commonly but not universally held. In general, however, there are several goals of classification. A classification is a common vocabulary designed to aid communication. A classification should be stable; names that are frequently changed become useless for communication. A classification should be predictive; if you know

the name of a plant, it should help you to learn more about it, and guide you to its literature.

Systematists generally agree about the goals of classification, but may disagree profoundly on how to reach those goals. In this text, we take a particular point of view, using phylogenetic classifications throughout. Thus, to the greatest extent possible, we have employed monophyletic and avoided paraphyletic or polyphyletic groups. In the few cases where a non-monophyletic family or order has not yet been divided into monophyletic units, we have placed the taxon name in quotation marks. The monophyly of many genera of angiosperms is questionable, but so few phylogenetic analyses are available at this level that possible or probable paraphyly or polyphyly of genera is not indicated.

The biological diversity on Earth is the result of genealogical descent with modification, and monophyletic groups owe their existence to this process. It is appropriate, therefore, to use monophyletic groups in biological classifications, so that we may most accurately reflect this genealogical history. Classifications based on monophyletic groups will be more predictive and of greater heuristic value then those based on overall similarity or idiosyncratic weighting of particular characters (Donoghue and Cantino 1988; Farris 1979). Phylogenetic classifications, because they reflect genealogy, will be the most useful in biological fields, such as the study of plant distributions (phytogeography), host/parasite or plant/herbivore interactions, pollination biology, and fruit dispersal, or in answering questions related to the origin of adaptive characters (Brooks and McLennan 1991; Forey et al. 1992; Humphries and Parenti 1986; Nelson and Platnick 1981). Because of its predictive framework, a phylogenetic classification can direct the search for genes, biological products, biocontrol agents, and potential crop species. Phylogenetic information is also useful in conservation issues. Finally, phylogenetic classifications provide a framework for biological knowledge and the basis for comparative studies linking all fields of biology (Funk and Brooks 1990).

Constructing a classification involves two steps, the first being the delimitation and naming of groups. In a phylogenetic classification this is uncontroversial: named groups must be monophyletic. The second step involves ranking the groups and placing them in a hierarchy. This remains problematical.

GROUPING: NAMED GROUPS ARE MONOPHYLETIC

A phylogenetic classification reflects evolutionary history and attempts to give names *only* to groups that are monophyletic—that is, an ancestor and all its descendants. In the example in Figure 2.6C, we infer that the Asteraceae (diamond plants) are monophyletic because they have flowers in heads. The square plants plus Asteraceae are also monophyletic because they share the derived character state of fused petals; this group has a name, the Asteridae (or the asterid clade). Similarly, the entire group of plants with tricolpate pollen (circle plants plus Asteridae) is monophyletic and is known as the eudicots (or the tricolpate clade). This group could be given a formal Latin name, but it does not have one at the moment.

In cladistic classification, paraphyletic groups are not named. In Figure 2.6C, a group made up of square plants plus circle plants would be paraphyletic. The most recent common ancestor shared by any square plant and a circle plant (dots on the diagram) is also the most recent common ancestor of any square plant and a diamond plant. In other words, the square plants are as distantly related to circle plants as any one of them is to diamond plants. If we name a group that included the square plus the circle plants, it would imply that the two plants are closely related, whereas they are not.

There are many examples in this book of named groups of plants that we now believe to be paraphyletic. One well-known example is "bryophytes," often used to refer to the the non-vascular land plants (liverworts, hornworts, and mosses; see Figure 1.1). But the liverworts, hornworts, and mosses are more distantly related to each other than the mosses are to the vascular plants (tracheophytes). If we refer to bryophytes (without quotation marks), the name implies a closer relationship than actually exists.

Several traditionally recognized plant families are paraphyletic; for example, Apocynaceae and Capparaceae. In this text, these have been recircumscribed so as to recognize monophyletic groups: Apocynaceae have been combined with Asclepiadaceae, and Capparaceae with Brassicaceae.

NAMING: NOT ALL GROUPS ARE NAMED

A phylogenetic classification attempts to name only monophyletic groups, but the fact that a group is monophyletic does not mean it needs to have a name. The reasons for this are practical. We could put every pair of species into its own genus, every pair of genera into its own family, every pair of families into its own superfamily, etc. Such a classification would be cumbersome; it also would not be stable, because our view of sister species would change each time a new species is described, and our view of the entire classification would have to shift accordingly. In practice, there are many monophyletic groups that are not named. For example, the genus *Liquidambar* (sweet gum) is monophyletic and contains four species. Although the relationships among the four species are quite clear, the pairs of species are not named, and few systematists would consider doing so. In another example, over half of the genera of the grass family fall into a single large clade which contains four traditionally recognized subfamilies. Although agrostologists refer to this clade as the PACC clade (an acronym for Panicoideae-Arundinoideae-Centothecoideae-Chloridoideae), it has no formal Latin name.

How do systematists decide which monophyletic groups to name? There is no codified set of rules, but several criteria have been suggested by various authors, and some criteria are in common use despite not being fully articulated. A major criterion—perhaps *the* major criterion—is the strength of the evidence supporting a group. Ideally, only clades linked by many shared derived characters should be formally recognized and named in classifications. This makes sense if a classification is to function as a common vocabulary. Names are most useful if they can be defined, and the more precise the definition the better. In other words, if a clade is to be named, it should have some set of characters by which it can be distinguished from other clades, or **diagnosed**. This also relates to nomenclatural stability. If the meaning of a name shifts every time a new phylogeny is produced or a new character is examined, then the name becomes effectively meaningless.

A second criterion is the presence of an obvious morphological character. Although systematists are not likely to agree on the importance of this criterion, it is an important extension of the idea of a well-supported group, and is also relevant to the use of classifications by non-systematists for identification purposes. If, for example, the only way a field biologist can identify an organism is by knowing whether it has an alanine or a serine at position 281 in its ribulose 1,5 bisphosphate carboxylase/oxygenase molecule, she may not find the classification of much help in making predictions about the organism. If, on the other hand, she knows that the organism is a grass with a particular spikelet structure, then she can easily and reliably infer many aspects of its biology. (Lack of an obvious morphological synapomorphy is one of several reasons that the PACC clade of the grasses is not given a name.) The characters used for classification do not have to be those used for identification, but many systematists prefer to name clades that are easily recognized morphologically.

Another criterion is size of the group. Human memory is easily able to keep track of small numbers of items (in the range of 3–7; Stevens, 1998), but to organize and remember larger number of items requires additional mnemonic devices. (As an example of this, consider how many 9-digit zip codes you can remember compared to the 5-digit variety, or to 7-digit telephone numbers.) Dividing a large group into smaller groups is a way to organize one's thinking about large numbers of taxa. In the words of Davis and Heywood (1963), "We must be able to place taxa in higher taxa so that we can find them again." The genus *Liquidambar* could be redefined to include only *Liquidambar styraciflua* and *L. orientalis*, and a new genus could be described to include *L. acalycina* and *L. formosana*. There seems little reason to do this, however, because four species is not a difficult number to keep track of. That said, there seems little reason to divide a large group if well-supported clades cannot be identified within it.

A fourth criterion is nomenclatural stability. A classification is ultimately a vocabulary, a means of communication. It cannot function this way if the meanings of the names continually change. Thus given a set of well-supported, diagnosable, monophyletic groups, ones that have been named in the past can—and we would argue should—continue to be named. This is yet another argument against formally naming the PACC clade of the grasses, in that it would entail an unnecessary set of changes affecting long-standing taxonomic usage. It is also an argument against dividing *Liquidambar* into two genera, even though both would be monophyletic and well-supported; both size of group and nomenclatural stability argue against the division (Backlund and Bremer 1998; Stevens 1998).

RANKING: RANKS ARE ARBITRARY

Having decided which monophyletic groups to name, there is still the question of exactly how to name them. The groups could, for example, be numbered, and a central index could list what is encompassed by the numbered group. This is similar to the system used by the telephone company to organize telephones. The difficulty, of course, is that without a telephone book (a central index) and/or an excellent memory the system is inaccessible. Biological classification attempts to provide a working vocabulary that conveys phylogenetic information, yet can be learned by biologists who are not themselves primarily systematists. Because a phylogeny is similar in structure to a hierarchy, in which small groups are included in larger groups, which themselves are included in still larger groups, it makes sense to reflect it as a hierarchy.

Botanical classification uses a system developed in the eighteenth century, in which taxa are assigned particular ranks, such as kingdom, phylum, class, order, family, genus, and species (i.e., Linnaean ranks; see Chapter 3 and Appendix 1). A classification of named monophyletic groups should be logically consistent with the phylogenetic relationships hypothesized for the organisms being classified (as expressed in the sequence of branching points in the cladogram). That is, the categorical ranks of a Linnaean classification can be used to express sister-group relationships. It is important to realize that, although monophyletic taxa are considered to represent real groups that exist in nature as a result of the historical process of evolution, the categorical ranks themselves are only mental constructs. They have only relative (not absolute) meaning (Stevens 1998). In other words, the familial level is less inclusive than the ordinal level and more inclusive than the generic level, but there are no criteria available to tell one that a particular taxon, such as the angiosperms, should be recognized at the level of phylum, class, or order.

In Figure 2.20, a cladogram of imaginary taxa A–E is first converted into a hierarchical classification using Linnaean ranks. Note that subgenus DE is nested within genus CDE, which is, in turn, nested within family

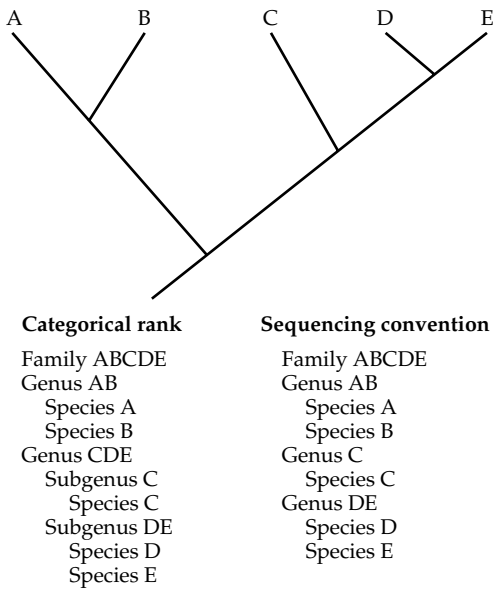


Figure 2.20 Alternative classifications based on the phylogeny of a hypothetical group of taxa ABCDE. One classification uses only three ranks (family, genus, species) plus a sequencing convention, whereas the other uses four ranks (family, genus, subgenus, and species).

ABCDE. (But we could have treated clade ABCDE as an order, with clade CDE as a family and clade DE as a genus.) These procedures often lead to difficulties because in order to fully express the sister group relationships (in the cladogram), one needs more ranks than are available (in the taxonomic hierarchy). Although additional ranks can be created by use of the prefixes *super-* and *sub-*, these may still be insufficient. Therefore, modifications to the method of classification outlined above have been proposed (Wiley 1979, 1981), such as the **sequencing convention**, which states that taxa forming an asymmetrical part of a cladogram may be placed at the same rank and arranged in their order of branching. The sequence of names in the classification denotes the sequence of branching in the cladogram. Note that this is the same as saying that not all monophyletic groups are given names.

Even though ranking is arbitrary, the criteria described above for deciding which groups to name can also be applied to deciding at what level to rank a group (see Stevens 1998 for full discussion). Nomenclatural stability again becomes important. Often one of the monophyletic groups that could be given the name of family already has a commonly used family name, so it makes sense to continue to use the name family for these taxa. For example, it has recently been shown that the earliest diverging lineage in the Poaceae includes only two extant genera, *Anomochloa* and *Streptochaeta*, so that the phylogeny looks like that in Figure 2.21. One could, in principle, create a new family for *Anomochloa* and *Streptochaeta*; after all, it would be monophyletic and would leave the Poaceae as also monophyletic. For the purposes of stability however, it makes sense to leave the two genera in Poaceae, where they have been given a subfamilial name, the Anomochlooideae.

For more discussion of the problems encountered in using the Linnaean system in phylogenetic classification, students should consult de Queiroz and Gauthier (1990, 1992); Forey et al. (1992); Wiley (1981); Wiley et al. (1991); and Hibbett and Donoghue (1998). Some systematists have proposed abandoning the Linnaean system altogether and replacing it with a "phylogenetic taxonomy" in which monophyletic groups would be given unranked names, defined in terms of common ancestry, and diagnosed by reference to synapomorphies (de Queiroz and Gauthier 1990, 1992). Full exploration of this possibility is beyond the scope of this text.

COMPARING PHYLOGENETIC CLASSIFICATIONS WITH THOSE DERIVED USING OTHER TAXONOMIC METHODS

Not all taxonomists use phylogenetic methods, although this is the majority approach. Some systematists have held the view that, although evolution has occurred, parallelism and reversal are so common that the details of evolutionary history can never be deciphered. This point of view led to a school of systematics known as **phenetics**. Pheneticists argued that, since evolutionary history could never be unequivocally detected, organisms might best be classified according to overall similarity. Thus, similar organisms were placed together in a group, while very different organisms were placed in different groups (Sneath and Sokal 1973).

One serious difficulty with the phenetic point of view was that many systematists produced treelike diagrams that grouped organisms by overall similarity, but these diagrams were then interpreted as though they reflected evolutionary history. Sometimes this led to results similar to

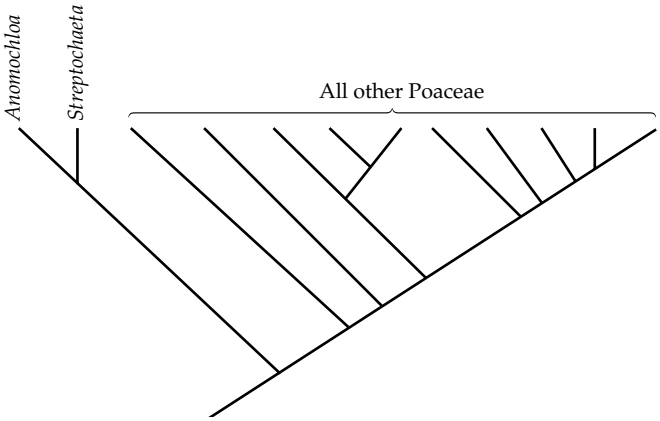


Figure 2.21 Phylogeny of Poaceae, showing the position of the genera *Anomochloa* and *Streptochaeta*.

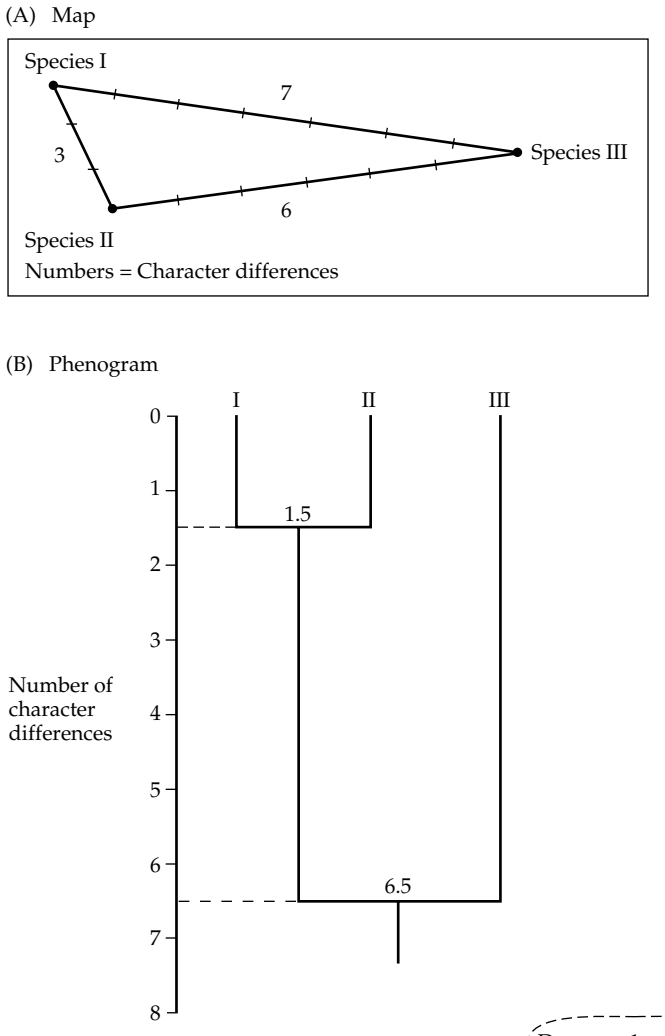


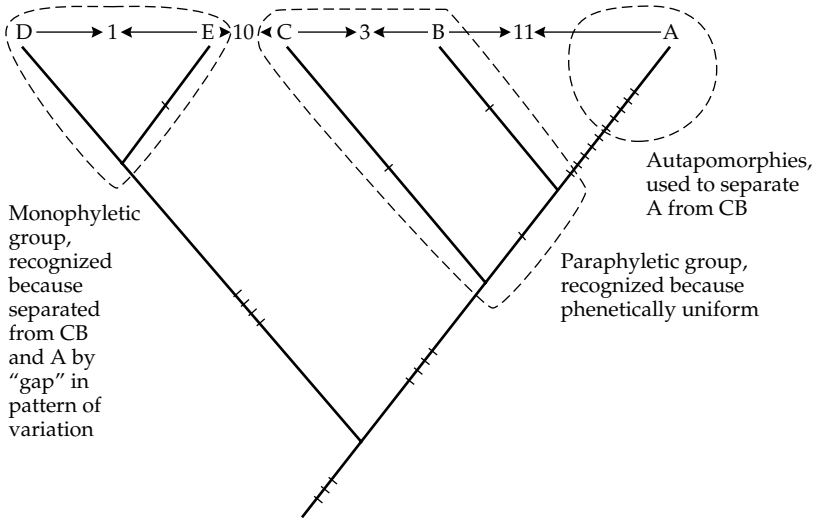
Figure 2.22 Two graphical means of expressing phenetic relationships. (A) Maplike diagram. (B) Phenogram.

those produced by a phylogenetic analysis, but sometimes it led to the production of "groups" made up of organisms that shared only the fact that they were different from everything else, including each other. Such groups have since proven to be paraphyletic or polyphyletic.

The development of phenetic methods was an important prelude to the acceptance and use of phylogenetic approaches. A taxonomist constructing a phenetic classification would first carefully observe as many characters as possible. These characters were divided into states, or the quantitative value of the character merely would be recorded (for example, a series of measurements of leaf length, with the mean recorded for each taxon). This information was arranged in a taxon by character matrix similar to that in Figure 2.8A. This matrix was converted to a similarity matrix (or taxon × taxon matrix) using any of several mathematical measures of similarity (or dissimilarity; see Sneath and Sokal 1973; Abbot et al. 1985). The systematist then grouped the taxa that were most similar, with the similarity relationships illustrated on either a maplike or treelike diagram (a phenogram; Figure 2.22). Phenograms were constructed using clustering algorithms, while maplike diagrams resulted from ordination studies employing multivariate statistical procedures (see Abbot et al. 1985).

Phenetic methods were used to produce classifications, many of which are useful for identification and information retrieval. These classifications were not designed to retrieve evolutionary history, however, and are thus not appropriate for asking evolutionary questions. Phenetic systems do not distinguish between synapomorphy and convergent or parallel evolution.

Evolutionary taxonomy differed from phylogenetic taxonomy in its approach to classification. The morphological similarity of a group was of utmost importance, and monophyly and paraphyly (in the strict cladistic senses of those words) were secondary. Thus a group could be recognized on the basis of some combination of derived and ancestral, unique and shared characters (Figure 2.23). Importance was given to the recognition of "gaps" in the pattern of variation among phylogenetically adjacent groups (Simpson 1961; Ashlock 1979; Cronquist 1987; Mayr and Ashlock 1991). Characters considered to be evolutionarily (or ecologically) significant



Classification

Family ABCDE
- Genus DE
  - Species D
  - Species E
- Genus CB
  - Species C
  - Species B
- Genus A
  - Species A

Figure 2.23 Phylogeny and a resulting non-phylogenetic classification produced according to the evolutionary school. The classification includes a mix of monophyletic and paraphyletic groups, separated from each other by morphological "gaps."

were stressed, and the expertise, authority, and intuition of individual systematists were considered to be significant. Finally, although evolutionary classifications usually referred to evolution, and the groups recognized in such classifications were often called monophyletic, the taxa were expected to be morphologically homogeneous, and to be separated from each other by discrete gaps (Ashlock 1979; Mayr and Ashlock 1991; Stevens 1986; Stuessy 1983, 1990).

It has been said that systematics is as much an art as a science (although this begs the question of how one might define art and science), in part because so many aspects of the discipline seemed to have no objective basis. One fortunate result of phylogenetic systematics is that at least one major aspect—the delimitation of groups—has become formalized such that there is general agreement on how it should be done. Whereas phenetic and evolutionary classifications were ambiguous about grouping criteria, phylogenetic classifications are precise. A named group can be taken as monophyletic, including all descendants of a single common ancestor.

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