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 plants.

There are many ways to 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 habitats (as they may be in some ecological classifications). A phylogeny-based classification, such as the one used 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, of a group of organisms. The second is basing the classification of the group 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 will then discuss briefly how one might construct a classification given that history.

How Are Phylogenies Constructed?

As described in Chapter 1, evolution is not simply descent with modification, but also involves the separation of lineages. This process can be visualized with diagrams such as those in Figures 1.3 and 1.4, but these are cumbersome to draw. Evolutionary history can be more conveniently summarized in a branching diagram (Figure 2.1A). (Some workers make distinctions between an evolutionary tree, a phylogeny, and a branching diagram or

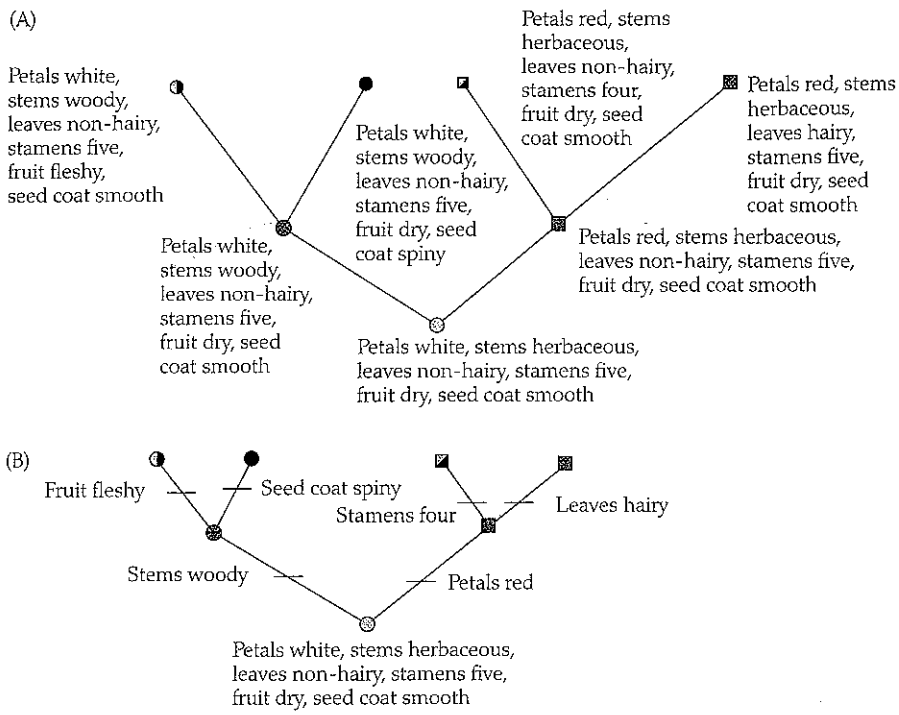


FIGURE 2.1 (A) A simple way to redraw the pattern of changes shown in Figure 1.4. Full descriptions are provided for each of the ancestors and their descendants. (B) A simpler way to redraw Figure 2.1A, showing only the changes that have occurred in the various lineages.

cladogram, but in this text the terms are used interchangeably.) To avoid repeating the ancestral character states retained in every group, systematists commonly note only the characters that have changed, and they place tick marks on the appropriate branches to indicate the relative order in which the character states originated (Figure 2.1B).

The shared derived character states in Figure 2.1B 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). This arrangement then leads 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 the shared derived character state of red petals and the ancestral character state of herbaceous stems, the other with the shared derived character state of woody stems and the ancestral character state of white petals. Each of these groups could also be divided into two groups. Thus the classification could 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 just 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, as by starting with the "primitive" families, such as Magnoliaceae and Nymphaeaceae. The latter families 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

In the examples shown in Figures 1.3, 1.4, and 2.1, we described evolution as though we were watching it happen. This is rarely possible, of course, so part of the challenge of systematics is that we must *infer* what went on in the past. The first step in making such inferences is to examine extant species closely for characters that are believed to be heritable. A **heritable character** is any aspect of the plant's morphology that can be passed down genetically through evolutionary time and still be recognizable. For example, a flowering plant's petal color, inflorescence structure, and habit (general growth pattern) are all known to be under genetic control, and these characters are generally stably inherited from one generation to the next. Many examples of such heritable characters are described in Chapters 4 and 5.

Systematics entails the precise observation of organisms. Without careful descriptions of characters, phylogeny reconstruction and descriptions of evolutionary history are meaningless. Without accurate comparative morphology, classification of any sort is impossible. The assessment of similarity is the basis of comparative biology, and of systematics in particular. In making such assessments, it may be harder than you think to determine which structures of one plant can usefully be compared to structures of a differ-

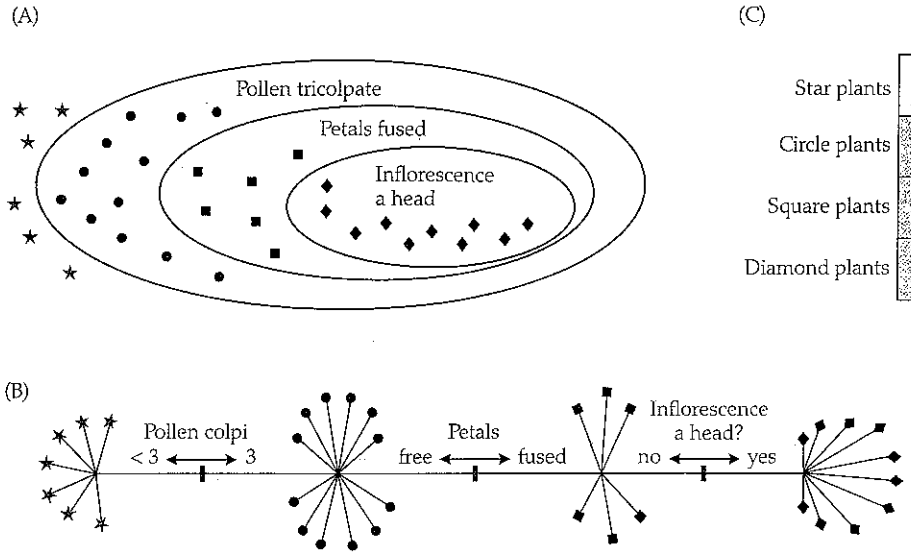


FIGURE 2.2 Each symbol represents a hypothetical pollen-producing plant species or group of species. A large subset of such plants have tricolpate pollen. Of that subset, a smaller group has fused petals, and of the plants with tricolpate pollen and fused petals, a subset has flowers arranged in a head. (A) The pattern described rendered as a Venn diagram of nested character states. (B) The pattern redrawn as an unrooted network; characters are indicated with bright green tick marks, with the different character states on either side. (C) The pattern redrawn as a matrix.

ent plant. Two structures may be deemed to be similar if (1) they are found in a similar position in both organisms; (2) they are similar in their cellular and histological structure; and/or (3) they are linked by intermediate forms of the structure (either by intermediates at different developmental stages of the same organism or by intermediates in different organisms). These three statements constitute **Remane's criteria** of similarity.

Remane (1952) actually called this list the "criteria of homology." In this book, however, we use the term *homology* in a more restricted sense, to mean **identity by descent**. In other words, if we say that a character is **homologous** among a group of species, we mean that all those species inherited that character from a common ancestor. Under this definition, observing similarity is only the first step in determining homology, since not every observed similarity is the result of homology.* (For example, structural similarities can evolve independently in unrelated plants that live in similar environments.) This text follows the viewpoint held by the many phylogenetic systematists who argue that homology can be determined only by constructing an evolutionary tree.

Characters, Character States, and Networks

From observations of heritable characters, plant groups that share particular character states can be identified. Suppose, for example, that pollen is observed to vary in the number of grooves on its surface (a character), and that the pollen in a large number of plant species has three grooves (a character state). These grooves are in fact germination furrows called *colpi* (singular *colpus*), and three-grooved pollen is described as *tricolpate*. Within the large group of plant species with tricolpate pollen is a smaller group whose petals (character) are fused (character state), and within this fused-petal group is a still smaller group with flowers arranged in a head. These nested groups can be depicted as a set of concentric ovals in a **Venn diagram**, as shown in Figure 2.2A.

The information in the Venn diagram can also be drawn as a **network** (Figure 2.2B). Here the characters are shown as vertical green lines, or "tick marks" (a convention that is seen in illustrations throughout this text). Whereas the shapes (species) to the left of the "pollen" line have fewer than three colpi, those to the right of the line have tricolpate pollen. Likewise, the line for "petals" indicates a shift between the character states free and fused, and the inflorescence line indicates a shift between flowers clustered in a head and flowers borne separately. We can count the number of changes along the network to determine its *length*: proceeding from right to left, there is one change each in inflorescences, petal fusion, and pollen colpi, so the network can be described as having a length of 3.

The same information can be presented as a **matrix** in which the rows correspond to plants and the columns correspond to characters (Figure 2.2C). The character states are then used to fill in the matrix. The changes in character state 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 2.2B represent three

*You should be aware that *homology* has several different meanings, and when reading the literature, it is worth checking what particular authors mean when they use the term.

changes in gene sequence (and thus in the resulting proteins) that altered the character states of some plants.

In Figure 2.2, all plants designated by the same shape are drawn as though they arose at the same time. This arrangement generally indicates ambiguity; for the purposes of this simplified example, we have not provided any information on their order of evolutionary origin. In addition, we have implied that determining the different character states is perfectly obvious. This is often not the case, however. When we describe the variation among similar morphological structures by dividing the character into character states, we are in fact putting forward a hypothesis of underlying genetic control, even though we rarely frame the assumption in these terms.

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 the genes underlying petal color have switched, over evolutionary time, to produce either red flowers from a blue-flowered ancestor or blue flowers from a red-flowered ancestor. In this instance, we know that there are in fact genes (e.g., components of the anthocyanin pathway) that control petal color, and thus the inference of two states controlled by a "genetic switch" is probably a reasonable one. In many cases, however, we have no idea of the genetic mechanisms that control the state of the structural characters observed. In proposing hypotheses about the nature of the underlying switches, often all we can do is be sure that the character states really are distinct. For quantitative characters such as leaf length or corolla tube width, this means graphing the quantitative data (i.e., the measurements) to be sure that the measurements of the species we are studying do not overlap.

For many characters, such measurements do overlap and also vary greatly, so much so that the assumption of underlying genetic switches—and therefore division into character states—is unsupported by any evidence. In these cases, the characters in question should be omitted from phylogenetic analysis (unless the overlap is caused by only a few individuals, in which case the character could be scored as polymorphic for that species and retained in the analysis). Even though such overlapping characters probably reflect genetic changes over evolutionary time, given our current state of knowledge, overlap makes it difficult to extract any reliable information on the underlying gene changes (although methods of dealing with plants with variable characters have been developed).

Variability and overlap in morphological characters are good reasons why many systematists have turned to molecular data in constructing phylogenies. With the emergence of nucleotide sequence data for many genes, the recognition of molecular character states (i.e., whether the nucleotide in a given position is A, T, G, or C) is often more precise. This may not be the case, however, if gene sequences are hard to align, or if restriction fragments are too similar in size. The use of molecular character states in plant systematics is detailed in Chapter 5.

Evolutionary Trees and Rooting

Figure 2.2 shows three different ways of recording and organizing observations about plants. Even though the network (Figure 2.2B) looks somewhat like a time line, it is not. It could be read from left to right, from right to left, or perhaps from the middle outward. To turn the network 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**. Rooting *polarizes* the character changes, giving them a specific direction.

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 different places. The network from Figure 2.2B is redrawn in Figure 2.3, 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—3—and that all the connections are the same, but that the order in which the character-change events occur differs considerably.

For example, in the rooting shown in Figure 2.3A, the ancestral plants had pollen with fewer than three colpi, petals not fused, and flowers not in heads, whereas we would conclude from Figure 2.3B that the ancestral plants had exactly the opposite character states. In Figure 2.3C, 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.

Rooting a phylogenetic tree is critical for interpreting how plants evolved, and different rootings suggest different patterns of change (different 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 an extinct plant has been fossilized does not mean that its lineage *originated* earlier than those of plants now living; we know only that it died out earlier.

In determining evolutionary history, we are interested in determining when lineages diverged from one another (that is, when taxa originated). When taxa die out is interesting to know, but that fact by itself does not help in determining their origins. (Fossils are, of course, extremely useful when included as additional taxa in a phylogeny. They often have combinations of character states that no longer occur in extant taxa and can affect the overall structure of the tree, sometimes in surprising and informative ways.)

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 all ingroup members (members of the group under study) are more closely related to one another 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 a network, the point at which it attaches is determined as the root of the tree.

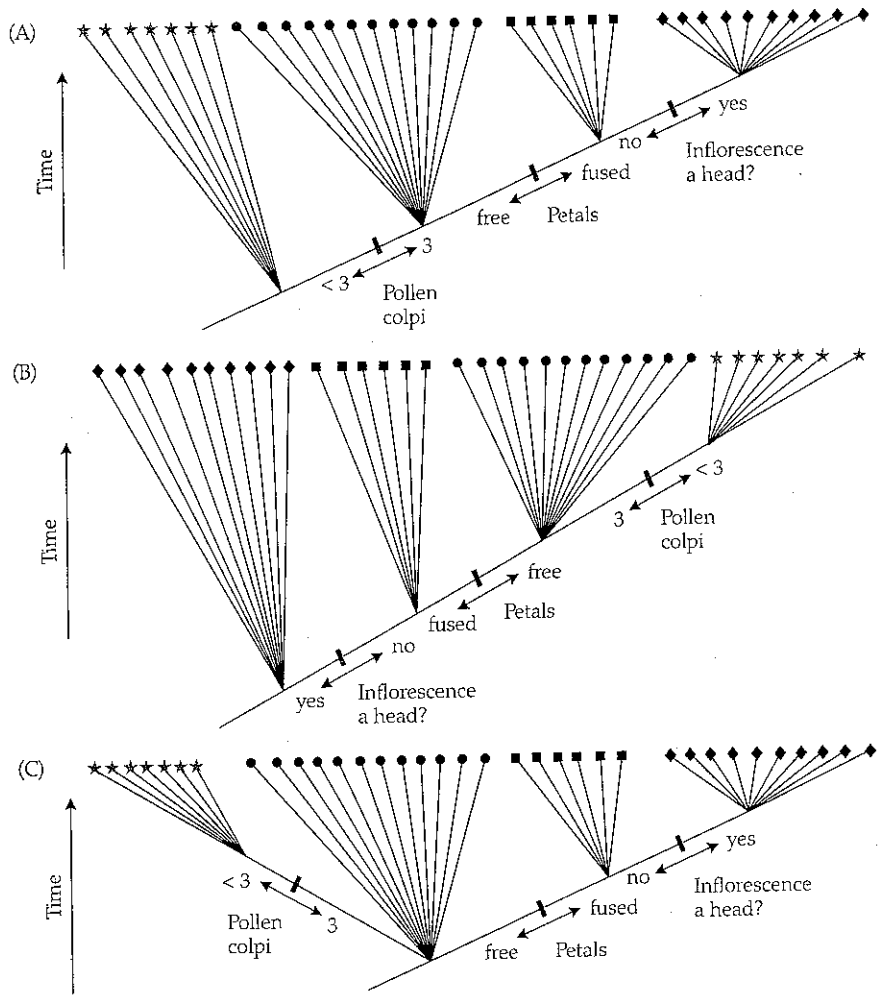


FIGURE 2.3 Three possible rootings of the network in Figure 2.2B. Note that in each case the number of evolutionary steps (character state changes) is the same as in the unrooted network.

In the case of Figures 2.2 and 2.3, the plants shown are all flowering plants (angiosperms), and their closest living relatives are the conifers, cycads, gnetophytes, or ginkgos, or a set of these (see Chapters 7 and 8). In Figure 2.4A, a conifer is added to the matrix from Figure 2.2C. (We could have used all gymnosperms as outgroups, but to keep the example simple we have chosen only one.)

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 2.4B. Because the conifer attaches among the star species, the tree can be rooted and redrawn as in Figure 2.4C. This tree corresponds to the rooted tree in Figure 2.3A and strengthens the hypothesis that Figure 2.3A accurately reflects evolutionary history.

Note that the tree can be drawn in different ways and still reflect the same evolutionary history. Comparing Figures 2.5A and B with Figure 2.4C shows that we can rotate the branches of the tree around any one of the branch points (**nodes**) without affecting the inferred order of events.

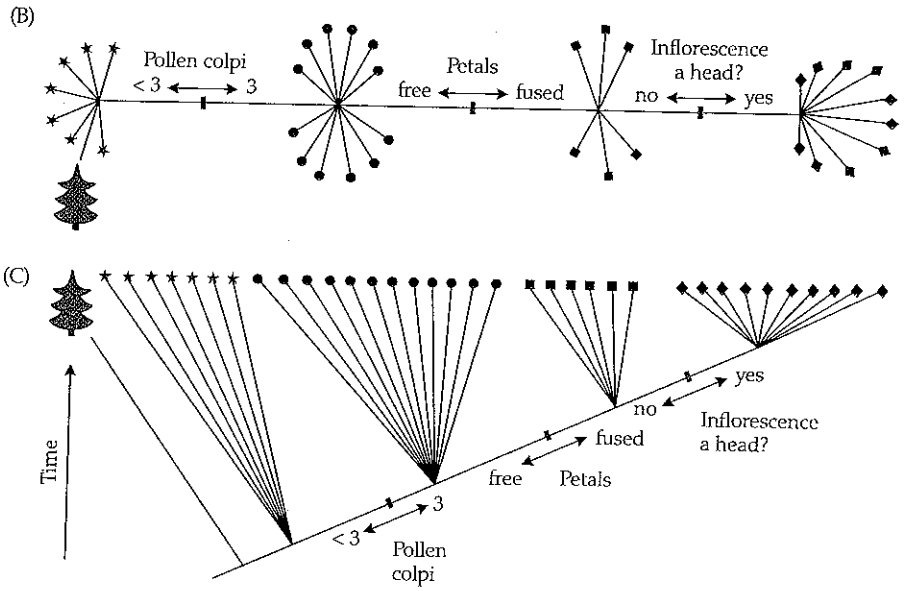
With a rooted tree (and only with a rooted tree), we can determine which groups are monophyletic (made up of an ancestor and *all* of its descendants). Therefore, in the example laid out in Figure 2.4C, the diamond plants are monophyletic (i.e., they form a clade). In fact, the flowering plants with 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 (i.e., is a shared derived character for, or 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 2.3B 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 and would 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 2.3B were correct, the diamond plus square species would not be a monophyletic group (as they are with the rooting in Figure 2.3A). Instead, they

FIGURE 2.4 (A) The matrix from Figure 2.2C, but with character states added for a conifer. (B) The unrooted network from Figure 2.2B, but with the conifer attached according to the character states in Figure 2.4A. (C) The network of Figure 2.4B rooted with the conifer. Note that the evolutionary history is now the same as in Figure 2.3A.

(A)

	Pollen colpi	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



would constitute a **paraphyletic** group, one that includes a common ancestor and some, but not all, of its descendants.

As mentioned earlier, a character state that is derived (synapomorphic) at one time may become ancestral later. In Figure 2.4B, 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 state. It is something that all of the species in the group inherited from their common ancestor and thus indicates nothing about their relationships with one another. Plesiomorphic character states cannot show evolutionary relationships in the group being studied because they evolved earlier than any of the taxa being compared and have merely have been retained in the group's various lineages.

Sometimes monophyly of a group is indicated by the fact that its character states 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 Poaceae 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 phylogenetic (sometimes called cladistic) analyses were done by hand until the mid-1980s. Characters are first divided into character states, as with any phylogenetic 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, each character is polarized, or given direction. The shared derived, or synapomorphic, character state can then be used as evidence of monophyly, and a cladogram can be constructed on the basis of synapomorphic character states (Box 2A). This kind of thinking is often useful in providing a first guess as to whether existing taxonomic groups are monophyletic and thus named appropriately.

Choosing Trees

As the preceding discussions have shown, determining the evolutionary history of a group of organisms is conceptually quite simple. First, characters are observed and divided into character states. Second, from the character states, a Venn diagram (see Figure 2.2A), a character × taxon matrix (see Figure 2.2C), or a branching network (see Figure 2.2B) can be constructed. Third, by inclusion of an outgroup, the net-

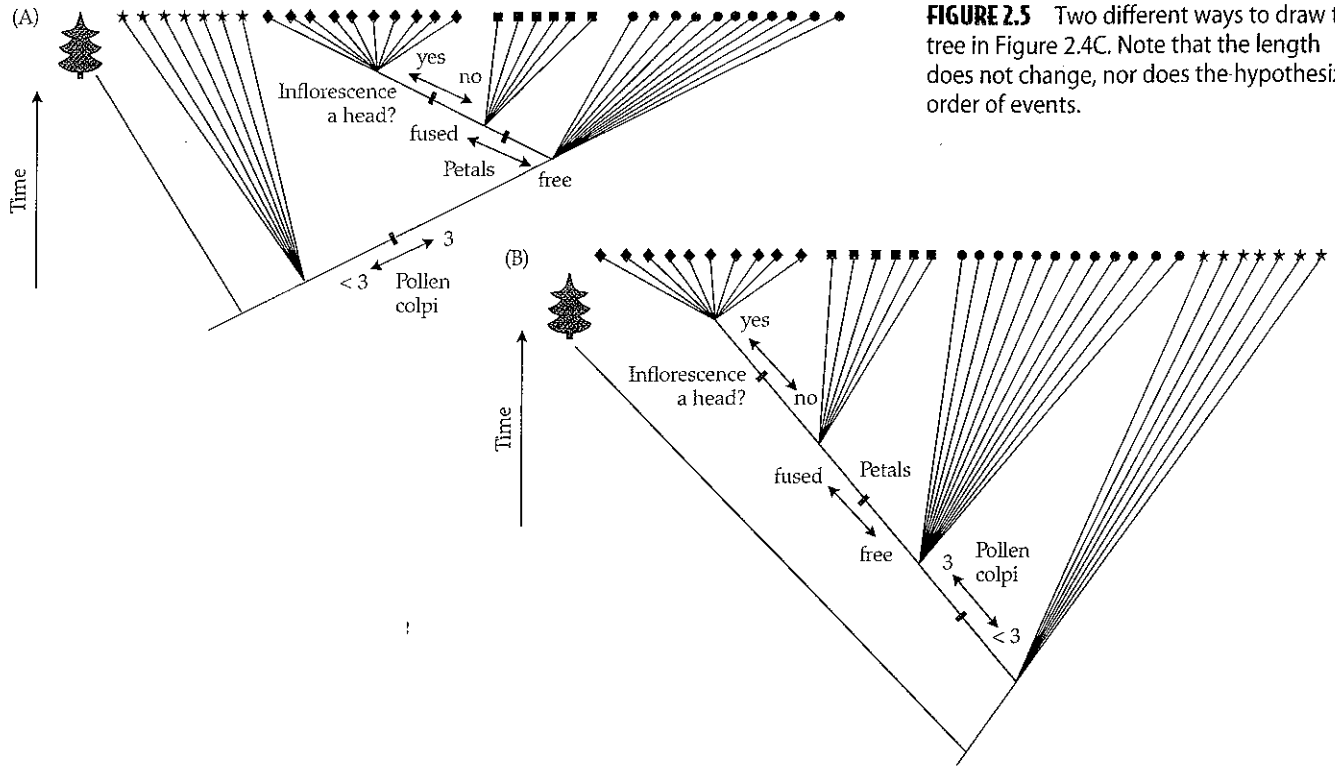


FIGURE 2.5 Two different ways to draw the tree in Figure 2.4C. Note that the length does not change, nor does the hypothesized order of events.

work 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 were the same.) A **reversal** occurs when a derived character state changes back to the ancestral state.

To provide a clear example, we will divide the group that we have called "star plants" into red star plants, gold star plants, and white star plants. Let us assume that the gold star and white star plants have only one cotyledon, whereas all the rest of the plants have more than one (including the conifer). Let us further assume that the white star plants have fused petals. We can add the character cotyledon number to the matrix in Figure 2.4A to create 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* parallel 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.

In this example, a group based on fused petals would be considered **polyphyletic**. Polyphyletic groups have two or more ancestral lineages in which the parallel character states evolved. (Although we distinguish here between paraphyletic and polyphyletic groups, many systematists have observed that the difference is slight and simply call any para- or polyphyletic group nonmonophyletic.) Petal fusion in this case is nonhomologous because 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 arises 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 can be converted into a phylogeny by rooting at the conifer, but they make different suggestions about how the plants have evolved. In Figure 2.8B, cotyledon number and number of pollen colpi have been stable over evolutionary time, whereas petal fusion has appeared twice, independently. In Figure 2.8C, 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 proposing a hypothesis about how evolution has happened—about which genetic changes have occurred, at what frequency, and in which order.

As the two networks show, our two hypotheses differ. How do we determine which one is correct? There is no way to be certain. 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?" By asking this question, we apply a rule that is used throughout science, known as

BOX 2A Hennigian Argumentation

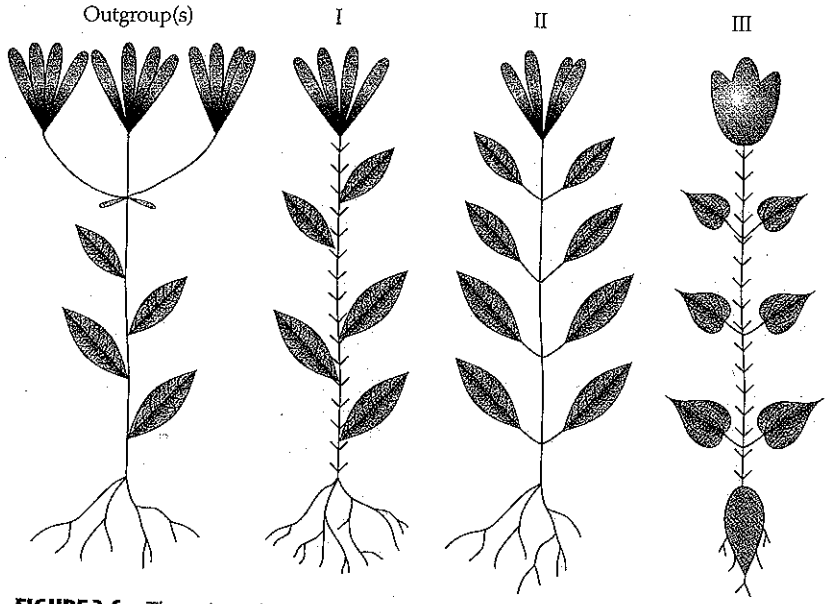


FIGURE 2.6 Three imaginary species (I, II, and III) and an outgroup.

In the examples presented thus far, a network is constructed and is then polarized by determining where the outgroup attaches. Some systematists, however, prefer to polarize the characters first, using one or several outgroups, and then construct the phylogeny. This goes back to the original concept of phylogenetic analysis proposed by Willi Hennig (see Chapter 3).

Consider, for example, the imaginary plants presented in Figure 2.6. In this case the character states of the outgroup are assumed to be ancestral (plesiomorphic) and are represented by 0; derived states are represented by 1 or higher numerals (Table 2.1). These character states are then used to produce a character × taxon matrix (Table 2.2).

Next a phylogenetic tree (or cladogram) is constructed in which the taxa are grouped (placed on the same branch) according to evidence provided

TABLE 2.1 States of morphological characters used in cladistic analysis of the three imaginary species in Figure 2.6.

	Character state[a]	
Morphological character	**Plesiomorphic**	**Apomorphic**
1. Roots	Less than 1 mm thick (0)	Greater than 5 mm thick (1)
2. Stems	Glabrous (0)	Pubescent (1)
3. Leaves	Alternate (0)	Opposite (1)
4. Venation	Pinnate (0)	Palmate (1)
5. Petiole	Lacking (0)	Present (1)
6. Base of blade	Acute (0)	Cordate (1)
7. Perianth parts	4 (0)	3 (1)
8. Perianth parts	Separate (0)	Fused (1)
9. Flowers[b]	In a group of 3 (0)	Solitary (1)

[a]Character state codings are given in parentheses.

[b]Note that inflorescence condition (flowers solitary versus flowers in groups of 3) cannot be polarized unless additional outgroups are employed.

Occam'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.

In the example we have presented here, in which there are few characters and little homoplasy, it is easy to construct the shortest network that can link the organisms. In most real cases, however, many networks are possible, 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 programs are PHYLIP (Felsenstein 1989), NONA (Goloboff 1993), and PAUP*4.0 (Swofford 2000). These programs either evaluate data over all possible trees (an exhaustive search) or make reasonable guesses about the topology of the shortest trees (branch-and-bound searches or heuristic searches).

If the taxa are numerous, 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 trees. For example, the possible relationships of three

TABLE 2.2 Character × taxon matrix for the three hypothetical species of Figure 2.6, based on characters in Table 2.1.

	Characters								
Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Species I	0	1	0	0	0	0	0	0	1
Species II	0	0	1	0	1	0	0	0	1
Species III	1	1	1	1	1	1	1	1	1
Outgroup(s)	0	0	0	0	0	0	0	0	0

by shared derived character states (synapomorphies). The presence of a derived character state (apomorphy) in two taxa suggests that they share a unique common ancestor in which the apomorphy first evolved; the two taxa are assumed to have inherited the apomorphy (or evolutionary novelty) from this ancestor. The cladogram, then, represents the simplest hypothesis that explains the pattern of derived character states, following the principle of parsimony.

A hypothesis of evolutionary relationships for species I, II, and III of Figure 2.6 is presented in Figure 2.7. Species II and III are hypothesized to share a unique common ancestor because they share the derived states of characters 3 and 5 (see Table 2.1). They both have opposite, petiolate leaves, which are hypothesized to have evolved in their common ancestor. Similarly, the shared possession of solitary flowers supports the recognition of a more inclusive monophyletic group containing species I, II, and III.

The presence of hairy stems in species I and III is homoplasious; that is, hairy stems are hypothesized to have evolved in parallel in these two species, so their similarity is not based on common ancestry. Note, however, that hairy stems could have evolved in the most recent common ancestor of all three species and then been lost (a reversal) in species II.

The sharing of pinnate leaves with acute (forming an angle less than 90°) bases and flowers with four separate perianth parts by species I and II is symplesiomorphic; these are shared ancestral characters. Such characters do not indicate relationship. In contrast, palmate venation, cordate (heart-shaped) leaf bases, and flowers with three fused perianth parts are derived character states unique to species III. These unique derived character states (autapomorphies) also tell us nothing about the phylogenetic relationships of species III.

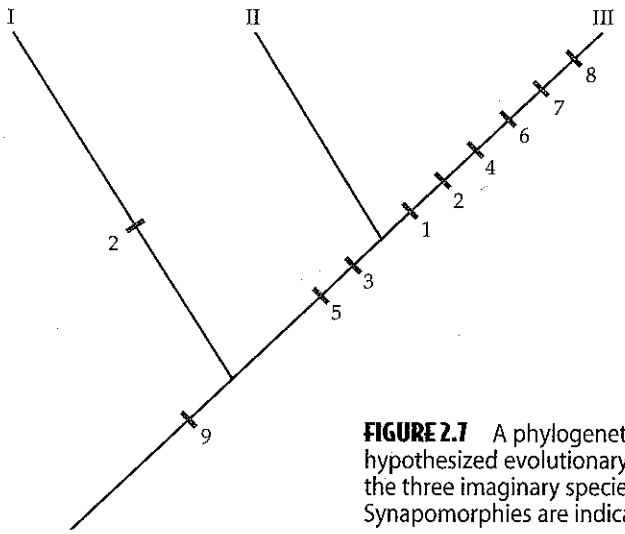


FIGURE 2.7 A phylogenetic tree illustrating hypothesized evolutionary relationships of the three imaginary species of Figure 2.6. Synapomorphies are indicated on the tree.

taxa can be expressed by only three rooted trees, [A(B,C)], [B(A,C)], and [C(A,B)]. But with larger numbers of taxa the number of potential trees expands rapidly; for example, four taxa yield 15 trees, five yield 105 trees, six yield 945 trees, and ten yield 34,459,425 trees!

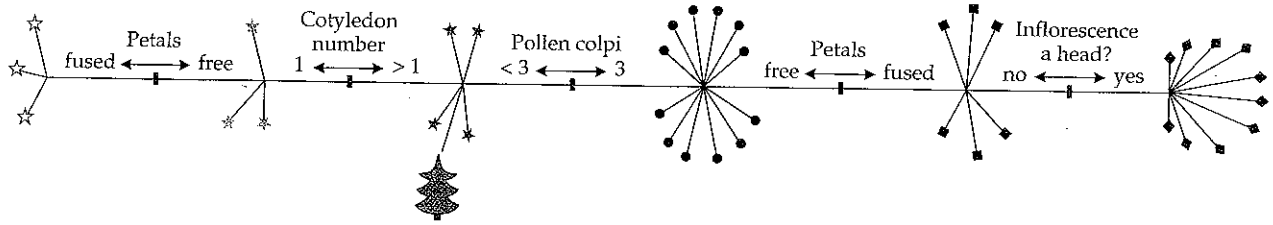
The parsimony method is widely used, easily applicable to morphological changes, and possibly also the most intuitive of tree reconstruction methods. Parsimony works well when evolutionary rates are slow enough that chance similarities (due to the evolution of identical derived character states independently in two or more lineages) do not overwhelm character states shared by the common ancestor. At higher rates of change, however, parsimony methods are susceptible to a phenomenon known as "long branch attraction" (Box 2B). Other methods of tree reconstruction use other criteria for choosing the preferred (optimal) tree. Instead of choosing the tree with the fewest evolutionary changes, one can convert the character × taxon 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 can develop theories about the probability of change

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

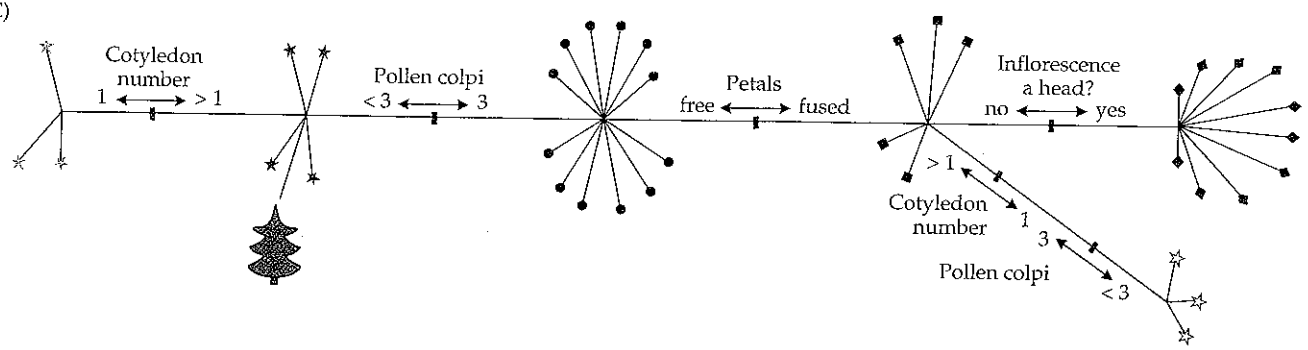
(A)

	Pollen colpi	Petals	Inflorescence in heads?	Cotyledon number
Red star plants	< 3	free	no	2
Gold 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

(B)



(C)



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, so this approach is known as the **maximum likelihood method** (Felsenstein 1981; Hillis et al. 1993; Huelsenbeck 1995; Swofford et al. 1996) (Box 2C). For brief descriptions of several current methods of phylogeny reconstruction, see Hall 2005.

Assessing Homoplasy

Parsimony analyses minimize the number of characters that change in parallel or reverse. If there are many such homoplasious characters, then the phylogenetic tree may be 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 in a phylogenetic tree 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 shown in Figure 2.2B, 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 those characters (petal fusion) changes twice on the tree, so the consistency index is 4/5 = 0.80.

Consistency indices may also be calculated for individual characters. In this case, the CI equals 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 (see Figure 2.8B) is 1/2 = 0.50. For a given character × taxon matrix, the shortest network or tree will also have the highest consistency index. Lower consistency indices indicate the presence of many characters that contradict the evolutionary tree.

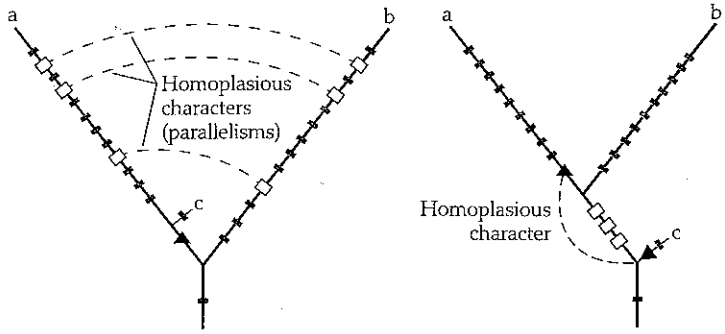
Comparing consistency indices across data sets is hazardous because the CI has some undesirable properties. For one thing, a character that changes once in only one taxon

BOX 2B Long Branch Attraction

Long branch attraction was identified originally by Felsenstein (1978) as a potential problem for phylogenetic analysis. If there are great differences in the rates of character evolution among lineages such that some lineages are evolving much more rapidly than others, and if characters have only a limited number of character states, then unusually long branches can be connected to each other in a tree whether or not they are actually closely related (Figure 2.9). This problem is particularly acute with DNA sequence data, for which each character has only four possible states, and for which mutation rates vary widely.

This phenomenon occurs because numerous random changes, some of which appear in parallel in the rapidly evolving lineages, outnumber the changes that provide information about common ancestry. The problem cannot be circumvented by adding more characters (base pairs, in DNA sequences); these merely add to the number of parallelisms linking the rapidly evolving lineages.

This situation can affect all methods of tree construction. With the correct model of evolution, however, maximum likelihood methods (see Box 2C) are less afflicted by this problem (although determining the correct model may be difficult). Long branch attraction is basically 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 2.9 Long branch attraction, a situation in which strongly unequal evolutionary rates cause parsimony to fail. (A) True phylogeny. Dashed 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.

will have a consistency index of 1.0, but such a character tells us nothing about relationships. Such a uniquely derived character is sometimes called an **autapomorphy**. For example, if one of the red star plants in Figure 2.8B had hairy leaves while all the other plants in the network had hairless ones, leaf hairiness would not be of any help in indicating the relationship of the hairy-leaved plant to the other plants. In other words, the character would be **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 it would give a misleading impression that many characters supported the tree. Uninformative characters, therefore, are often omitted before the consistency index is calculated.

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 relationship is true of both molecular and morphological data and of analyses of species, genera, or families.

The **retention index (RI)** circumvents the problems summarized in the previous two paragraphs, and also another limitation of the CI (Wiley et al. 1991; Forey et al. 1992). The CI should vary from near 0 (a character that changes many times on the tree) to 1.0 (a character that changes only once), but often the real range is much less. For example, in the matrix in Figure 2.8A, only two groups—the white star plants and the gold star plants—have a single cotyledon. If the one-cotyledon plants are all on a single branch of the network, 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 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 RI then equals the maximum length minus the actual length, divided by the maximum length minus the minimum length:

$$(L_{max} - L_{actual})/(L_{max} - L_{min})$$

In Figure 2.8B, then, the RI is $(9 - 5)/(9 - 4) = 4/5 = 0.80$.

BOX 2C Maximum Likelihood and Bayesian Methods

Parsimony analyses remain very common in phylogenetic analysis, but for analyses using DNA sequences as characters, maximum likelihood and Bayesian methods are becoming routine. These methods rely on the assumption that mutations in a DNA sequence are random. Over a particular period of evolutionary time, if the probability of a particular nucleotide base mutating is 1/100, then in a DNA sequence 100 bases long, we expect that one of the bases will mutate. We don't know which particular base will change, just that one of them will. If the period of evolutionary time is doubled, then we expect two mutations in our hypothetical sequence. In general, the expected number of changes will be equal to the mutation rate multiplied by time; this formula is often symbolized by μt. Over longer and longer periods, more and more of the bases will change until at some point a second mutation will occur at a site that has already mutated. Again, we will not know which particular site has undergone the second mutation, but we can estimate that it must have occurred because of the total number of mutations seen in the sequence. Basic probability theory allows us to estimate the number of "extra" mutations at the site. The branch lengths used in creating the phylogenetic tree then incorporate these extra mutations that we infer must have occurred. All of our assumptions about the probabilities of particular mutations together constitute a **model of evolution**. Both maximum likelihood and Bayesian methods are known as model-based methods because they incorporate ideas about the probability of change.

The theoretical statistical underpinnings of maximum likelihood and Bayesian approaches are very different. As a practical matter, however, a major distinction is simply computational speed. Maximum likelihood analyses take a long time to run, and bootstrap analyses require a high-performance computer. Bayesian methods estimate support for the tree at the same time as the tree is computed and so are faster.

Summarizing Evolutionary Trees

Parsimony analyses often 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 evolutionary histories, for the same taxa. In addition, studies using different kinds of characters (e.g., gene sequences, morphology) may find different trees. Rather than choosing among the trees in these cases, systematists may 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 in a consensus tree.

Strict consensus trees contain only monophyletic groups that are common to all trees. For example, analyses of different sets of data have produced different ideas about the relationships among the early angiosperms. A study of the sequences of 4 genes led to the evolutionary tree shown in Figure 2.10A (which has been simplified for the purposes of this example) (Rydin et al. 2002). Adding more gene sequences, and analyzing them in a different way, led to the tree in Figure 2.10B (Burleigh and Mathews 2004). The trees both show that the angiosperms are sister to the gymnosperms and that the gymnosperms are monophyletic. Both trees also show that the Gnetales and the conifers (Pinaceae plus non-Pinaceae conifers) are closely related. The strict consensus of the two cladograms (Figure 2.10C) therefore shows the gymnosperms as monophyletic and the Gnetales plus conifers as a clade.

There are differences between the two evolutionary hypotheses, however. The 4-gene tree suggests that the Gnetales are sister to all conifers, but the 13-gene tree indicates that the Gnetales are sister only to the Pinaceae, which constitute a subset of the conifers. In the strict consensus tree (Figure 2.10C), the Gnetales, Pinaceae, and the non-Pinaceae conifers appear as though they arose at the same time. This means that the available data cannot tell us whether they arose together or one after the other, nor can we determine the order in which they arose.

Having multiple lineages arising at the same apparent node in the diagram is usually an expression of ambiguity. The 13-gene tree suggests that the cycads are sister to all other gymnosperms, but in the 4-gene tree, the cycads, ginkgo, and the clade containing the rest of the gymnosperms all look as though they arose at the same time. The ambiguity in the 4-gene tree leads us to conclude that we really do not know which gymnosperm lineages appeared first. This uncertainty is reflected in the strict consensus tree by the fact that all those lineages are drawn as though they arose at the same time.

When many trees are 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** shows 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 consensus tree (along with an indication of 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 in comparisons of phylogenies with slightly different terminal taxa or constructed 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

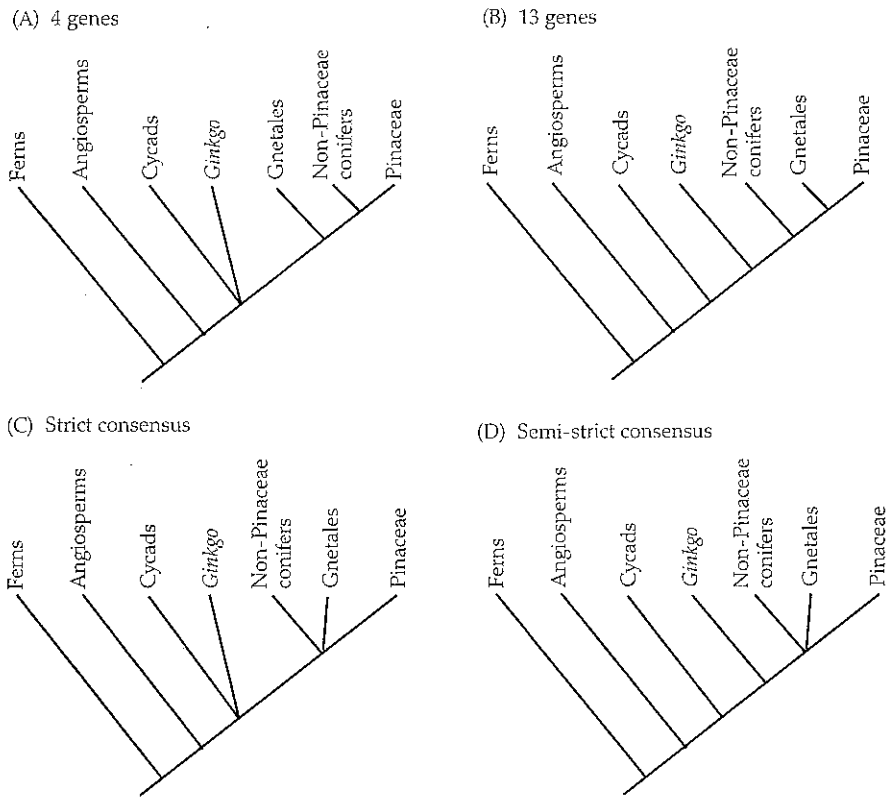


FIGURE 2.10 (A) Phylogeny of seed plants based on DNA sequence data from 4 genes. (B) Phylogeny of seed plants based on DNA sequence data from 13 genes. (C) Strict consensus of the trees in A and B. (D) Semi-strict consensus of the trees in A and B. (A based on Rydin et al. 2002; D modified from Burleigh and Mathews 2004.)

relationships among the species. The semi-strict consensus tree then indicates all relationships supported by one tree or both trees and not contradicted by either.

For example, although the 4-gene tree (Figure 2.10A) does not give us any information about the order in which cycads, ginkgo, and the remaining gymnosperms originated, the 13-gene tree (Figure 2.10B) does. The two trees are not really conflicting; the 13-gene tree just provides more precise information. The semi-strict consensus tree thus follows the 13-gene arrangement of those three groups (Figure 2.10D).

The Probability of Evolutionary Change in Characters

In trying to infer the evolutionary history of a group, we depend on an implicit or explicit description (model) of the evolutionary process (see Box 2C). The more accurately the description reflects the underlying process, the more accurately we will be able to estimate the evolutionary history. This is particularly important for very divergent species in molecular phylogenies, for which parsimony methods often produce misleading results (see Box 2B). For nucleotides in a DNA sequence, mutation is assumed to be random, although this assumption is often modified to reflect hypothesized mechanisms of molecular evolution.

Developing a 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 those genes lead to different character states. Nonetheless, certain assumptions must be made if one is to proceed at all. (And, we note, *no* methods are entirely free of assumptions!) The major assumptions have to do with the likelihood of particular changes in 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 (binary) characters are interpreted as representing a single genetic switch—"on" producing one state (e.g., tricolpate pollen), and "off" resulting in the other state (e.g., one-grooved, or monosulcate, pollen). Over evolutionary time, of course, such characters 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 colpi in a matrix containing some taxa with pantoporate pollen, that character would now have three states: monosulcate, tricolpate, and pantoporate. Pollen colpi would now be a multistate character, in contrast to the binary characters discussed previously. Multistate characters raise a difficult question: how many genetic switches are involved?

It is possible that monosulcate pollen changed to tricolpate pollen, which then changed to pantoporate pollen; this progression matches what we think happened in the angiosperms over evolutionary time (Figure 2.11A). (Recall that the outgroup does not have tricolpate pollen.) This scenario implies two genetic switches. It also implies that they must have occurred in order; that is, pantoporate pollen could arise only after tricolpate pollen did. If we accept this

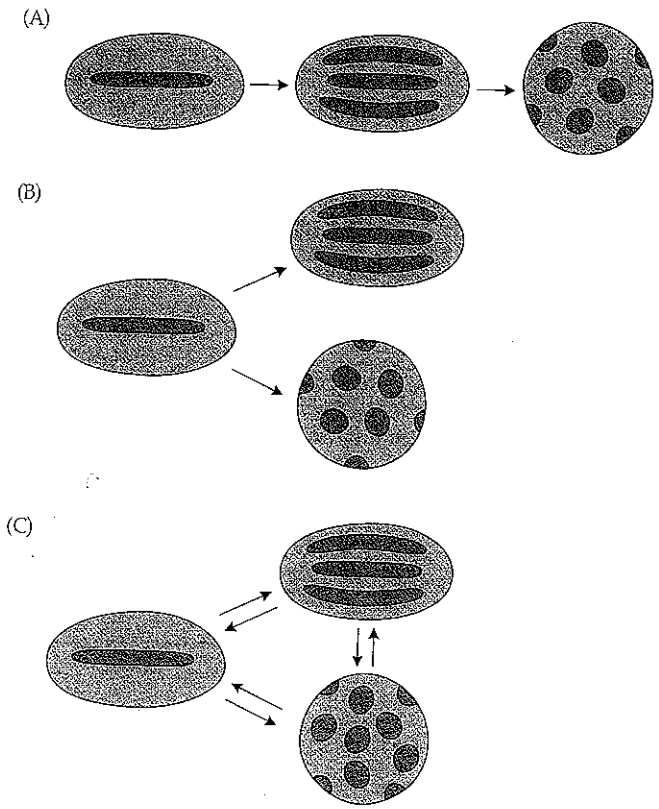


FIGURE 2.11 Three alternative hypotheses about the evolution of pollen morphology. (A) Monosulcate changed to tricolpate, which then changed to pantoporate. As drawn, the character is ordered and irreversible. (B) Monosulcate changed to tricolpate, and then independently changed to pantoporate. Again,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

series of events, the multistate character is considered **ordered**.

If we decide to allow for reversals of character states—that is, if we consider the possibility that pantoporate pollen might switch back to tricolpate and tricolpate to back monosulcate—the character is still ordered. It requires two evolutionary (genetic) steps to go from monosulcate to pantoporate pollen, and two steps 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 want to consider the possibility that monosulcate pollen changed to tricolpate pollen, and in an independent event, monosulcate pollen changed to pantoporate pollen (Figure 2.11B). This sequence would suggest that there is a genetic switch that allows change from monosulcate to tricolpate pollen, as well as a switch that allows change from monosulcate to pantoporate pollen, but that a change from tricolpate to pantoporate pollen is impossible. The character in this case is still ordered, but in a different way from what Figure 2.11A shows. If reversals are possible, then two steps are required to get from tricolpate to pantoporate pollen, 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 method 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 cotyledon 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 C, therefore, we are comparing two hypotheses: (1) that mutations in the genes leading to petal fusion have happened more than once and (2) that 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 approach 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 in order for a morphological structure to be created, but only one of those genes needs to change for the structure to be lost.

We can build Dollo's law 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**. (Defining 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 phylogenetic analyses. This weighting reflects the assumption that certain characters should be harder to modify 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 they 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); the 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, in DNA sequence analyses, 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 to be easier to reverse. Restriction site gains may be weighted over restriction 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 easier for selection to modify over evolutionary time.

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

Underlying every discussion of weights is the assumption that all characters of organisms evolve independently. This assumption requires that change in one character not increase the probability of change in another character. Like the previous assumption, this one may be violated frequently; for example, a change in flower color might well lead to a shift in pollinators, which would then increase the probability that corolla shape would change. Violations of this assumption obviously affect character weighting, in that the likelihood of change for any 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. 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 has the maximum likelihood, or the minimum distance. Trees can be evaluated more precisely, however. For the purposes of this discussion, we will continue to focus on phylogenies generated by parsimony methods (i.e., with the fewest evolutionary steps).

With parsimony methods, the shortest available tree is preferred over one that is longer. However, some parts of the tree may be more reliable than others. This will be the case if reversals and parallelisms (or simple misinterpretations 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 support for a particular part of a tree 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, a phylogeny of some members of the Ericaceae based on DNA sequence data (Figure 2.12; Kron and Judd 1997) found 18

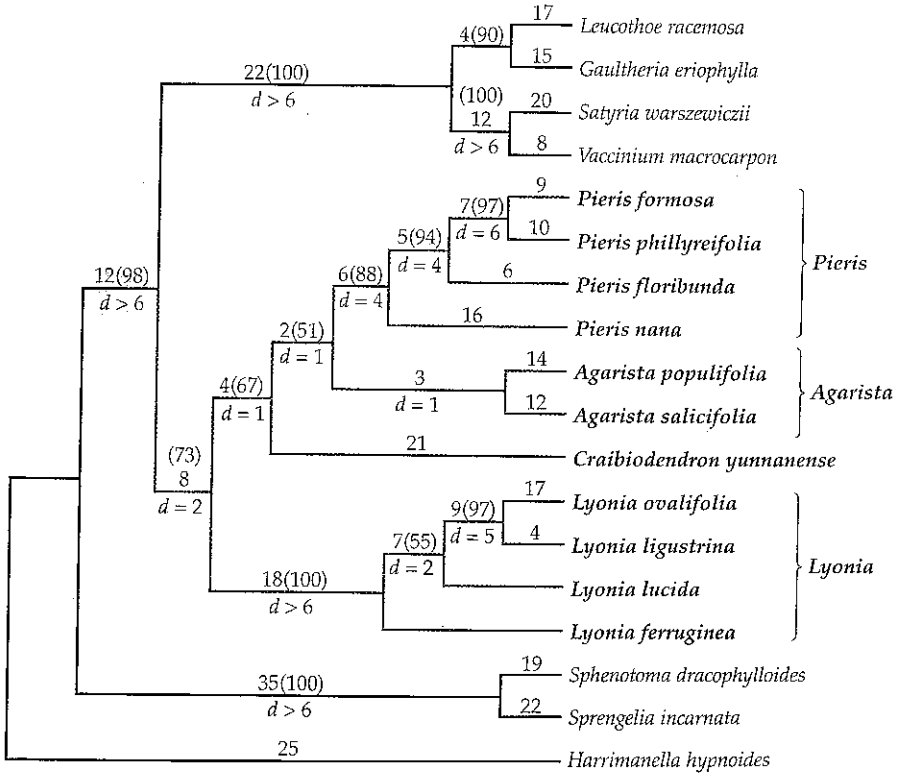


FIGURE 2.12 The single most parsimonious tree found in analysis of *Lyonieae* (taxa in boldface type, lines in blue) using data from sequences of the *matK* gene. Branch lengths appear above lines; bootstrap values are in parentheses; decay indices (*d*) appear below lines. Length = 425, consistency index = 0.60. (From Kron and Judd 1997.)

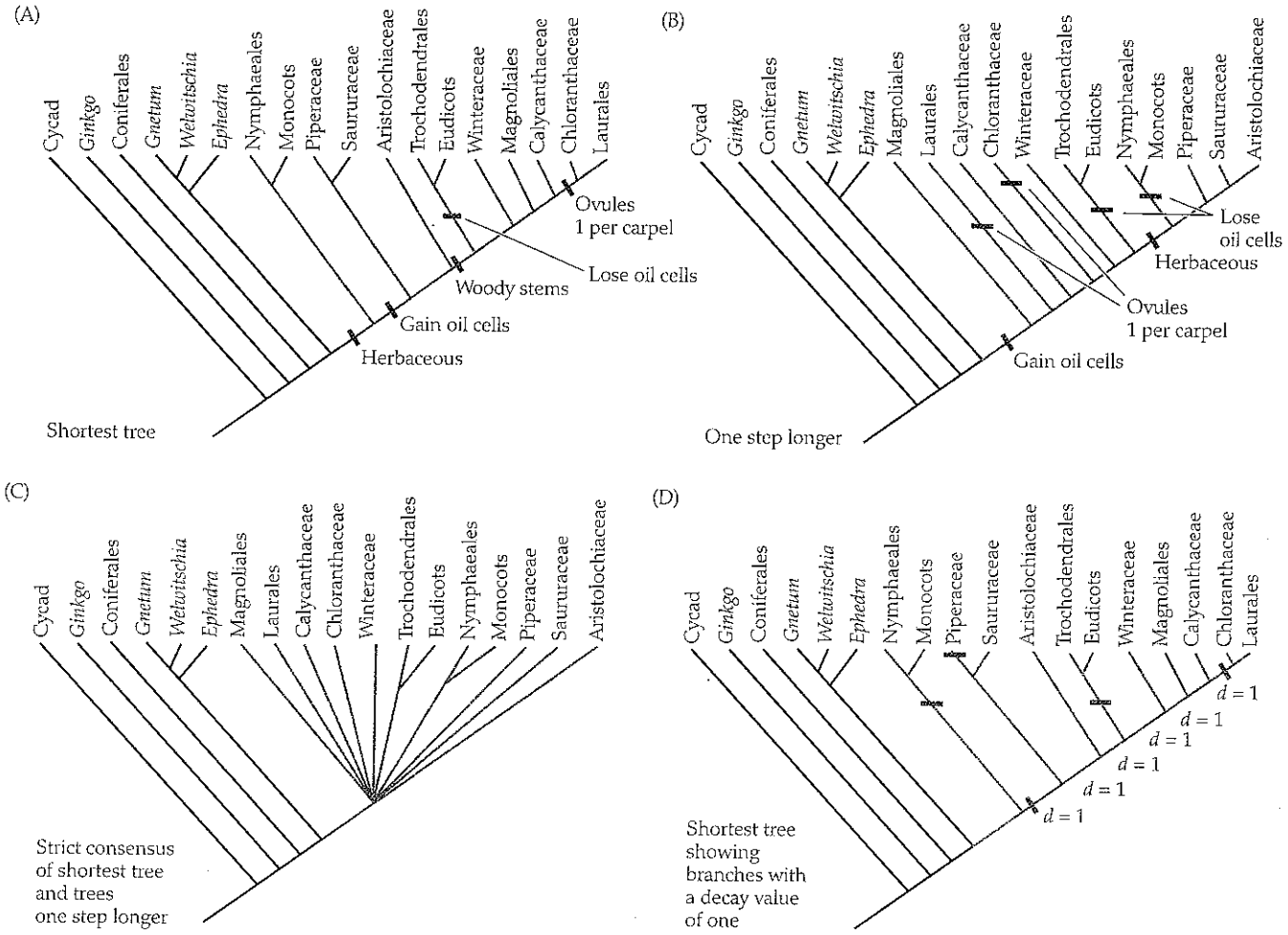


FIGURE 2.13 (A) Phylogeny of the angiosperms (blue lines), indicating patterns of change in the presence or absence of oil cells, ovule number per carpel, and plant habit (woody or herbaceous). (B) An alternative tree, only one step longer than the tree in A, showing patterns of change in the same characters. 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 tree and trees one step longer (Figures 2.13A and B). (D) The same tree as in A, showing branches with a decay value of 1. (Data from Doyle et al. 1994.)

changes on the branch leading to the *Lyonia* clade. In an analysis of morphological characters for the same taxa, there were four characters that changed along the *Lyonia* branch and nowhere else on the tree. In other words, a number of the changes that occurred during the origin of the *Lyonia* clade produced novel characteristics, found nowhere else in the family. Groups like the *Lyonia* clade that share numerous characters that do not change elsewhere on the cladogram are more believable than groups that share 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. In other words, suppose we ask whether there are other ways to analyze the homoplasious characters that lead to trees that are one, two, or three steps longer.

For example, in a study of angiosperm diversification (Doyle et al. 1994), the shortest tree indicated that the earliest-diverging lineages in the angiosperms were the monocots and the water lilies (Nymphaeaceae; see Chapter 9). This implied that the character herbaceous stems was gained once and then lost, whereas reduction in the number of ovules per carpel to one occurred only once, and oil cells were gained once and lost once (Figure 2.13A). On the other hand, trees one step longer, in which the earliest angiosperm lineages led to the magnolias, suggested 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, we can hypothesize that some characters are less homoplasious, but some are more so. If we now take the strict consensus of all the trees, including the shortest ones and those one step longer, all 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).

You can see that many of the branches in the shortest trees do not appear in trees one step longer. Thus all those branches are not drawn in the strict consensus; in other words, they "collapse", or "decay." We can indicate this by placing a 1 next to each of the collapsing branches of the shortest tree (Figure 2.13D). This 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 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 expectations, 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 from the original matrix to become the first column of the new matrix. Then choose another column 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 method is usually described as random sampling with replacement.

Figure 2.14 shows the matrix in Figure 2.8A randomly sampled with replacement; note that the first character from the original matrix (pollen colpi) has been selected twice, whereas the third character (inflorescence a head) has been missed by the random selection process. Multiple randomized matrices are constructed, and the most parsimonious trees are found for each new matrix. This process is used to create a set of at least 100 trees, which can be summarized by a consensus tree (see pages 24–25). In the bootstrap consensus tree, a clade with a bootstrap value of, say, 95% was present in 95% of the trees generated in the bootstrap analyses.

The phylogeny in Figure 2.12 shows both bootstrap and decay values, along with branch lengths. 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 decay value of 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, 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 morphology, chloroplast gene sequences, and nuclear gene sequences.

	Pollen colpi	Petals	Pollen colpi	Cotyledon number
Red star plants	< 3	free	< 3	2
Gold 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.

Comparing trees is often particularly intriguing when the data come from different genes, as will be discussed in more detail 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 kind of data can produce alone. Morphological phylogenies assume that hybridization has not occurred, or at least is rare (Box 2D); multiple molecular trees can often test this assumption.

Describing Evolution: Mapping Characters on Trees

Phylogenies can 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 evolutionary history, however, careful attention must be given to the characters and character states used in the description. In the discussion that follows we will focus

BOX 2D Phylogenetic Analysis Assumes that Evolution Can Be Diagrammed as a Branching Tree

Phylogenetic studies assume that after two lineages diverge, 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; Wagner 1980, 1983; Bremer 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, 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).

on morphological characters, but many of the points apply to any kind of character.

Consider a group of plants for which the phylogenetic tree is known; a good example is the Ericaceae, for which much information is available (Figure 2.15). Assume for the purposes of this discussion that this tree is an accurate reflection of history and that each of the terminal genera really is monophyletic, as demonstrated by studies of 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. In addition, when constructing classifications, one frequently wants to know what morphological characters can be attributed to and distinguish a particular monophyletic group.

Figure 2.15 shows 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 once in the lineage leading to *Ledum* (Labrador tea) and again in the lineage leading to *Vaccinium* sect. *Oxycoccum* (cranberries). Phrased 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 2.16A). There must have been one genetic change, but it could have happened as easily in the lineage leading to the cranberries (sect. *Oxycoccum*) as in the lineage leading to the blueberries (other *Vaccinium*).

Only by reference to the outgroup *Epacris* can we 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 2.16B). 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, 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 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 be the

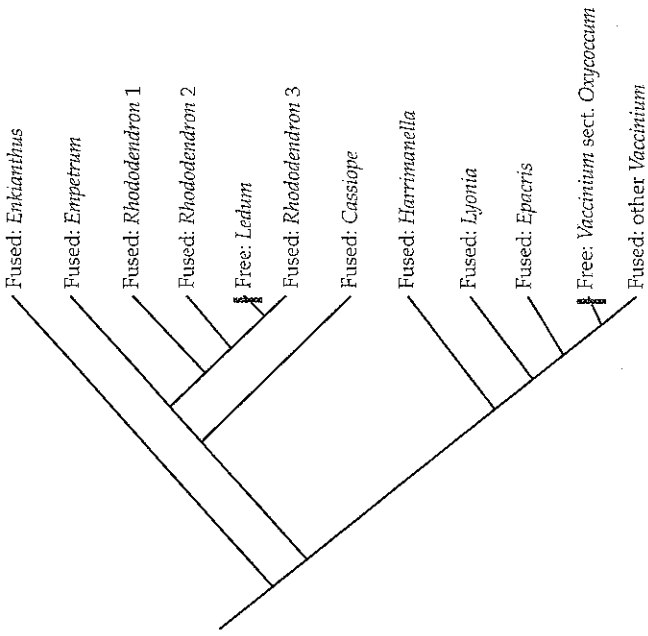


FIGURE 2.15 Phylogeny of a portion of the Ericaceae. The genus *Rhododendron* is paraphyletic and is represented by three separate lineages, numbered 1 to 3. Two changes to free petals are hypothesized. (Data from Stevens 1998.)

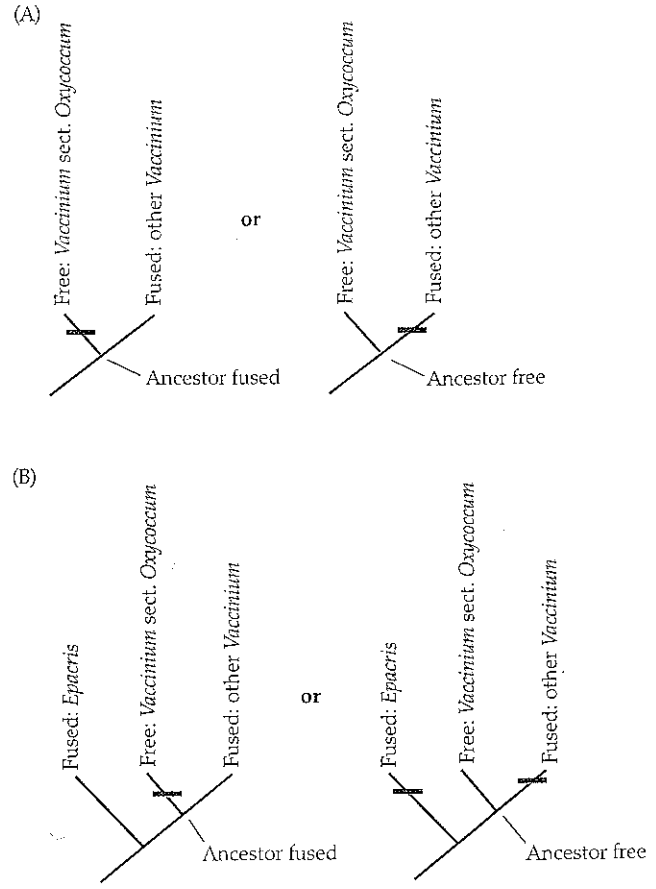


FIGURE 2.16 (A) Two *Vaccinium* taxa differ in character states. It is impossible to determine from this information alone what the character state of the ancestor was because either assumption involves one change in one descendant lineage. (B) The addition of an outgroup determines the character state of the ancestor. In this case, it is simpler (requires fewer steps) to assume that the ancestor had fused petals.

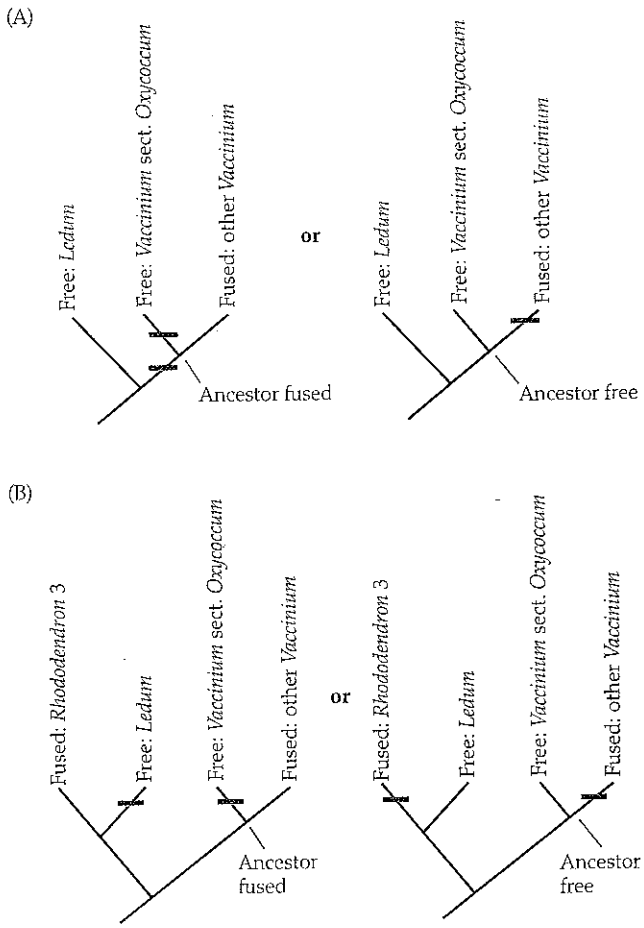


FIGURE 2.17 (A) Analysis of character state change in *Vaccinium* using a different outgroup. Note that the inference of the ancestral state is exactly the opposite of that reached when *Epacris* is used as an outgroup. (B) Analysis of character state change in *Vaccinium* using two outgroups that differ in state. It is now impossible to determine the character state of the ancestor.

case if material of the other genera were hard to obtain, or if those genera 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 2.17A). *This is exactly the opposite conclusion from the one reached in the previous example,* and the only difference is the genera included in the analysis.

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 2.17B). It is as simple to postulate that the ancestor of the group had fused petals and that there were two changes to free petals as it is to postulate that the ancestor had free petals and that there were two changes to fused.

These two choices are known as **equally parsimonious reconstructions**. 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 is sister to the rest of *Vaccinium* (Figure 2.18). In addition, suppose that it is unclear whether the petals are fused or free. (This type of ambiguity is more common than 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.) In this example we do not know what the ancestral state was for *Vaccinium,* so we cannot make any hypothesis about the direction of evolutionary change. Nor can we be sure that the character "petals fused" is a synapomorphy for the genus.

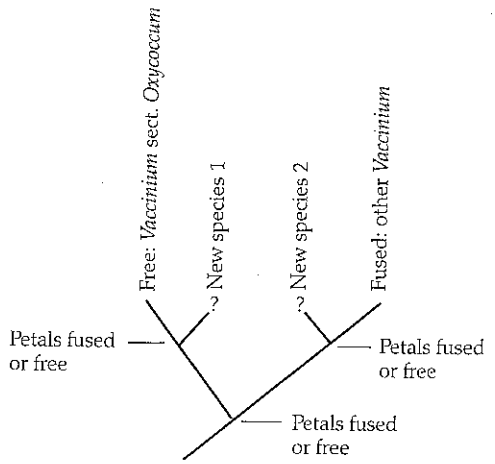


FIGURE 2.18 Addition of species for which the character state is unknown can prevent any inference about the ancestral state.

Various algorithms have been developed to assign character state changes to particular portions of trees (see Chapters 3 and 4 of Maddison and Maddison 2000 for a lucid and comprehensive discussion of these). Depending on the algorithm used, the character state changes can be biased in favor of parallelisms (the "delayed transformation," or DELTRAN, algorithm) or in favor of reversals ("accelerated transformation," or ACCTRAN). The results can have implications—sometimes major ones—for hypotheses about the evolutionary process, and they may also affect how organisms are described in a classification.

Constructing a Classification

The theory of classification is a topic with which systematists have been wrestling for centuries, and their struggles have led 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, classification has several goals. A classification is a common vocabulary designed to aid communication. Therefore a classification should be stable; names that are frequently changed become useless for communication. In addition, a classification should be predictive; that is, the name of a plant should help you to learn more about that plant and guide you to its literature.

Systematists generally agree about the goals of classification, but they may disagree profoundly about how to reach those goals. In this text we take a particular point of view, using phylogenetic classifications throughout. Thus, as far as possible, we recognize monophyletic and avoid paraphyletic or polyphyletic groups. In the few cases in which a nonmonophyletic 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 relatively few phylogenetic analyses are available at this level, so generally we have not tried to indicate possible or probable paraphyly or polyphyly of genera.

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 are more predictive and of greater heuristic value then those based on overall similarity or on idiosyncratic weighting of particular characters (Farris 1979; Donoghue and Cantino 1988).

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 (Nelson and Platnick 1981; Humphries and Parenti 1986; Brooks and McLennan 1991; Forey et al. 1992). 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 for making conservation decisions. Finally, phylogenetic classifications provide a framework for biological knowledge and a basis for comparative studies linking all fields of biology (Cracraft and Donoghue 2004).

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

Grouping: Named Groups Are Monophyletic

A phylogenetic classification reflects evolutionary history and attempts to give names *only* to groups that are monophyletic—that is, composed of an ancestor and all its descendants. In the example in Figure 2.4C, we infer that the Asteraceae (the 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 also 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 and may not actually need one.

In phylogenetic classification, paraphyletic groups are not named. In Figure 2.4C, 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 is also the most recent common ancestor of any

circle plant and a diamond plant. In other words, the circle plants are as distantly related to square plants as any one of them is to diamond plants. Naming a group that included the square plus the circle plants would imply that the two plants are closely related even though 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," a group that traditionally includes the nonvascular land plants (liverworts, hornworts, and mosses; see Figure 1.1). But the liverworts, hornworts, and mosses are more distantly related to one another than the mosses are to the vascular plants (tracheophytes). Without quotation marks, the name *bryophytes* implies a closer relationship than actually exists.

Several traditionally recognized plant families, such as Apocynaceae and Capparaceae in the broad sense, are paraphyletic. In this text these families have been recircumscribed so as to recognize monophyletic groups: Apocynaceae has been combined with Asclepiadaceae, and Capparaceae has been combined with Brassicaceae (although some systematists divide it into Capparaceae s.s. and Cleomaceae).

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, and so on. But such a classification would be cumbersome; in addition, it would not be stable because our view of sister species would change each time a new species was described, and our view of the entire classification would have to shift accordingly.

In practice, many monophyletic groups are not named. For example, the genus *Stenanthium* (Melanthiaceae) is monophyletic and contains four species (Zomlefer et al. 2001; Zomlefer and Judd 2002; Wofford 2006). Although it is clear that these four species fall into two monophyletic pairs, the two 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 that contains four large, traditionally recognized subfamilies plus two smaller ones. Although agrostologists refer to this clade as the PACCAD clade (an acronym for Panicoideae-Arundinoideae-Centothecoideae-Chloridoideae-Aristidoideae-Danthonioideae), it has no formal Latin name.

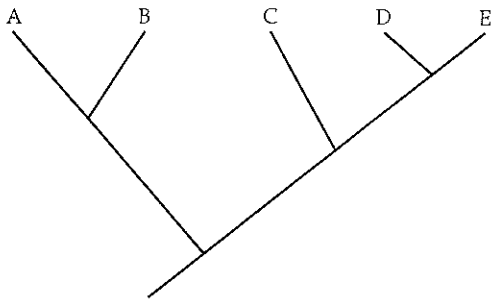
How do systematists decide which monophyletic groups to name? There is no codified set of rules, but several criteria have been suggested, and some criteria are in common use despite not being fully articulated. A major criterion—perhaps *the* major criterion—is the strength of the evidence supporting 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 a set of characters by which it can be distinguished from other clades, or **diagnosed**. This criterion is also important to nomenclatural stability: if the meaning of a name shifts every time a new phylogeny is produced or a new character is examined, the name becomes effectively meaningless.

A second criterion is the presence of an obvious morphological character. Although systematists may not 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 nonsystematists 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 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, she can easily and reliably infer many aspects of its biology. (Lack of an obvious morphological synapomorphy is one of several reasons that the PACCAD 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 to 7) (Stevens 1998), but to organize and remember larger numbers of items requires additional mnemonic devices. (As an example, consider how many 9-digit zip codes you can remember compared with the 5-digit variety, or with 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: 83), "We must be able to place taxa in higher taxa so that we can find them again." The genus *Stenanthium* could be redefined to include only *Stenanthium gramineum* and *S. diffusum*, and a new genus could be described to include *S. densum* and *S. leimanthoides*. 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, groups 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 PACCAD clade of the grasses, in that it would entail an unnecessary set of changes affecting long-standing taxonomic usage (Backlund and Bremer 1998; Stevens 1998).



Categorical rank	Sequencing convention
Family ABCDE	Family ABCDE
Genus AB	Genus AB
Species A	Species A
Species B	Species B
Genus CDE	Genus C
Subgenus C	Species C
Species C	Genus DE
Subgenus DE	Species D
Species D	Species E
Species E	

FIGURE 2.19 Alternative classifications based on the phylogeny of a hypothetical group of taxa A, B, C, D, and E. The classification on the left uses four categorical ranks (family, genus, subgenus, and species); the one on the right uses only three ranks (family, genus, and species) plus a sequencing convention.

Ranking: Ranks Are Arbitrary

Having decided which monophyletic groups to name, we still have 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 each numbered group. This approach is similar to the system used by the telephone company to organize telephone numbers. 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 for the classification 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 are used to express sister-group relationships.

Even though 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 no criteria are available to indicate that a particular taxon, such as the angiosperms, should be recognized at the level of phylum, class, or order.

In Figure 2.19, a cladogram of imaginary taxa A through E is first converted into a hierarchical classification according to Linnaean categorical ranks. Note that subgenus DE is nested within genus CDE, which is in turn nested within family ABCDE. (But we could have treated clade ABCDE as an order, clade CDE as a family, and clade DE as a genus.) Often, however, to fully express the sister-group relationships (in the cladogram), one needs more ranks than are available (in the taxonomic hierarchy), even after creating additional ranks by use of the prefixes *super-* and *sub-*.

One modification to the method of classification outlined here is the **sequencing convention**, which states that taxa that form an asymmetrical part of a cladogram may be placed at the same rank and arranged in their order of branching (Wiley 1979, 1981). Thus in Figure 2.19, AB, C, and DE could all be designated as genera. 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 here for deciding which groups to name can also be applied to deciding the level at which to rank a group (see Stevens 1998 for full discussion). Nomenclatural stability again becomes important here. For example, it has recently been shown that the earliest-diverging lineage in the family Poaceae includes only two extant genera, *Anomochloa* and *Streptochaeta* (Figure 2.20). Thus 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: Anomochlooideae.

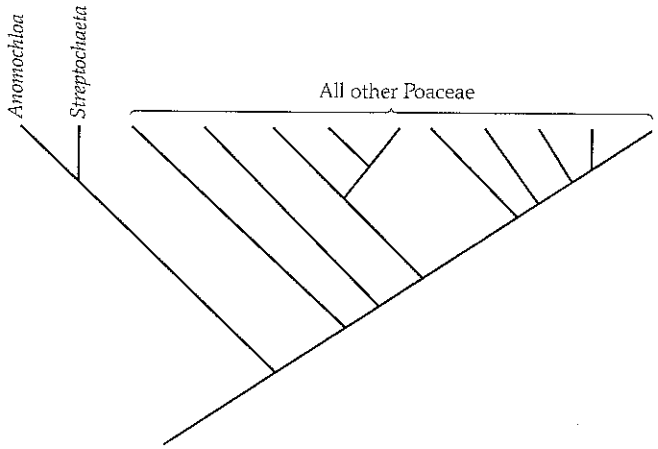


FIGURE 2.20 Phylogeny of Poaceae, showing the position of the genera *Anomochloa* and *Streptochaeta*.

Some systematists have proposed abandoning the Linnaean system altogether and replacing it with a "phylogenetic taxonomy." Full exploration of this possibility is beyond the scope of this text, but we will briefly address the arguments against the use of Linnaean ranks here.

Because rank is arbitrary, a genus (group of species) in one family may not be the same age as, encompass the same amount of variation as, or indeed have anything in common at all with a genus in another family—other than the fact that they are both monophyletic groups. Trained systematists are generally aware of this (Darwin was, for example) and realize that genera, families, and so on are not comparable units (Stevens 1997). Some scientists, however, frequently use such categories as though they were real. For example, it is common to measure plant diversity by listing the number of families represented by a local flora, even though the unit *family* does not mean anything in particular.

If rank is arbitrary, then one logical step would be to eliminate ranks altogether. Taxa would be placed in named groups, but the groups would not be designated as genus, family, order, or any other rank. Such categorization already exists informally, particularly among groups above the level of orders. The eudicots, for example, are widely recognized as monophyletic, but are not given a particular Linnaean rank. Similarly, few systematists worry about whether the angiosperms should be recognized as a division, class, subclass, superorder, or other rank; they are clearly monophyletic and designated by the non-Linnaean name *angiosperm*.

Eliminating ranks becomes more problematic among orders, families, and genera. Groups assigned to those ranks are familiar and their names are in common use, so an entirely new sort of nomenclature is unlikely to be accepted rapidly or without protest. Nonetheless, an alternative system of phylogenetic nomenclature, known as the *PhyloCode*, is being developed. The PhyloCode is designed entirely outside the rules of the International Code of Botanical Nomenclature (ICBN), which governs the use of Linnaean ranks and has long been used by all plant taxonomists (see Appendix 1). It is an alternative nomenclatural system rather than a revision of the existing system (see the PhyloCode Web site at www.ohiou.edu/phylocode).

Another result of phylogenetic studies is the observation that many phylogenies are only partially resolved, so that precise placement of taxa is impossible given the available data. This means that some species cannot be placed for certain in a genus, and some genera cannot be reliably assigned to a family. The current system allows uncertain placements above the rank of species to be reflected by the category *incertae sedis*—literally, "of uncertain position." An alternative would be a rank-free system, in which neither placement in a larger group nor naming all branches of a dichotomy or polytomy is necessary.

The authors of this text have been involved in reclassifications of genera, families, and orders on the basis of phylogenetic data and have found that—as long as the phylogeny is clear—use of the standard Linnaean hierarchy is quite easy (especially when it is supplemented by unranked informal names). When the phylogeny is unclear, it is usually reasonable to wait for more data before modifying the classification.

For more discussion of the problems encountered in using the Linnaean system in phylogenetic classification, consult Wiley 1981; de Queiroz and Gauthier 1990, 1992; Wiley et al. 1991; Forey et al. 1992; and Hibbett and Donoghue 1998.

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 have been 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 because 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 approach 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 approach led to results similar to 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 one another. Such groups have since proved 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 first carefully observed as many characters as possible. These characters were divided into states, or the quantitative value of the character was recorded (e.g., a series of measurements of leaf length was taken and the mean recorded for each taxon). This information was arranged in a character × taxon matrix similar to that in Figure 2.8A. The matrix was converted to a similarity (taxon × taxon) matrix by the use of 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 and illustrated the similarity relationships with either a maplike or a treelike diagram (a phenogram) (Figure 2.21). Phenograms were constructed using clustering algorithms, while maplike diagrams resulted from ordination studies employing multivariate statistical procedures (see Abbot et al. 1985).

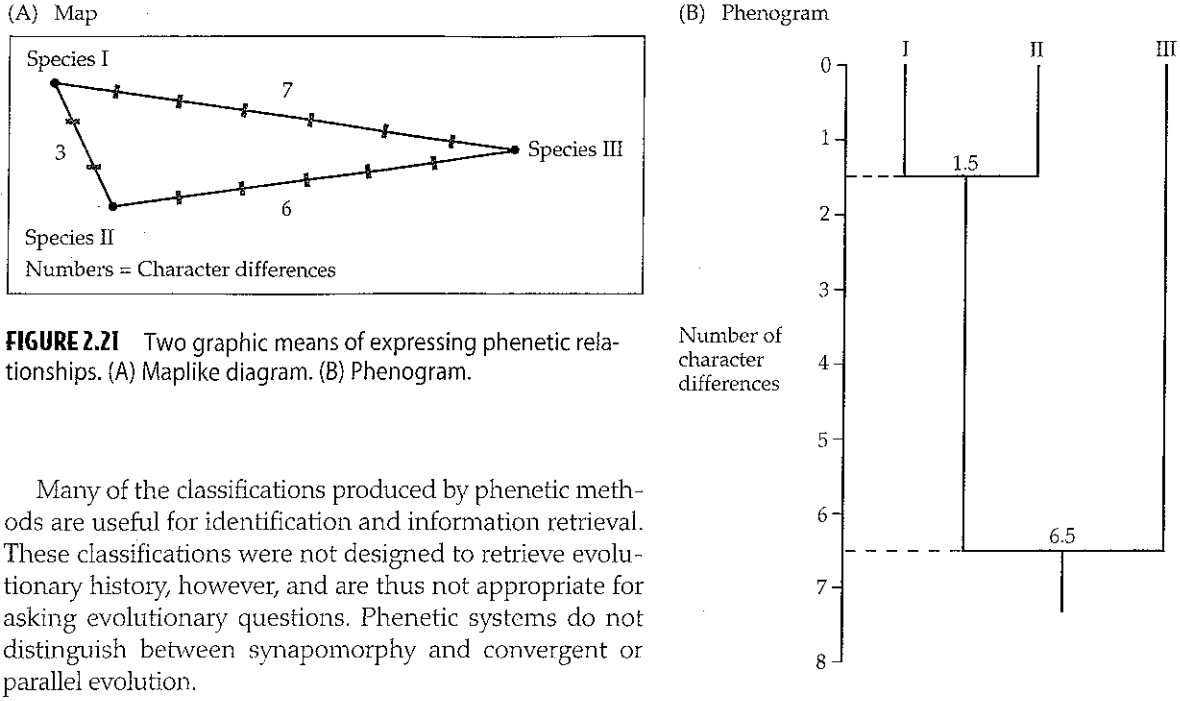


FIGURE 2.21 Two graphic means of expressing phenetic relationships. (A) Maplike diagram. (B) Phenogram.

Many of the classifications produced by phenetic methods 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 also 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 2.22). 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 were stressed, and the expertise, authority, and intuition of individual systematists were central. Finally,

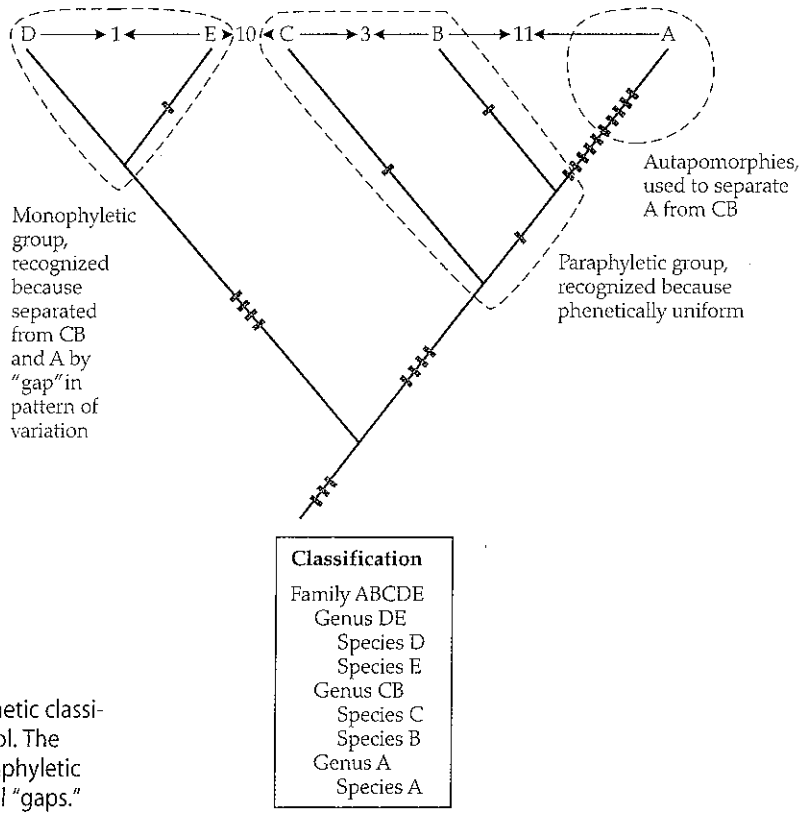


FIGURE 2.22 Phylogeny and a resulting nonphylogenetic classification produced according to the evolutionary school. The classification includes a mix of monophyletic and paraphyletic groups, separated from one another by morphological "gaps."

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 one another by discrete gaps (Ashlock 1979; Stuessy 1983, 1990; Stevens 1986; Mayr and Ashlock 1991).

It has been said that systematics is as much an art as a science (although this statement 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 of systematics—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.

LITERATURE CITED AND SUGGESTED READINGS

Items marked with an asterisk are especially recommended to those readers who are interested in further information on the topics discussed in this chapter.

Abbot, L. A., F. A. Bisby and D. A. Rogers. 1985. *Taxonomic analysis in biology*. Columbia University Press, New York. [An introduction to various phenetic methods.]

Albert, V. A., A. Backlund, K. Bremer, M. W. Chase, J. R. Manhart, B. D. Mishler and K. C. Nixon. 1994. Functional constraints and *rbcL* evidence for land plant phylogeny. *Ann. Missouri Bot. Gard.* 81: 534–567.

Ashlock, P. D. 1979. An evolutionary systematist's view of classification. *Syst. Zool.* 28: 441–450. [Presentation of an easy-to-follow explicit method to construct an evolutionary taxonomic classification.]

*Backlund, A. and K. Bremer. 1998. To be or not to be: Principles of classification and monotypic plant families. *Taxon* 47: 391–400.

Bremer, K. 1983. Angiosperms and phylogenetic systematics. *Verh. Naturwiss. Verein Hamburg* 26: 343–354. [Discussion of reticulate evolution and cladistics.]

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803. [Decay indices.]

Bremer, K. and H.-E. Wanntorp. 1979. Hierarchy and reticulations in systematics. *Syst. Zool.* 28: 624–627.

*Brooks, D. R. and D. A. McLennan. 1991. *Phylogeny, ecology, and behavior*. University of Chicago Press, Chicago. [Excellent presentation of biological uses of phylogenetic hypotheses.]

Burleigh, J. G. and S. Mathews. 2004. Phylogenetic signal in nucleotide data from seed plants: Implications for resolving the seed plant tree of life. *Am. J. Bot.* 91: 1599–1613.

Cantino, P. D., H. N. Bryant, K. de Queiroz, M. J. Donoghue, T. Eriksson, D. M. Hillis and M. S.Y. Lee. 1999. Species names in phylogenetic nomenclature. *Syst. Biol.* 48: 790–807.

*Cracraft, J. and M. J. Donoghue. 2004. *Assembling the tree of life*. Oxford University Press, Oxford and New York.

Cronquist, A. 1987. A botanical critique of cladism. *Bot. Rev.* 53: 1–52.

*Dahlgren, R. and F. N. Rasmussen. 1983. Monocotyledon evolution: Characters and phylogenetic estimate. In *Evolutionary biology*, vol. 16, M. K. Hecht, B. Wallace and G. T. Prance (eds.), 255–395. Plenum Press, New York. [Contains a simple introduction to cladistic methods.]

Davis, P. D. and V. H. Heywood. 1963. *Principles of angiosperm taxonomy*. Krieger, New York.

*de Queiroz, K. and J. Gauthier. 1990. Phylogeny as a central principle in taxonomy: Phylogenetic definitions of taxon names. *Syst. Zool.* 39: 307–322. [Proposal to abandon the Linnaean system.]

de Queiroz, K. and J. Gauthier. 1992. Phylogenetic taxonomy. *Annu. Rev. Ecol. Syst.* 23: 449–480.

de Queiroz, A., M. J. Donoghue and J. Kim. 1995. Separate versus combined analyses of phylogenetic evidence. *Annu. Rev. Ecol. Syst.* 26: 567–581.

*Donoghue, M. J. and P. D. Cantino. 1988. Paraphyly, ancestors, and the goals of taxonomy: A botanical defense of cladism. *Bot. Rev.* 54: 107–128.

Doyle, J. A., M. J. Donoghue and E. A. Zimmer. 1994. Integration of morphological and ribosomal RNA data on the origin of angiosperms. *Ann. Missouri Bot. Gard.* 81: 419–450.

*Eldredge, N. and J. Cracraft. 1980. *Phylogenetic patterns and the evolutionary process: Methods and theory in comparative biology*. Columbia University Press, New York.

*Farris, J. S. 1974. Formal definitions of paraphyly and polyphyly. *Syst. Zool.* 23: 548–554. [A polyphyletic group is defined as "a group in which the most recent common ancestor is assigned to some other group and not the group itself."]

Farris, J. S. 1979. The information content of the phylogenetic system. *Syst. Zool.* 28: 458–519.

Farris, J. S. 1989. *Hennig86*, Version 1.5. Port Jefferson Station, NY.

Felsenstein, J. 1978. Cases in which parsimony and compatibility methods will be positively misleading. *Syst. Zool.* 27: 401–410.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* 17: 368–376.

Felsenstein, J. 1989. *PHYLIP 3.2 manual*. University of California Herbarium, Berkeley.

*Forey, P. L., C. J. Humphries, I. L. Kitching, R. W. Scotland, D. J. Siebert and D. M. Williams. 1992. *Cladistics: A practical course in systematics*. Oxford University Press, Oxford. [Summary of then-current cladistic methods.]

*Frohlich, M. W. 1987. Common-is-primitive: A partial validation by tree counting. *Syst. Bot.* 12: 217–237. [The principle that says that given an ingroup and an outgroup, both with states A and B of a homologous character, if state A is much more common than state B *in the outgroup*, then state A is likely to be ancestral *within the ingroup*.]

Funk, V. A. 1985. Phylogenetic patterns and hybridization. *Ann. Missouri Bot. Gard.* 72: 681–715.

Gift, N. and P. F. Stevens. 1997. Vagaries in the delimitation of character states in quantitative variation: An experimental study. *Syst. Biol.* 46: 112–125.

*Givnish, T. J. and K. J. Sytsma. 1997. Homoplasy in molecular vs. morphological data: The likelihood of correct phylogenetic inference. In *Molecular evolution and adaptive radiation*, T. J. Givnish and K. J. Sytsma (eds.), 55–101. Cambridge University Press, Cambridge.

Goloboff, P. A. 1993. *NONA*, Version 1.5.1. P. A. Goloboff, Tucumán, Argentina.

Hall, B. G. 2005. *Phylogenetic trees made easy*, 2nd ed. Sinauer Associates, Sunderland, MA.

*Hennig, W. 1966. *Phylogenetic systematics*. University of Illinois Press, Urbana.

Hibbett, D. and M. J. Donoghue. 1998. Integrating phylogenetic analysis and classification in fungi. *Mycologia* 90: 347–356.

Hillis, D. M., M. W. Allard and M. M. Miyamoto. 1993. Analysis of DNA sequence data: Phylogenetic inference. *Methods Enzymol.* 224: 456–487.

*Huelsenbeck, J. P. 1995. Performance of phylogenetic methods in simulation. *Syst. Biol.* 44: 17–48. [Comparison of performance in computer simulations of parsimony, maximum likelihood, and overall similarity methods of tree construction.]

*Huelsenbeck, J. P. and D. M. Hillis. 1993. Success of phylogenetic methods in the four-taxon case. *Syst. Biol.* 42: 247–264. [Several types of parsimony methods are relatively successful in reconstructing phylogeny.]

Humphries, C. J. and V. A. Funk. 1982. Cladistic methodology. In *Current concepts in plant taxonomy* (Systematics Association Special Volume, No. 25), V. H. Heywood and D. M. Moore (eds.), 323–362. Academic Press, London. [Introduction to basic cladistic methods.]

Humphries, C. J. and L. R. Parenti. 1986. *Cladistic biogeography*. Clarendon, Oxford.

Kellogg, E. A. 1989. Comments on genomic genera in the Triticeae. *Am. J. Bot.* 76: 796–805.

Kellogg, E. A., R. Appels and R. J. Mason-Gamer. 1996. When genes tell different stories: The diploid genera of Triticeae (Gramineae). *Syst. Bot.* 21: 321–347.

Kitching, I. J., P. L. Forey, C. J. Humphries and D. M. Williams. 1998. *Cladistics: The theory and practice of parsimony analysis*, 2nd ed. Oxford University Press, Oxford. [Summary of parsimony methods for constructing phylogenetic hypotheses, including issues dealing with characters.]

Kron, K. A. and W. S. Judd. 1997. Systematics of the *Lyonia* group (Andromedeae, Ericaceae) and the use of species as terminals in higher-level cladistic analyses. *Syst. Bot.* 22: 479–492.

*Maddison, D. R. and W. P. Maddison. 2000. *MacClade: Analysis of phylogeny and character evolution*, Version 4.0. Sinauer Associates, Sunderland, MA. [A useful program for exploring patterns of character change on a cladogram.]

Maddison, W. P., M. J. Donoghue and D. R. Maddison. 1984. Outgroup analysis and parsimony. *Syst. Zool.* 33: 83–103.

Mayr, E. and P. D. Ashlock. 1991. *Principles of systematic zoology*, 2nd ed. McGraw-Hill, New York.

*McDade, L. A. 1990. Hybrids and phylogenetic systematics. I. Patterns of character expression in hybrids and their implications for cladistic analyses. *Evolution* 44: 1685–1700.

*McDade, L. A. 1992. Hybrids and phylogenetic systematics. II. The impact of hybrids on cladistic analyses. *Evolution* 46: 1329–1346.

McDade, L. A. 1997. Hybrids and phylogenetic systematics. III. Comparison with distance methods. *Syst. Bot.* 22: 669–683.

Nelson, G. and N. Platnick. 1981. *Systematics and biogeography*. Columbia University Press, New York.

Pankhurst, R. J. 1991. *Practical taxonomic computing*. Cambridge University Press, Cambridge. [Use of computers in databases, identification, and phenetic and cladistic analyses.]

*Quicke, D. L. J. 1993. *Principles and techniques of contemporary taxonomy*. Blackwell, London.

Remane, A. 1952. *Die Grundlagen des naturlichen Systems, der vergleichenden Anatomie and der Phylogenetik* (The principles of the natural system, comparative anatomy, and phylogenetics). Geest & Portig, Leipzig.

Rydin, C., M. Källersjó and E. M. Friis. 2002. Seed plant relationships and the systematic position of Gnetales based on nuclear and chloroplast DNA. *Int. J. Plant Sci.* 163: 197–214.

*Sanderson, M. J. and M. J. Donoghue. 1989. Patterns of variation in levels of homoplasy. *Evolution* 43: 1781–1795.

Schuh, R. T. 2000. *Biological systematics: Principles and applications*. Cornell University Press, Ithaca, NY. [Detailed coverage of cladistic methods and application of cladistic results in classification, biogeography, ecological investigations, and conservation.]

Simpson, G. G. 1961. *Principles of animal taxonomy*. Columbia University Press, New York.

Sneath, P. H. A. and R. R. Sokal. 1973. *Numerical taxonomy*. Freeman, San Francisco.

Sokal, R. R. 1986. Phenetic taxonomy: Theory and methods. *Annu. Rev. Ecol. Syst.* 17: 423–442.

*Sokal, R. R. and F. J. Rohlf. 1980. An experiment in taxonomic judgment. *Syst. Bot.* 5: 341–365.

Stevens, P. F. 1980. Evolutionary polarity of character states. *Annu. Rev. Ecol. Syst.* 11: 333–358.

*Stevens, P. F. 1984. Homology and phylogeny: Morphology and systematics. *Syst. Bot.* 9: 395–409.

Stevens, P. F. 1986. Evolutionary classification in botany, 1960–1985. *J. Arnold Arbor.* 67: 313–339.

*Stevens, P. F. 1991. Character states, morphological variation, and phylogenetic analysis: A review. *Syst. Bot.* 16: 553–583.

Stevens, P. F. 1997. How to interpret botanical classifications: Suggestions from history. *BioScience* 47: 243–250.

Stevens, P. F. 1998. What kind of classification should the practising taxonomist use to be saved? In *Plant diversity in Malesia III: Proceedings of the 3rd International Flora Malesiana Symposium 1995*, J. Dransfield, M. J. E. Coode and D. A. Simpson (eds.), 295–319. Royal Botanical Gardens, Kew, London.

Stuessy, T. F. 1983. Phylogenetic trees in plant systematics. *Sida* 10: 1–13.

Stuessy, T. F. 1990. *Plant taxonomy*. Columbia University Press, New York.

*Swofford, D. L. 1993. *PAUP: Phylogenetic analysis using parsimony*, Version 3.1.1. Distributed by the Illinois Natural History Survey, Champaign.

*Swofford, D. L. 2000. *PAUP*: Phylogenetic analysis using parsimony and other methods*, Version 4.0. [Beta-test edition, distributed by Sinauer Associates, Sunderland, MA.]

*Swofford, D. L., G. J. Olsen, P. J. Waddell and D. M. Hillis. 1996. Phylogenetic inference. In *Molecular systematics*, 2nd ed., D. M. Hillis, C. Moritz and B. K. Mable (eds.), 407–514. Sinauer Associates, Sunderland, MA. [Excellent summary of methods of tree construction.]

Sytsma, K. J. and J. C. Pires. 2001. Plant systematics in the next 50 years—Re-mapping the new frontier. *Taxon* 50: 713–732.

*Wagner, W. H., Jr. 1980. Origin and philosophy of the groundplan-divergence method of cladistics. *Syst. Bot.* 5: 173–193.

Wagner, W. H., Jr. 1983. Reticulistics: The recognition of hybrids and their role in cladistics and classification. In *Advances in cladistics: Proceedings of the second meeting of the Willi Hennig Society*, N. I. Platnick and V. A. Funk (eds.), 63–79. Columbia University Press, New York.

Watrous, L. E. and Q. D. Wheeler. 1981. The outgroup comparison method of character analysis. *Syst. Zool.* 30: 1–11.

Weins, J. J., ed. 2000. *Phylogenetic analysis of morphological data*. Smithsonian Institution Press, Washington, DC.

Wiley, E. O. 1979. An annotated Linnaean hierarchy, with comments on natural taxa and competing systems. *Syst. Zool.* 28: 308–337.

*Wiley, E. O. 1981. *Phylogenetics*. Wiley, New York. [Detailed discussion of cladistic principles.]

*Wiley, E. O., D. Siegel-Causey, D. R. Brooks and V. A. Funk. 1991. *The compleat cladist: A primer of phylogenetic procedures* (University of Kansas, Museum of Natural History, Special Publication, No. 19). University of Kansas, Museum of Natural History, Lawrence. [Summary of then-current cladistic methods.]

Wofford, B. E. 2006. A new species of *Stenanthium* (Melanthiaceae) from Tennessee, U.S.A. *Sida* 22: 447–459.

Zomlefer, W. B. and W. S. Judd. Resurrection of segregates of the polyphyletic genus *Zigadenus* s.l. (Liliales: Melanthiaceae) and resulting new combinations. *Novon* 12: 299–308.

Zomlefer, W. B., N. H. Williams, W. M. Whitten and W. S. Judd. 2001. Generic circumscription and relationships in the tribe Melanthieae (Liliales, Melanthiaceae), with emphasis on *Zigadenus*: Evidence from ITS and *trnL-F* sequence data. *Am. J. Bot.* 88: 1657–1669.