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PHYLOGENIES AND THE ANALYSIS OF EVOLUTIONARY SEQUENCES, WITH EXAMPLES FROM SEED PLANTS[1]

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Abstract.—Studies of character evolution have frequently relied on ahistorical correlations rather than on phylogenies. However, correlations do not estimate the number of times that a trait evolved, and they are insensitive to the direction or the temporal sequence of character transformation. In contrast, cladograms can provide this information. A cladistic test of the hypothesis that the evolution of dioecy is favored in animal-dispersed plants indicates that dioecy may have originated somewhat more often in such lineages. Nevertheless, differences in rates of speciation or extinction must largely account for the observed species-level correlation between dispersal and breeding system.

In considering the evolution of individual traits, cladograms help identify the context in which a feature evolved and specify which organisms should be compared in evaluating the causes of character change. Determining whether a feature and a performance advantage were strictly historically correlated or followed one another in sequence helps to distinguish whether the trait is an adaptation or an exaptation for the function. For example, cladograms of seed plants suggest that double fertilization arose incidentally prior to the origin of angiosperms and that the resulting product was later co-opted and elaborated as a nutritive tissue for the developing embryo.

The order of character assembly in a lineage also bears on the evolution of functional and developmental interdependencies. In particular, it may be possible to trace the evolution of a character's "burden" from an initial period, during which change is more likely, through later stages, wherein successful modification is less likely owing to the evolution of dependent characters. The evolution of vessels and of floral phyllotaxis in angiosperms may exemplify this pattern. Recognition that the likelihood of character transformation may change during the evolution of a group warns against character weighting in phylogenetic analysis.

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Phylogenetic trees appear only rarely in *Evolution, The American Naturalist,* or *Paleobiology,* and have seldom been integrated into any general approach to evolution, despite suggestions that this be done (e.g., Lauder, 1981, 1982; Huey, 1987; Wake and Larson, 1987). Rather than take advantage of phylogenies, evolutionists have tended to ignore history altogether or have gone to great lengths to try to factor it out (methods for doing so are reviewed by Pagel and Harvey [1988]). Perhaps the main reason for this neglect is that evolutionists have doubted the ability to reconstruct evolutionary history accurately. This attitude has been fueled by the view that natural selection can obliterate all traces of the past—that convergent evolution is so pervasive that attempts to reconstruct phylogeny are doomed from the outset.

Taxonomists themselves have sometimes fostered this skeptical outlook, but there has

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recently been a dramatic renewal of interest in phylogeny reconstruction. This is primarily a function of fundamental advances in logic (especially Hennig's [1966] deduction that only synapomorphies provide evidence of monophyly) coupled with better methods for analyzing character data (e.g., Farris, 1970; see Felsenstein, 1982). These developments, combined with new character information (especially molecular sequence data and developmental information on morphological traits), have rendered phylogenies far more worthy of serious attention.

Even if we assume, however, that a reasonably accurate picture of phylogeny can be achieved, another and perhaps more fundamental question needs to be resolved. Evolutionists are rightly concerned with formulating general laws about the evolutionary process, and it is not entirely clear how phylogenies can be put to use in studying general properties of evolution. What, if anything, can be gained from phylogenies that cannot be obtained otherwise?

Here, I consider the use of phylogenies in studying character evolution, that is, the causes and consequences of the evolutionary transformation of characters. In particular, I focus on the role of cladograms in elucidating sequences of character change and the bearing of such sequences on the choice among alternative evolutionary explanations. By way of illustration, I draw on examples from seed plants and angiosperms, relying heavily on the results of recent cladistic studies (Doyle and Donoghue, 1986; Donoghue and Doyle, 1989). Rather than detail these analyses here, I will simply assume that they provide an accurate assessment of phylogenetic relationships and will concentrate instead on how the results might be used in studying the evolutionary process.

Correlations and the Evolution of Dioecy

Studies of character evolution have often relied heavily on correlations between a trait of interest and other traits or environmental variables. Unfortunately, this ahistorical comparative approach may give misleading results (e.g., Ridley, 1983; Clutton-Brock and Harvey, 1984; Felsenstein, 1985; Huey, 1987). In particular, it cannot provide an estimate of the number of times that a trait evolved, and it is insensitive to the direction of evolution and the order in which characters were assembled in a lineage. To illustrate these points, I will focus on a single example, namely Givnish's studies of the relationship between dioecy and animal dispersal of fleshy propagules in seed plants (Givnish, 1980, 1982; also see Bawa [1980]).

Givnish (1980) observed that 339 species of nonangiospermous seed plants ("gymnosperms") are monoecious and have dry seeds dispersed by wind, 402 are dioecious and have fleshy propagules dispersed by animals, and relatively few species have either of the other combinations (45 are monoecious with fleshy propagules; 18 are dioecious with dry propagules). This highly significant association ($X^2 = 569.89$, $d.f. = 1$, $P < 0.0001$) led to a consideration of its adaptive significance. In particular, Givnish (1980) devised a model to explain why the evolution of dioecy would be favored in animal-dispersed plants more than in wind-dispersed plants, focusing on ecological mechanisms related to pollination and dispersal, rather than on the benefits of outcrossing (cf. Darwin, 1876). He argued that increased investment in female function by plants with fleshy propagules would yield a disproportionate increase in fitness owing to increased attraction of dispersal agents (especially birds) and the resulting increases in the number of seeds dispersed and the number of offspring successfully established. Under these circumstances, a mutant producing only seeds would spread in a population, and plants that were not as successful in producing seeds would be favored if they produced only pollen.

The basic assumptions of Givnish's (1980) model are perhaps questionable (e.g., Herrera, 1982), but here I wish to reconsider the correlation that motivated his model in the first place. Givnish tallied the number of species in each category, which effectively assumes that each species provides independent evidence of the relationship. As Felsenstein (1985) and others have pointed out (cf. Harvey and Mace, 1982), this procedure implicitly assumes a phylogeny that is not resolved into a series of dichotomies but is instead an explosive radiation with all species arising simultaneously from a single ancestor. That is, it ignores the likely alternative that some species are close relatives and share traits that evolved only once in their common ancestor.

Givnish (1980) recognized this problem and tried to compensate for it by examining the relationship using genera ($X^2 = 44.70$, $d.f. = 1$, $P < 0.0001$) and then families (Fisher's exact test, $P < 0.005$) instead of species. However, finding significant correlations at higher taxonomic levels does not solve the problem, because genera and families might be related. For example, inasmuch as the cycads probably inherited dioecy and fleshy seeds from their common ancestor, Givnish's recognition of three families of cycads overestimates the number of origins of dioecy and fleshiness.

Furthermore, a positive correlation between dioecy and fleshiness does not establish the order in which they evolved (Fig. 1). In effect, Givnish (1980) assumed that dioecy evolved after fleshiness, but perhaps it was the other way around in some lineages (Baker and Cox, 1984 p. 250), in which case a very different explanation would be called for. Indeed, establishing the order of origination of traits is generally critical in choosing among possible evolutionary explanations. To cite a second example involving breeding systems in plants, the explanation for the origin of heterostyly depends on whether it evolved before or after the self-incompatibility system with which it is associated (that is, either to promote outcrossing or for greater efficiency of pollen transfer between compatible forms [Ganders, 1979]).

Finally, the association might be spurious, because both dioecy and fleshiness may have evolved independently in response to some third factor that was not considered. In fact, Muenchow (1987) makes exactly this argument. Although she accepts that dioecy and fleshiness are weakly correlated in angiosperms, she proposes that this is a function of the independent evolution of both traits in perennial plants living in understory habitats, where limitations on pollination and dispersal may obtain. Phylogenies, by specifying which characters were already present (and which were not) when the character of interest evolved, might help in identifying additional relevant factors (e.g., perhaps the variable labeled "*X*" in Fig. 1).

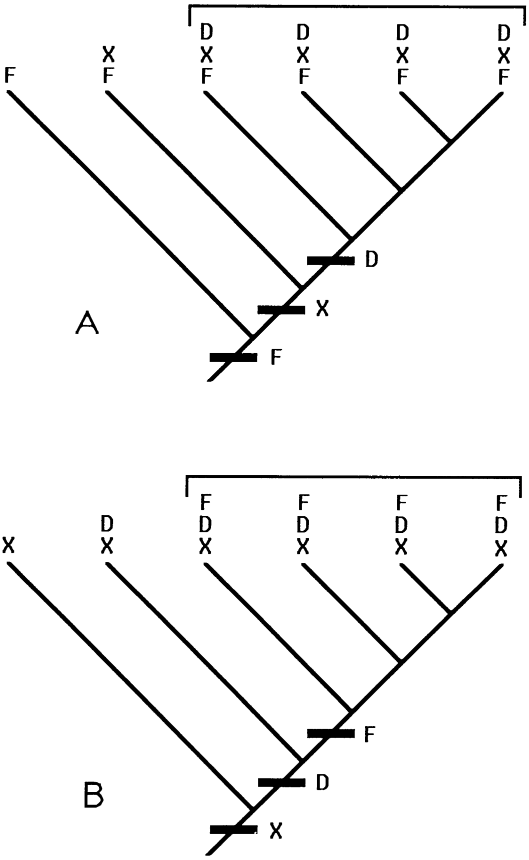

FIG. 1. Hypothetical patterns of character evolution illustrating that correlations do not establish the sequence of evolutionary events. A) The evolution of fleshy propagules (F) before dioecy (D); B) the evolution of dioecy (D) before fleshy propagules (F). In both cases, a strong positive association is evident between F and D. A third character, *X*, might have influenced the evolution of dioecy and/or fleshy propagules.

These points also apply to many of the arguments stimulated by the Givnish-Bawa dioecy hypothesis. Thomson and Barrett (1981) reasoned that if ecological rather than outcrossing explanations accounted for the evolution of dioecy, this breeding system should be found in approximately equal frequencies in self-compatible and self-incompatible plant groups. They favored an outcrossing explanation based on comparative studies by Baker (1959, 1967) indicating that dioecy rarely occurs in groups that are self-incompatible. In response, Givnish (1982) calculated the correlation using angiosperm families and genera, concluding that there is not a significant negative relationship between dioecy and self-incompatibility.

As Givnish (1982 p. 853) noted, his tests assumed that "a trait originating in one family or familial ancestor is unlikely to 'infect' another family." This assumption is violated, because some (perhaps many) angiosperm families are not monophyletic. For example, the small family Garryaceae, consisting entirely of dioecious species, is probably the sister group of the dioecious genus *Aucuba,* which is generally placed in Cornaceae. A single origin of dioecy would account for its occurrence in both families, and counting Garryaceae as a separate instance of the evolution of dioecy (as Givnish [1982] did) would overestimate originations. Another of Givnish's assumptions, namely that "the appearance of a trait in several genera within a family is due to one or a few initial innovations" (Givnish, 1982 p. 853), is also violated in many cases. His analysis surely underestimates the number of origins of dioecy in large families, such as Asteraceae and Rubiaceae, and in some smaller families as well (e.g., dioecy probably originated several times within Cornaceae [Eyde, 1988]). Furthermore, the co-occurrence of dioecy and self-incompatibility within a family or genus does not mean that dioecy actually originated from self-incompatibility in such cases (Simpson, 1989). For example, as Baker (1984) pointed out, dioecy and heterostyly are very rare in the legume genus *Bauhinia* (which Givnish [1982] cited) and probably arose independently from self-compatibility within separate sections of the genus.

Phylogenies and Evolutionary Sequences

The dioecy example exposes several ways in which ahistorical correlations, by failing to take into account phylogenetic relationships, might give a misleading estimate of quantities that are often central to evolutionary arguments. Several statistical methods have been devised to deal with the lack of independence caused by phylogenetic relationship without actually taking phylogenies into account (Pagel and Harvey, 1988). These involve nested analysis of variance or multiple regression aimed at identifying an appropriate taxonomic level for comparison (e.g., Harvey and Mace, 1982; Stearns, 1983, 1984) or analyses that are otherwise tied to some equivalency of taxonomic ranks (e.g., Gittleman, 1981; the phylogenetic connectivity matrix of Cheverud et al. [1985]). While these indirect approaches may reduce the problem, focusing on taxonomic ranks is at best a substitute (perhaps often a poor substitute) for taking direct account of phylogeny (Huey, 1987). But, if a phylogeny were available, how exactly could it be used?

Felsenstein (1985) devised a method to test the independence of changes in two continuous characters evolving under Brownian motion. This approach, which requires both a phylogenetic tree and information on the duration of lineages, was utilized by Sessions and Larson (1987) in analyzing the relationship between genome size and development in salamanders. Huey and Bennett (1987) sidestepped both the Brownian-motion model and the need to know the ages of lineages in their analysis of the "coadaptation" of continuous variables (thermal preference and optimal sprint temperature in lizards) by estimating values at the internal nodes of a tree using an iterative approach to minimize the total amount of change along the branches. Neither of these methods is appropriate in the dioecy case, where the variables are more or less discrete. Furthermore, these tests are for a general correlation between character changes, that is, whether changes in two characters are in the same direction and of the same magnitude (Maddison, 1990). They do not, by themselves, specifically address the issue of the sequence of changes, which is crucial in evaluating Givnish's dioecy hypothesis.

Ridley (1983) developed an approach for discrete variables based on parsimoniously mapping characters of interest onto relevant cladograms to establish the number of independent evolutionary events (also see Lauder [1981]). Curiously, Ridley (1983, 1986), Pagel and Harvey (1988), and others have described the assessment of states at internal nodes as a process of successive outgroup comparison, but this procedure may not correctly identify all most-parsimonious solutions (Maddison et al., 1984); character-optimization methods should be used in analyzing character change in "known" phylogenies (Swofford and Maddison, 1987). In any case, to test for the association between changes in two characters, Ridley (1983) suggested the following procedure: 1) each time a change occurs in a character of interest, simply note which state of the other character is present on that branch in the tree; 2) assemble the results in a contingency table showing the number of times that each of the four combinations of the two characters is realized; and 3) assess the probability of the observed distribution (e.g., using chi-square or Fisher's exact tests).

Although Felsenstein (1985 p. 8) acknowledged that this approach is "immeasurably superior to simply treating the species as if independently evolved," he criticized it on the grounds that character optimization under parsimony minimizes the number of origins of character states. In particular, if sister lineages share a state, it will be interpreted as having arisen once in their common ancestor rather than in parallel. In contrast, under the maximum-likelihood approach adopted by Felsenstein (1985), two separate origins might be preferred. Felsenstein's method is tied to a specific model of evolutionary change (Sober, 1984*a*); given uncertainty over the choice among such models, systematists have generally preferred parsimony both in choosing among trees and in reconstructing ancestral character states (Farris, 1983). Although the use of parsimony is not free of evolutionary assumptions, these appear to be limited in the case of tree construction (Farris, 1983; Sober, 1984*a*; Felsenstein and Sober, 1986), and parsimony is a robust (and statistically consistent) procedure over a wide range of evolutionary circumstances (though not all [Felsenstein, 1978]). Where parsimony is employed in studies of character evolution, however, it is important to recognize that it provides a minimum estimate of the number of state changes and that this could conceivably bias statistical tests for character association. As Felsenstein (1985) noted, the circumstances under which such a bias would significantly affect the outcome have not been explored. However, parsimony should generally give conservative results, biasing (if at all) against detecting significant associations (Ridley, 1983). It should not give misleading results unless the location of the changes detected by parsimony positively misrepresent the location of any extra steps not detected by parsimony.

Ridley's (1983) approach has another difficulty—one that is especially relevant to the dioecy problem. Simply recording the number of times that a particular combination of states appears in a cladogram may obscure information on the sequence of character origination. Thus, an entry in the matrix under dioecy and fleshiness might represent a change to dioecy in a lineage that is already fleshy or the origination of both dioecy and fleshiness along a single branch in the cladogram. In the latter case, the order of origination is unclear, and it may be inappropriate to count such cases in favor of a particular sequence hypothesis. If Ridley's test is performed in the manner described by Ridley (1986 p. 1854) and Pagel and Harvey (1988 p. 419), wherein one identifies changes in both characters and combines this information in a single contingency table, then the sequence is further confounded. In this case, an entry under dioecy and fleshiness might also represent the evolution of fleshiness in a dioecious lineage.

An approach that explicitly keeps track of the sequence of change in discrete characters was employed by Sillén-Tullberg (1988) in analyzing the evolution of gregariousness (G) and aposematic (warning) coloration (W) in butterfly larvae (also see Carothers [1984]). By inferring phylogenies from classifications of several butterfly groups, she was able to determine the minimum number of origins of W after G, of G after W, and of W and G along the same branch. Leaving aside the latter, on the grounds that the sequence could not be determined, she found that warning coloration evolved nine times in solitary larvae and never in gregarious larvae and that gregariousness

FIG. 2. A composite cladogram of seed plants based on Doyle and Donoghue (1986), Hart (1987), and Donoghue and Doyle (1989), showing parsimonious reconstructions of the evolution of fleshy propagules (animal dispersal) and dioecy (MacClade, version 2.1 [W. P. Maddison and D. R. Maddison, unpubl.]). Black branches indicate fleshy propagules, white branches indicate dry propagules, and striped branches represent equivocal conditions. Stippling reflects uncertainty concerning the mode of dispersal in taxa marked by "?." Taxa known to be dioecious are marked by asterisks; black crossbars indicate the origin of dioecy, open bars indicate reversal to monoecy. Maddison's (1990) statistical test was applied to two optimizations (see text): in optimization 1, stippled and striped branches are assigned dry propagules (colored white); in optimization 2, stippled and striped branches are assigned fleshy propagules (colored black). Additional information on the cladograms, terminal taxa, and characters is provided in the Appendix.

evolved more often in warningly colored than in cryptic larvae. From this, she concluded (without statistical test) that the evolution of unpalatability predisposed butterflies to the evolution of gregarious larvae.

Phylogenies and the Evolution of Dioecy

Recent cladistic studies of major groups of seed plants make it possible to use the type of analysis developed by Sillén-Tullberg (1988) to test the Givnish-Bawa hypothesis that fleshy, animal-dispersed propagules promote the evolution of dioecy. A composite cladogram of seed plants, based on the cladistic analyses of Doyle and Donoghue (1986), Hart (1987), and Donoghue and Doyle (1989), is shown in Figure 2. This also shows the distribution and optimization of a propagule/dispersal character (fleshy/animal-dispersed vs. dry/not animal-dispersed) and a breeding-system character (dioecious vs. monoecious or perfect). The three component cladograms and the two characters of interest are discussed in the Appendix (which highlights the kinds of difficulties that are likely to be encountered in any study adopting an explicitly historical approach). Uncertainties remain concerning some cladistic relationships, and there are significant difficulties in character optimization, especially owing to uncertainty over the traits of extinct groups. Consequently, the following exercise cannot yet be viewed as a critical cladistic test of the Givnish-Bawa hypothesis. It is presented simply to illustrate a phylogenetic approach to the problem and to encourage a more complete solution as better information becomes available on phylogenetic relationships and character distributions.

Assessing the historical association between breeding system and dispersal is complicated by the fact that the mode of dispersal is highly questionable in some fossil groups, especially those taxa marked by "?" in Figure 2 (Tiffney, 1986). Although the seeds of these groups appear to have had a fleshy or leathery outer coat (sarcotesta), these were not necessarily dispersed by animals; they were often small in size and sometimes had wings. Furthermore, because birds presumably did not exist in the Paleozoic, it is unclear what animals would have served as dispersal agents. Owing to this uncertainty, I consider two optimizations of the dispersal character. In the first (optimization 1), I assume that the questionable taxa were dispersed by wind, in which case the stippled branches in Figure 2 are optimized as dry/not animal-dispersed (colored white). In this case, I also assume that all equivocal (striped) branches are wind-dispersed, thereby minimizing fleshiness along the branches and maximizing the number of origins of animal dispersal. In optimization 2, I assume the reverse, namely that the questionable taxa were animal-dispersed and that, therefore, the stippled branches are colored black. To maximize the difference between the two optimizations, in the second case I also consider the equivocal branches to be animal dispersed; this maximizes fleshiness and minimizes separate origins.

The number of origins of dioecy in relation to fleshy propagules can be determined by inspection of Figure 2. Under optimization 1, dioecy is seen to arise twice after the evolution of fleshy propagules and three times in lineages with dry propagules; two cases of apparent reversion to monoecy are seen within conifers, both in lines with fleshy propagules. In five cases, dioecy and fleshiness arise along the same branch, and the order of origination is therefore unclear. These numbers do not provide compelling evidence for the view that the evolution of dioecy is favored in plants that are animal dispersed. Indeed, within the "gymnosperms" considered by Givnish (1980) there are no unequivocal cases of this sequence. However, if the five "simultaneous" appearances are counted as instances of the evolution of dioecy after fleshiness, the relationship is strengthened considerably. Optimization 2 is much more supportive of the hypothesis that animal dispersal promotes the evolution of dioecy. In this case, dioecy originates seven times in fleshy clades (or eight times if the one simultaneous appearance is counted) and only two times in dry clades.

The significance of these results is difficult to evaluate, because it is unclear whether the observed frequencies of change differ from the null expectation that dioecy evolved randomly with respect to dispersal. This depends critically on the distribution of the independent variable, in this case the commonness or rarity of animal dispersal. If most seed plants were animal dispersed (most branches colored black), then dioecy might be expected to originate more often in such lines by chance alone. On the other hand, if animal dispersal were very rare (most branches white), there might be a highly significant relationship between dioecy and animal dispersal, even if dioecy originated an equal number of times in fleshy and in dry lines.

Maddison (1990) recognized this point and devised a statistical test to determine whether or not a discrete state of one character has evolved *surprisingly* often with respect to another. His test functions under the null hypothesis that gains and losses of the first character are randomly distributed among the branches, without regard to the condition of the second character. Although it does not rely on a specific model of character evolution or require knowledge of the duration of lineages (as does Felsenstein's [1985] method), Maddison's test does assume that the states of ancestors (internal nodes) are "known." Obviously, in the present case, significant uncertainties exist concerning both phylogenetic relationships and character optimization. Nevertheless, Maddison's test can be applied to the two optimizations discussed above (Fig. 2), in the hopes of providing some bounds on the significance of the association. Specifically, we can ask whether dioecy has evolved significantly more often than expected by chance alone in plants that are animal-dispersed than in those that are not.

When Maddison's test is applied to the "gymnosperm" portion of the cladogram (to facilitate comparison with Givnish's [1980] correlations) under optimization 1, and when simultaneous occurrences are counted as instances of dioecy evolving after fleshiness, dioecy does appear to be significantly concentrated in animal-dispersed lines. Indeed, the probability of observing five or more gains of dioecy in fleshy lines, given seven gains and two losses overall, is 0.01. On the other hand, if simultaneous appearances are not counted in favor of the sequence hypothesis, then it appears that dioecy has not evolved significantly more often after fleshiness. Indeed, in this case there are no instances of this sequence. Application of Maddison's test to the "gymnosperm" portion under optimization 2 gives basically the same results. Again, counting simultaneous appearances in favor of the evolution of dioecy after fleshiness yields a significant relationship ($P = 0.018$), whereas if these cases are not counted, the relationship is not significant ($P = 0.13$).

These results highlight the fact that support for the sequence hypothesis (and hence for the model of cause and effect) depends critically on how the simultaneous appearances are interpreted. This is a special difficulty in this case, because such a high percentage of the associations between dioecy and fleshiness are due to co-origination along the same branch. In fact, under optimization 1, all five origins of dioecy are strictly coincident with origins of fleshiness, a highly improbable arrangement according to Maddison's test ($P = 0.00007$). One possible explanation for this pattern is that species with one but not the other trait, whose addition to the analysis might establish the sequence, are now extinct and unknown. Alternatively, the traits might have originated simultaneously (perhaps as a function of a single developmental shift) or in very rapid succession, in which case finding organisms with intermediate character combinations would be impossible or highly unlikely. Paradoxically, then, when the relationship between changes in two characters is very tight, such that a change in one strongly promotes a rapid change in the other, it will be especially difficult to sort out the order of origination using cladistic tests.

This analysis also focuses attention on the need for better information on the breeding and dispersal systems of early seed plants. The sensitivity of the test to differences in the treatment of the dispersal character is evident in comparing the results for the two optimizations. Despite the fact that there are more unequivocal origins of dioecy after fleshiness under optimization 2 (and, hence, the relationship seems stronger using Sillén-Tullberg's [1988] step-counting method), statistical significance is not as great in this case; because more of the branches are optimized as fleshy (colored black), the probability is increased that dioecy will evolve in such lines by chance alone. Indeed, a statistically significant result would not have been obtained in this case with just one fewer origin of dioecy in a fleshy line—for example, if the line leading to Gnetales (*Ephedra, Welwitschia,* and *Gnetum*) were assigned dry seeds (colored white). The best hope of achieving an unequivocal solution may be to extend this type of analysis to angiosperms, where there are perhaps fewer uncertainties and many more instances of the origin of dioecy and animal dispersal to examine. In angiosperms, there may also be fewer instances of "simultaneous" appearances resulting from extinction of taxa with intermediate character combinations.

This exercise does unequivocally demonstrate that separate origins of dioecy (or animal dispersal) are not largely responsible for the highly significant species-level correlation observed by Givnish (1980). Indeed, regardless of exactly how these characters are optimized on seed-plant cladograms, the number of origins is certainly far fewer than the number of species (or even genera or families) showing these traits. Thus, the use of cladograms in exploring Givnish's correlation argument has focused attention on a new question: if not a function of originations, then why are there such a large number of dioecious, animal-dispersed species? Perhaps these traits, alone or in combination, favor a higher rate of speciation or a lower rate of extinction. At least among nonangiospermous seed plants, some clades in which both dioecy and animal dispersal have evolved (e.g., cycads, Podocarpaceae) do appear to have been highly "successful," at least judging by the number of species. However, appropriate sister-group comparisons (cf. Vrba, 1980) are difficult to make in these cases, either due to uncertain relationships or problems in estimating the numbers of species in fossils lineages. Nevertheless, the genus *Juniperus* (with approximately 60 species) provides a good example of a dioecious, animal-dispersed group that is far more speciose than any possibly related groups with the ancestral conditions (e.g., *Cupressus* with 12 species, *Chamaecyparis* with six species). Here, it is difficult to determine whether this pattern is a function of increased speciation or of decreased extinction, and the role played by animal dispersal or dioecy is unclear.

In this context, it is noteworthy that, according to Carlquist's (1974) calculations, dioecious and bird-dispersed plants have speciated more in the Hawaiian islands than plants with other breeding and dispersal systems. Bird dispersal (in combination with extreme topographic diversity) may be primarily responsible for increased speciation in this case, because nondioecious bird-dispersed groups also appear to be speciose. Thus, the unusually high incidence of dioecy in the Hawaiian flora (Bawa, 1982; Baker and Cox, 1984) may be simply an incidental effect of its association with bird dispersal.

The dioecy example illustrates how a phylogenetic approach can yield insights into evolutionary problems that could not be obtained otherwise, especially in establishing appropriate evolutionary questions and in narrowing the set of alternative evolutionary explanations. Moreover, it demonstrates that progress along these lines is possible even in the face of considerable uncertainty regarding relationships and character distributions (also see Moran [1988]). At the very least, the exercise itself focuses attention on what additional data are needed to perform a critical test.

Sequences, Adaptation, and Endosperm

The dioecy case focuses attention on tests for character association that rely on convergence. But what if we were concerned with individual instances of the evolution of such traits or features that evolved only once? How might phylogenies, and sequences in particular, be useful in evaluating the causes and consequences of the evolution of such characters?

Whether a trait is an adaptation, rather than simply adaptive, depends on the cause of its evolution (Williams, 1966; Lewontin, 1978; Burian, 1983; Sober, 1984*b*). Specifically, the assertion that a property is an adaptation is a claim that its evolution (the historical event) was brought about by natural selection for some particular function. Greene (1986) and Coddington (1988) have explored the use of cladograms in testing hypotheses of adaptation (also see Wanntorp [1983]). They argue that adaptation is supported when the evolution of a derived morphology is seen to be strictly coincident with the origin of a derived function or performance advantage, as determined by appropriate comparisons with related organisms having the ancestral trait (Fig. 3A). Coddington (1988) considers this pattern to be evidence that natural selection was responsible for the derived morphology, whereas Greene (1986) urges that adaptation simply be equated with this pattern, regardless of its cause (in other words, that we dispense with the inference of natural selection). More importantly for present purposes, Greene (1986) also discussed cases in which the origin of a feature and a performance advantage follow one another in sequence. If the feature originated before its performance advantage (Fig. 3B), then it is considered to be an "exaptation" for that task (Gould and Vrba, 1982), whereas if the function predated the trait (Fig. 3C), the trait may be simply irrelevant to the task.

A consideration of endosperm evolution illustrates the use of cladograms in analyzing the sequence of events in the origin of a unique feature (M. J. Donoghue and S. M. Scheiner, unpubl.). In nonangiospermous seed plants, one product of the meiotic division in the ovule develops into a large, multicellular female gametophyte, which functions as a nutritive tissue for the developing embryo and later the seedling (Foster and Gifford, 1974). In contrast, the female gametophyte in angiosperms is extremely reduced. In fact, at maturity it generally consists of only eight nuclei, two of which (the polar nuclei) are situated in a large central cell. Two sperms are delivered by the pollen tube directly to the female

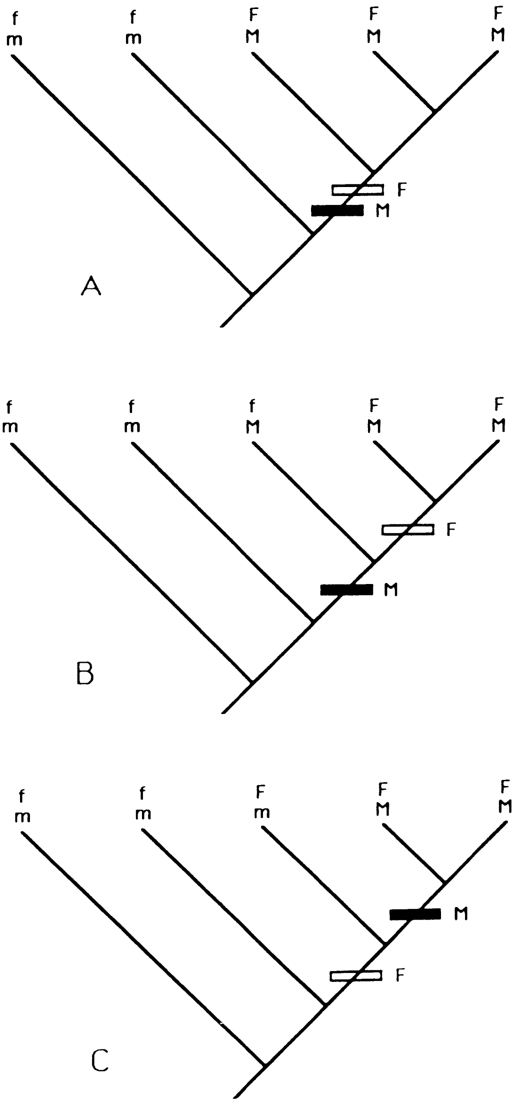

FIG. 3. The role of cladograms in evaluating hypotheses of adaptation (after Greene [1986] and Coddington [1988]). A) A cladogram depicting the coincident evolution of a new morphological state (change from m to M) and a new function or performance advantage (change from f to F); this pattern is consistent with the hypothesis that M is an adaptation (was selected) for F. B) The derived morphological trait (M) evolves before the function or performance advantage (F) with which it later becomes associated; this is consistent with the hypothesis that M is an "exaptation" (Gould and Vrba, 1982). C) The derived morphological trait (M) arises after the evolution of the function or performance advantage (F); the trait may be irrelevant to the task.

gametophyte (siphonogamy), whereupon double fertilization takes place: one sperm fuses with the egg (which develops into the diploid embryo); the other fuses with the two polar nuclei to form a triploid nucleus. The latter develops into a triploid tissue, endosperm, which serves the nutritive function in the seed. The challenge, then, is to explain how and why the angiosperm condition evolved.

Traditionally, endosperm is thought to have been favored as the nutritive tissue because heterozygosity results in greater vigor and because triploidy allows an increased rate of protein synthesis (e.g., Brink and Cooper, 1947; Stebbins, 1974, 1976). Alternative theories focus on the genetic relatedness of various seed tissues and invoke parent-offspring conflict and kin selection in explaining the transition to the angiosperm condition (e.g., Westoby and Rice, 1982; Queller, 1983, 1984). The details of these more recent models are not critical here, except to note that alternative explanations assume different sequences leading to the evolution of triploidy. For example, fusion of the second sperm with one female nucleus, followed by the evolutionary addition of a second female nucleus, is compatible with the view that the second female nucleus was added "as a means of decreasing the impact of double fertilization" (Willson and Burley, 1983 p. 81). The alternative evolutionary sequence, namely the fusion of two female nuclei followed by fusion with the second sperm, is obviously incompatible with this hypothesis.

Cladistic studies of seed plants (Crane, 1985; Doyle and Donoghue, 1986) may have an important bearing on this issue (Fig. 4). These analyses indicate that Gnetales (*Ephedra, Welwitschia,* and *Gnetum*) are the closest living relatives of angiosperms. The significance of this result from the standpoint of endosperm evolution is that double fertilization has been reported and may occur regularly in Gnetales (Land, 1907; Herzfeld, 1922; Kahn, 1943; Moussel, 1978). In the past, because these plants were not considered to be close relatives of angiosperms (e.g., Eames, 1952), this was viewed as a curious case of convergence, with no bearing on the angiosperm condition (but see Meeuse [1963]). Now it appears that double

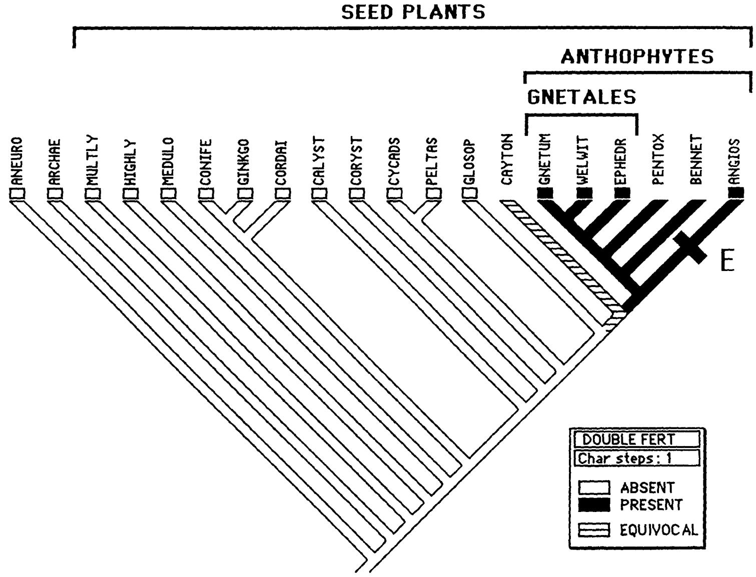

FIG. 4. A cladogram of seed plants (Doyle and Donoghue, 1986), showing the possible evolution of double fertilization (black branches) and triploid endosperm (crossbar labeled E). The condition in fossil groups (e.g., BENNET, PENTOX, and CAYTON) is uncertain; double fertilization might therefore have evolved sometime before or after the origin of anthophytes. In any case, double fertilization may be an exaptation, arising incidentally and later being co-opted and elaborated in the angiosperm line (see text). Abbreviations for terminal taxa are defined in the Appendix with the following additions: CONIFE = conifers; ANGIOS = angiosperms.

fertilization in Gnetales and angiosperms may be homologous and that a closer look at fertilization in Gnetales might shed light on the evolution of endosperm. This, of course, depends in part on the condition in extinct anthophytes (Bennettitales and *Pentoxylon*) and Mesozoic "seed ferns" (e.g., *Caytonia*). Although this condition is uncertain, these plants had small seeds that might have developed rapidly (Tiffney, 1986) and, thus, may also have been predisposed to retain the ventral canal nucleus until fertilization.

In *Ephedra,* which most closely approximates the basal embryological condition in Gnetales (*Welwitschia* and *Gnetum* share a host of derived embryological traits [Doyle and Donoghue, 1986]), the sister nucleus of the egg (the ventral canal nucleus) is often present when the two sperms are delivered, whereas in most "gymnosperms" it has disintegrated by this stage. In this case, the second sperm may fuse with the second female nucleus. Although the diploid product of this second fertilization may undergo several divisions, any derivative nuclei soon abort, and the female gametophyte, which is fully developed at the time of fertilization, continues to function as the nutritive tissue.

Taken together, these observations raise the intriguing possibility that double fertilization originated (whether one or more times) as an incidental consequence of the evolution of siphonogamy (delivery of sperm to the female gametophyte by a pollen tube) coupled with the availability of a second female nucleus at the time of fertilization (M. J. Donoghue and S. M. Scheiner, unpubl.). Perhaps, as Kahn (1943 p. 374) suggested, it arose simply as "the natural outcome of a tendency towards fusion between any two nuclei of opposite sexual potencies that happen to lie free in a common chamber." If this scenario is basically correct, the second fusion product, which was initially functionless, must later have been co-opted and elaborated as the nutritive tissue in the angiosperm line. Thus, if we apply Greene's (1986) cladistic test (Fig. 3), double fertilization is best interpreted as an exaptation, having arisen before the performance advantage with which it later became associated (Gould and Vrba, 1982; Greene, 1986). In this case, then, the cladistic result that

Gnetales are closely related to angiosperms helps tease apart the order of events in the evolution of endosperm and establishes the need to consider separate explanations for double fertilization and triploidy.

Sequences, Burden, and Character Weighting

Phylogenies and character sequences can also be put to use in studying the evolution of what Riedl (1978 p. 80) called "burden," or "the responsibility carried by a feature or decision." Burden is a function of the position of a trait in relation to other features and is measured by the number of attributes that are functionally dependent upon its existence. Much the same idea has been developed by Wimsatt (e.g., Wimsatt, 1986; Wimsatt and Schank, 1988) under the name "generative entrenchment." The significance of this concept for evolution concerns the likelihood of character change. Changes in heavily burdened elements (often those expressed earlier in development) should have larger and more pervasive effects, and successful modifications of such elements should be less likely than modifications of less heavily burdened traits.

Riedl (1978) emphasized that a feature's burden is not a static attribute—it evolves. Characters that are now heavily burdened probably did not start out that way. In most cases, the burden associated with a character is low at first and builds up as other characters evolve that are functionally and developmentally dependent upon it. Initially, while burden is low, changes (even loss) may not have deleterious consequences, whereas later, when burden has increased, successful modifications become less likely. Thus, according to Riedl (1978) the evolution of a feature might follow a characteristic trajectory, from an initial period of relative freedom through later periods of constancy or fixation. Of course, some characters may remain relatively unburdened, and burden can even decrease as a result of the loss or modification of a dependent character.

This conception of the evolution of burden can be evaluated using cladograms in combination with functional and developmental information. Riedl himself provided several examples of the phylogenetic "fixation path" of a character (e.g., the paired anterior appendages of vertebrae [Riedl, 1978 p. 151–158]), but this program of research has seldom been pursued rigorously (but see Lauder [1981, 1982], Schaefer and Lauder [1986], and Emerson [1988]). Below, I briefly outline three examples from seed plants of the effect of the sequence of character assembly on burden and the likelihood of character change.

Double Fertilization.—The first case builds on the endosperm example discussed above. If the origin of double fertilization preceded the evolution of triploid endosperm, then little (perhaps nothing) depended on the second fertilization when it first appeared. The burden associated with double fertilization was low, and its occurrence or failure to occur may have had few consequences. With the evolution of endosperm, however, double fertilization became a critical element in a system upon which the life of the embryo depended. Although at one time in its history double fertilization might have been lost without major deleterious effects, its loss in modern angiosperms with normal endosperm development is highly unlikely. In effect, double fertilization has been locked-in by the evolution of processes dependent on its occurrence; it might be "a frozen accident."

Vessel Evolution.—The evolution of vessels provides a second example. Vessels are water-conducting tubes in the xylem composed of a series of vessel cells (elements) connected end-to-end. Vessel elements were apparently derived by a modification of the developmental program giving rise to tracheids, such that pit membranes were lost between end walls of adjacent cells. Within seed plants, vessels are found only in Gnetales and in angiosperms. If Gnetales are related directly to angiosperms (Crane, 1985), vessels may have evolved only once. However, if the extinct groups Bennettitales and *Pentoxylon* are inserted between Gnetales and angiosperms, it is most parsimonious to suppose that vessels originated independently in Gnetales and in angiosperms (Fig. 5; Doyle and Donoghue, 1986). This arrangement is consistent with the view that vessel perforations represent modified cir-

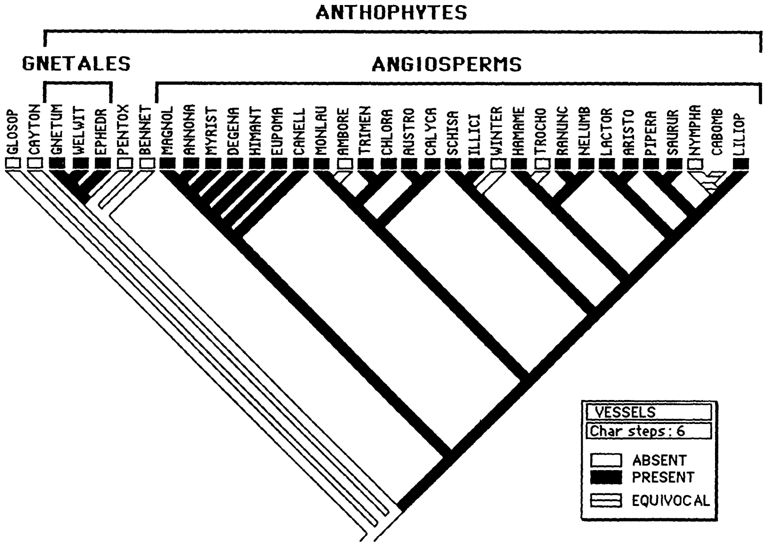

FIG. 5. A cladogram of anthophytes based on Doyle and Donoghue (1986) and Donoghue and Doyle (1989), showing the evolution of vessels (black branches). Vessels appear to have arisen independently in Gnetales and angiosperms and to have been lost at least four times early in angiosperm evolution. Note that this cladogram shows only the basal radiation of angiosperms; "higher dicot" groups are thought to be derived from the hamamelid line (Donoghue and Doyle, 1989). Abbreviations for terminal taxa are defined in the Appendix.

cular bordered pits in Gnetales and modified scalariform pits in angiosperms (Thompson, 1918; Bailey, 1944; but see Muhammad and Sattler [1982]). In any case, it appears that the first Gnetales and the first angiosperms lived in similar seasonally arid or disturbed environments (perhaps as stream-side "weeds"; Stebbins, 1974; Doyle et al., 1982; Crane, 1987). This observation, coupled with evidence that vessels provide a more efficient means of water transport (e.g., Huber, 1956), suggests that vessels evolved as an adaptation to aridity, as suggested for Gnetales (though not for angiosperms) by Carlquist (1975).

Some angiosperms that lack vessels (e.g., Winteraceae) are widely believed to be primitively vesselless, in which case vessels must have evolved within angiosperms, probably several times independently (Bailey, 1944; Cheadle, 1953). However, cladistic analyses support the alternative suggestion of Young (1981) that the common ancestor of extant angiosperms had vessels and that these were lost in several early lines (Fig. 5; Donoghue and Doyle, 1989). This parsimony argument has been sharply criticized, primarily on the grounds that it fails to appreciate the adaptive value of vessels and the dire consequences of vessel loss in terrestrial environments (Carlquist, 1983, 1987). However, claims about the likelihood of vessel loss should be evaluated in an historical context.

The first vessels were presumably of a primitive type (with numerous scalariform partitions) and limited in number, and tracheids probably continued to function in water conduction (as they do in Gnetales [Carlquist, 1975]). Under such circumstances of low burden, the loss of vessels may not have been difficult, entailing only the retention of pit membranes (intermediate conditions are known [Carlquist, 1983, 1987]), and the functional consequences may not have been great, especially in the wet upland environments where vesselless angiosperms are found today. Vessel loss might even have been adaptive in some environments. While vessels are more efficient, tracheids are "safer," because pit membranes confine air embolisms (brought about by drought or freezing) to single cells, whereas such cavitation would disable an entire vessel (Carlquist, 1975). Indeed, Carlquist (1988) interpreted instances of the virtual loss of vessels in *Ephedra* growing in cold alpine deserts as an adaptive rise in the frequency of tracheids.

These observations show that it is at least conceivable that vessels were lost in several early lines of angiosperms, as suggested by cladistic studies. It is certainly true, however, that the loss of vessels elsewhere within angiosperms has been exceedingly rare. This pattern might be explained by the evolution of dependent traits that increased the functional burden of vessels. Indeed, the presence of vessels probably facilitated the evolution of such traits. For example, vessels may have been a prerequisite for the evolution of large, undissected leaves in tropical climates (Doyle et al., 1982; Doyle and Donoghue, 1986), because such leaves would tend to overheat unless vessels were present to allow rapid transpiration. In keeping with this argument, floras in apparently tropical climates prior to the rise of angiosperms were dominated by plants with small or xeromorphic leaves (Doyle et al., 1982). This idea is also supported by the independent occurrence of the same sequence of events in Gnetales: the evolution of vessels (probably under arid conditions) appears to have preceded the evolution of dicot-like leaves in the tropical genus *Gnetum* (Doyle and Donoghue, 1986).

Floral Phyllotaxis.—A final example, based directly on ideas developed by Endress (1987), concerns the number and arrangement of flower parts in angiosperms. Endress has carefully documented the exceptional range in floral phyllotaxis found among presumably primitive angiosperms ("magnoliids") as compared to that found in derived lines, including monocots (with whorls of three) and "higher dicots" (whorls of four and five). Cladistic analyses confirm that modifications in floral phyllotaxis were very common early in angiosperm evolution and that some patterns evolved independently in several lines (Donoghue and Doyle, 1989). Thus, several changes occurred from the spiral arrangement of a large and indefinite number of parts to whorled patterns with few and definite numbers of parts (e.g., the evolution of trimery [Kubitzki, 1987]). However, there also may have been reversals from whorled to spiral phyllotaxis and from few to many parts (for example, within water-lilies [Nymphaeales]).

Whatever the reason for the transition to fewer parts in whorls (e.g., reduction of the floral axis for more rapid flower development [Stebbins, 1974]), this change set the stage for the fusion of adjacent parts within each whorl. The evolution of fused parts, perhaps especially the union of carpels and the resulting fusion of style tissue into a common passageway for pollen tubes (Carr and Carr, 1961; Endress, 1982), would in turn have increased the burden on whorled phyllotaxis, as the arrangement of parts in whorls is a prerequisite for proper fusion (Endress, 1987). Consequently, changes in floral phyllotaxis would be far less likely to succeed in any clade in which, for example, fused carpels happened to have evolved. Substantial phyllotactic modifications would be even less likely following the evolution of the fusion of parts in adjacent whorls, such as the adnation of stamens to the corolla tube or of stamens to the gynoecium (e.g., in Orchidaceae). Indeed, in such cases, the basic phyllotactic pattern is virtually invariant, and plasticity is evident at other levels of organization (cf. Robinson, 1985). Thus, in composites (Asteraceae), variability is displaced to the level of the inflorescence, and in milkweeds (Asclepiadaceae), tissues derived from the fusion of parts are often highly modified (e.g., "hoods" and "horns").

Endress (1987) also cites several cases of apparent release from constraint followed by a return (or "decay") to spiral phyllotaxis. For example, in *Quercus rubra,* the staminate flowers show a spiral arrangement, whereas the parts are whorled in carpellate flowers. Endress (1987) hypothesizes that the whorled arrangement is retained in female flowers because this is necessary for the proper fusion of carpels, whereas a reversion to a spiral arrangement occurred in male flowers following the loss of carpels. In other words, the burden on whorled phyllotaxis was lifted by the loss of a dependent character: fused carpels.

Character Weighting.—In each of these examples, the evolution of one trait (double fertilization, vessels, or whorled phyllotaxis) appears to have facilitated the evolution of a dependent character (endosperm, large leaves, or fusion of parts). In turn, the evolution of the second trait increased the burden on the first, thereby decreasing the likelihood that it would be successfully modified. Cladograms are necessary in documenting the sequence of character change in each case, but they also provide a means of testing ideas concerning changes in flexibility during evolution. For example, if the potential for modification is greatest when a character first evolves and later decreases, this might be reflected in patterns in the distribution of homoplasy. In particular, most instances of parallelism and reversal should be localized in the vicinity of the origin of the trait. Although inspection of angiosperm cladograms appears to bear this out in the case of vessels and floral phyllotaxis (i.e., there are more changes early in angiosperm evolution than later), quantitative methods need to be devised to search for and express patterns in the location of character changes in phylogenies.

These observations have an important bearing on the problem of differentially weighting characters in phylogeny reconstruction. The basic idea of character weighting is to reflect differences in the likelihood of change, on the view that characters that are less likely to undergo change are probably more reliable indicators of phylogenetic relationship (e.g., Felsenstein, 1981). Accepting this premise, the question then becomes how best to assess the likelihood of character change. Perhaps, if a character's burden could be measured, this could be used to assign a weight. However, as we have seen, the degree of burden and, hence, the likelihood of modification very probably changes during the evolution of a group. If so, assigning any one weight would surely be inappropriate; in fact, this practice would seriously jeopardize the use of cladograms in evaluating hypotheses about character evolution. For example, if the vessel character were weighted heavily (on the grounds that vessels are now heavily burdened), there would be a strong bias against phylogenetic arrangements necessitating any extra gains or losses of vessels. This would effectively preclude discovery of whether there was ever a time (perhaps early in angiosperm evolution) when changes might have occurred. It seems, then, that weights must either be allowed to vary so as to reflect changes in burden (and it is not clear how this could be done) or be abandoned altogether. The latter may be a reasonable option if all characters traverse some range of propensities to undergo change and if phases of high and low propensity in different characters are more or less uncoupled.

Conclusions

Futuyma (1988 p. 225) hoped that evolutionists would "identify and define rigorously questions to which both synchronic and historical evolution can make indispensable contributions." Perhaps the suggestions made here regarding the use of phylogenies in analyzing sequences in character evolution are a step in this direction. There are, of course, many other avenues to be explored. Although most studies of character evolution have focused on particular traits and groups, as more cladograms become available it may be possible to extract very general patterns through the comparison of disparate groups (cf. Lauder, 1981, 1982). For example, an analysis of homoplasy (parallelism and reversal) in cladistic data sets demonstrates that the level of homoplasy is strongly related to the number of taxa included in a study but is not correlated with taxonomic rank or the number of characters considered (Sanderson and Donoghue, 1989). Furthermore, the level of homoplasy does not seem to differ significantly between plant and animal data sets, contrary to the widespread view that plants (because they are relatively simple and grow indeterminately) are more prone to homoplasy than are "higher" animals (e.g., Cronquist, 1987).

Making direct use of cladograms is preferable to employing comparative methods devised to "reduce the effects of phylogeny" (Pagel and Harvey, 1988 p. 418). The latter are certainly better than ignoring history altogether, but they fail to take advantage of information on the direction and temporal sequence of character change that only trees can provide. However, the difficulties associated with a direct phylogenetic approach must not be underestimated. Phylogenies cannot simply be extracted from traditional classifications, because these very often misrepresent cladistic relationships (Dobson, 1985; Donoghue and Cantino, 1988). Cladistic analysis is a time-consuming and onerous task, and even in the best of cases, uncertainties are likely to persist concerning both cladogram topology and character optimization. Felsenstein (1985) and Maddison (1990) note that such uncertainties might affect statistical tests for character association, and more effort will need to be devoted to this issue. More attention must also be paid to the effects of the inclusion or exclusion of taxa on character optimization (Doyle and Donoghue, 1987). In the meantime, cladistic tests can set limits on the significance of historical character correlations and can help in evaluating sequence hypotheses that are so often critical in evolutionary explanation. Perhaps the greatest impediment to progress along these lines is simply the limited number of carefully conducted phylogenetic analyses. One hopes that, as awareness of the uses of cladograms increases, so too will a commitment to basic phylogenetic research.

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Appendix

Cladograms Shown in Figure 2

The seed-plant portion (bottom) of the tree shown in Figure 2 was obtained from Doyle and Donoghue (1986); the conifer portion (upper left) is based on analyses by Hart (1987) and has been substituted for the single conifer taxon utilized in the seed-plant study; and the angiosperm portion (upper right) is taken from Donoghue and Doyle (1989) and has been substituted for the single angiosperm taxon. Combining separately derived cladograms in this fashion is a suspect procedure from the standpoint of achieving a globally parsimonious solution (Maddison et al., 1984). However, this is not likely to be a significant problem in this case. The monophyly of angiosperms and of conifers is well supported, and it is therefore unlikely that either group would be dismembered in a simultaneous cladistic analysis. Furthermore, seed-plant cladograms of Doyle and Donoghue (1986) were utilized by Hart (1987) and by Donoghue and Doyle (1989) in choosing outgroups for assessing character polarities within conifers and angiosperms, respectively.

In each case, only one tree was selected from among a number of equally parsimonious trees. Ideally, other equally and nearly equally parsimonious cladograms would also be examined in testing character associations, but this becomes impractical as the number of trees increases. In the present case, inspection of equally parsimonious cladograms suggests that their use would not significantly affect the outcome.

Terminal Taxa Shown in Figure 2 (Listed from Left to Right Within Each Portion)

Seed-Plant Cladogram (Bottom) (Doyle and Donoghue, 1986).—ANEURO = *Aneurophyton* s.l.; ARCHAE = *Archaeopteris*; MULTLY = protostelic lyginopterids; HIGHLY = "higher" lyginopterids; MEDULO = *Medullosa*; conifers (see below); GINKGO = Ginkgoales; CORDAI = Cordaitales; CALYST = *Callistophyton*; CORYST = Corystospermaceae; CYCADS = Cycadales; PELTAS = *Peltaspermum*; GLOSOP = Glossopteridales; CAYTON = *Caytonia*; GNETUM = *Gnetum*; WELWIT = *Welwitschia*; EPHEDR = *Ephedra*; PENTOX = *Pentoxylon*; BENNET = Bennettitales; angiosperms (see below).

Conifer cladogram (upper left) (Hart, 1987).—LEBACH = Lebachiaceae; PTSLAR = *Ptsuga* and *Larix*; CEDRUS = *Cedrus*; AIBKET = *Abies, Keteleeria,* and *Pseudolarix*; TSUGA = *Tsuga*; PICEA = *Picea*; PINCAT = *Pinus* and *Cathaya*; SAXEGO = *Saxegothaea*; DECUSS = *Decussocarpus*; DACFAL = *Dacrycarpus* and *Falcatifolium*; PARASI = *Parasitaxus*; LEPIDO = *Lepidothamnus*; LAGARS = *Lagarostrobus*; MICROS = *Microstrobus*; TORAME = *Torreya* and *Amentotaxus*; TAXPST = *Taxus* and *Pseudotaxus*; AUSTRO = *Austrotaxus*; CEPHAL = *Cephalotaxus*; ARAUCA = Araucariaceae; SCIADO = *Sciadopitys*; ATHROT = *Atherotaxis*; SEQSEQ = *Sequoia* and *Sequoiadendron*; TAIWAN = *Taiwania*; CRYCUN = *Cryptomeria* and *Cunninghamia*; METTAX = *Metasequoia* and *Taxodium*; MICPLA = *Microbiota* and *Platycladus*; THUTHU = *Thuja* and *Thujopsis*; JUNIPE = *Juniperus*; CHACUP = *Chamaecyparis* and *Cupressus*; CALOCE = *Calocedrus*; TETRAC = *Tetraclinus*; CALACT = *Callitris* and *Actinostrobus*; AUSLIB = *Austrocedrus* and *Libocedrus*; PILGER = *Pilgerodendron*; DISFIT = *Diselma* and *Fitzroya.*

Angiosperm Cladogram (Upper Right) (Donoghue and Doyle, 1989).—MAGNOL = Magnoliaceae; ANNONA = Annonaceae; MYRIST = Myristicaceae; DEGENA = Degeneriaceae; HIMANT = Himantandraceae; EUPOMA = Eupomatiaceae; CANELL = Canellaceae; MONLAU = Monimiaceae s.l., Gomortegaceae, Hernandiaceae, and Lauraceae; AMBORE = Amborellaceae; TRIMEN = Trimeniaceae; CHLORA = Chloranthaceae; AUSTRO = Austrobaileyaceae; CALYCA = Calycanthaceae; SCHISA = Schisandraceae; ILLICI = Illiciaceae; WINTER = Winteraceae; HAMAME = Hamamelidales; TROCHO = Trochodendrales; RANUNC = Ranunculidae; NELUMB = Nelumbonaceae; LACTOR = Lactoridaceae; ARISTO = Aristolochiaceae; PIPERA = Piperaceae; SAURUR = Saururaceae; NYMPHA = Nymphaeaceae; CABOMB = Cabombaceae; LILIOP = Liliopsida (monocotyledons).

Characters Shown in Figure 2

Propagule Type/Mode of Dispersal: Fleshy/Animal-Dispersed Versus Dry/Not Animal-Dispersed.—The three portions of the tree shown in Figure 2 were produced using MacClade, version 2.1 (W. P. Maddison and D. R. Maddison, unpubl. [a phylogenetics computer program distributed by the authors, Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138]). The state of the propagule character in each terminal taxon is shown by a small box below the name: a black box indicates fleshy propagules, and a white box symbolizes dry propagules. Where a box is absent, the basic condition in the terminal taxon was scored as "unknown." In conducting Maddison's test (see text), these taxa were assigned the most-parsimonious state based upon their position in the cladogram. Stippled boxes indicate those taxa whose state assignments differ between the two character optimizations considered (see text).

Parsimonious optimizations of the propagule character, obtained using MacClade (W. P. Maddison and D. R. Maddison, unpubl.; Swofford and Maddison, 1987), are shown by shading of the branches. Black branches signify fleshy/animal-dispersed propagules and white branches indicate dry/not animal-dispersed propagules. Stripes indicate that the condition along the branch is equivocal, that is, there are equally parsimonious optimizations that allow the branch to be fleshy or dry. Owing to uncertainty regarding the mode of dispersal of some fossil seed plants (Tiffney, 1986), two different optimizations were considered (see text). Under optimization 1, taxa marked by "?" are scored as dry, and both stippled and striped branches are assumed to be wind-dispersed. In contrast, under optimization 2, questionable taxa are scored as fleshy, and stippled and striped branches are considered to be animal-dispersed.

This character was not included in Doyle and Donoghue's (1986) analysis of seed plants, but Hart (1987) and Donoghue and Doyle (1989) each included a character reflecting propagule type. Although it is unlikely that inclusion of the propagule character in these studies substantially influenced the cladograms obtained, the character(s) of interest would ideally be excluded from the data set used in generating the cladograms. In a global analysis of all seed plants, this character would not be included, because "fleshy" and "dry" would not pass an initial test of homology based on similarity in position and development (Patterson, 1982). For example, in angiosperms these terms generally apply to carpels/fruits, whereas in "gymnosperms" they apply to seeds (or associated structures). These distinctions are not critical from the standpoint of evaluating Givnish's (1980) hypothesis, because the factor of interest in his model is dispersal by animals (specifically endozoochory). "Fleshy" and "dry" are here considered to be functional indicators of the mode of dispersal, regardless of the derivation of the relevant tissues.

Breeding System: Dioecious Versus Monoecious or Perfect.—Whether seed and pollen organs were borne on the same or on separate plants is not known with certainty in many extinct groups, because fossil reproductive structures are often found detached. In this case, a conservative approach has been adopted, dioecy being assigned only to taxa in which it is known to occur. The name of each dioecious taxon is marked by an asterisk.

The presumed evolution of the breeding-system character is shown by crossbars in Figure 2; a black crossbar along a branch indicates the origin of dioecy, and a white crossbar indicates a reversal from dioecy to monoecy. The precursor of dioecy in different lineages may differ. In most "gymnosperms," monoecy is the likely precursor, whereas within anthophytes (Bennettitales, Gnetales, *Pentoxylon,* and angiosperms [Doyle and Donoghue, 1986]) and especially within angiosperms, dioecy may have arisen directly from perfect (bisexual) reproductive structures. Breeding system was not included as a character in the cladistic analyses of seed plants (Doyle and Donoghue, 1986) or angiosperms (Donoghue and Doyle, 1989) but was included in Hart's (1987) analysis of conifers.