15

The Suitability of Molecular and Morphological Evidence in Reconstructing Plant Phylogeny

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Renewed interest in phylogenies over the last few decades coincides with a growing sense that it will actually be possible to obtain an accurate picture of evolutionary history. Indeed, the prospects of retrieving phylogeny now seem better than ever, owing to basic theoretical advance (due mainly to Hennig, 1966), the availability of computer programs that can handle large data sets, and the accessibility of new sources of evidence, especially molecular characters.

It is our impression that methods of phylogenetic inference—their assumption and reliability—have received more attention than the data upon which phylogenies are based. However, the rapidly increasing use of molecular techniques has focused attention on the pros and cons of molecular versus morphological evidence. Hillis (1987) and Patterson (1987) have reviewed the main arguments, and a summary of results for a number of major groups is available in the proceedings of the recent Nobel symposium (Fernholm et al., 1989). Regarding plant phylogeny in particular, only Sytsma (1990) has attempted a general survey and comparison of molecular and morphological studies. However, reviews of the use of particular molecules (e.g., Palmer et al., 1988, on chloroplast DNA) include useful discussion of molecular versus morphological results, and comparisons have been made within several angiosperm families (e.g., see in this volume, Chapter 10 by Doyle et al. on Fabaceae, Chapter 11 by Jansen et al. on Asteraceae, and Chapter 13 by Sytsma and Smith on Onagraceae).

This chapter is not a general review. Few generalizations seem possible at present, because there are too few careful morphological and molecular cladistic studies of the same groups of plants. We anticipate that much more detailed comparisons will be possible within a few years, although (surprisingly) the limiting factor is likely to be the number of solid morphological analyses. In the meantime, our aim is to examine the view that an accurate picture of phylogeny can be obtained solely on the basis of molecular data—that morphological data can be set aside safely at the outset of an analysis and mapped onto the molecular phylogeny later.

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This view seems to be popular. Sibley and Ahlquist (1987, p. 118), for example, assert that "the molecules can reconstruct the phylogeny with a high degree of accuracy. Given the phylogeny, the morphologist will be able to interpret structure and to separate similarities due to common ancestry from those resulting from convergence." Much the same view has been expressed by Gould (1985), and it appears to be widespread in botanical circles. For example, in reference to cases of adaptive radiation, Sytsma et al. (1991) specifically recommend a "two-step process" in which trees based only on molecular data are used to interpret the evolution of morphological characters. Morphological evidence, they say, should be avoided, because it "can often be phylogenetically uninformative or even misleading because of the operation of strong selection resulting in homoplasy, difficulty in ordering or even polarizing character states, the high number of autapomorphies, and the lack of well defined synapomorphies." Likewise, Gottlieb (1988, p. 1170) contends that "the molecular data are self-sufficient in that their usefulness does not depend on concordance with other lines of phenotypic evidence." Rather than being relevant in assessing relatedness, "the data of morphology, the traditional source of information about phylogeny, should be viewed as relevant to studies of plant development." Much the same view has been expressed by other botanists and is, we believe, widely held.

Here we consider theoretical arguments and selected empirical studies of plant phylogeny that bear on the view that phylogenetic hypotheses based on molecular data alone are more reliable than those based on morphology or on a combination of evidence. We conclude that, at best, this outlook is premature, and, at worst, it will stand in the way of achieving an accurate picture of phylogeny. We will deal only in passing with some kinds of difficulties that might arise in interpreting molecular results (e.g., different modes of inheritance of different genomes, or inadvertent analysis of paralogous genes; Doyle, 1987; Kawata, 1987; Patterson, 1987; Avise, 1989), or arguments in favor of morphological data (e.g., the likelihood of more thorough sampling of organisms/taxa; Hillis, 1987; Donoghue et al., 1989). Almost all of our discussion concerns broad (or "higher level") phylogenetic questions, both for practical purposes (to narrow the scope), and because these are the sorts of problems we have personally pursued. However, we believe that our general conclusions also apply to population and "species-level" problems, which are the focus of several chapters in this book.

We will be disappointed if our analysis is interpreted as a reaction against molecular approaches to phylogeny reconstruction. Nothing could be further from

the truth, as should be evident from the fact that both of us are pursuing molecular studies (MJD on *Viburnum*, MJS on *Astragalus*). Our argument is not against the use of molecular data; rather, it is against ignoring relevant morphological evidence. On the positive side, we hope to focus attention on a set of issues that must be confronted in adopting the view that *both* morphological and molecular data should be used in reconstructing phylogeny.

Homoplasy

Here we consider a series of issues related to homoplasy (convergence, parallelism, reversal) and its impact on phylogeny reconstruction. The first four sections deal mainly with theoretical arguments on homoplasy in relation to reliability, selection, character complexity, and environmental variation. We have deferred a discussion of levels of homoplasy seen in real data sets to the last section, because the homoplasy reported in cladistic studies may have a variety of causes.

Homoplasy and Reliability

Many arguments for the superiority of molecular data rest on the assumption that homoplasy is directly related to reliability. If more reliable results are obtained when there is less homoplasy and if molecular data are less homoplastic than morphological data, then it follows that molecular data are superior. We consider the first part of this equation to be questionable; that is, the relationship between level of homoplasy and reliability or confidence is weak at best.

The standard intuition about the effect of homoplasy stems in part from the view that it is merely a "mistake" (Mickevich and Weller, 1990), which implies that homoplastic characters cannot be useful in reconstructing phylogeny. However, this is clearly false, because independent gains (or losses) can certainly function as synapomorphies of the two or more clades in which they evolved (e.g., the independent evolution of an inferior ovary, or of a particular structural rearrangement of the genome; see below). A homoplastic character might be *misleading*, but this depends in a complicated way on how it interacts with other characters in the data set. Homoplasy—even a large amount of it—does not by itself guarantee an inaccurate tree, particularly in large studies, which tend to have high levels of homoplasy simply by virtue of the number of taxa involved (Sanderson and Donoghue, 1989).

The overall amount of homoplasy is not as critical as its distribution (Jansen et al., 1990). Thus, it is not difficult to construct data sets in which there is a high level of homoplasy *and* a high level of confidence (Sanderson and Donoghue, 1989). This is true, at least, if confidence is estimated by resampling techniques such as the bootstrap (Felsenstein, 1985; Sanderson, 1989). Conversely, because reliability is a function of the weight of multiple, independent synapomorphies

(Hennig, 1966; Sanderson, 1989), confidence can be low even if homoplasy is low or nonexistent, such as when only one synapomorphy supports each clade.

Homoplasy and Selection

The second assumption of the argument presented above is that molecular characters are less homoplastic than morphological characters. This view is typically linked to two other assumptions: (1) selectively neutral characters are less likely to show homoplasy than those subject to selection (a view advanced initially by morphological systematists to aid in character selection/weighting; e.g., Mayr, 1969), and (2) molecular data as a whole are less likely to be subject to selection than morphological data. The second assumption implies that most morphological changes are adaptations (but see Gould and Lewontin, 1979) and that most molecular changes are neutral (but see Kreitman and Aguade, 1986). Nevertheless, instances of apparent sequence conservation are generally interpreted as reflecting selective constraints (e.g., Kimura, 1983; Patterson, 1988).

But even the first assumption is misguided. That is, the selective value of a character does not necessarily bear any particular relationship to the amount of homoplasy it exhibits. Features that are or were subject to selection need not have evolved more than once and may be highly conserved. It is not difficult, for example, to think of morphological traits that are presumed to be adaptations and are also thought to have evolved only once (e.g., the closed carpel of angiosperms). Some morphological characters might actually become less likely to undergo change (including homoplasy) owing to increased "burden," that is, by virtue of the evolution of dependent traits that constrain further evolution (Riedl, 1978; Donoghue, 1989).

Just as selection need not result in a high level of homoplasy, neutrality does not insure a low level. Mutations can occur at the same nucleotide site during the evolution of a lineage and, all things being equal, the probability of such multiple hits increases with time. When the number of possible states is highly constrained, as it is in the case of nucleotides, the chance that mutations at a particular site will result in homoplasy is quite high (Mishler et al., 1988). One might therefore expect high levels of homoplasy in neutral molecular characters given a sufficient amount of time. Archie (1989b) has shown that the consistency indices of two plant nucleotide data sets (derived by Bremer, 1988, from the amino acid studies of Martin et al., 1983, 1985) do not differ from those expected in randomly generated data, perhaps indicating near saturation with homoplastic multiple hits.

Homoplasy and Character Complexity

In the case of complex morphological features, it has long been argued that homology can be reliably determined at the outset through a detailed comparison of position, structure, and development (i.e., Remane's primary criteria of homol-

ogy; Remane, 1952; Kaplan, 1984); any characters that have passed such a rigorous examination are unlikely to be homoplastic. The key to this argument is "complexity" (Donoghue, 1991). In the case of "simple" characters (e.g., effectively lacking development) the determination of homology rests more or less completely on congruence with other characters (i.e., Remane's auxiliary criteria; Remane, 1952). Patterson (1988) used this reasoning to reach the unexpected conclusion that similarity is a better guide to homology than congruence in the case of molecular data. But it is important to recognize that his assessment applies only to the comparison of whole sequences, where the level of complexity is sufficient (but not too great) to establish probabilities of convergence (Donoghue, 1991). It does not apply to individual nucleotide sites, where homology is established in the act of aligning sequences.

Based on similar logic, structural modifications of the genome (e.g., inversions, large insertions and deletions/transfers) have been touted as especially reliable indicators of phylogenetic relationship (e.g., Palmer et al., 1988; but see Doyle, 1987). Given the very large number of possible rearrangements, and the possible functional consequences of such modifications, it is highly unlikely that the same one would arise more than once. Furthermore, any doubt regarding homology could be resolved by sequencing through the critical regions to determine if the similarity extends to the individual nucleotide level. It is now clear, however, that even major structural rearrangements are not infallible guides to phylogeny—they too can arise independently. Perhaps the most obvious example is the (presumably) independent loss of one copy of the chloroplast DNA (cpDNA) inverted repeat in conifers (Strauss et al., 1988), within legumes (Lavin et al., 1990), and within Geraniaceae (Downie and Palmer, Chapter 2, this volume).

A second example demonstrates that molecular and morphological systematists employ the same logic in such cases. Downie et al. (1991; Downie and Palmer, Chapter 2, this volume) have shown that the loss of the cpDNA *rpl*2 intron, which was originally thought to be unique to caryophillids (Zurawski et al., 1984; Palmer et al., 1988), has also occurred in several other taxa (Convolvulaceae, *Cuscuta, Drosera,* two genera of Geraniaceae, *Menyanthes,* and Saxifragaceae *sensu stricto*). Although this distribution suggests a number of independent origins, Downie and coauthors argue that the loss of this intron can still be a powerful indicator of relationships *within* the different lines in which it has occurred. This parallels an argument made by Donoghue (1983) concerning the evidential significance of a morphological feature, *Adoxa*-type embryo sac development. Although its presence in *Adoxa, Sambucus,* and some species of *Tulipa, Erythronium,* and *Ulmus* strongly suggests that it has evolved a number of times, it still might provide evidence of a direct connection between *Sambucus* and *Adoxa,* in view of the other characters that also suggest a close relationship. Nevertheless, even at this level, homology is ultimately judged by congruence with other data (Patterson, 1982). It could still turn out

that the *Adoxa*-type embryo sac evolved independently in *Adoxa* and *Sambucus,* even if they are closely related, or that the *rpl*2 intron was lost several times within Geraniaceae, for example.

Homoplasy, Environmental Variation, and Subjectivity

Morphological traits may be subject to considerable variation solely as a function of environment. This seems especially true in plants, which are notorious for plasticity in such features as body and leaf size (Stebbins, 1950; Schlichting, 1986; Sultan, 1987). Although it is conceivable that this kind of variation would lead a phylogenetic analysis astray, we are unaware of any example where this has actually occurred. There appear to be several reasons why. First, systematists working on higher level phylogenetic problems generally deal with characters that are not prone to environmental variation. Plants of *Astragalus,* for example, always produce zygomorphic flowers; subjecting them to more water or light will not induce actinomorphy. Second, occasional variation is recognized for what it is in most cases; for example, the presence of a four-merous flower in a lineage characterized by parts in fives. Plant systematists pursuing morphological studies at the generic level or below typically examine hundreds or thousands of individual specimens and will often see plants in a variety of habitats in the field, prior to deciding on appropriate characters for analysis. The availability of such extensive information about variation and plasticity seems to compensate to some extent for lack of information about the genetic and developmental basis of morphological characters. In particularly difficult cases, the extent of plasticity can be tested (e.g., Davis, 1983, 1987).

A concrete indication that the problem of plasticity has not had a major impact on phylogeny reconstruction is seen in comparing studies of plants and animals. If plants tend to be more plastic than animals, as is commonly believed, and if plasticity leads to problems in phylogenetic analysis, one might expect to see more homoplasy in plant studies than in animal studies. In fact, Fig. 15.1 indicates that the level of homoplasy in plant and animal studies does not differ significantly (Sanderson and Donoghue, 1989; but see Syvanen et al., 1989, who we believe were misled by their comparison of trees based only on cytochrome c). This suggests that plant systematists are not really being fooled by plasticity; rather, they have become adept at delimiting characters even in the face of considerable environmental variation.

Environmental variation is related to the issue of subjectivity in delimiting states, since it might blur discrete differences into a continuum. Alternatively, apparently discrete states may represent an underlying continuous variable subject to a threshold effect. This observation highlights a more general problem, namely the appropriate way in which to subdivide a system into characters and states (or the necessity of doing so at all; Felsenstein, 1988). In morphology there are certainly constraints on subdividing characters into states. Thus, as Wagner

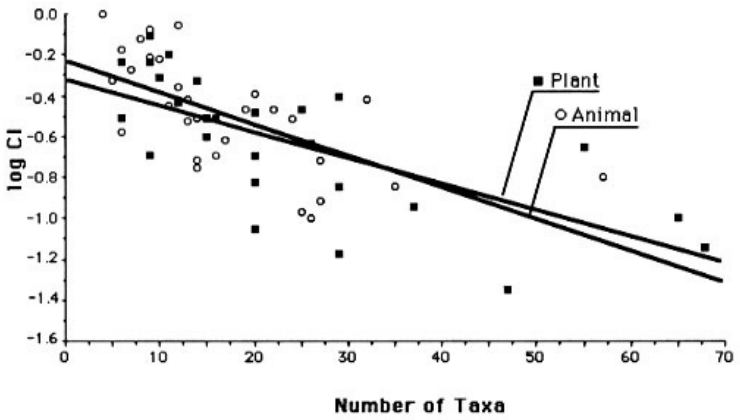

Figure 15.1. Log of consistency index versus number of taxa for 26 plant and 30 animal cladistic studies; redrawn from Sanderson and Donoghue (1989).

(1989) has emphasized, some degree of developmental individuation is critical. As an example, he discussed the morphology of the androecium in *Convolvulus*, in which stamens of varying length within the corolla are arranged in just two patterns that are mirror images of one another. Under these circumstances it does not make sense to treat the length of particular stamens as character states, because this attribute lacks its own genetic or developmental basis.

In the case of molecular data the appropriate characters and states have seemed more obvious, but it is not completely clearcut. Thus, in sequence data it is generally assumed that each nucleotide position (where homology is assessed in the alignment phase) is a character and the four possible bases are the appropriate states. But there are other ways to delimit the states at each site; for example, bases might be partitioned into purines and pyrimidines.

Even if there were some one "correct" or "natural" atomization, this does not mean that the wrong tree will be obtained by subdividing in another way. If this were not so, it is hard to imagine how any progress could have been made in reconstructing phylogeny, since idiosyncracies of individual investigators would have muddled the whole picture. Whether, and under what circumstances, different atomizations will yield different results has not been explored directly, but could easily be tested using sequence or morphometric data. It may turn out to be rather difficult to concoct circumstances under which different subdivisions give very different results.

Levels of Homoplasy in Real Data Sets

Leaving aside theoretical expectations, we can now ask whether there actually appears to be more homoplasy in cladistic studies based on morphological versus molecular data. A comparison of 42 morphological and 18 molecular cladistic studies (Sanderson and Donoghue, 1989) indicated no significant difference in homoplasy as measured by the consistency index (Kluge and Farris, 1969). As shown in Fig. 15.2, when the number of taxa is taken into consideration it is not possible to conclude that one type of data consistently shows more homoplasy than the other.

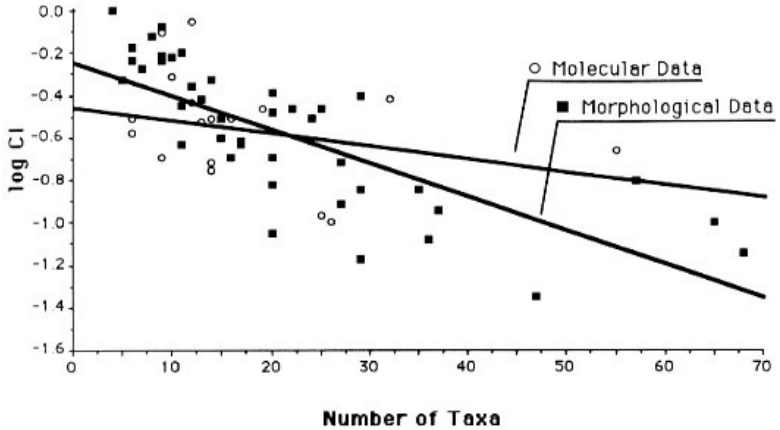

Figure 15.2. Log of consistency index versus number of taxa for 42 morphological and 18 molecular cladistic studies; redrawn from Sanderson and Donoghue (1989).

It is important to note, however, that our analysis is very preliminary and that there were too few molecular studies to draw a statistically robust conclusion. More molecular studies are needed, especially analyses that include many terminal taxa. Furthermore, as more cladistic analyses become available, it would be desirable to subdivide the molecular studies (as well as the morphological studies). It is possible, for example, that a different pattern would emerge in comparing only analyses based on cpDNA, or those based on protein electrophoresis. Thus, we note that for small numbers of taxa the lowest consistency indices are found in electrophoretic studies, whereas the highest consistency index for a large number of taxa is based on cpDNA restriction site data (Jansen et al., 1990). It may also turn out that restriction fragment, nucleotide sequence, and amino acid data sets will differ significantly in consistency, since the processes governing the tempo and mode of evolution may be different in each. Additional parameters will also need to be considered, such as the relationship between the amount of homoplasy and the degree of resolution of relationships.

Neutrality and Rates of Evolution

Besides its supposed connection to lower levels of homoplasy, neutrality has also been regarded as an advantage of molecular data because neutral characters are more likely to evolve in a clocklike fashion. From the outset of molecular studies of phylogeny, clocklike evolution has been considered among the most

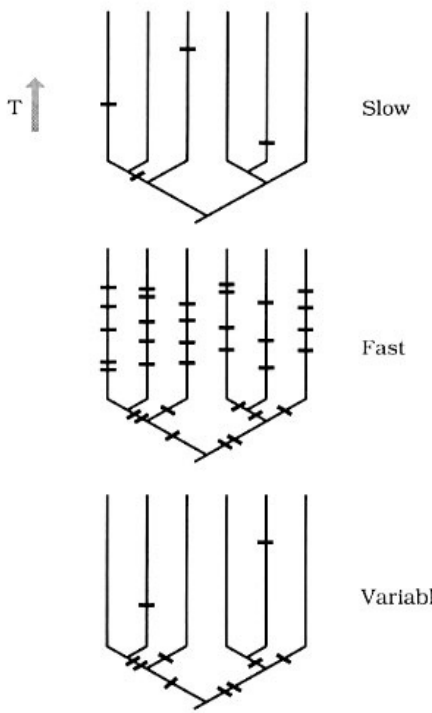

Figure 15.3. Clocklike evolution (whether slow or fast) is not desirable when reconstructing a rapid, ancient radiation. Character changes are shown as bars on branches.

important benefits of molecular evidence (see references in Clegg, 1990; Melnick, 1990). One reason for this is that variable rates of evolution could mislead some methods of inference, particularly those based on overall similarity. This argument is no longer very compelling, because methods are now available that are far more robust to variation in rates of evolution—cladistic parsimony methods with outgroup rooting, for example (e.g., Hillis, 1987; Sober, 1988). In any case, the molecular clock has since been called into question (e.g., Britten, 1986; Gillespie, 1986; Doyle et al., 1990; Melnick, 1990; Scherer, 1990).

However, even if we knew at the outset that a particular molecule evolved in a clocklike manner, this might not be desirable from the standpoint of phylogeny reconstruction (as opposed, for example, to age estimation). To appreciate why, consider Fig. 15.3, wherein we depict a group that radiated over a short period of time in the distant past, with little subsequent cladogenesis. Under these circumstances, a molecule evolving at a slow but constant rate may show too few changes during the critical period of diversification (Fig. 15.3, top). The result, commonly observed, will be an unresolved tree. On the other hand, in the case of a rapidly evolving molecule (Fig. 15.3, center), changes might mark each of the branches of the radiation, but continued rapid evolution would yield an increasingly "noisy" picture (Lanyon, 1988; Donoghue et al., 1989). That is,

homoplasy is likely to increase and be distributed so as to eventually overwhelm the signal in the data (Felsenstein, 1978). This might account, for example, for difficulties in assessing cyanophyte phylogeny (Bremer and Bremer, 1989), relationships among major lines of land plants (Mishler et al., 1990), and even among major seed plant groups (Zimmer et al., 1989).

Ironically, changes of rate might actually be desirable—even necessary—to achieve a resolved and accurate assessment of relationships (Lanyon, 1988; Donoghue et al., 1989). Ideally, we would like to focus on characters that evolved like those shown in Fig. 15.3 (bottom); that is, those that underwent sufficient change during the radiation, but changed very little after that time. It might be argued that this pattern of evolution is more likely in the case of morphological characters, where there may be selection associated with cladogenetic events (Olmstead, 1989) and/or subsequent constraints on change as a function of selection (Lanyon, 1988) or increasing burden (Donoghue et al., 1989). Thus, morphological characters could more faithfully retain information on the sequence of events in ancient, rapid radiations, especially when it is possible to include fossils, since these can provide relatively unmodified branches near the base of the radiation of interest (Donoghue et al., 1989).

It is now widely appreciated that different molecules and different parts of the same molecule can evolve at different rates, which means that choices must be made regarding which data are appropriate for which phylogenetic problems (e.g., Palmer et al., 1988; Sytsma, 1990). It is not entirely obvious, however, how to evaluate the limits of particular molecules (especially in view of the usually limited sampling within terminal taxa), or how to identify and treat rate variation within a molecule (Mishler et al., 1988). In some cases, limits are imposed by the method of analysis. Thus, in the case of restriction fragment comparisons, reliable estimates of homology become virtually impossible in considering very distantly related species (e.g., Palmer et al., 1988). In other cases, an initial analysis of the data might serve as a warning of unreliable results; for example, if the level of homoplasy is similar to that found in random data sets (Archie, 1989a) or if incongruent results are obtained when different subsets of the data are analyzed. Smith (1989) used well-supported morphological cladograms and divergence dates for echinoderms to test the limits of RNA sequence data, and concluded that 18S rRNA data yield reliable results for divergences within the last 100 m yrs, whereas beyond this point estimations are too prone to error due to saturation effects (regardless of whether one analyzes paired sites, unpaired sites, or transversions).

Independence

Character Independence and Weighting

The independence of morphological features from one another may be doubtful in some cases. If two or more "characters" are linked in the sense that they always

undergo evolutionary change together, then they should be counted as only one bit of evidence to prevent them from outweighing a smaller number of truly independent characters. The argument is sometimes made that the same difficulties do not plague molecular data or that the problem is much reduced. Thus, it is commonly assumed that changes at different nucleotide sites are independent of one another and that each is to be counted as a single bit of evidence.

In response to this argument, it is important to recognize that, although the situation may be difficult in the case of morphological data, it is by no means impossible to establish independence. Sepal size and petal size may be strictly correlated in a particular taxon, but petal size, type of stomate development, and pollen exine structure are quite likely to be evolutionarily uncoupled, and each of these traits can legitimately provide evidence on phylogeny. Moreover, it is possible to evaluate hypotheses of morphological independence by gathering appropriate genetic and developmental data.

Although some molecular characters are very probably independent of one another (e.g., third sites in different codons), there are also well-known exceptions. The application of some techniques is very likely to result in correlations; for example, the use of restriction fragment patterns derived from random probes of the nuclear genome (e.g., Song et al., 1988). Of much more general importance are the effects of secondary structure. Thus, there may be compensatory base changes where nucleotides are paired, as in the stem regions of rRNA molecules (Hixson and Brown, 1986; Wolters and Erdmann, 1986; Steele et al., 1988; Wheeler and Honeycutt, 1988), or gene conversion activity promoting homogenization of the inverted repeats of cpDNA (Palmer, 1985, 1987). In the absence of information on secondary structure, paired sites may inadvertently be treated as independent, thereby overweighting what is basically a single underlying change (although mismatches are sometimes maintained). Thus, Wheeler and Honeycutt (1988) suggested that nucleotide positions in paired regions of rRNA "should be downweighted, perhaps by one-half, or even excluded." In contrast, Patterson (1989) and Smith (1989) report instances in which paired sites actually appear to perform better than unpaired sites and suggest that this may be related to divergence time.

More complicated problems arise through interactions among sites that may be separated by some distance in the sequence (cf., Appels and Honeycutt, 1988). For example, changes in one site can initiate selection for a compensatory change at a distant site, perhaps even in a gene coding for a separate but functionally interrelated protein. Such effects might be especially important in enzymes such as RUBISCO, which are constructed from separately encoded subunits. Indeed, in this case a change in the cpDNA might influence selection in nuclear DNA or vice versa. In most cases we are still blissfully unaware of such functional constraints on evolution.

It should be clear from the foregoing discussion that the issue of character independence can be rephrased in terms of character weighting. The argument is

sometimes made that it is possible to establish objective, *a priori* weighting schemes for molecular data based on the likelihood of character change, and that this allows the use of methods such as maximum likelihood. From time to time, the same has been said of morphological data, and quantitative methods have even been devised (e.g., Riedl, 1978). However, whereas most morphologists have been suspicious of such procedures (tending to reject weighting altogether or make use of *a posteriori* weighting, such as the successive approximations method; Farris, 1969; Carpenter, 1988), molecular systematists have been much more confident that the relative likelihood of character state changes can be derived from first principles or prior empirical data. It is widely accepted, for example, that third sites within a codon are less constrained (Kimura, 1983), that transitions are more likely than transversions (Lake, 1987), and that an independent loss of a restriction site is more likely than an independent gain (DeBry and Slade, 1985; Templeton, 1983).

Before proceeding, it is important to consider that even if these observations about the relative likelihood of change are correct, it is not clear when and how differential weighting will affect the outcome of phylogeny reconstruction. Nor is it clear that not weighting characters differently will render the outcome especially sensitive to differences in likelihood of character change. Despite earnest efforts to comprehend the relationship between likelihood of character change and parsimony, this connection remains poorly understood (Sober, 1988).

In any case, hypotheses about likelihood of change are not as easy to translate into a weighting scheme as they may appear. Thus, the rate of evolution of third-position sites can be influenced by a number of factors. For example, such changes might be constrained by the presence of a functional open reading frame encoded on the opposite strand (c.f., Zurawski and Clegg, 1987). It has also been noted that transition-transversion ratios differ in different genomes (e.g., from 30:1 in animal mitochondrial DNA to 1.5–2:1 in cpDNA; Palmer, 1987; Zurawski and Clegg, 1987), and even in different genes within a genome (Zurawski and Clegg, 1987; Wheeler, 1990). However, such conclusions have only rarely been tested empirically (using cladistic analysis), and the results of such studies have not always fit the preconceptions. For example, Doyle (1991) constructed phylogenies based on 16 glutamine synthetase sequences. He found few differences in the level of homoplasy or confidence in comparing subsets of the data consisting of transitions versus transversions or third versus first and second sites within codons. Furthermore, methods designed to deal with differences in likelihood of change make their own assumptions. Thus, Lake's method of invariants ("evolutionary parsimony"; Lake, 1987; Holmquist et al., 1988) assumes that transversions of the two types are balanced, and it may be sensitive to deviations from this assumption resulting, for example, from differences in G+C content (Gouy and Li, 1989; Sidow and Wilson, 1990).

In the case of restriction sites, it is quite clear that independent gains can occur. It is on this basis, in fact, that Albert et al. (Chapter 16, this volume) argue

against the use of Dollo parsimony. Their derivation of a likelihood weighting scheme for restriction site data demonstrates the assumptions inherent in such calculations. Some of these are clearly unrealistic, for example, a constant rate of substitution across lineages (e.g., Wu and Li, 1985). It also emerges that such weighting schemes are not generalizable (to other genomes, for example, or possibly even to different genes in the same genome) since they are tied to particular estimates of substitution rate, which themselves are dependent on assumptions about cladistic relationships and divergence times (Brown et al., 1979; Wolfe et al., 1987). The most that one can hope for under such circumstances is what Albert et al. actually report, namely that weights fall within relatively narrow limits over the entire range of phylogenetic problems to which the data in question are applicable.

Another weighting problem concerns the relative value of structural mutations versus site mutations. Palmer et al. (1988, p. 1188) argued that "the extreme rarity and lack of homoplasy of major rearrangements makes each one a single character in a way that nucleotide substitutions, which inevitably will be afflicted with certain levels of homoplasy, can never be," and that such rearrangements "should be weighted much more heavily than a single nucleotide substitution or restriction site mutation." However, it is unclear exactly which structural rearrangements warrant such treatment and how much more they should be weighted (Olmstead et al., 1990). It is clear that the outcome of an analysis can be substantially affected by such decisions. This sensitivity is illustrated by the analyses of prochlorophyte relationships conducted by Morden and Golden (1989a, b) based on *psb*A sequence data. When the presence or absence of a seven-amino-acid domain is treated as the equivalent of one or two amino acid substitutions, *Prochlorothrix* may be nested among blue-green algal groups (consistent with the 16S rRNA result of Turner et al., 1989). However, when it is weighted any more heavily, *Prochlorothrix* appears as the sister group of green chloroplasts (consistent with morphological and pigment evidence, Miller and Jacobs, 1989).

The greatest difficulties are caused by cases in which the likelihood of character-state change is itself subject to change during the evolution of a group. Especially troublesome are instances in which a character is independent when it first evolves but later becomes coupled with another character, or vice versa. Where there are such changes in the degree of independence during the evolution of a group, there does not appear to be any straightforward way to code or weight characters to reflect their changing evidential significance (Donoghue, 1989). We suspect that this is a very real problem in some morphological studies. In seed plants, for example, it appears that leaf and sporophyll evolution may have been strictly coupled in some parts of the phylogeny (simultaneous reduction in the evolution of coniferopsids) and uncoupled in other parts (e.g., within anthophytes) (Doyle and Donoghue, 1986). We are uncertain how often this problem will arise in molecular data, but there are ample opportunities for it to occur. For example, evolutionary changes in the secondary structure of a molecule could change pairing and functional relationships and hence the degree of independence.

Taken together, these observations indicate that the supposed differences between molecular and morphological data in terms of character independence and weighting are a matter of degree. In view of how little we still know about the relevant mechanisms of molecular evolution, the assumption that all sites are independent rests largely on faith. Just as genetic and developmental studies have revealed complex relationships among morphological traits, as we learn more about molecular mechanisms we are likely to discover many more (and even more subtle) forms of interdependence. For example, there is the possibility that some structural changes may have a "destabilizing" effect (Palmer et al., 1987), or that the transfer of a functional gene (Baldauf and Palmer, 1990) will result in a release from selective constraint and changes in substitution rate. In the meantime, it is brash to imply that weighting of morphological characters can never be justified or that the weighting of molecular characters is easily accomplished.

Independence of Data Sets

Another reason given for focusing exclusively on molecular characters is the desire to avoid circularity in studying morphological evolution (e.g., Olmstead, 1989; Sytsma, 1990). It would, of course, be circular to reach conclusions about the evolution of any trait in a phylogeny based exclusively on that character. But the desire to study the evolution of one or several morphological traits does not justify omitting all morphological characters. Surely there are other morphological traits that are independent and can help in establishing the phylogeny, and failure to consider such characters may yield an unresolved or inaccurate tree. Even the character of interest has some bearing on the inference of phylogeny. If the addition of this character to the analysis results in a change in topology, then it is not acceptable to leave it out and proceed to interpret its evolution. One simply needs more data.

This outlook may be clarified by turning the argument around. Suppose that one were interested in studying the evolution of a particular portion of the genome. An extreme form of the independence argument implies that molecular data should be eliminated altogether—that the phylogeny must be based solely on other data, perhaps from morphology. We assume that most readers will balk at this suggestion. Why, it will be asked, is it necessary to omit all molecular data in order to study some single aspect of molecular evolution? Furthermore, the tree based solely on morphology might be inaccurate—after all, it does not take into account the molecular data. These are precisely the points we made in the preceding paragraph with regard to morphology.

In order to obtain an accurate picture of the evolution of a given feature, the phylogeny should be based on all of the relevant evidence, rather than ignoring some (perhaps sizable) portion of the data on the grounds that it is similar in some

respect to the trait of interest. The issue is the independence of the phylogeny from the character(s) of interest, not whether a trait happens to be classified as morphological or molecular. In any case, in view of the great concern that characters be independent (see above), it is ironic that the independence of data sets has been used as an argument for keeping them separate. If morphological and molecular characters really are independent of one another, this is a powerful argument for putting them together (Barrett et al., 1991). Exceptions arise when there are entities under consideration that have separate evolutionary histories, and the aim is to compare these histories. Thus, in studying hybridization, one might wish to construct separate trees based on cpDNA and on morphology or nuclear genes.

The Number of Characters

The beauty of molecular data is that there is potentially so much of it—and increased numbers add evidential weight and statistical power to phylogenetic inferences. In general, it appears that the more characters there are per taxon, the higher the level of confidence, at least as measured by the bootstrap (Sanderson, 1989). And this relationship is unaffected by the amount of homoplasy present, which tends to vary independently of the number of characters (Sanderson and Donoghue, 1989; but see Archie, 1989a).

In response to this argument, it should be remembered that it is possible to gather more and better morphological data than we have now, for example, through studies of development. Furthermore, morphological data can be gathered from more organisms/taxa. Even if DNA will soon be routinely obtained from herbarium specimens, it is unlikely that much molecular evidence will ever be obtained from fossils, notwithstanding the success of Golenberg et al. (1990) in sequencing the *rbc*L gene of a Miocene *Magnolia*. It may be that a clear picture of phylogeny will require a very good sample of taxa, including fossils, in which case there would be a significant advantage to morphological data (Donoghue et al., 1989). In this regard, the simulation studies of Wheeler (1991) are especially intriguing. Wheeler found that the number of characters used in the reconstruction accounted for most of the variation in cladogram resolution (although this also depended on whether evolution was assumed to be clocklike), but that cladogram accuracy depended largely on the number of taxa included in the analysis (either 4 or 12 in his study).

It is also important to note that increased clarity is by no means guaranteed to come cheaply. The ratio of phylogenetically uninformative (constant or autapomorphic) characters to potentially informative characters has typically been very high in molecular data, entailing a significant investment of time and money to obtain a moderate amount of relevant information. In the case of hominoid primates, only 54 potentially informative sites were discovered in a sample of 10,939 sites in three sequences from nuclear and mitochondrial DNA (Holmquist et al., 1988), and some of these potentially informative sites are homoplastic on the most parsimonious tree. It may be more efficient in many cases to concentrate on morphological data, especially considering the evident congruence of data sets discussed below.

For our purposes, the question is whether the number of molecular characters that might be amassed can justify ignoring whatever morphological data are available. In practice, there have seldom been so many more molecular characters that one would seriously entertain abandoning the morphological evidence. Thus, although Olmstead (1989) made a point of the large number of molecular characters that could be acquired, morphology still accounted for over one third of the (binary-coded) characters he analyzed in *Scutellaria*. Moreover, some morphological studies contain a very large number of characters, and it is unlikely that they will be outnumbered by informative molecular characters any time soon. The most impressive numbers, however, are found in vertebrates; for example, Gauthier et al. (1989) considered 972 potentially informative characters in analyzing relationships among 83 tetrapod taxa.

But even if many more molecular characters were available, should morphological evidence be abandoned? Doing so effectively assumes that the relatively few morphological traits would be overpowered by the molecular traits in such a way that they could have no effect on the outcome. In turn, this seems to imply that there is no significant variation in the level of support for different clades in molecular studies. If, on the contrary, some nodes happened to be supported by only a few character changes (perhaps as a function of the tempo of evolution rather than sampling error), then the addition of only a few morphological characters might tip the balance in favor of a new tree, as shown in Fig. 15.4. This effect would be even more pronounced if the few molecular characters in question showed some homoplasy.

In fact, in molecular studies conducted to date, there is often considerable variation in the level of support for different clades and considerable homoplasy. In some cases, the result is an almost complete lack of resolution, especially when trees in the neighborhood of the most parsimonious cladogram(s) are taken into consideration. This has been demonstrated by Bremer (1988) for amino acid data on angiosperm families (also see Archie, 1989b), and by Bremer and Bremer (1989) for rRNA oligonucleotide catalog data on blue-green algae. Lack of resolution of some clades is also apparent in many studies with cpDNA restriction site data, even when there is little homoplasy (Olmstead et al., 1990). The analysis of *Clarkia* by Sytsma et al. (1990) provides an example of this problem: although the traditional sections of the genus are well marked, resolution of relationships among the sections is completely lost in the consensus of the 25 trees within one step of the most parsimonious trees. Sytsma et al. (1990) postulate a rapid radiation early in the history of the genus to account for this pattern, as did Sytsma and Smith (1988) for sections of *Fuchsia*, and Chase and Palmer (in Palmer et al., 1988) for lack of resolution among several lineages within Oncidii-

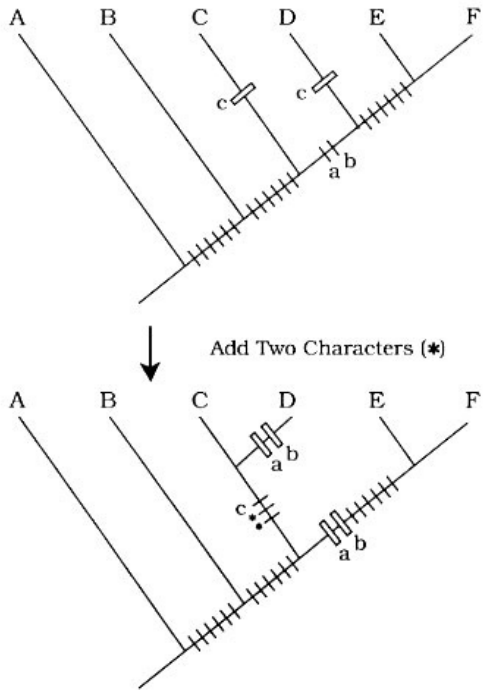

Figure 15.4. When character support is not uniformly distributed, it is possible for the addition of a small number of characters to change a topology based on a large number of characters. The upper cladogram (based on 21 characters) requires 22 steps, with homoplasy in character c. The addition of two new characters (*), with derived states in taxa C and D, yields the lower cladogram, requiring 25 steps and homoplasy in characters a and b.

nae (Orchidaceae). In all of these cases, the addition of even a few morphological characters could help resolve relationships.

In general, there is nothing to be lost (and potentially much to be gained) by taking into account the morphological data. It may not have any effect on the outcome, either because it is congruent with the molecular data (but adds no resolution) or because whatever incongruence exists is resolved in favor of the molecular characters. On the other hand, the addition of morphological characters might help to resolve with greater confidence some unresolved portion of the molecular tree or might result in a change in topology in an area that is not strongly supported by molecular data. These effects are evident in the examples discussed by Miyamoto (1983), Hillis (1987), and Kluge (1989), and they are also apparent in a preliminary analysis of major seed plant groups based on a combination of rRNA sequence and morphological data (Donoghue, Zimmer, and Doyle, unpublished data). In the worst scenario, morphological data may be so noisy that they lower the resolution of an otherwise highly resolved molecular data set. We know of no cases in which this has occurred. Of course, the reverse might also occur, namely that noisy molecular data might obscure the signal in morphology. For example, it is possible that 5S rRNA data would obscure the

picture of green plant relationships based on morphological characters (Bremer et al., 1987). If one suspected such a case, it would be valuable to explore whether the level of homoplasy in one or both of the separate data sets deviated from that seen in random data (cf., Archie, 1989b), and to examine confidence levels in the separate and combined data sets.

Patterns in Empirical Studies

Most of what we "know" about phylogeny is based on morphology, and most of that knowledge is probably not far off the mark. If nothing else, this has been confirmed time and again by molecular studies. Thus, it is not surprising that molecular data show tobacco and pea to be more closely related than either is to corn or to a liverwort (Ritland and Clegg, 1987). By now it is clear that congruence is the rule, not the exception, even in more controversial cases. Thus, Jansen and Palmer's cpDNA results for Asteraceae (Jansen and Palmer 1987; Palmer et al., 1988; Jansen et al., 1990; Jansen et al., Chapter 11, this volume) agree in many ways with Bremer's (1987) morphological evidence, especially as regards the monophyly of Asteroideae and the position of Barnadesiinae. We note that although Bremer (1987) included one molecular character in his analysis (a 22-kb inversion), the same result is obtained when this character is omitted (Bremer, personal communication). The monophyly of the Gnetales is also strongly supported both by morphological (Crane, 1985; Doyle and Donoghue, 1986) and rRNA sequence data (Zimmer et al., 1989; Hamby and Zimmer, Chapter 4, this volume). Many other examples of congruence are documented in this volume.

In other cases, the molecular and morphological data give consistent results, but one provides better resolution than the other. In the case of seed plant phylogeny, the morphological data provide rather strong support for the anthophyte clade, with Gnetales being more closely related to angiosperms than any other extant group (Crane, 1985; Doyle and Donoghue, 1986). In the rRNA sequence data, this relationship is less clear, with several alternative placements of Gnetales seen within one or two steps of the most parsimonious trees (Zimmer et al., 1989; Hamby and Zimmer, Chapter 4, this volume). In other instances, molecular data favor one of several alternatives based on morphological data; *Fuchsia* section *Skinnera* provides a good example (Sytsma et al., 1991; Sytsma and Smith, Chapter 13, this volume), and the question of the placement of the root of the angiosperms might also fall in this category (Donoghue and Doyle, 1989).

Cases of genuine disagreement are hard to find. In fact, we know of no case in which cladistic analysis of morphological data *strongly* supports a conclusion that is *strongly* contradicted by cladistic analysis of molecular data (but see Sytsma and Smith, 1988, for a different interpretation). Moreover, apparent discordance seems to be as common in comparing different morphological studies

of the same group, or different molecular studies, as it is in comparing molecular versus morphological results (Wyss et al., 1987).

Reports of incongruence are largely based on comparing results obtained by different methods of analysis or, more often, one sort of data has been rigorously analyzed and the other has not. The *Clarkia-Heterogaura* example (Sytsma and Gottlieb, 1986; Sytsma and Smith, 1988, and Chapter 13, this volume) falls in this category: the morphological data have not yet been treated cladistically, and it is inappropriate to interpret the traditional classification as a phylogeny (Donoghue and Cantino, 1988; Doyle et al., 1990). We suspect that morphological results will be consistent with molecular results in this case but that the molecular data will provide a clearer resolution of the position of *Heterogaura* within *Clarkia* (but see Sytsma, 1990, for a different perspective). Other apparent instances of incongruence involve weak support for particular relationships in analyses based on one or both data sets; for example, the apparent difference between cpDNA and morphology regarding the status of the Lactucoideae (aside from Barnadesiinae). Although Jansen et al. (1990) favor the monophyly of Lactucoideae (based largely on Dollo parsimony), the Wagner parsimony analysis of their data set also produced trees in which Lactucoideae are paraphyletic, consistent with Bremer's (1987) result. Although Bremer's (1987) analysis did not strongly support the paraphyly of Lactucoideae (see Sanderson, 1989), additional morphological data have strengthened this hypothesis (Bremer, personal communication).

Other instances of apparent incongruence may be a function of the sample of taxa considered, which can certainly influence tree topology (Donoghue et al., 1989; Wheeler, 1991). For example, initial analyses of rRNA sequence data indicated that monocots were polyphyletic, with *Sagittaria* and *Potamogeton* arising within different dicot lines (Zimmer et al., 1989; Donoghue and Doyle, 1989). But these arrangements were only weakly supported, and with the addition of taxa to the analysis they are now seen to be less parsimonious (Hamby and Zimmer, Chapter 4, this volume). Similarly, apparent disagreement between Hamby and Zimmer's (1988) rRNA analysis of grasses and the morphological analysis of Kellogg and Campbell (1987) may be a function of the coverage of taxa in the two studies. Congruent results are obtained when the morphological data set is reduced to just those taxa included in the molecular study (Kellogg, personal communication), and the addition of taxa to the rRNA study might bring it in line with the larger morphological study (Hamby and Zimmer, Chapter 4, this volume).

The foregoing review of empirical studies, aside from confirming our expectations based on the theory of evolution, provides as strong an argument in favor of morphological data as it does in favor of molecular data. And if there is disagreement, it is not clear, without further evidence and/or analysis, which result (if any) is correct (Doyle, 1987). As Cracraft and Mindell (1989, p. 398) pointed out, "it is one of the ironies of our time" that another conclusion has been

drawn by some molecular systematists: the reliability of molecular data are judged by congruence with morphological phylogenies, and then it is claimed that molecular data are more informative than morphology whenever there appears to be a conflict (e.g., Ahlquist et al., 1987). If morphology is so untrustworthy, why should it be used at all in evaluating molecular results? And if it is trustworthy enough to use in this capacity, why should we not trust it when conflicts arise? It is also ironic that even those who are most wary of morphological data nevertheless lean on it heavily in designing their own research, namely in choosing which groups to work on, which subgroups to sample, and so on. If it is admitted that morphological evidence has been useful in establishing phylogenetic relationships, then what excuse can there be for setting it aside?

Consequences of Ignoring Data

The temptation to ignore data is evidently great, but experience suggests that this should be resisted. Thus, we are suspicious of assertions to the effect that some forms of data are useless; for example, the claim that continuous variables cannot provide evidence on phylogeny (Pimentel and Riggins, 1987). In the same vein, molecular systematists will want to consider carefully the pronouncement by Wilson et al. (1989) that restriction site mapping is now outmoded and should be abandoned in favor of sequencing.

Here, the controversy over the use of fossils is especially instructive. Patterson (1981) and others have implied that it is acceptable to ignore fossils in reconstructing phylogeny. After all, fossils are generally incomplete and are therefore unlikely to have much effect on an analysis compared to extant organisms. As reasonable as this may seem on the surface, it is simply wrong. Inclusion of fossils can and does make a difference, both in terms of tree topology and the interpretation of character evolution (Donoghue et al., 1989). This is true even when data on fossils are limited. The fact that there are more data on extant organisms does not insure that this information will overwhelm the limited fossil evidence. Completeness and relevance to resolving a particular phylogenetic problem are not the same thing.

Although the case of fossils has to do with taxa, and only indirectly with characters, we trust that the parallel to the molecule versus morphology debate will be obvious. Morphological data, even when limited in comparison with molecular data, may be highly relevant. Just as with fossils, this may be especially true in the case of ancient, rapid radiations. As we noted in connection with Figs. 15.3 and 15.4, in such instances molecular data may be limited or very noisy, and the addition of even a few morphological characters—characters associated with early branching events that have since become fixed—could make a big difference (see above; Olmstead, 1989). What purpose is served in denying such possibilities at the outset? Why not try to make use of both morphological and molecular data?

Conclusions

The arguments presented above lead us to the conclusion that it is a mistake to set morphological data aside and base phylogeny reconstruction only on molecular evidence. As we have tried to show, arguments that appear to support this outlook are illogical, otherwise unconvincing, or rest largely on faith. It is obvious that there are highly desirable attributes associated with molecular data, especially the large number of characters that can potentially be obtained rather readily. But these attributes do not justify ignoring morphological characters, which have some fortunate properties of their own, such as their availability from a larger sample of organisms, including fossils. Of course, in most ways molecular and morphological data are quite similar—differences are mainly a matter of degree and do not map neatly onto the division of characters into molecular versus morphological.

Theoretically, there appear to be good reasons to retain both types of data, and empirical studies indicate not only that both types of data are relevant, but that failure to consider all of the data might lead to unresolved or even inaccurate results. Why, then, have we not analyzed all of the data? Undoubtedly, part of the problem is uncertainty over how best to proceed. In particular, it has been unclear whether to analyze data sets separately and find the consensus of the resulting trees, or to combine data sets and analyze them simultaneously. The pros and cons of these alternatives have been discussed at length elsewhere (see Miyamoto, 1985; Hillis, 1987; Cracraft and Mindell, 1989; Kluge, 1989; Barrett et al., 1991). In most cases we believe that it is best to combine data sets, rather than separate results. At least this is our recommendation when the same phylogenetic question is being addressed by the two data sets, which need not be the case if there are entities involved that have different modes of inheritance and hence more-or-less independent phylogenies (see Kawata, 1987; Avise, 1989). For example, as illustrated in several chapters in this volume, the study of hybridization is facilitated by tracing organelle and organism phylogenies separately. At the very least, it is important to recognize, as Barrett et al. (1991) have shown, that consensus trees (even strict consensus trees, which contain only those components that appear in all of the trees being compared) can be *positively incongruent* with the tree(s) based on an analysis of the pooled data. Thus, contrary to popular opinion, consensus techniques are not even a means of playing it safe, and it behooves the investigator to determine whether a consensus tree is really sanctioned by all of the data. This requires a combined analysis.

The prospect of combining molecular and morphological data sets raises a set of difficult issues, especially regarding character weighting and differences in the nature of the sampling of terminal taxa. Here, we can offer only a few comments on these issues. The feeling that it will be necessary to weight characters differentially in a combined analysis arises mainly from the worry that the data set with more characters (usually molecular) will overwhelm the data set with fewer characters (e.g., Kluge, 1983). As we stressed above, the sheer number of characters of a particular type is not as important as the nature of the character support and the distribution of homoplasy. Based on the overall congruence seen in empirical studies, complementarity may be the usual result.

The second problem—differences in the sampling of taxa—is not as widely appreciated. In morphological studies the character states assigned to a terminal taxon are usually based on information from many organisms, whereas in molecular studies there may be just a single representative of a taxon (which might be well nested within that taxon). Thus, the terminal taxon "conifers" in the Doyle and Donoghue (1986) analysis of seed plants is scored as a presumed basal state based on virtually all modern and fossil conifers, whereas in the rRNA sequence studies of Zimmer and colleagues (Zimmer et al., 1989; Hamby and Zimmer, Chapter 4, this volume) conifers are represented by sequences from just a few accessions. Some practical means of handling such discrepancies are explored elsewhere (Donoghue, Zimmer, and Doyle, unpublished data). Although it may seem that consensus techniques sidestep these issues, the problems are only hidden or arbitrarily resolved (Barrett et al., 1991).

In part, the reluctance to combine data sets may have a sociological basis. Enthusiasm over a new source of evidence is understandable, as are exaggerated claims on its behalf. But too often in the history of systematics the rising popularity of one sort of data takes place at the expense of another, which remains insufficiently explored. This is not just an incidental effect of limited resources—such replacement has often been actively pursued. That is, it is felt that the best way to promote the new data is to find fault with the old, and what could be better than to claim that the old data are worthless? But rhetoric of this sort, and the fads that it encourages, are unhealthy from the standpoint of our common goal, namely reconstructing the phylogeny of plants. Molecular data, when gathered carefully and analyzed in an appropriate manner, are obviously very useful in understanding evolutionary history, and the same can be said of morphological data. Both are, we believe, extremely promising avenues to pursue, and neither has come close to achieving its full potential. Ultimately, however, our efforts to reconstruct phylogeny will be judged by their success in integrating *all* of our observations, which means that more attention should be devoted to combining molecular and morphological evidence.

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