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Phylogenetic Inference, DNA Sequence Analysis, and the Future of Molecular Systematics

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The Emergence of Phylogenetic Systematics

Comparative biology has had a long and noble history (Rieppel, 1988). Its central goal has been to understand the bewildering diversity of form observed across the world’s organisms. Concepts such as taxa (in particular, species) and homology have been the core intellectual instruments for facilitating this understanding. Thus, comparative biologists have labored to sort the world’s organisms into species (however construed), largely based on their characteristics of form (but viewed broadly, including any intrinsic attribute), and to describe their similarities and differences. For several hundred years now, this effort to compare has led to the realization that similarities and differences among species are best ordered in terms of a hierarchy of relationships, which we now take to represent the primary pattern of life’s history as it has diversified over the last 4 billion years.

Systematics is the science of comparative biology and the primary goal of systematists is to describe taxic diversity and to reconstruct the hierarchy, or phylogenetic relationships, of those taxa (Hennig, 1966; Eldredge and Cracraft, 1980; Nelson and Platnick, 1981; Wiley, 1981). The overall importance of this goal is that corroborated hypotheses of relationship are the prerequisites for all inferences regarding what needs to be explained (i.e., various patterns) within the context of an historical perspective. This might involve repeated patterns of geographic distribution from one group to another (Nelson and Platnick, 1981), the distribution across lineages of certain behavioral, ecological, or morphological attributes, or the sharing among taxa, or perhaps among spatially segregated populations, of variation at the level of DNA. Just as importantly, it is only by having an hypothesis for the hierarchical pattern of shared attributes—with its im-
plications for understanding character polarity—that one can begin to formulate explanations for derived novelties that have arisen and become fixed in populations.

Procedures for constructing phylogenetic hypotheses have been most fully developed by the discipline of phylogenetic systematics, or cladistics (Hennig, 1966), which presently dominates the field of systematics (Hull, 1989). The emergence of cladistics can be related to its direct connection to genealogy in that only shared derived characters (synapomorphies) are used as evidence to support hypotheses about phylogenetic relationships. With respect to those hypotheses, similarities due to the retention of primitive features (symplesiomorphies) are ignored because they are uninformative regarding relationships. The cladistic approach is thus conceptually coherent in that only synapomorphies are considered to provide evidence for monophyly, and only monophyletic groups have objective reality as historical entities.

The increasing acceptance of cladistics can also be attributed to its placement of systematic methodology within a broader scientific context. Thus, one of the more important philosophical advances has been to require that phylogenetic hypotheses conform to observations as closely as possible (Farris, 1983). Stated differently, those hypotheses which make the fewest ad hoc assumptions about shared patterns of character-state distributions are to be preferred (Eldredge and Cracraft, 1980; Wiley, 1981), yet the hypotheses themselves will always remain vulnerable to testing by additional observations. This philosophical and methodological approach, known as parsimony, has become an essential component of modern systematic methodology (Farris, 1983; Sober, 1983, 1988), and as we shall discuss below, also provides the rationale for another important component of phylogenetic inference: congruence analysis.

The Growth of Comparative Sequence Analysis

Prior to the 1960s, most systematic studies utilized morphological characters as evidence for relationships. However, over the last 20 to 30 years, the contributions of molecular approaches to phylogenetic research have increased steadily (Hillis, 1987; Patterson, 1987; Doolittle, 1990; Hillis and Moritz, 1990). At this time, DNA sequences are becoming the preferred database for molecular systematics. Nuclear genomes, coupled with their extranuclear counterparts, are exceedingly large and complex, thereby offering the systematist an almost endless array of characters with different structural/functional properties, mutational/selectional biases, and evolutionary rates (Li et al., 1985; Nei, 1987; Li and Graur, 1991). Therefore, the potential exists for using the evolutionary characteristics of particular genomic regions to match those sequences to specific systematic questions. This permits the use of sequences to address a broad range of systematic questions. Thus, studies of population variation can be investigated with mitochondrial DNA (mtDNA) sequences from the noncoding control re-
gion, a part of the molecule which often shows significant variation, even among individual organisms (Greenberg et al., 1983). At the other extreme, the phylogenetic relationships within and among kingdoms can be evaluated with highly conserved stretches of coding DNA, for example, those for ribosomal RNAs (rRNAs) (Field et al., 1988; Lake, 1988; Sogin et al., 1989; Mindell and Honeycutt, 1990). The tremendous flexibility in their resolving power ensures the future importance and widespread acceptance of DNA sequences in comparative research.

Several recent advances have now made it possible for systematists, even those with little formal training in molecular biology, to obtain DNA sequence data on a routine basis. Although DNA sequencing has become a simple laboratory procedure (Sanger et al., 1977; Sanger, 1981), the polymerase chain reaction (PCR), without question, is most responsible for the increased interest in using DNA sequences in comparative work (Saiki et al., 1988; Erlich, 1989; Kocher et al., 1989). Pairs of oligonucleotide primers are chosen to flank the region of DNA that is to be amplified by PCR. Following cycles of DNA denaturation by heat, primer annealing by cooling, and strand extension with a thermostable enzyme such as Taq polymerase, microgram quantities of double-stranded DNA can be synthesized from nanogram amounts of template. By modifying the ratios of the two primers, additional amplification cycles can produce single-stranded DNA (ssDNA), which can then be directly sequenced to obtain nucleotide data (Gyllensten and Erlich, 1988). As very little template is required (theoretically, only one molecule), the original samples for PCR need not be fresh or frozen tissue (Pääbo et al., 1988; Kocher et al., 1989). Thus, DNA can be amplified from alcoholic and formalin specimens, as well as from skins, feathers, bones, single hairs, and mummified material (Higuchi et al., 1984; Pääbo et al., 1988, 1989; Pääbo, 1989). PCR has therefore greatly enhanced the value of samples which were previously not accessible to the molecular systematist. The ability to use museum specimens is of special importance, particularly since natural biological diversity continues to be lost at an alarming rate. Furthermore, PCR studies of museum specimens collected at different times promise to add a temporal component to phylogenetic and evolutionary analysis (Thomas et al., 1990).

PHYLOGENETIC INFERENCE USING DNA SEQUENCES: METHODOLOGICAL PROBLEMS

Despite the spectacular technical advances in obtaining comparative sequence data, many difficulties still exist for the investigator, not only during the process of acquiring the sequence information itself, but also in subsequent analyses undertaken to generate phylogenetic hypotheses for the taxa of interest. Some of these difficulties and limitations are discussed below.
Acquisition of Comparative Sequence Data

Most comparative sequence data that have been used in molecular systematics were obtained by conventional cloning procedures (Sambrook et al., 1990), although many investigators have now adopted asymmetrical PCR as the method of choice (Gyllensten and Erlich, 1988; Allard et al., 1991). While PCR is relatively simple in theory and practice, many laboratories attempting to obtain comparative sequence data using asymmetrical amplification have often experienced variable success in producing high quality ssDNA, especially when trying to amplify fragments over 800 to 1000 base pairs (bp) in length. Yet, other factors may be as important as fragment length in influencing the quality of single-stranded product from asymmetrical PCR, and reaction conditions must be carefully optimized (Gyllensten, 1989). These difficulties may be only transitory, however, since new procedures for obtaining single-stranded template from double-stranded PCR reactions have the potential for greatly facilitating large-scale comparative studies (Higuchi and Ochman, 1989; Mitchell and Merrill, 1989).

Only after it becomes possible to obtain large amounts of comparative sequence information rapidly, accurately, and at reasonable cost, will DNA sequences fulfill their potential for systematics. In order to answer systematic questions, the most important objective is to obtain character-state data, which can often be accomplished most effectively by aligning, in tandem, those segments of sequence that are easiest to collect; for example, by using several so-called universal PCR primer-pairs (Kocher et al., 1989). Thus, it will sometimes be the case that systematist may not bother to sequence both complementary strands of DNA, much less, complete genes. In instances such as this, the goal of the systematist will frequently conflict with that of the molecular evolutionist who may be interested in different questions involving the evolutionary patterns seen across entire genes. Such conflicts are regrettable, given the general importance of DNA sequences to fields other than molecular systematics (Fickett and Burks, 1989).

Once DNA sequences have been determined, they are deposited in permanent data banks that can be accessed by others for many different purposes. Given their general utility, the accuracy of these sequences should therefore be verified by checking both complements against one another. For a similar reason, nucleotide sequencing using RNA templates should be avoided in favor of direct DNA analysis (Hillis et al., 1990).

A major concern of molecular evolution is the relationship between the higher-level structure of genes and their products (including, for example, proteins and structural RNAs), and their primary sequences (Gerbi, 1985; Gautheret et al., 1990; Johnson et al., 1990). Such information is also relevant to molecular systematics. For example, knowledge about the secondary structure of rRNA may provide an important basis for assigning weight to different regions of the molecule in a phylogenetic analysis (Wheeler and Honeycutt, 1988; Smith, 1989). Consequently, obtaining complete gene sequences across a spectrum of taxa becomes important, because data
of this kind will permit the detailed analyses of molecular structure, function, and evolution necessary to justify the weighting (Irwin et al., 1991).

Acquiring sequence data is labor intensive; therefore, molecular systematists generally use only single individuals to represent each taxon in their study. As a consequence, the assumption is made (usually implicitly) that polymorphism does not affect the phylogenetic analysis because within-group variation is negligible. However, this assumption may not be well-founded, especially when the branch points under consideration are of a relatively recent age, as would be true in the case of a recent radiation of species within a clade (Nei, 1986). Under these circumstances, the sequences may not reflect the true species relationships of the group, even if a single branching pattern is strongly supported by the data. Instead, the strong support might reflect a gene phylogeny that may not be congruent with the cladistic history of the taxa themselves. Such misleading situations arise because of random sorting of ancestral polymorphic alleles by drift after cladogenesis. The only way to test whether a gene phylogeny is congruent with the species phylogeny is to compare the current results against data from other individuals and for unlinked genes (Nei, 1986; Felsenstein, 1988).

Sequence Alignment

The first step in analyzing nucleotide data is to align the sequences against one another (Waterman, 1984, 1989; see also papers in Doolittle, 1990). This initial step is crucial, as all subsequent analyses are dependent on the final alignment. In many instances (e.g., involving protein-coding sequences of mtDNA), sequence alignment is easy and can be done by eye, without the aid of an alignment algorithm. On the other hand, many kinds of sequences vary so much across taxa that computer-assisted alignment is essential to minimize the differences among them. Most computer procedures use some measure of similarity (or dissimilarity) to search for the best alignment for a given pair of sequences. Different pairwise comparisons are then combined to produce the final overall result. Because of their indirect approach to the problem, such strategies may not reveal the optimal alignment for multiple sequences. Algorithms for the simultaneous comparison of multiple sequences related by a given tree topology have been developed, but are limited by their dependence on the tree topology itself and by the need for large amounts of computer time (Sankoff, 1975; Sankoff and Cedergren, 1983; see also Waterman et al., and Mindell, this volume).

In molecular systematics, sequence alignment is essentially a problem of homology\(^1\) of character-state data that are the individual nucleotides at each base position. Morphologists have traditionally recognized homolo-

\(^1\) Here, sequence homology is equated with orthology (common ancestry by speciation) and paralogy (ancestry by gene duplication) is not included in the definition (Fitch, 1970; Patterson, 1988). In phylogenetic analysis, paralogous comparisons are avoided, because they provide evidence on the order of gene duplications, but not of speciation. Thus, only orthologous sequences are of importance in reconstructing species relationships.
gies by the criteria of similarity and congruence (Patterson, 1988). Homologies themselves are hypotheses of synapomorphy that are first postulated on the basis of observed similarity (Eldredge and Cracraft, 1980). After phylogenetic reconstruction, these assessments are then evaluated according to their agreement, or disagreement, with the most-parsimonious solution (i.e., with respect to their congruence with other characters). Those similarities which conflict with the most-parsimonious distribution of the entire suite of characters are reinterpreted as homoplasies (convergences, parallelisms, and reversals).

Homology has been largely equated with similarity by comparative molecular biologists (see Patterson, 1988). Unfortunately, this emphasis on similarity has obscured the conceptual and methodological relationship between homology and character support for a genealogical hypothesis. Even though methods of sequence alignment may maximize overall similarity prior to a phylogenetic analysis, hypotheses of base-position homology nevertheless remain falsifiable by character congruence on the tree that most parsimoniously explains the distribution of all the data across the taxa. The same tests of similarity and congruence that apply to morphological characters, therefore, apply to sequence data as well.

The importance of sequence alignment to comparative analysis is highlighted by the use of large-subunit rRNA sequences to resolve relationships among prokaryotes and eukaryotes. The original study of these groups by Lake (1988) emphasized small-subunit rRNA sequences and concluded that eocytes and eukaryotes are each other's closest living relatives. A major implication of this arrangement was the possibility that eukaryotes originated from a sulfur-metabolizing, thermophilic, anucleate ancestor. However, both Lake (1990) and Gouy and Li (1990) have noted that the results for large-subunit rRNA sequences are dependent on the particular alignment used as well as the choice of species. The instability of the large-subunit rRNA results reemphasizes the need for more rigorous and efficient methods of aligning multiple sequences in which the tasks of alignment and phylogenetic analysis are more fully integrated (Hein, 1989, 1990; see also Waterman et al., and Mindell, this volume).

Reconstructing Phylogenetic History

That nucleotide sequences provide a rich source of data for resolving phylogenetic relationships is no longer disputed. What is being debated, rather, is not so much how phylogenetic hypotheses can be constructed from those data (many procedures are capable of generating such hypotheses), but which method should be preferred and how we might objectively assess the veracity, or reliability, of the result. These questions define a host of critical problems within systematics in general, many of which bear on the nature of comparative evidence and its application to evaluating alternative hypotheses of relationship. Entangled with these problems are a series of debates over methods of phylogenetic inference that take as their focus
what investigators either assume or infer about the evolutionary dynamics of nucleotide sequences. None of these controversies, however, appear to be unique to molecular data. For years, morphologists have debated whether it is desirable or possible to use assumptions or inferences about the evolutionary characteristics of morphological features and whether one can apply these to evaluate the reliability of those characters as evidence for phylogenetic inference.

A good deal is known about the evolutionary dynamics of DNA (e.g., see the summaries of Li et al., 1985; Nei, 1987; Li and Graur, 1991), and assumptions about the nature of its evolution have been incorporated into all methods of phylogenetic inference in the form of weighting schemes, correction factors for multiple mutations, and so forth. Thus, it is not the use of these assumptions per se that would lead one to prefer one method over another inasmuch as it is generally possible to incorporate any given assumption into most tree-building algorithms (except in those special cases when certain assumptions, such as constant rates, are a logical corollary of the method). Justification for a particular method will therefore have to come from elsewhere, and various possibilities have been proposed. Felsenstein (1988), for example, identifies two approaches to justification: "hypothesico-deductive," which he equates with the application of parsimony; and statistical. Other investigators have sought to justify the choice of method by simulation analysis in which DNA sequences evolve under various assumptions and according to "known" phylogenies. The method of phylogenetic inference that recovers the "true" phylogeny, given the particular a priori assumptions of the simulation, is preferred over those which do not (Sourdis and Krimbas, 1987; Sourdis and Nei, 1988; Saitou and Imanishi, 1989).

There are at least two general sets of methods of phylogenetic inference that can be applied to sequence data (we appreciate the fact that others may categorize these differently) (Felsenstein, 1981, 1988; Swofford and Olsen, 1990). The first set utilizes discrete character data and includes two well-known approaches; Hennigian cladistics (often called the parsimony method, but this term can be applied more broadly to other procedures as well; see below), and maximum likelihood. A second set of procedures clusters intertaxon similarity/dissimilarity distance measures derived from paired comparisons of the sequences. Categorizing methods in this way mirrors some of the most intense debates seen in the recent systematic literature, and also emphasizes certain theoretical and methodological problems that should concern all workers undertaking phylogenetic inference.

It is probably the case that the majority of nucleotide sequence data sets have been analyzed using distance methods. Surprisingly, however, few investigators who apply distance algorithms have addressed the extensive literature criticizing and defending the use of distances in phylogenetic inference (Farris, 1981, 1983, 1985, 1986a, b; Felsenstein, 1984, 1986). Distance analysis receives its justification from the argument that phylo-
genetic inference must be treated as a case of statistical inference (Felsenstein, 1984, 1986), yet as the debates have highlighted, this is not entirely self-evident (Farris, 1983). Indeed, the conflict between using methodological parsimony and statistical inference as the basis for justifying any tree-building method will undoubtedly remain a controversial issue within systematics for some years to come.

If one chooses to apply a statistical approach, then the data must possess certain properties in order for the statistical procedures to have validity. This is the basis for much of the disagreement: it is well known that systematic data, including DNA sequences, often do not satisfy the underlying assumptions of statistical models (Sanderson, 1989; Swofford and Olsen, 1990). This appears to be a problem for the statistical viewpoint but not for methodological parsimony. In principle, at least, methodological parsimony may be applied no matter what the underlying characteristics of the data. It is certainly the case that the statistical structure of data is very much a concern for all methods of analysis—even in cladistics one assumes independence of characters—but the exact nature of that structure is itself transparent to methodological parsimony. This forms the basis for the suggestion that methodological parsimony is a more general approach to hypothesis evaluation than is statistical inference.

Methodological parsimony is a general criterion in science for adjudicating the effectiveness of alternative hypotheses in accounting for data (Farris, 1983; Sober, 1983). It applies to all methods of phylogenetic inference which rely on an optimality criterion (Swofford and Olsen, 1990). Some quantity is being minimized or maximized in all of these methods, and the decision to choose the minimum or maximum solution forms the basis for the application of parsimony. It must be understood, however, that this approach does not address the truth of the resulting hypotheses. The application of statistical methods has been offered as one way to evaluate the efficacy, or reliability, of hypotheses relative to others. If one chooses not to apply a statistical approach, however, the most-parsimonious solution remains the single best hypothesis for explaining the data, given the criterion forming the basis for the parsimony decision. To claim otherwise is to argue that our choice of hypothesis does not have to conform to evidence, a claim that will lead scientific discovery nowhere.

As noted, it has become popular to use simulations to compare the properties of various tree-building algorithms. The reliability of a method is then judged on whether it accurately identifies the “true” tree used in the simulation. No one would suggest that a given approach will always find the true phylogenetic relationships for a set of real taxa, and few would suggest that simulations are not informative regarding when a particular

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2 We distinguish here between parsimony methods and methodological parsimony. The former is generally equated with cladistic algorithms in which the parsimony criterion is the minimization of homoplasies. Methodological parsimony is a more general, philosophical criterion, and keeping the distinction in mind may help clarify the debate over the role of statistics versus parsimony in phylogenetic inference.
procedure might fail to find the correct tree. But there are some fundamental philosophical and empirical differences between simulations of fictitious taxa and their DNA sequences, on the one hand; and real-world taxa and their sequence characteristics, on the other. These differences place important limitations on the general significance of simulated results.

The results of simulation are logically dependent upon the model of nucleotide evolution used to generate the sequences, along with the parameters of the model such as generation time, time to cladogenesis, and sequence length, as well as the algorithm used in the specific analysis. The evolutionary models used in many simulation studies are exceedingly simple, and even though they will surely become more sophisticated (e.g., more “realistic”) in the future, such studies will still face a credibility gap. There is no single evolutionary model, no matter how complicated, that can mimic all of the historical complexities of sequence data. Even as models increase in sophistication, no one who studies DNA would expect that a particular model of sequence change will necessarily hold across different clades of taxa. If the assumptions of these models lack veracity with respect to real-world situations, their relevance to empirical cases will continue to be questioned.

In short, because of the immense complexities of the real world, simulation studies can only be expected to identify some of the limitations of a given method of phylogenetic inference. It is unrealistic to expect any computer model to capture all of the nuances of the molecular evolutionary process. Thus, the successful reconstruction of a simulated phylogeny by a particular method does not necessarily guarantee similar success when the method is applied to actual data. Some important parameter of the actual data may have been overlooked in designing the simulation, thereby limiting the general significance of its conclusions. There is a need, therefore, for an approach to evaluate methods and phylogenetic results in the absence of truth about the real world. In the next section, we suggest that congruence analysis, an extension of methodological parsimony, offers one such approach for comparing the reliability of competing methods and/or empirical results.

CONGRUENCE ANALYSIS AS ARBITER

Congruence analysis is a time-honored tradition of science, in general, and of systematics, in particular. Unfortunately, its importance has been overlooked within molecular systematics, especially by those searching for a means to evaluate the reliability of phylogenetic methods. As a principle, the notion of congruence applies broadly, from instances of pattern recognition to expectations of theory. Thus, it is congruence of observations that signifies the presence of a pattern and implies the need for a common explanation; it is congruence of evidence that allows us to prefer one hypothesis over another. In both instances, the use of concordance can be viewed as an extension of the application of methodological parsimony.
In principle, the true phylogenetic relationships for a given set of taxa will never be known with certainty. In the absence of such knowledge, systematics has long relied on studies of congruence among data sets to assess the reliability of its phylogenetic hypotheses. Well-corroborated patterns of relationship are accepted as the best estimates of the true phylogeny because it is more parsimonious to accept a common explanation—common descent, for example—than to conclude that two (or more) identical results were obtained independently. For such reasons, studies of congruence have been viewed by systematists as a powerful way to resolve difficult phylogenetic questions (Penny et al., 1982; Kluge, 1989).

Congruence analysis provides an important mechanism with which to evaluate the reliability of different tree-building methods (see also Mickevich, 1978). Given a common data set, those approaches which lead to congruent results should be preferred over those that do not. Furthermore, such comparisons can then serve as a basis for investigating why a particular method has failed to converge on a congruent pattern that is supported by different sets of data and/or other methods of phylogenetic inference. Studies of congruence, therefore, can provide insights into the limitations and assumptions of different tree-making algorithms.

We suggest that empirical investigations of congruence among actual data sets using different tree-building procedures, along with varying the assumptions of those methods, will likely provide more insight into the efficacy of these approaches than will simulation analysis, which is generally far removed from the real world. One approach might rely on phylogenetic hypotheses of major groups of organisms that are highly corroborated by a variety of molecular and nonmolecular data, such as higher-level relationships within the primates (Miyamoto and Goodman, 1990). Those methodological approaches which lead to congruence with well-corroborated hypotheses should be preferred over those that rarely do. In this way, the reliability of different tree-building methods can be evaluated against our best estimates of phylogenetic relationship without interference from oversimplifications of the real world and from individual bias during the selection of parameters in simulation analyses.

Congruence analysis also permits one to evaluate the reliability of different weighting schemes of character transformations for use in a phylogenetic study. In their phylogenetic investigation of cervid and other artiodactyl mtDNA sequences, Kraus and Miyamoto (1991) found that cladistic analysis of all mutations (transitions, transversions, and gap events) resulted in a phylogeny supporting the monophyly of antlered deer in the family Cervidae. In contrast, when only transversions were counted, the same method (i.e., parsimony) led to a solution whereby antlered deer were not monophyletic. The former arrangement favoring antlered deer monophyly is corroborated by morphological data, unlike its alternative solution. Thus, congruence suggests in this case that the use of all mutations provides more reliable results than analysis of transversion differences alone. The failure of transversion parsimony to obtain a reliable result can
be attributed to the loss of cladistic information offered by transitions at these lower hierarchical levels.

The primary limitation on obtaining knowledge of phylogenetic history has not been so much with difficulties encountered with any particular method, but often with the actual data (Kraus and Miyamoto, 1991). We either have too few data or the evidence is of such poor quality (because of extensive homoplasy, intraspecific polymorphism, paralogy) that no method can use them effectively. Conversely, when the data are highly structured, the same groupings will very likely emerge no matter what method of phylogenetic inference is employed. The real problem occurs when different methods yield incongruent results for the same data. Is this the result of the methods per se, the choice of underlying assumptions used in the approach (e.g., correction factors, weighting schemes), or because the data are ambiguous? By relying on studies of congruence, answers to such questions will continue to accumulate for real-world situations.

THE FUTURE OF MOLECULAR SYSTEMATICS

The growing importance of DNA sequences for phylogenetic inference and for analysis of evolutionary processes at the molecular level requires that investigators strive for complete gene sequences whose accuracy has been verified by sequencing both complements. Such efforts will enhance the overall value of these comparative data to biology as a whole. With regard to systematics, a more detailed understanding of molecular evolution will facilitate the development of improved methods of phylogenetic reconstruction. Because large amounts of sequence data will be needed to address problems about the extent of intraspecific polymorphism, and to resolve many vexing phylogenetic questions, we might expect that cooperative research projects involving different laboratories will be required.

Continued technological developments (with respect to collecting sequence data), coupled with the vast phylogenetic information contained in nuclear and extranuclear genomes, virtually guarantees that nucleotide sequences will become the primary source of systematic data in the near future. Before the full potential of these data is realized, however, many of the problems noted above will need to be resolved. We have proposed that congruence analysis will play an important role in resolving controversies over systematic methods and phylogenetic relationships. Congruence provides the ultimate test of reliability in the absence of revealed truth, and as such, adoption of this methodological criterion can be regarded as crucial to the continued development of the field. Despite the growing importance of sequence data, it cannot be stressed strongly enough that there remains a pressing need to enlarge our nonmolecular database of systematic characters. If we are to gain meaningful insights into the "whats," "hows," and "whys" of the history of life, phylogenetic studies
will have to rely on all available comparative information, both molecular and nonmolecular.

ACKNOWLEDGMENTS

We thank M. W. Allard, D. P. Mindell, and M. R. Tennant for their helpful suggestions about the manuscript. This research was supported by grants from the National Science Foundation to MMM (BSR-8857264 and BSR-8918606) and JC (BSR-8805957 and BSR-9007652).

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