Ann. Rev. Ecol. Syst. 1987. 18:23–42

MOLECULAR VERSUS MORPHOLOGICAL APPROACHES TO SYSTEMATICS

David M. Hillis

Department of Zoology, University of Texas, Austin, Texas 78712

INTRODUCTION

In the past two decades, molecular investigations of systematic problems have progressed from uncommon curiosities to a standard means of elucidating phylogenetic history. This relatively sudden rise of biochemical systematics has precipitated predictable debates between the traditional morphological and the new molecular camps. Despite warnings about potential pitfalls of each approach by proponents of the other, few attempts have been made to examine the degrees of conflict and consensus between these techniques over the large and rapidly accumulating myriad of comparative studies.

A primary objective of phylogenetic studies is to reconstruct the evolutionary history of a group of organisms. Because the organisms under study have a single history, systematic studies of any set of genetically determined characters should be congruent with other such studies based on different sets of characters in the same organisms. Congruence between studies is strong evidence that the underlying historical pattern has been discovered; conflict may indicate theoretical or procedural problems in one or both analyses, or it may indicate that additional data are needed to resolve the phylogenetic relationships in question.

In this review, I first outline the advantages of both morphological and molecular approaches to systematics. I then discuss some common differences in assumptions and methods of analysis that can lead to spurious conflict between studies, especially those concerning phylogenetic reconstruction. A major impediment in comparing the two approaches is that

0066-4162/87/1120-0023$02.00

the histories of the application of the two techniques to systematic problems differ to a large extent. Molecular systematics grew mostly out of population genetics, whereas morphological systematics stemmed largely from comparative anatomy. Although this difference in background has presented numerous problems in comparing many past studies, recent advances in systematic theory have transcended traditional boundaries and have been applied with equal success to both morphological and molecular data sets (19, 52, 59, 141). Finally, I discuss ways in which conflicting studies can be reconciled, and I argue for the increased combination of molecular and morphological data in order to maximize phylogenetic information.

ADVANTAGES OF EACH TECHNIQUE

Although arguments for the general superiority of one type of systematic data over another have appeared periodically (46, 86, 99), most systematists recognize that morphological and molecular approaches each have distinct advantages (124). Although the output from molecular systematists has increased manyfold in both quality and quantity during the last several decades (29), this has certainly not been accompanied by a decrease in output from morphologists. To a large extent, this is because systematists from each camp are able to address questions and problems that cannot be addressed by systematists from the other. Because of this complementarity, collaborations between morphological and molecular systematists often produce analyses that transcend the usefulness of separate studies (e.g. 12, 35, 55, 91, 93, 107, 123).

Advantages of Molecular Methods

SIZE OF THE DATA SETS Perhaps the greatest advantage of molecular data is the extent of the data set. Because all heritable information of an organism is encoded in DNA, the set of morphological data with a genetic basis is a small subset of molecular information. The maximum number of independent characters of an organism is limited by the number of nucleotide pairs in its DNA. This number ranges from about 5×10^3 for the smallest viruses to nearly 4×10^{11} for some eukaryotes (23, 60, 115). However, except for a number of viruses, only a small fraction of this sequence information has been examined in any organism.

In recent years, available DNA sequence information has been compiled in the GenBank data base under contract with the US National Institutes of Health. Release 44.0 (August 1986) summarizes 11,413 reports and lists approximately 4×10^6 base pairs (bp) from eukaryote nuclear genomes, 5×10^5 bp from eukaryote organelles, 1×10^6 bp from bacterial genomes, and 2×10^6 bp from viral sequences. The information, however, is highly variable

by taxonomic group, and over half the sequence data has been obtained from fewer than 10 species. Of eukaryote nuclear sequences, 74% have been obtained from vertebrates; mammalian sequences alone account for 64% of the total, and sequences from one species, *Homo sapiens*, represent 30% of the eukaryote data base. Advances in DNA sequencing technology undoubtedly will result in a tremendous increase in the DNA sequence data base, which represents the largest possible set of characters for systematic analysis.

PHYLOGENETIC LIMITS In principle, neither molecular nor morphological methods are limited by phylogenetic scale in their application. In practice, however, few morphological characters are shared among major groups of organisms (eukaryotes versus eubacteria, for instance). In contrast, biomolecules provide a phylogenetic record from very recent time to the origin of life on Earth, because of the size and diversity in rates of change of different portions of the genome (15, 111).

Among the most rapidly evolving DNA sequences are those found in the mitochondria of eukaryotes (mtDNA) (6, 15, 16). Studies of mtDNA have been useful for studying population phylogeny within species (5, 7, 20, 80), as well as for recovering phylogenies of closely related complexes of organisms (17, 130). In addition to mtDNA, allozymes have been widely used to detect cryptic species and to recover phylogenies of morphologically similar organisms (4, 19, 70). In some cases, reproductively isolated species are morphologically nearly or completely indistinguishable; their reproductive isolation became clear to systematists only after they were studied by molecular methods (35, 36, 61, 70, 71, 73, 74).

On the other end of the phylogenetic spectrum, some gene sequences that are involved in basic life processes evolve so slowly that homologies can be established throughout living organisms. Chief among these are the ribosomal RNA genes (rDNA), which have been used to reconstruct the basic outlines of organic evolution (111). Numerous other DNA sequences evolve at intermediate rates between mtDNA and rDNA, so that virtually any level of phylogenetic question can be addressed by choosing the correct molecular segments. In addition to the coding regions, some gene arrays contain a diversity of transcribed and nontranscribed spacers that provide a record of evolutionary history from very recent to ancient times (67, 68).

EXTENT OF NONHERITABLE VARIATION In order for comparative data to be useful for phylogenetic reconstruction, the characters under study must represent heritable variation. Environmental influences on the phenotype must be sorted from genetic variation. For some groups, environment seems to have little influence on phenotype, but for other groups the effects of

environment are great (see 2, 79, 88, 108). Although methods have long been in use for estimating heritability (39, 109, 117, 134), in many cases the effects of environment are simply assumed to be minimal unless shown otherwise.

Although nonheritable variation is primarily a problem of morphological characters, some molecular characters can be confounded by this problem as well. Although DNA sequence data are nearly or completely free of nonheritable variation, the expression of gene products can be affected by environment (e.g. see 71). In addition, molecular analyses can be affected by degradation of the gene products over time due to inadequate or inappropriate storage (32). In general, however, biomolecular data are confounded less by environmental influences than are morphological data.

Advantages of Morphological Methods

APPLICABILITY TO MUSEUM SPECIMENS One of the greatest advantages of morphological over molecular approaches to systematics is the much greater applicability of the former approach to the extensive collections of preserved specimens in museums. Although some molecular information can be obtained from traditionally preserved specimens (114, 132), the majority of molecular techniques require fresh or cryopreserved material (31). For many groups of poorly known organisms, the only known specimens of many species are represented merely by the holotype or type series. Collecting additional material can be prohibitive because of rarity of the species, inaccessibility of the habitat, destruction of known collection localities, legal protection of the habitat or species, or high costs of procurement. A high percentage of recently extant species have been exterminated in this century by human activities, especially through the destruction of tropical rainforests (97). Because of this high extinction rate, a majority (or at least a large fraction) of described species may never be collected again and will remain known only from traditionally preserved specimens.

Efforts to establish and maintain collections of cryopreserved specimens have increased dramatically. A recent review of frozen tissue collections lists nearly 100 such collections in 18 countries throughout the world (30). However, unlike traditional museum collections, material is rarely "loaned" from frozen tissue collections, primarily because tissues are modified and usually destroyed during analysis. A more satisfying solution is beginning to appear with the establishment of gene libraries that have been cloned into bacterial cultures. Gene libraries can be stored indefinitely and can be shared by any number of researchers. It is surprising that the systematics community has done virtually nothing to encourage the proliferation of such collections, considering that gene libraries can contain an inexhaustible record of virtually all genetic information about an organism.

APPLICABILITY TO FOSSIL SPECIES Paleontology always has been primarily a morphological endeavor, and the low percentage of biomolecules that are preserved in fossils ensures that this will continue to be the case. The special importance, or lack thereof, of fossils to phylogenetic reconstruction has been debated extensively (26, 27, 41, 59, 103, 112, 120, 141). Whether or not fossils provide any special insights into phylogenetic history, at minimum they represent a set of taxa that provides potential information about evolution. This information is nearly limited to morphological analysis. Moreover, an understanding of morphological variation in fossils requires an understanding of the morphology of living species. Obviously, the ability to incorporate relevant information from fossils is a great advantage of morphological analyses.

Although relatively little molecular information has been obtained from extinct species, a few molecular techniques have been applied with considerable success to well-preserved fossil specimens. DNA has been cloned from skins of recently extinct species (62), from Egyptian mummies (110), and from human brain tissue buried underwater for 8000 years (33). Some structural proteins, such as collagen, are extremely stable and can be analyzed from recent fossils (3). Analyses of collagen have been conducted on humanoid remains as old as 1.9 million years (82). However, except in unusual circumstances, most fossils remain within the domain of morphological study.

USE OF ONTOGENETIC INFORMATION Two methods are commonly used to distinguish phylogenetically informative data (apomorphies) from phylogenetic noise (plesiomorphies) in systematics: the outgroup criterion and the ontogeny criterion (43, 77, 78, 84, 116, 127, 138). Much has been written about the relative merits of these two methods. Nelson (104) in particular has argued for the superiority of the ontogeny criterion, primarily because the ontogenetic states can be observed directly and the method need not make a priori assumptions of relationships. Furthermore, Nelson (104) has criticized the outgroup method as indirect (in that transformations are not directly observable) and as requiring some prior knowledge of relationships. Others have addressed these criticisms (14, 28, 77), and many systematists favor the outgroup criterion even when ontogenetic information is available (14, 45, 84).

The argument over outgroup versus ontogeny criteria has not involved molecular systematists, because most molecules lack ontogenetic development. Gene sequences are either present or absent; timing of protein expression may vary, but for the most part proteins go through little ontogenetic change. Some proteins (e.g. the components of hemoglobin) are encoded by multiple genes that are expressed at different times during development (37), but this information is of limited use in character analysis. On the other hand,

morphological ontogeny is under genetic and epigenetic control, so advances in developmental genetics should help clarify the ontogeny question. Nevertheless, use of ontogenetic information in systematics is likely to be largely restricted to morphological systematists for some time.

COST A major obstacle to increased use of molecular techniques in systematics is cost. Although modern morphological methods can be expensive, some morphological data can be collected with minimal expenditures on supplies and equipment. The greatest barrier for molecular systematists probably is the initial set-up of a laboratory. Costs vary by specific discipline, but most molecular laboratories require tens of thousands to hundreds of thousands of dollars to establish and maintain. Because systematics is a relatively poorly funded subdiscipline of biology (81), these costs can be prohibitive. However, the value and need for molecular data in systematics is recognized (128), in spite of the expense.

CONFLICT

Conflict between morphological and molecular studies of phylogeny may be spurious or real. Reasons for spurious conflict include differences in assumptions about the evolutionary process and differences in methods of analysis. However, if the methods of analysis of two studies are the same and are appropriate for both, but results from the two studies differ, then the conflict can be considered real.

Differences in Assumptions

RATES OF CHANGE Change within monophyletic groups, whether morphological or molecular, obviously is positively correlated with time, even though some individual lineages may remain more or less static (in morphology and/or biochemistry) throughout much of their history (9, 38, 54). However, the degree of constancy of change through time is a point of considerable debate (13, 51, 131, 136). This debate relates to phylogenetic reconstruction in two important ways: (*a*) Are rates of change constant enough that they can be assumed a priori to be equal for purposes of analysis, and (*b*) are rates of change constant enough that a "clock" model can be applied to dating lineage divergence?

Systematists have answered negatively to both of the above questions as they relate to morphology, but molecular systematists are split in their answers to these questions as they relate to molecules (131). Early proponents of molecular systematics were mostly proponents of a molecular clock (98, 119, 142), so in the minds of many, the data base and the assumptions about equal rates of change have become closely linked. However, many recent molecular

systematists have taken a more conservative approach and have chosen methods of analysis that do not assume constant rates of change (e.g. 8, 11, 19, 52, 65, 90, 96, 111). Such methods are applicable to rate-constant as well as rate-variable data, and in fact provide a potential test of the molecular clock hypothesis.

A considerable number of studies have begun to accumulate that address the predictive value of the correlation between time and molecular divergence. A recent review by Britten (13) shows that average rates of DNA change-through-time differ by a factor of five in some of the monophyletic groups thus far studied. Because this represents a comparison of average changes between groups, the disparity between the slowest-evolving lineages and the fastest-evolving lineages must be even greater. Moreover, rates of change of different portions of the genome are decoupled; for instance, average rates of divergence of nuclear and mitochondrial DNA may be similar or very different within a particular lineage (136). Gillespie (51) has shown that methods that have been used to assess the fit of data to molecular clock models have tended to mask the variability in rates of molecular evolution. When the masking effect is removed, molecular evolution appears to be highly episodic (51). These studies suggest that molecular clock models have limited predictive value.

Assumptions of a molecular clock have produced considerable spurious conflict between morphological and molecular data for two reasons. As discussed in a later section, this assumption has influenced the methods of tree construction that have been selected to analyze molecular data. In addition, in an attempt to relate molecular data to time since divergence, many molecular systematists have converted character-state data into single numerical summaries, or "genetic distances." Unfortunately, such conversions usually result in considerable loss of information, and the formulation of genetic distances is a point of extensive debate (19, 42, 58, 63, 92, 131, 133). Additionally, most distance measures have attributes that make them unacceptable for use in phylogenetic analysis (42, 63).

Much of the apparent conflict between molecular and morphological studies of phylogeny appears to be directly or indirectly related to a priori acceptance of the molecular-clock hypothesis. Several studies have shown consensus of morphological and molecular data when the restrictions of this hypothesis were removed, even though the data sets initially appeared to be in conflict (65, 90, 91). The existence of such examples should be sufficient to demand the use of rate-independent methods of analysis.

SPECIES CONCEPTS Although differences among systematists in their species concepts have had little effect on phylogenetic analyses, these differences have contributed to the view that molecular and morphological data are in

conflict. The problem stems primarily from the requirement by some systematists that "acceptable" species should be morphologically distinct, and the converse view by other systematists that species should exhibit some minimal level of genetic divergence (10, 18, 57, 87). Both views clearly are typological, and historically distinct evolutionary lineages may not show any particular level of divergence at either the morphological or molecular level. Complete reproductive isolation (a sufficient, though not necessary, condition of most species concepts) can be achieved by simple changes that may not affect morphology or a particular measure of genetic divergence (49, 107, 135, 140). Therefore, disagreement among morphological and molecular systematists over species definitions usually represents a disagreement of typology rather than fact.

Differences in Design and Methods of Analysis

SAMPLE SIZES Variation of sample sizes in phylogenetic studies is extensive, but a large portion of the variance occurs between morphological and molecular studies. Many morphologists analyze large samples of each species from throughout its range, and they may examine all known specimens in order to determine the extent of intraspecific variation of a morphological character. Although extensive geographical analyses exist for some molecular characters, sample sizes in molecular studies are usually much smaller than in morphological studies (often as small as a single individual). Analyses of large sample sizes in molecular studies are often limited by availability of specimens (because of the inapplicability of museum specimens) and/or expense of analysis.

The use of small sample sizes in allozymic studies has been shown to have a minimal effect on genetic distances as long as the number of loci examined is relatively large (53, 100, 102). Although these studies pertain specifically to genetic distance analyses, numerous investigators have cited this information as justifying small sample sizes in cladistic analyses of allozymes (70, 72, 93, 94, 125). Swofford & Berlocher (129) have argued that the effects of small sample sizes on presence/absence studies are potentially much greater than on analyses of genetic distances, and they have proposed a novel method for analyzing polymorphic characters. The commonest argument against small sample sizes is the relatively high probability of not observing polymorphic characters that occur in relatively low frequency (129). However, in many species allozymic variation is partitioned mostly among, rather than within, local populations (122). Therefore, a strategy of collecting small samples from throughout the range of a species is likely to encompass a greater proportion of allelic diversity than is the strategy of collecting a large sample from a single locality (19).

The problem of missing some alleles at polymorphic loci due to small sample sizes will have a strong effect on phylogenetic analyses only if polymorphic synapomorphies (that is, synapomorphies that are not fixed within the defined group of species) are relatively common compared to fixed synapomorphies. In fact, studies that have expressly distinguished fixed from polymorphic allozymic synapomorphies have found fixed synapomorphies overwhelmingly more common than polymorphic synapomorphies (e.g. 35, 65). An example of the effect of small sample sizes in phylogenetic analyses of allozymes can be drawn from an allozymic study of the lizard genus *Pholidobolus* (65). In this study, 29 gene loci were examined among 8 species of lizards: 5 species of *Pholidobolus* (the ingroup) and 3 species of *Proctoporus* (the outgroup). Sample sizes varied depending on the rarity of the species, from a single individual of *Ph. annectens* (which is known from only a few specimens) to 20 individuals from 5 populations of *Ph. montium*. In total, 57 specimens were examined, and a single shortest cladogram was derived from the data. If only single specimens from each species had been examined, the study would have required only one-seventh the cost in materials and time for data collection. What was the benefit of this seven-fold increase in cost? There are few enough samples and taxa in this example that an assessment based on exhaustive individual sampling is possible. There are 100,800 possible combinations of individuals of each species that can be examined from the complete data set. I have analyzed all of these combinations, and 100% produced the same cladogram topology as did the full data set (Figure 1). Of the 24 synapomorphies described in the original study (65), only 2 differed among the 100,800 combinations of individuals. Furthermore, in 40,320 combinations (40%), all of the same synapomorphies were found; 2 synapomorphies were lost in only 14,112 combinations (14%).

The above example demonstrates that increased sample sizes and increased geographic representation of samples (as well as accompanying increased expense) do not necessarily translate into increased phylogenetic information in allozymic studies. However, as the ratio of fixed to polymorphic synapomorphies decreases, so does the need for increased sample sizes. Some gene sequences are known to evolve in a concerted fashion, so that polymorphic states are rapidly fixed within species (34). In such sequences, polymorphisms are restricted to single species, because rates of fixation generally exceed rates of speciation (25). Sequences that evolve in this concerted fashion are ideal for phylogenetic reconstruction, because polymorphic synapomorphies are rare or absent, which thus justifies the use of small sample sizes (67). It is clear more examples must be studied to determine the extent of polymorphic synapomorphies in a variety of organisms.

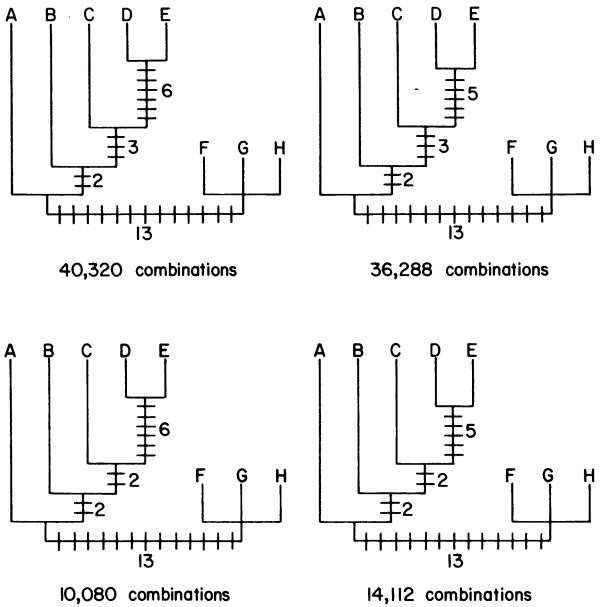

Figure 1 Cladograms of *Pholidobolus* and *Proctoporus* from all 100,800 combinations of individuals in reference 65. Autapomorphies are not shown for simplicity; number of synapomorphies for each branch are indicated. The cladogram in the upper left is also the cladogram for the complete data set. Letters designate the following taxa: A: *Ph. affinis;* B: *Ph. macbrydei;* C: *Ph. prefrontalis;* D: *Ph. montium;* E: *Ph. annectens;* F-H: three species of *Proctoporus.*

TREE CONSTRUCTION A common reason, discussed earlier, for spurious conflict between morphological and molecular studies is the use of rate-dependent methods of analysis. In recent years, methods of tree construction for the purposes of producing phylogenetic hypotheses have proliferated rapidly. For the purposes of discussing spurious conflict between studies, I divide these methods of tree construction into rate-dependent and rate-independent methods. Rate-dependent methods are much more likely to produce spurious conflict than are rate-independent methods (65, 90, 91).

Tree construction techniques can be rate-dependent for two distinct reasons: (*a*) a rate-dependent clustering algorithm; or (*b*) a rate-dependent rooting algorithm. It is widely recognized that rate-dependent clustering algorithms, such as the unweighted pair-group method of arithmetic averages (UPGMA; 126), are inappropriate for reconstructing phylogenies (40). Nonetheless, many molecular systematists use UPGMA trees to summarize average genetic distances among taxa; they often are careful to note that the clustering of taxa does not represent an hypothesized phylogeny (e.g. 65). However, the method is easily abused because of its computational simplic-

ity, and many biologists seem to be unaware of the limitations of the resulting phenograms (e.g. see the conclusions in 75 and 137 as criticized in 50 and 66).

Numerous methods have been developed that avoid rate-dependent clustering problems by producing an unrooted network of taxa that can be connected by branches of varying lengths (65). In order for these networks to be interpreted in a hierarchical fashion, they must be directed or "rooted." Many systematists assume that because network construction is rate-independent, trees that are rooted from such networks are also free of rate assumptions. However, a common method for rooting trees in molecular studies, midpoint rooting, requires an expressly rate-dependent assumption (65). If the assumption (viz. that average rates of change between the most diverse taxa are equal) is not true, then the rooted tree may be a poor representation of phylogeny (65). Other methods, such as outgroup (43, 84, 138) and Lundberg rooting (83, 89) are rate-independent and are much less likely to produce spurious conflict among data sets. Because of this phenomenon, the use of midpoint rooting algorithms should be consciously avoided.

Differences in Results

Real conflict of results among studies requires methods that can be used to reconcile the differences. Two primary approaches have been developed to deal with conflicting phylogenetic data: consensus techniques and combination techniques. Consensus techniques emphasize stability and common information, whereas combination techniques emphasize descriptive power and global parsimony (95).

Several methods of constructing consensus cladograms have been developed (1, 85, 105, 106, 113). The most commonly used methods are Adams consensus (1), strict consensus (105), and majority consensus (85). The first two approaches are contrasted in an example in Figure 2. In an Adams consensus tree, conflicting clades are collapsed to the first node of agreement between the competing phylogenetic hypotheses. Adams consensus trees are useful for identifying taxa that are responsible for conflict (47). However, this technique can result in trees that are not indicative of any of the original cladograms (Figure 2). Strict consensus trees consist of groups of species that are defined by every data set and represent the most conservative method of reconciling conflicting phylogenetic data. Majority consensus is similar to strict consensus but identifies monophyletic groups recognized in the majority (rather than all) of the competing hypotheses of relationships.

Miyamoto (95) has criticized consensus approaches because they do not take into account the relative strengths of support for various groups by the different data sets. Furthermore, he argued that consensus trees do not

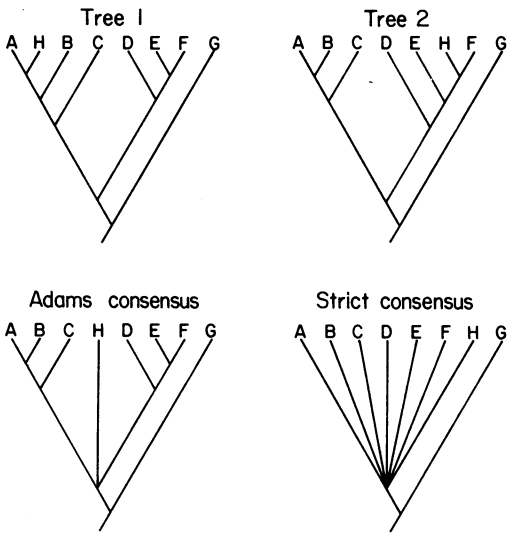

Figure 2 Comparison of Adams consensus and strict consensus. Note that tree 1 and tree 2 are identical except for the placement of taxon H. Adapted from Funk (47).

represent parsimonious solutions to character transformation and thus they sacrifice considerable descriptive power. The alternate approach, namely combination of data sets for reanalysis, has the advantages of greater information content and global parsimony (95). However, some morphologists (e.g. 76) have argued that this approach biases the results in favor of molecular data because of the great number of character states in many molecular studies (e.g. DNA sequences). Theoretically, it is possible to weight characters in combined data sets according to their complexity or probability of evolving, but in practice this is largely a subjective procedure.

An example that contrasts consensus and combination techniques is presented in Figure 3. This example is drawn from allozymic, morphological, and karyological studies of frogs of the *Eleutherodactylus rugulosus* group (94). Figure 3 shows the relationships of species held in common among these studies as supported by each data set. As shown by the consensus cladogram, all three data sets support the monophyly of two clades: one consisting of *E. taurus, E. rugulosus,* and *E. punctariolus,* and the other consisting of *E. fleischmanni, E. angelicus,* and *E. escoces.* However, different relationships are suggested within each of these groups by the morphological and allozymic data (the karyological data are uninformative beyond the support of the two groups). The consensus tree summarizes the information that is common to all data sets and therefore ignores portions of the tree for which conflicting data are available. In contrast, the combination of data sets results in an evaluation of the strength of the conflicting data, and it resolves the tree in favor of the

strongest information. In this case, the tree is resolved in favor of the morphological information.

Both consensus trees and combined trees provide useful information when conflicting data sets need to be evaluated and reconciled. When used simultaneously, as in Figure 3, the two approaches immediately contrast the information in common and the relative strengths of the data sets. Classifications are best based on information in common among multiple data sets (i.e. consensus trees), whereas the best estimate of phylogeny and best estimate of character evolution are represented in the analysis of the combined data sets.

CONSENSUS

Although molecular techniques have added considerably to our systematic knowledge, most of the systematic information from molecules has illuminated terra incognita, and more often than not, substantiated earlier work by morphologists. Many systematists are realizing the value of multidisciplinary studies and are combining as many sources of information as possible in order to maximize information, explanation, and stability. No single systematic data set can be expected to be informative at all phylogenetic levels simultaneously. Some techniques are useful for resolving questions of phylogeny among closely related species, whereas others are useful across ancient time spans (67). Often, several different techniques are required to maximize phylogenetic resolution within a group of interest.

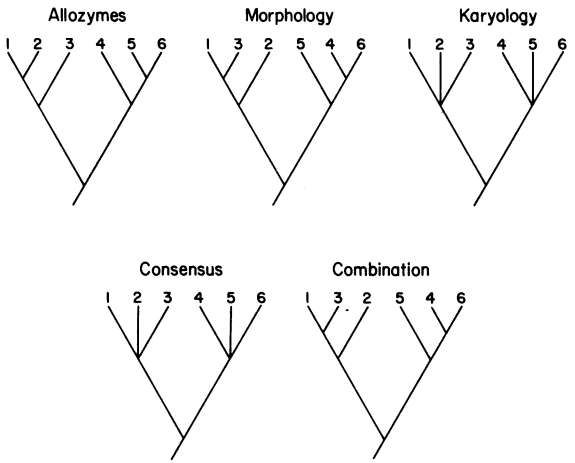

Figure 3 Contrast of consensus and combination approaches to reconciling conflict (adapted from 94). Taxa codes: 1: *Eleutherodactylus taurus;* 2: *E. rugulosus;* 3: *E. punctariolus;* 4: *E. angelicus;* 5: *E. fleischmanni;* 6: *E. escoces.*

An example of consensus and complementarity among morphological and two types of molecular data is presented in Figure 4. The three data sets (extracted from 21, 24, 44, 56, 64, 67, 69, 70, 139) all concern relationships of species groups of Holarctic and Neotropical frogs of the genus *Rana*. There are no conflicts among the data sets, but no single data set fully resolves the relationships among the species groups. The allozymic data set is most informative among closely related groups (e.g. the *Rana pipiens* complex: species groups 7–10 in Figure 4), whereas the morphological and rDNA data sets are more informative at lower levels of the tree. By combining any two of the data sets, a greater portion of the phylogeny can be ascertained. If all three data sets are combined, the tree is resolved fully (Figure 4).

Not all examples of combination of molecular and morphological data are as successful as the example in Figure 4. On the other hand, conflict in systematics is not limited to comparisons between molecules and morphology. Conflicts among morphological (e.g. 118 versus 121) or among molecular (see 113) studies are probably as common as real conflicts between morphological and molecular studies.

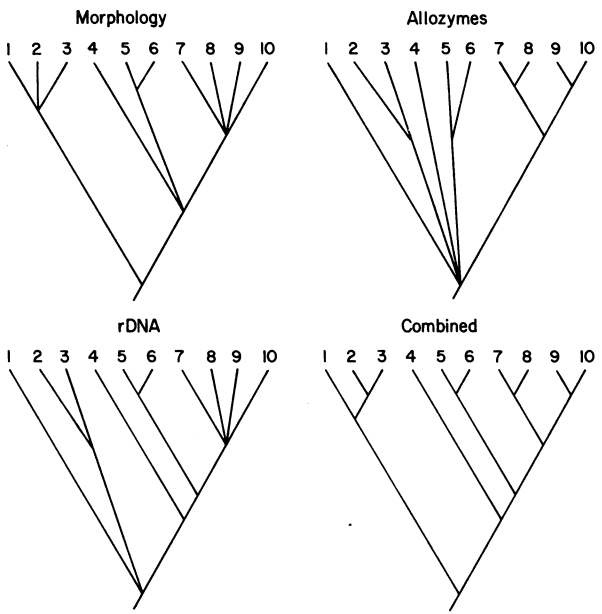

Figure 4 Consensus and complementarity among morphological and molecular data sets that pertain to Holarctic and Neotropical species groups of *Rana* (adapted from 67). Taxa codes: 1: *R. boylii* group; 2: *R. sylvatica;* 3: *R. temporaria* group; 4: *R. catesbeiana* group; 5: *R. palmipes* group; 6: *R. tarahumarae* group; 7: *R. montezumae* group; 8: *R. areolata* group; 9: *R. pipiens* group; 10: *R. berlandieri* group.

CONCLUSIONS

Morphological and molecular systematic techniques each have distinct advantages for phylogenetic reconstruction. Morphological techniques are applicable to an enormous range of museum and fossil material, and a large portion of the Earth's organisms will continue to be studied primarily or exclusively from morphological information. On the other hand, the potential molecular data set is incredibly extensive and, when fully utilized, should provide a detailed record of the history of life. Studies that combine the two approaches can thereby maximize both information content and usefulness. However, it is important to select methods of analysis that are as assumption-free as possible and also are amenable to combination of data sets. This requires rate-independent methods of network construction and tree rooting, as well as use of character-state data rather than distance summaries whenever possible. Such combinations of molecular and morphological studies should provide a truly comprehensive view of biotic evolution.

ACKNOWLEDGMENTS

This work has been supported by National Science Foundation grants BSR-8614622 and BSR-8657640. I thank Brian Crother, Maureen Donnelly, Marc Hayes, Peter Lahanas, Jay Savage, and Steve Werman for their suggestions on various drafts of this manuscript.

Literature Cited

1. Adams, E. N. III. 1972. Consensus techniques and the comparison of taxonomic trees. *Syst. Zool.* 21:390–97
2. Allee, W. C., Schmidt, K. P. 1951. *Ecological Animal Geography*. New York: Wiley. 2nd ed.
3. Armstrong, W. G., Halstead, L. B., Reed, F. B., Wood, L. 1983. Fossil proteins in vertebrate calcified tissues. *Phil. Trans. R. Soc. London* B301:301–43
4. Avise, J. C. 1975. Systematic value of electrophoretic data. *Syst. Zool.* 23:465–81
5. Avise, J. C., Giblin-Davidson, C., Laerm, J., Patton, J. C., Lansman, R. A. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. *Proc. Natl. Acad. Sci. USA* 76:6694–98
6. Avise, J. C., Lansman, R. A. 1983. Polymorphism of mitochondrial DNA in populations of higher animals. See Ref. 101, pp. 147–64
7. Avise, J. C., Lansman, R. A., Shade, R. O. 1979. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. *Genetics* 92:279–95
8. Avise, J. C., Patton, J. C., Aquadro, C. F. 1980. Evolutionary genetics of birds. I. Relationships among North American thrushes and allies. *Auk* 97:135–47
9. Avise, J. C., Patton, J. C., Aquadro, C. F. 1980. Evolutionary genetics of birds. II. Conservative protein evolution in North American sparrows and relatives. *Syst. Zool.* 29:323–34
10. Ayala, F. J. 1975. Genetic differentiation during speciation. *Evol. Biol.* 8:1–78
11. Baverstock, P. R., Cole, S. R., Richardson, B. J., Watts, C. H. S. 1979. Electrophoresis and cladistics. *Syst. Zool.* 28:214–19
12. Best, T. L., Sullivan, R. M., Cook, J. A., Yates, T. L. 1986. Chromosomal,

genic, and morphological variation in the agile kangaroo rat, *Dipodomys agilis* (Rodentia: Heteromyidae). *Syst. Zool.* 35:311–24
13. Britten, R. J. 1986. Rates of DNA sequence evolution differ between taxonomic groups. *Science* 231:1393–98
14. Brooks, D. R., Wiley, E. O. 1985. Theories and methods in different approaches to phylogenetic systematics. *Cladistics* 1:1–11
15. Brown, W. M. 1983. Evolution of animal mitochondrial DNA. See Ref. 101, pp. 62–88
16. Brown, W. M., George, M. Jr., Wilson, A. C. 1979. Rapid evolution of animal mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* 76:1967–71
17. Brown, W. M., Wright, J. W. 1979. Mitochondrial DNA analyses and the origin and relative age of parthenogenetic lizards (genus *Cnemidophorus*). *Science* 203:1247–49
18. Burger, W. C. 1975. The species concept in *Quercus*. *Taxon* 24:45–50
19. Buth, D. G. 1984. The application of electrophoretic data in systematic studies. *Ann. Rev. Ecol. Syst.* 15:501–22
20. Cann, R. L., Stoneking, M., Wilson, A. C. 1987. Mitochondrial DNA and human evolution. *Nature* 325:31–36
21. Case, S. M. 1978. Biochemical systematics of members of the genus *Rana* native to western North America. *Syst. Zool.* 27:299–311
22. Cavalier-Smith, T., ed. 1985. *The Evolution of Genome Size*. Chichester, UK: Wiley
23. Cavalier-Smith, T. 1985. Eukaryote gene numbers, noncoding DNA and genome size. See Ref. 22, pp. 69–103
24. Chantell, C. J. 1970. Upper Pliocene frogs from Idaho. *Copeia* 1970:654–64
25. Coen, E. S., Strachan, T., Dover, G. A. 1982. Dynamics of concerted evolution of ribosomal DNA and histone gene families in the *melanogaster* species subgroup of *Drosophila*. *J. Mol. Biol.* 158:17–35
26. Cracraft, J. 1974. Phylogenetic models and classification. *Syst. Zool.* 23:71–90
27. Crowson, R. A. 1970. *Classification and Biology* London: Heineman
28. de Queiroz, K. 1985. The ontogenetic method for determining character polarity and its relevance to phylogenetic systematics. *Syst. Zool.* 34:280–99
29. Dessauer, H. C., Hafner, M. S., eds. 1984. *Collections of Frozen Tissues: Value, Management, Field and Laboratory Procedures, and Directory of Existing Collections*. Lawrence, KS: Assoc. Syst. Coll.
30. Dessauer, H. C., Hafner, M. S. 1984. Holdings in existing collections of frozen tissues. See Ref. 29, pp. 46–61
31. Dessauer, H. C., Hafner, M. S., Goodman, M. 1984. Value of frozen tissue collections for studies in evolutionary biology. See Ref. 29, pp. 3–5
32. Dessauer, H. C., Menzies, R. A. 1984. Stability of macromolecules during long term storage. See Ref. 29, pp. 17–20
33. Doran, G. H., Dickel, D. N., Ballinger, W. E. Jr., Agee, O. F., Laipis, P. J., Hanswirth, W. W. 1986. Anatomical, cellular and molecular analysis of 8,000-yr-old human brain tissue from the Windover archaeological site. *Nature* 323:803–6
34. Dover, G. A. 1982. Molecular drive: A cohesive mode of species evolution. *Nature* 299:111–17
35. Duellman, W. E., Hillis, D. M. 1987. Marsupial frogs (Anura: Hylidae: *Gastrotheca*) of the Ecuadorian Andes: Resolution of taxonomic problems and phylogenetic relationships. *Herpetologica* 43:135–67
36. Echelle, A. A., Mosier, D. T. 1981. All-female fish: A cryptic species of *Menidia* (Atherinidae). *Science* 212: 1411–13
37. Efstratiadis, A., Posakony, J. W., Maniatis, T., Lawn, R. M. et al 1980. The structure and evolution of the human β-globin gene family. *Cell* 21:653–68
38. Eldredge, N., Gould, S. J. 1972. Punctuated equilibrium: An alternative to phyletic gradualism. In *Models in Paleobiology*, ed. T. J. M. Schopf, pp. 82–115. San Francisco: Freeman/ Cooper
39. Falconer, D. S. 1960. *Introduction to Quantitative Genetics*. New York: Ronald
40. Farris, J. S. 1971. The hypothesis of nonspecificity and taxonomic congruence. *Ann. Rev. Ecol. Syst.* 2:277–302
41. Farris, J. S. 1976. Phylogenetic classification of fossils with recent species. *Syst. Zool.* 25:271–82
42. Farris, J. S. 1981. Distance data in phylogenetic analysis. See Ref. 48, pp. 3–23
43. Farris, J. S. 1982. Outgroups and parsimony. *Syst. Zool.* 31:328–34
44. Farris, J. S., Kluge, A. G., Mickevich, M. F. 1980. Paraphyly of the *Rana boylii* group. *Syst. Zool.* 28:627–34
45. Fink, W. L. 1982. The conceptual relationship between ontogeny and phylogeny. *Paleobiology* 8:254–64
46. Frelin, C., Vuilleumier, F. 1979. Biochemical methods and reasoning in

systematics. *Z. Zool. Syst. Evolutionsforsch.* 17:1–10
47. Funk, V. A. 1985. Phylogenetic patterns and hybridization. *Ann. Missouri Bot. Gard.* 72:681–715
48. Funk, V. A., Brooks, D. R., eds. 1981. *Advances in Cladistics: Proceedings of the First Meeting of the Willi Hennig Society.* Bronx: NY Bot. Gard.
49. Ghiselin, M. T. 1974. A radical solution to the species problem. *Syst. Zool.* 23:536–44
50. Giles, E., Ambrose, S. H. 1986. Are we all out of Africa? *Nature* 322:21–22
51. Gillespie, J. H. 1986. Rates of molecular evolution. *Ann. Rev. Ecol. Syst.* 17:637–65
52. Goodman, M., Czelusniak, J., Moore, G. W., Romero-Herrera, A. E., Matsuda, G. 1979. Fitting the gene lineage into its species lineage, a parsimony strategy illustrated by cladograms constructed from globin sequences. *Syst. Zool.* 28:132–63
53. Gorman, G. C., Rezi, J. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. *Copeia* 1979:242–49
54. Gould, S. J., Eldredge, N. 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. *Paleobiology* 3:115–51
55. Gould, S. J., Woodruff, D. S., Martin, J. P. 1974. Genetics and morphometrics of *Cerion* at Pongo Carpet: A new systematic approach to this enigmatic land snail. *Syst. Zool.* 23:518–35
56. Green, D. M. 1986. Systematics and evolution of western North American frogs allied to *Rana aurora* and *Rana boylii:* Electrophoretic evidence. *Syst. Zool.* 35:283–96
57. Harper, R. S. 1923. The species concept from the point of view of a morphologist. *Am. J. Bot.* 10:229–33
58. Hedrick, P. W. 1983. *Genetics of Populations.* Boston: Sci. Books Int.
59. Hennig, W. 1966. *Phylogenetic Systematics.* Urbana: Univ. Ill. Press
60. Hedman, M. 1985. The evolution of bacterial genomes. See Ref. 22, pp. 37–68
61. Highton, R. 1979. A new cryptic species of salamander of the genus *Plethodon* from the Southeastern United States (Amphibia: Plethodontidae). *Brimleyana* 1:31–36
62. Higuchi, R., Bowman, B., Freiberger, M., Ryder, O. A., Wilson, A. C. 1984. DNA sequences from the quagga, an extinct member of the horse family. *Nature* 312:282–84
63. Hillis, D. M. 1984. Misuse and modification of Nei's genetic distance. *Syst. Zool.* 33:238–40
64. Hillis, D. M. 1985. Evolutionary genetics and systematics of New World frogs of the genus *Rana:* An analysis of ribosomal DNA, allozymes, and morphology. PhD thesis. Univ. Kans., Lawrence. 304 pp.
65. Hillis, D. M. 1985. Evolutionary genetics of the Andean lizard genus *Pholidobolus* (Sauria: Gymnophthalmidae): Phylogeny, biogeography, and a comparison of tree construction techniques. *Syst. Zool.* 34:109–26
66. Hillis, D. M. 1986. Out of Africa—through a genetic bottleneck. *Nature* 323:208
67. Hillis, D. M., Davis, S. K. 1986. Evolution of ribosomal DNA: Fifty million years of recorded history in the frog genus *Rana. Evolution* 40: 1275–88
68. Hillis, D. M., Davis, S. K. 1987. Evolution of the 28S ribosomal RNA gene in anurans: Regions of variability and their phylogenetic implications. *Mol. Biol. Evol.* 4:117–25
69. Hillis, D. M., Frost, J. S., Webb, R. G. 1984. A new species of frog of the *Rana tarahumarae* group from southwestern Mexico. *Copeia* 1984:398–403
70. Hillis, D. M., Frost, J. S., Wright, D. A. 1983. Phylogeny and biogeography of the *Rana pipiens* complex: A biochemical evaluation. *Syst. Zool.* 32:132–43
71. Hillis, D. M., Patton, J. C. 1982. Morphological and electrophoretic evidence for two species of *Corbicula* (Bivalvia: Corbiculidae) in North America. *Am. Midl. Nat.* 105:74–80
72. Honeycutt, R. L., Greenbaum, I. F., Baker, R. J., Sarich, V. M. 1981. Molecular evolution and vampire bats. *J. Mammal.* 62:805–11
73. Hubby, J. L., Throckmorton, L. H. 1968. Protein differences in *Drosophila.* IV. A study of sibling species. *Am. Nat.* 102:193–205
74. Johnson, W. E., Selander, R. K., Smith, M. H., Kim, Y. J. 1972. Biochemical genetics of sibling species of the cotton rat *(Sigmodon). Stud. Genet. VII. Univ. Tex. Publ.* 7213:297–305
75. Jones, J. S., Rouhani, S. 1986. How small was the bottleneck? *Nature* 319:449–50
76. Kluge, A. G. 1983. Cladistics and the classification of the great apes. In *New Interpretations of Ape and Human Ancestry.*, ed. R. L. Ciochan, R. S. Corruccini, pp. 151–77. New York: Plenum

77. Kluge, A. G. 1985. Ontogeny and phylogenetic systematics. *Cladistics* 1: 13–27
78. Kluge, A. G., Strauss, R. E. 1985. Ontogeny and systematics. *Ann. Rev. Ecol. Syst.* 16:247–68
79. Knight, H. H. 1924. On the nature of the color patterns in Heteroptera with data on the effects produced by temperature and humidity. *Ann. Entomol. Soc. Am.* 27:258–74
80. Lansman, R. A., Shade, R. O., Shapira, J. F., Avise, J. C. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. *J. Mol. Evol.* 17:214–26
81. Lillegraven, J. A., Stuessy, T. F. 1979. Summary of awards from the systematic biology program of the National Science Foundation, fiscal years 1973–1977. *Syst. Zool.* 28:123–31
82. Lowenstein, J. M. 1980. Species-specific proteins in fossils. *Naturwissenschaften* 67:343
83. Lundberg, J. G. 1972. Wagner networks and ancestors. *Syst. Zool.* 18:1–32
84. Maddison, W. P., Donoghue, M. J., Maddison, D. R. 1984. Outgroup analysis and parsimony. *Syst. Zool.* 33:83–103
85. Margush, T., McMorris, F. R. 1981. Consensus *n*-trees. *Bull. Math. Biol.* 43:239–44
86. Maxson, L. R., Wilson, A. C. 1974. Convergent morphological evolution by studying proteins of tree frogs in the *Hyla eximia* species group. *Science* 185:66–68
87. Mayr, E. 1963. *Animal Species and Evolution*. Cambridge: Harvard Univ. Press
88. McFarlane, J. E. 1964. Factors affecting growth and wing polymorphism in *Gryllodes sigillatus* (Walk.): Dietary protein level and a possible effect of photoperiod. *Can. J. Zool.* 42:767–71
89. Meacham, C. A. 1984. The role of hypothesized direction of characters in the estimation of evolutionary history. *Taxon* 33:26–38
90. Mickevich, M. F., Farris, J. S. 1981. The implications of congruence in *Menidia*. *Syst. Zool.* 30:351–70
91. Mickevich, M. F., Johnson, M. S. 1976. Congruence between morphological and allozyme data in evolutionary inference and character evolution. *Syst. Zool.* 25:260–70
92. Mickevich, M. F., Mitter, C. 1981. Treating polymorphic characters in systematics: A phylogenetic treatment of electrophoretic data. See Ref. 48, pp. 45–58
93. Miyamoto, M. M. 1981. Congruence among character sets in phylogenetic studies of the frog genus *Leptodactylus*. *Syst. Zool.* 30:281–90
94. Miyamoto, M. M. 1983. Frogs of the *Eleutherodactylus rugulosus* group: A cladistic study of allozyme, morphological, and karyological data. *Syst. Zool.* 32:109–24
95. Miyamoto, M. M. 1985. Consensus cladograms and general classifications. *Cladistics* 1:186–89
96. Miyamoto, M. M., Goodman, M. 1986. Biomolecular systematics of eutherian mammals: Phylogenetic patterns and classification. *Syst. Zool.* 35:230–40
97. Myers, N. 1986. Tropical deforestation and a mega-extinction spasm. In *Conservation Biology: The Science of Scarcity and Diversity*, ed. M. E. Soule, pp. 394–409. Sunderland, Mass: Sinauer
98. Nei, M. 1971. Interspecific gene differences and evolutionary time estimated from electrophoretic data on protein identity. *Am. Nat.* 105:385–98
99. Nei, M. 1975. *Molecular Population Genetics and Evolution*. Elsevier: North Holland
100. Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–90
101. Nei, M., Koehn, R. K., ed. 1983. *Evolution of Genes and Proteins*. Sunderland, Mass: Sinauer
102. Nei, M., Roychoudhoury, A. K. 1974. Sampling variances of heterozygosity and genetic distance. *Genetics* 76:379–90
103. Nelson, G. J. 1974. Classification as an expression of phylogenetic relationships. *Syst. Zool.* 22:344–59
104. Nelson, G. 1978. Ontogeny, phylogeny, paleontology, and the biogenetic law. *Syst. Zool.* 27:324–45
105. Nelson, G. 1979. Cladistic analysis and synthesis: Principles and definitions, with a historical note on Adanson's *Familles des Plantes* (1763–1764). *Syst. Zool.* 28:1–21
106. Nelson, G., Platnick, N. 1981. *Systematics and Biogeography: Cladistics and Vicariance*. New York: Columbia Univ. Press
107. Nixon, S. E., Taylor, R. J. 1977. Large genetic distances associated with little morphological variation in *Polycelis*

coronata and *Dugesia tigrina* (Planaria). *Syst. Zool.* 26:152–164
108. Njoku, E. 1956. Studies on the metamorphosis of leaves. II. The effect of light intensity on leaf shape in *Ipomoea caerulea*. *New Phytol.* 55:91–110
109. Osborne, R. H., DeGeorge, F. V. 1959. *Genetic Basis of Morphological Variation*. Cambridge: Harvard Univ. Press
110. Pääbo, S. 1985. Molecular cloning of ancient Egyptian mummy DNA. *Nature* 314:644–45
111. Pace, N. R., Olsen, G. J., Woese, C. R. 1986. Ribosomal RNA phylogeny and the primary lines of evolutionary descent. *Cell* 45:325–26
112. Patterson, C., Rosen, D. E. 1977. Review of ichthyodectiform and other Mesozoic teleost fishes and the theory and practice of classifying fossils. *Bull. Am. Mus. Nat. Hist.* 158:81–172
113. Penny, D., Foulds, L. R., Hendy, M. D. 1982. Testing the theory of evolution by comparing phylogenetic trees constructed from five different protein sequences. *Nature* 297:197–200
114. Ranker, T., Werth, C. R. 1986. Active enzymes from herbarium specimens: Electrophoresis as an afterthought. *Am. Fern J.* 76:102–13
115. Rees, H. 1972. DNA in higher plants. In *Evolution of Genetic Systems*, ed. H. H. Smith, pp. 394–418. New York: Gordon & Breach
116. Rieppel, O. 1979. Ontogeny and the recognition of primitive character states. *Z. Zool. Syst. Evolutionsforsch.* 17:57–61
117. Robertson, F. W. 1957. Studies in quantitative inheritance. XI. Genetic and environmental correlation between body size and egg production in *Drosophila melanogaster*. *J. Genet.* 55:428–43
118. Rosen, D. E., Forey, P. L., Gardiner, B. G., Patterson, C. 1981. Lungfishes, tetrapods, paleontology and plesiomorphy. *Bull. Am. Mus. Nat. Hist.* 167:159–276
119. Sarich, V. M., Wilson, A. C. 1967. Rates of albumin evolution in primates. *Proc. Natl. Acad. Sci. USA* 58:142–47
120. Schaeffer, B., Hecht, M. K., Eldredge, N. 1972. Phylogeny and paleontology. *Evol. Biol.* 6:31–46
121. Schultze, H.-P. 1981. Hennig und der Ursprung der Tetrapoda. *Palaont. Z.* 55:71–86
122. Selander, R. K., Whittam, T. S. 1983. Protein polymorphism and the genetic structure of populations. See Ref. 101, pp. 89–114
123. Shaklee, J. B., Tamaru, C. S. 1981. Biochemical and morphological evolution of Hawaiian bonefishes *(Albula)*. *Syst. Zool.* 30:125–46
124. Shoshani, J. 1986. Mammalian phylogeny: Comparison of morphological and molecular results. *Mol. Biol. Evol.* 3:222–42
125. Sites, J. W. Jr., Bickham, J. W., Pytel, B. A., Greenbaum, I. F., Bates, B. A. 1984. Biochemical characters and the reconstruction of turtle phylogenies: Relationships among batagurine genera. *Syst. Zool.* 33:137–58
126. Sneath, P. H. A., Sokal, R. R. 1973. *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. San Francisco: Freeman
127. Stevens, P. F. 1980. Evolutionary polarity of character states. *Ann. Rev. Ecol. Syst.* 11:333–58
128. Stuessy, T. F., Thompson, K. S., eds. 1981. *Trends, Priorities and Needs in Systematic Biology*. Lawrence, Kans: Assoc. Syst. Coll. 2nd ed.
129. Swofford, D. L., Berlocher, S. H. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. *Syst. Zool.* In press
130. Templeton, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37:221–44
131. Thorpe, J. P. 1982. The molecular clock hypothesis: Biochemical evaluation, genetic differentiation and systematics. *Ann. Rev. Ecol. Syst.* 13:139–68
132. Thorpe, R. S., Giddings, M. R. 1981. A novel biochemical systematic technique for herpetology based on epidermal keratin. *Experientia* 37:700–2
133. Throckmorton, L. H. 1978. Molecular phylogenetics. In *Biosystematics in Agriculture*, ed. R. H. Foote, L. Knutson, P. L. Lentz, pp. 221–39. New York: Wiley
134. Turesson, G. 1922. The genotypical response of the plant species to the habitat. *Hereditas* 3:211–350
135. Turner, B. J. 1974. Genetic divergence of Death Valley pupfish species: Biochemical versus morphological evidence. *Evolution* 28:281–94
136. Vawter, L., Brown, W. M. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. *Science* 234:194–95
137. Wainscoat, J. S., Hill, A. V. S., Boyce,

A. L., Flint, J., et al. 1986. Evolutionary relationships of human populations from an analysis of nuclear DNA polymorphisms. *Nature* 319:491–93
138. Watrous, L. E., Wheeler, Q. D. 1981. The outgroup method of character analysis. *Syst. Zool.* 30:1–11
139. Webb, R. G. 1978. A systematic review of the Mexican frog *Rana sierramadrensis* Taylor. *Contrib. Sci. Mus. Nat. Hist. Los Angeles Co.* 300:1–13
140. Wiley, E. O. 1978. The evolutionary species concept reconsidered. *Syst. Zool.* 27:17–26
141. Wiley, E. O. 1981. *Phylogenetics: The Theory and Practice of Phylogenetic Systematics*. New York: Wiley
142. Wilson, A. C., Carlson, S. S., White, T. J. 1977. Biochemical evolution. *Ann. Rev. Biochem.* 46:573–639