LETTERS TO THE EDITOR

Re: Molecules and Morphology in Amniote Phylogeny

To the Editor:

A continuing debate in the systematic literature centers on the analysis of multiple data sets in phylogeny estimation. Advocates of the consensus approach (also called "taxonomic congruence") suggest that different data sets should be analyzed separately, as long as they have different "biological properties" (e.g., Miyamoto and Fitch, 1995). At the other extreme, proponents of the total evidence approach (also called "character congruence") advocate combining all characters in a single analysis (e.g., Kluge, 1989). Intermediate positions also have been taken (Bull *et al.,* 1993; Chippindale and Wiens, 1994). However, the possibility that some data sets inherently have greater potential for conveying phylogenetic information than other data sets usually is not an issue that is addressed. Certainly, multiple morphological data sets have been combined in the past, long before the total evidence approach was introduced, without generating any controversy. It is the combining of molecular *and* morphological data sets in a single analysis that has caused the greatest attention because it implies that each class of data is equally effective in conveying phylogenetic information. We disagree and believe that there is a qualitative distinction between molecular and morphological data that argues for their independent treatment in evolutionary studies (Nei, 1987; Woese, 1987; Maxson, 1992; Graur, 1993; Avise, 1994; Hedges and Sibley, 1994). Here, we focus on a recent study that combined molecular and morphological data from amniote vertebrates and present our arguments against this approach.

Eernisse and Kluge (1993) examined amniote relationships by combining published morphological and molecular data in a single parsimony analysis. Their study was prompted by the results of our 18S ribosomal RNA (rRNA) sequence analysis of amniotes in which birds and mammals clustered rather than birds and crocodilians (Hedges *et al.,* 1990). Although we reported that our results regarding the bird–mammal grouping were inconclusive and that additional data were needed, Eernisse and Kluge (1993) suggested that the resolution of amniote relationships was available with existing data. Their total evidence approach resulted in a tree essentially identical to the classical phylogeny based on morphological data alone. They justified the total evidence approach by stating that it resulted in a tree that was "more highly resolved and robust." We believe that careful examination of their

study reveals the underlying weaknesses of the total evidence approach.

Size of the Data Sets

It has been suggested that the phylogenetic signal of larger data sets will overwhelm that of smaller data sets in a combined analysis (Miyamoto, 1985). Eernisse and Kluge (1993) counter that criticism by suggesting that character covariation of the different data sets is more important than the number of characters in each data set. However, if characters covary, then they will support the same topology. It is the characters in two data sets that support conflicting topologies that are of concern. In such cases, the parsimony criterion dictates that a data set with more characters supporting one topology will prevail over a data set with fewer characters supporting an alternative topology.

Although sequence data are most likely to overwhelm morphological data in a combined analysis, the opposite situation occurred in Eernisse and Kluge's (1993) analysis. In that case, a large number of parsimony-informative morphological characters supporting a bird–crocodilian grouping (Gauthier *et al.,* 1988) were combined with a small number of parsimony-informative characters supporting a bird–mammal grouping (Hedges *et al.,* 1990). It was not surprising that the combined analysis also supported a bird–crocodilian grouping. That outcome could have been predicted beforehand and provided no new insight into the differences between the two data sets.

Statistical Significance

Because nearly any data set will yield a bifurcating tree, an important aspect of modern phylogenetic analysis is to ascertain whether the results are significant. Eernisse and Kluge (1993) rejected alternative methods of phylogenetic analysis as well as "statistical assessments" for inferring confidence and argued that parsimony "does not depend on the special nature of the model chosen" (Eernisse and Kluge, 1993, p. 1174). However, statistics is integral to phylogeny estimation; all methods have their strengths and weaknesses, and all are model-dependent (Felsenstein, 1988).

In the case of the 18S rRNA data set (Hedges *et al.,* 1990), the number of total sites (1.8 kb) is large, but the total number of parsimony sites supporting alternative phylogenetic arrangements within the amniotes is relatively small and those sites differ in the type of nucleotide substitutions. There are five unambiguous and two additional changes to Gs and Cs that support a bird–

mammal grouping, whereas the two sites supporting a bird–crocodilian grouping are changes to an A or U (Hedges *et al.,* 1990; Hedges and Maxson, 1992). Any weighting scheme that favors As and Us (e.g., Marshall, 1992; Lockhart *et al.,* 1994) clearly will result in support for birds+crocodilians. Although we obtained strong (100% bootstrap confidence) support for the monophyly of lissamphibians and of amniotes, the bird–mammal grouping was not statistically significant. In fact, it was the conclusion of our study that more data would be needed to resolve the origin of birds. By focusing only on the most-parsimonious topology, without reference to statistical significance, little insight can be gained from the analyses of Eernisse and Kluge (1993).

Molecules versus Morphology

The practice of combining molecular and morphological data in a single phylogenetic analysis recently has become popular (e.g., Eernisse and Kluge, 1993; Hillis *et al.,* 1993; Lydeard *et al.,* 1995). However, we believe that these two classes of data provide a greater contribution to evolutionary studies if they are treated independently: molecular data for estimating the phylogeny and nonmolecular data for mapping evolutionary changes on that phylogeny. Otherwise, if both molecular and morphological traits are used to construct the phylogeny, then interpreting the evolution of morphological traits on that phylogeny becomes circular.

The qualitative difference between molecular and morphological data involves adaptive convergence. Morphological data are more susceptible to adaptive convergence, as evidenced by the many striking cases of camouflage and mimicry (Cott, 1940; Wickler, 1968) and therefore are less desirable for phylogeny estimation. This is not to say that adaptive convergence has erased the phylogenetic signal from most morphological characters. Clearly, that is not the case, and much of our present classification of organisms (based on morphology) has been corroborated by molecular evidence (Avise, 1994).

Nonetheless, genuine sequence convergence, involving long chains of amino acids of similar sequence, is not known to occur and is not predicted (Doolittle, 1994). This is due to the redundancy of the genetic code, the presence of large amounts of noncoding DNA, and the fact that many regions of genes are selectively neutral (Hedges and Sibley, 1994). Of the adaptive changes at the molecular level, a small number of substitutions may occur in parallel in unrelated organisms and it has been claimed that this constitutes "selective convergence" that may affect phylogenetic analysis (Hillis, 1995). However, this is misleading because so few parallel changes, against a backdrop of unaffected amino acid variation, are unlikely to result in strong support for an incorrect phylogeny. In fact, a reexamination of the frequently cited case of sequence convergence involving stomach lysozymes (Stewart *et al.,* 1987), using additional taxa, revealed that the true phylogeny is not obscured (Doolittle, 1994). Apparently, this is because some of the residues originally thought to be convergent now are being found in lysozymes of other species.

In the case of amniote phylogeny, there are at least 20 morphological characters supporting a bird–mammal relationship (e.g., single aortic trunk, folded cerebellum, loop of Henle, etc.) and a large number that also support a bird–crocodilian relationship (Gauthier *et al.,* 1988). Some morphological studies have concluded that mammals are the closest relatives of birds (Gardiner, 1982; Løvtrup, 1985; Jamieson and Healy, 1992), and some of the same characters used to support birds+crocodilians recently have been reinterpreted to support birds+mammals (Gardiner, 1993). For a variety of reasons, it is interesting to know, based on an independent phylogeny, how these characters have evolved. For example, is endothermy (and its related traits) in birds and mammals the result of convergence or shared ancestry? Or, alternately, are the many traits shared by birds and reptiles the result of some similar behavioral or environmental factors?

A much larger molecular data set was assembled recently to address those questions, and the result favored a bird–crocodilian relationship with a high degree of statistical significance (Hedges, 1994). In addition, it was discovered that the several genes that provided some earlier support for a bird–mammal relationship (e.g., hemoglobin-β, 18S rRNA, myoglobin) are in the minority; most genes individually support birds+crocodilians. This finding now permits us to interpret the evolution of those morphological traits in amniotes, and it indicates that the endothermy in birds and mammals, and many associated traits, is the result of convergence.

The Multigenic Approach

Because the accuracy of phylogeny estimation is known to be positively correlated with the size of the data set (Nei, 1991), it is advantageous to combine sequences of multiple genes. We believe that such a *multigenic* approach is preferred over the consensus approach (Miyamoto and Fitch, 1995) because typical genes encode proteins of only a few hundred amino acid residues and usually are unable to resolve most of the nodes in a tree with statistical significance (e.g., Baldauf and Palmer, 1993: Fig. 2). Unless there is some reason to exclude a particular gene from such analysis (e.g., Frye and Hedges, 1995) and as long as any substitution or base composition biases are taken into account, the multigenic approach should provide the best estimate of phylogeny for a group. This method differs from the total evidence approach in that morphological data are not considered in the phylogeny estimation,

but rather are used to better understand phenotypic evolution in the context of an independent phylogeny (e.g., Felsenstein, 1985). In this sense, morphological data have attained a higher intrinsic value, now that we can better understand the evolution of these complex traits through comparative analyses.

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