

Points of View

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An Approach to the Analysis of Comparative Data When a Phylogeny Is Unavailable or Incomplete

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Recent years have seen widespread acceptance of the idea that comparative analyses must be conducted in a phylogenetic context. However, many comparative biologists work on groups for which no robust phylogenetic hypothesis exists and thus are faced with the choice of either using an available taxonomy as if it were a phylogeny or ignoring phylogeny altogether. Ahistorical (=nonphylogenetic) analyses have a high probability of being inaccurate (Felsenstein, 1985; Grafen, 1989; Martins and Garland, 1991). Using taxonomies is perhaps less risky, but many available taxonomies probably poorly reflect phylogeny and do not resolve the relationships among taxa within a category (e.g., genus, family).

An alternative, as yet unexplored possibility is to turn the question on its head and ask whether the results of the statistical analysis vary depending on the phylogeny used. If analyses using each conceivable phylogeny for the group all yield significant results, then it is safe to conclude that, regardless of what the correct phylogeny is, the analysis will be significant. Similarly, if analyses using all conceivable phylogenies all result in negative findings, then the conclusion that no significant relationship exists is warranted. In

some (perhaps many) instances, the outcome of the statistical analysis will depend on the phylogeny; for some potential phylogenetic resolutions, the results will be significant, and in others they will be non-significant. This situation can be presented in a positive light as well by identifying which of the universe of possible phylogenies leads to a significant result. Stating the results in this manner may induce other researchers to conduct systematic studies on the group.

METHODS

In the absence of a phylogeny, the methodology proposed here involves conducting statistical analyses on a large number of possible phylogenies. For small data sets, it may be possible to try all possible phylogenetic configurations, although for larger data sets the large number of possible phylogenies precludes trying each one. Further, many statistical comparative methods (e.g., independent contrasts [Felsenstein, 1985], weighted squared-change parsimony [Martins and Garland, 1991], autocorrelation [Cheverud et al., 1985; Miles and Dunham, 1992]) require information on branch lengths, but for any tree there is an infinite number of possible branch length combinations. Consequently, in most cases it will be necessary to examine a large number of possible trees and branch lengths and assume that such

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a sample is representative of all possible permutations.

In the examples below, I used computer simulations to generate a large sample of possible phylogenies. Then, for each phylogeny, I asked whether the relationship between two variables was statistically significant. A study begins with trait values for extant species but no phylogeny for these species. A simulation run begins with the extant taxa and chooses two taxa to be sister taxa. These taxa are then replaced by a hypothetical ancestral taxon in the pool of available taxa. The process then repeats itself until only one hypothetical taxon remains; the phylogenetic tree is thus specified by the relationships among the extant taxa and ancestral nodes. Then, using the tree and trait values, a statistical analysis is conducted. This process is then repeated to generate a large number of trees (for heuristic purposes, I created 250 simulated trees, but one might want to use a much greater number, particularly for large trees). The program to conduct these simulations is available from the author.

These simulations require two assumptions about models of evolutionary diversification. The first assumption concerns the manner in which cladogenesis occurs. Most discussions have favored a Markovian model of evolutionary diversification in which during each branching event each extant taxon is equally likely to speciate (e.g., Simberloff, 1987; Slowinski and Guyer, 1989; Heard, 1992). Accordingly, probabilities of speciation per species per "generation" in the simulation were set low enough ($P \leq 0.01$) such that the branching process is essentially Markovian (Losos and Adler, in press). Speciation probabilities were adjusted so that the expected time between each speciation event is constant.

The second assumption concerns the model of character evolution, of which there are a number of possibilities (see discussion in Garland et al., 1993). In the following examples, I use two sets of assumptions. First, I assume that evolution only occurs during speciation events (speciational evolution of Garland et al., 1993). Second, I assume that evolutionary change

proceeds gradually and in proportion to branch lengths in units of time (=number of generations in the simulation).

The purpose of these simulations is not to argue that either cladogenesis or character evolution occurs in a particular way. Rather, for heuristic purposes, in the simulations I have used what would seem to be reasonable first guesses. However, there are many other possible models for both cladogenesis (e.g., vicariant events leading to all lineages speciating simultaneously) or character evolution (e.g., variation in rates of character change). Probably the most prudent course would be to conduct simulations embodying a wide variety of assumptions. To the extent that results are not affected by the particular models used, results are likely to be robust.

In the examples that follow, I will use the independent contrasts method (Felsenstein, 1985; Harvey and Pagel, 1991; Garland et al., 1992), although the approach outlined here is applicable to any phylogenetic comparative method (e.g., Martins and Garland, 1991). In the contrast analyses, branch lengths were either the number of generations along each branch in the gradual evolution simulations or were all set equal to 1 in the speciational evolution analysis. The independent contrasts method assumes that all contrasts are drawn from a distribution with the same mean and variance. If a nonspeciational model of evolution is specified, then the expected absolute value of the contrasts will be a function of their branch lengths; consequently, the contrasts must be standardized. For example, if characters evolved in a gradual manner, then a relationship should exist between degree of differentiation (=contrast value) and branch length; standardization should remove this relationship. Garland et al. (1992) developed methods to test whether contrasts have been adequately standardized. A significant relationship between the contrasts and their branch lengths indicates that the contrasts are not adequately standardized and, consequently, that a gradual model of evolutionary change is not consistent with the phylogeny, branch lengths, and character

values of extant taxa. Using this method for checking the adequacy of standardization for the non-speciational evolution simulations, I found in many cases that a significant regression existed for the relationship between the absolute value of a contrast and its standard deviation (i.e., the branch length for that contrast). In almost all cases in the simulations, the significant regression had a negative slope. Consequently, following Garland et al. (1992), the contrasts were recalculated using $\log_e(\text{branch length})$. If this transformation failed to adequately standardize the contrasts, I concluded that the results of that simulation were inconsistent with a gradual model of evolutionary change and discarded that simulation run. Given that all but one of the simulations in any study must give an incorrect tree and that evolutionary change may not be gradual, it is not surprising that many of the simulation runs provided results inconsistent with a gradual model. Other means by which branch lengths might be standardized could be explored as well (see Garland et al., 1992).

EXAMPLES

These examples illustrate the utility of the simulation approach for interpreting comparative analyses when no phylogeny is available. The first example involves the relationship between fore- and hind limb length among a sample of 16 Cuban and Hispaniolan species of *Anolis* lizards. Previous studies indicate that fore- and hind limb length should be strongly related, which is not surprising given that both increase with body size (Losos, 1990). As expected, an ahistorical analysis indicates that the two variables are strongly and positively related ($r^2 = 0.91$, $F_{1,14} = 144.25$, $P < 0.001$; Fig. 1). However, no reliable phylogeny exists for Cuban and Hispaniolan anoles as a whole, although we do have some idea of the relationships among particular subsets of this group. Therefore, we can reasonably ask, are there any conceivable phylogenies in which statistical analyses would not indicate a significant relationship between these two variables?

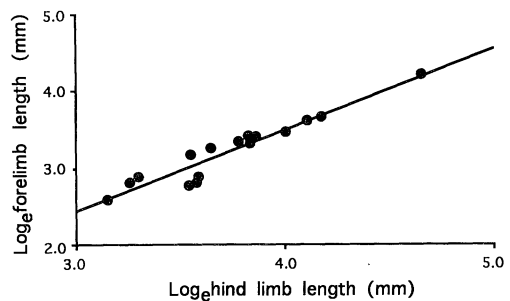


FIGURE 1. $\log_e(\text{forelimb length})$ versus $\log_e(\text{hind limb length})$ in 16 species of *Anolis* lizards.

For each of the 250 simulation phylogenies assuming speciational evolution, the relationship between forelimb and hind limb contrasts was significant at the $P < 0.05$ level (all were significant at $P < 0.0005$). For the gradual evolution analyses, I ran 350 simulations, 270 of which were adequately standardized. In only one of these 270 phylogenies was the contrast analysis not significant. Thus, the data strongly suggest a significant relationship exists between fore- and hind limb length regardless of the true phylogeny. Further, examination of the one nonsignificant phylogeny indicates that this phylogeny is unlikely to be correct. That phylogeny indicates a deep basal split between two clades, one containing *A. cybotes* and *A. barahonae* and the other containing the remaining 14 species. However, *A. cybotes* is surely more closely related to the three other members of the *cybotes* species complex included in this analysis than it is to *A. barahonae* (Wyles and Gorman, 1980). Of course, when possible it will be advantageous a priori to incorporate whatever phylogenetic information is available in the simulations.

The second example is a somewhat contrived analysis of the relationship between $\log_e(\text{perch height})$ and $\log_e(\text{mass})$ in eight species of *Anolis* (Fig. 2). Ahistorical analysis indicates a significant relationship ($r^2 = 0.42$, $F_{1,6} = 6.01$, $P < 0.05$). However, a considerable fraction of the simulation phylogenies yield nonsignificant results for both speciational (96/250) and gradual (164/370; 30 trials not adequately stan-

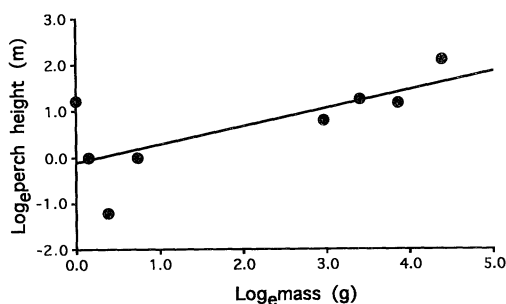


FIGURE 2. $\text{Log}_e(\text{mass})$ versus $\text{log}_e(\text{perch height})$ for eight species of *Anolis*.

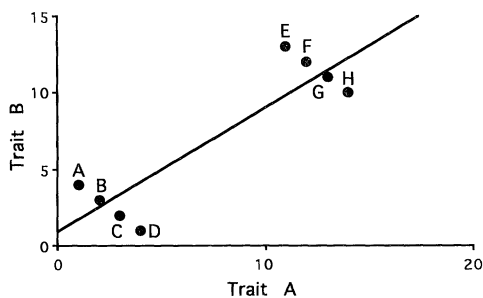


FIGURE 3. Trait A versus trait B for eight hypothetical species.

standardized) models. Consequently, in this case it is not possible to determine whether a significant relationship exists until a robust phylogenetic hypothesis is obtained.

The third example is entirely hypothetical. Consider the values for eight species (Fig. 3). Traits A and B are strongly positively correlated ($r = 0.89$). However, the species values are distributed in two clumps. If these clumps represent monophyletic groupings, then the observed relationship between the two variables could stem from a single evolutionary event at the base of the phylogeny (cf. Felsenstein, 1985; Garland et al., 1993). By contrast, if each species were most closely related to a species from the other cluster, then the observed relationship would have resulted from multiple instances of correlated evolution.

In the gradual evolution simulations for these eight hypothetical species, 19 of 287 simulations were nonsignificant. As expected, several of the phylogenies yielding nonsignificant contrast correlations reflected situations in which phenotypically similar species were generally closely related. However, the simulations indicated that most cases of nonsignificance resulted from a second situation. If a pair of phenotypically similar species were sister taxa and if their divergence was much more recent than most other divergences in the tree (e.g., Fig. 4), then contrasts from this pair would be weighted much more highly than would other contrasts (because contrasts are standardized by dividing by the square root of branch length in the gradual

evolution model). Because traits A and B are negatively related among phenotypically similar species (Fig. 3), a relatively large weight given to a contrast between two phenotypically similar species would have a large negative value and would preclude finding a significant positive relationship between the two traits among all contrasts (for a real example of this phenomenon, see Garland and Janis, 1993: fig. 6b). Thus, the simulations indicate that most phylogenetic topologies are consistent with a significant relationship between the two variables. Nonetheless, if the true phylogeny corresponds to one of two types of conceivable phylogenetic topologies, then a significant relationship would not be found between the two variables. This is a clear example of how the simulations may provide information that might not otherwise have been intuitively obvious.

DISCUSSION

By examining a large number or all of the possible phylogenies, one can get an idea of how likely it is that the outcome of an analysis is dependent on the correct phylogeny. The following rules may help guide interpretation of such investigations.

1. If all analyses give the same result (significant or nonsignificant), then one may conclude that the given result is likely to obtain regardless of what the true phylogeny is.
2. If a substantial minority of phylogenies

give different results from the majority, then the outcome of the analysis will depend on what the correct phylogeny is. In some cases, careful inspection of the simulation phylogenies will reveal general patterns about how particular phylogenetic groupings will lead to particular results.

3. The most difficult situation to interpret will occur when all but a few phylogenies give the same result. If the few phylogenies that give contradictory results are unlikely to be correct, either because of the phylogenetic relationships or relative length of branches, then one may with confidence accept the majority result. In other cases, however, two opposing viewpoints could be taken. On one hand, one could argue that if an overwhelming proportion (say, 95%) of the simulation phylogenies support one result, then that result is most likely to be correct. This view would consider each possible phylogeny to be equally likely. On the other hand, in the absence of other phylogenetically useful information, one could argue that the most conservative course would be to assume that taxa that are phenotypically similar in the characters of interest are closely related. These phylogenies, by minimizing the number of evolutionary events, are most likely to provide nonsignificant results in statistical analyses. Thus, the fact that such phylogenies are a small minority of all possible phylogenies would not be considered important. This argument assumes that contradictory results come from the phylogenies in which phenotypically similar species group together. In cases in which the phylogenies providing contradictory findings are those with particular branch length distributions, it would be difficult to provide a plausible reason why the most conservative course would be to consider those phylogenies most likely to be correct.

Extensions of the Approach

This approach can also be applied to situations in which some phylogenetic in-

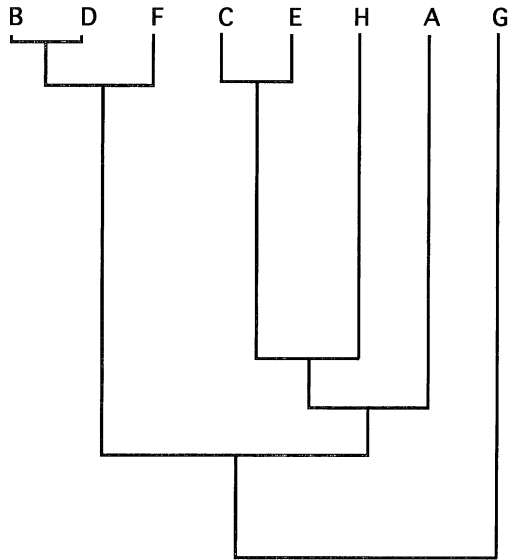


FIGURE 4. One of the phylogenies produced for the species in Figure 3 in the gradual evolution simulations. Vertical distance of each branch is proportional to time between speciation events. Because the branch lengths in the contrast involving species B and D are considerably shorter than branch lengths of any other contrast, the B-D contrast will be weighted substantially more than any of the other contrasts.

formation is available. Often, one knows that particular species are sister taxa or that a set of species form a monophyletic clade. This information can be included by considering in the simulations only phylogenies consistent with what is known. For example, the simulations could be constrained so that two species are always sister taxa. Or, a set of species could be constrained to always form a monophyletic group, even if relationships within that group were allowed to vary. As an example, Figure 5 provides a phylogeny for the Hispaniolan and Cuban *Anolis* species discussed in the first example. Although most of the tree is unresolved, three monophyletic groups are recognized, one of which is divided into two sets of sister taxa. Analyses on simulations constrained to be consistent with this topology yielded significant results in all 250 speciation trials and all 335 (out of 500) gradual trials that were adequately standardized.

At the extreme, one may have a gener-

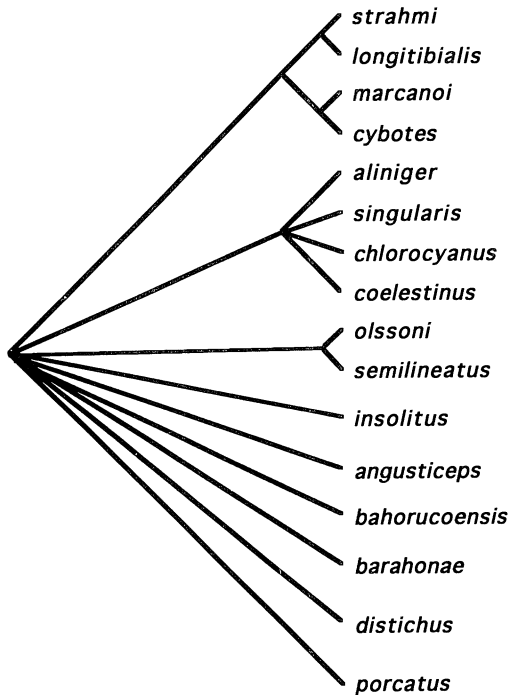


FIGURE 5. Phylogeny for the 16 species of Hispaniolan and Cuban *Anolis* lizards in Figure 1. Phylogenetic relationships follow Williams (1976) and Schwartz (1979).

ally well-resolved phylogeny except for one or more polytomies. At present, there are a number of algorithms available to handle such a situation, but these methods either make particular assumptions (e.g., that phenotypically similar species are closely related) and/or try to minimize the importance of a polytomous node by reducing it to a single contrast (e.g., Grafen, 1989; Maddison, 1989; Harvey and Pagel, 1991; Purvis and Garland [1993] dealt primarily with parameter estimation rather than hypothesis testing, although they also argued that with respect to hypothesis testing, the degrees of freedom for a polytomous node should be bounded rather than set to their most conservative value). A more thorough approach would be to try all possible resolutions of the polytomy (Harvey and Pagel, 1991; Harvey and Purvis, 1991), or at least a reasonably large sample of them, as advocated here. Interpretation would follow the same rules dis-

cussed above. The method proposed herein could also be used if one had a fully resolved phylogeny but no information on branch lengths and one wanted to use one of the statistical methods requiring branch length information.

Guidelines

One might wonder whether it is possible, a priori, to predict whether the results of a particular statistical analysis are likely to be dependent on the phylogeny. Although much work remains to be done, three factors are likely to be important.

1. How strong is the relationship between the variables? The more tightly the variables are related, the less likely it will be that the relationship, based on ahistorical analysis, is an artifactual consequence stemming from the pattern of phylogenetic relationships. At the extreme, if two variables are perfectly related ($r^2 = 1.0$), then regardless of the phylogeny, it is clear that evolutionary change in one variable is always completely predicted by change in the second variable.
2. How tightly clustered are the data points? If data points clearly fall into two (or more) groups, then closely related species may be phenotypically similar, and thus the number of evolutionary changes would be greatly overestimated by ahistorical analysis. By contrast, if the data points are evenly dispersed, then closely related species will not be phenotypically similar regardless of the true phylogeny. Consequently, evolutionary change must have occurred on all branches of the phylogeny, and phylogenetic effects will be minimal.
3. How likely is it that some branches of the phylogenetic tree will be considerably shorter than the rest? That is, was there a time of radiation in which the time between speciation events was probably very short? Have any extant species only very recently diverged? One point that example no. 3 made clear is that in analyses assuming a gradual

model of evolutionary change, the branch lengths may be as important as the phenotypes of extant species in determining contrast values. In any such analysis, it is always possible that sister taxa separated by short branch lengths and not conforming to the general pattern of relationship of the variables may exist (e.g., variables negatively, rather than positively, related; see example no. 3). Because these contrasts will have relatively great values, they will be outliers and may be sufficient to cause the overall relationship to be nonsignificant.

CONCLUSIONS

It is no longer acceptable to analyze comparative data in an ahistorical context. The ideal solution when conducting a comparative study on a group lacking a well-corroborated phylogeny is to go out and work on resolving the phylogeny; in many cases, however, this may not be practical. I have illustrated how even in the absence of a fully resolved phylogeny or lacking a phylogeny altogether one may still be able to draw conclusions or at least identify how resolution of the phylogeny, when it occurs, will affect interpretations of the relationship of the traits in question.

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