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Phylogenetic uncertainties and sensitivity analyses in comparative biology

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SUMMARY

Phylogenetic comparative analyses combine information on character states and phylogenetic relationships of taxa to test hypotheses regarding character evolution. These studies encounter uncertainties at various steps, including uncertainty in the topology of phylogenetic trees, the scoring of characters, and the addition of taxa that have not explicitly been included in phylogenetic analyses. Here we highlight a variety of sensitivity tests designed to explore the robustness of comparative conclusions to changes in underlying assumptions. These include the examination of character correlations on a set of plausible phylogenetic hypotheses (including alternative rootings and 'neighbouring' trees), as well as under alternative character codings. TreeBASE – a prototype relational database of phylogenetic data – should prove useful in accessing alternative hypotheses.

1. INTRODUCTION

In recent years it has become increasingly clear that knowledge of phylogenetic relationships is crucial in extracting historical patterns and possible evolutionary causes from comparative data (e.g., Brooks & McLennan 1991; Harvey & Pagel 1991). Phylogenetic trees provide concrete hypotheses about the chronicle of evolutionary events, including the sequence of splitting events during the evolution of a group and the sequence of character changes (e.g. O'Hara 1988; Donoghue 1989; Maddison & Maddison 1992). Reconstructing character changes helps us avoid trying to explain things that never really happened (Wanntorp 1983), and is necessary in assessing whether changes in different characters were significantly correlated. Although these points are now widely appreciated, there are still few studies of plant ecological traits that have explicitly incorporated phylogenetic trees.

Attempts to put the theory and methods of comparative biology to use raise a wide range of practical issues that need more attention if comparative studies are going to be convincing. In particular, as Harvey & Pagel (1991, pp. 70–71) emphasized, 'Comparative biologists should be aware of the fact that they may well be working with the wrong tree!' It is highly likely, in fact, that virtually every phylogenetic tree found in the literature *is* wrong in one way or another. Does this mean that phylogenetic hypotheses should be ignored? Obviously not! After all, scientists *always* rely on prior inferences that are themselves subject to error. Instead, as Harvey & Pagel (1991, p. 203) rightly concluded, 'Comparative methods need to be developed that take into account the uncertainty about the phylogeny.' Some attention has been paid recently to these issues, mainly focusing on the use of simulated trees in establishing confidence in particular comparative results (Losos 1995; Martins 1996). Such approaches may be of use when virtually nothing is known at the outset about phylogenetic relationships. Here we focus instead on exploring the implications of a set of proposed and plausible phylogenetic hypotheses, under the view that in practice there are often a relatively small number of alternative topologies deemed worthy of serious consideration. We also emphasize the need for sensitivity tests to address uncertainties that are more directly associated with carrying out a comparative study, especially in scoring taxa for the characters of interest and accounting for taxa that were not actually included in the phylogenetic analysis. Our aim is to provide some practical suggestions for dealing with several sources of phylogenetic uncertainty. In doing so we hope to encourage the development and use of sensitivity analyses to assess the robustness of conclusions derived from comparative studies.

2. COMPARATIVE BIOLOGY

Several of the examples discussed below focus on the evolution of a single character or even a single evolutionary event, and these will seem out of place if 'comparative biology' is equated with particular statistical methods for examining character correlations. This observation compels us to comment briefly on the circumscription of comparative biology, and especially on the distinction made recently between the 'convergence' and 'homology' approaches (for contrasting views, see Coddington 1994; Pagel 1994; Wenzel & Carpenter 1994).

The convergence approach relies on repeated instances of the evolution of a particular kind of

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characteristic (e.g. Harvey & Pagel 1991). The basic idea is that repeated instances are needed to establish whether there is a significant pattern of association between various traits or between a trait and an environmental variable. Under this view, the study of individual evolutionary events, no matter how detailed, is seen as simply lacking the statistical power to establish anything general. The homology approach, in contrast, focuses on the analysis of the circumstances surrounding individual evolutionary changes (e.g. Coddington 1988; Donoghue 1989; Baum & Larson 1991). Under this view, characteristics derived independently in different lineages are not the same (i.e. homologous); or, rather, they are the same only by virtue of having been categorized as such by particular investigators. In any case, it is argued that general patterns observed in multiple lineages will have little bearing on the explanation offered in any particular instance.

This contrast, while perhaps of some heuristic value, will be counterproductive if it tempts us to equate 'real' comparative biology with one approach and dismiss the other. Phylogenetic trees play a central role in both approaches, and each provides valuable and complementary insights (Coddington 1994). In fact, the most satisfying studies will be those that iterate between approaches. Preliminary comparisons may suggest a set of manipulative experiments, which might in turn suggest a refined adaptive hypothesis, which might then be tested by reference to repeated instances, and so on. The role played by phylogenies is fundamentally the same along the spectrum from 'homology' to increasing 'convergence'. That is, trees allow us to infer whether a particular kind of change occurred once or a number of times, whether change has been in one direction or another, whether change in one character has been associated with change in another, whether associated changes have occurred in a particular sequence, and whether particular changes are associated with shifts in diversification rate (e.g. Sanderson & Donoghue 1994). These are the issues that unite comparative biology, as we conceive it, rather than the use of any particular method (e.g. independent contrasts; Felsenstein 1985).

3. UNCERTAINTIES AND SENSITIVITY ANALYSES

Ideally one would begin a comparative analysis with perfect knowledge of phylogenetic relationships (including extinct lineages), accurate information on the characters of interest for all populations, and models of character evolution that would provide accurate inferences about character changes on the tree. Obviously, real comparative studies are far from this ideal. We never know the true tree, we have limited knowledge about character distributions, and we lack reliable models of character evolution. For the most part, comparative analyses have ignored such uncertainties and have instead proceeded by examining a single tree, a single scoring of characteristics, and so on. Whereas this may suffice for demonstration purposes, it leaves nagging doubts about the robustness of the results. How, one wonders, would the results differ with a somewhat different tree, or scoring, or evolutionary model?

Here we will mainly consider uncertainties and sensitivity analyses relating to tree topology, drawing on examples from our own work, and with an emphasis on broad analyses of angiosperms. We also touch briefly on several uncertainties that arise in coding characters and in trying to accommodate mismatches between the available comparative data and the available phylogenetic information. Simulation studies of the independent contrasts method have examined the effect of choosing different models of character evolution (Martins & Garland 1991; Diaz-Uriarte & Garland 1996), and we will not address this problem explicitly here. Ours is by no means an exhaustive treatment of the possible sources of uncertainty or sensitivity tests; instead, we have tried to provide ideas on how to proceed, which might then be adapted to the circumstances surrounding any particular comparative study.

(*a*) *Uncertainties about tree topology*

Estimates of phylogeny may be erroneous for a variety of reasons, only a few of which are mentioned here. First, and most obviously, the available data may be too few or too noisy to yield an accurate estimate of relationships. Second, a perfectly accurate gene tree might not reflect phylogenetic relationships among species, owing to hybridization, lineage sorting and/or lateral transfer (Pamilo & Nei 1988; Doyle 1992; Clark *et al.* 1994; Maddison 1995). Third, estimation methods (e.g. parsimony, maximum likelihood, etc.) may be statistically inconsistent under some evolutionary circumstances (e.g. high and uneven rates of change), such that, in the worst cases, the addition of data leads to greater confidence in the wrong relationships (e.g. Felsenstein 1978; Penny *et al.* 1992; Huelsenbeck & Hillis 1993). Fourth, even when methods are consistent, optimal solutions may not have been found. This problem relates to the fact that many phylogenetic problems are computationally challenging (Maddison *et al.* 1992; Rice *et al.* 1995, in prep.; Swofford *et al.* 1996). For example, exact solutions cannot generally be obtained for parsimony problems involving more than 20 or 30 taxa. Instead, larger problems rely on heuristic search algorithms (generally involving some form of branch swapping), and some or all optimal solutions may not be obtained owing to the limitations of hill-climbing algorithms (finding local, not global, optima) and practical constraints on computer time.

A pertinent example of computational limitations (and perhaps all four problems) is provided by the preliminary analysis of 500 seed plant *rbc*L sequences conducted by Chase *et al.* (1993). In the case of their B series trees (judged by them to be the more reliable), searches were conducted for approximately one month on a Macintosh Quadra computer using PAUP (Swofford 1993), resulting in the discovery of 3900 trees of 16538 steps (all characters included). A reanalysis of this dataset by Rice *et al.* (1995, in prep.;

http://herbaria.harvard.edu/~rice/treezilla), running on several SUN workstations for a total of approximately 9 months, yielded many shorter trees, including 8975 trees five steps shorter than the published trees. Longer searches would undoubtedly yield even shorter trees.

Comparative studies based on the Chase *et al.* (1993) analysis have tended to use the single published B-series tree (e.g. Silvertown & Bond, this issue). The consequences of this choice have not been examined. As a first step in this direction we have conducted a simple simulation study to explore the differences between the single Chase *et al.* tree, the 7670 fully resolved most parsimonious trees found by Rice *et al.* (1995; 1305 trees have polychotomies due to collapsed zero-length branches), and a set of 100 trees of 500 taxa generated using MacClade's random tree function (Maddison & Maddison 1992; see Martins 1996, for a thorough discussion of random tree generation).

Our simulations consisted of the following steps. First, one fully resolved tree was arbitrarily chosen from among the most parsimonious trees of Rice *et al.* to be the 'true tree' (we used the single tree available on the treezilla web site). We then simulated the evolution of two continuous characters on this topology, starting from the root and proceeding upward to the 500 terminal taxa. At each node, the changes in the two characters along each daughter branch were selected at random from a bivariate normal distribution with correlation coefficients (C_I; = the 'input correlation' of Martins & Garland 1991) of 0 and 0.5 in our two simulation runs. This corresponds to a 'speciational' model of character evolution in which expected change is independent of branch length. From the resulting character states we then calculated: (i) the ahistorical correlation (C_A; 'tip correlation' of Martins & Garland 1991) based on the character states across all 500 taxa; (ii) the 'observed' historical correlation (C_O) based on independent contrasts calculated from actual character values during the simulation; and (iii) the reconstructed historical correlation (C_R), calculated by inferring the history of character change using the squared change parsimony algorithm of Maddison (1991), and again calculating the correlation coefficient from the independent contrasts at each node. The use of squared change parsimony differs from methods employed in other implementations of the independent contrasts approach (e.g. Martins & Garland 1991; Purvis & Rambaut 1995), and the effect of this difference has not been systematically investigated (cf. Diaz-Uriarte & Garland 1996). The program used to carry out these simulations (available from D. Ackerly) accepts trees coded in standard NEXUS-format parenthetical notation, which may be especially useful in carrying out sensitivity analyses over many trees.

As expected, the observed and reconstructed evolutionary correlations on the 'true tree' were virtually identical in each of the two simulations, and these two parameters were also fairly close to the input correlations (simulation A: $C_I = 0.5$, $C_O = 0.536$, $C_R = 0.519$, $C_A = 0.363$; B: $C_I = 0.0$, $C_O = -0.021$, $C_R = -0.032$, $C_A = -0.020$). Comparison of the ahistorical and historical correlations (especially for simulation A) illustrate how different these can be even when large numbers of taxa are considered, illustrating the utility of the independent contrasts method. Of most importance for the present discussion, the results over the 7670 most parsimonious *rbc*L trees were clustered very tightly around the value observed for the one 'true tree', with a total range of less than 0.02 in both simulations, and the correlations observed for the slightly less parsimonious tree of Chase *et al.* (1993) were similar to these in both cases (figure 1). We do not know whether the relative consistency in the outcomes that we observed here will hold for other characters or other types of analyses; in fact, we expect that it will not hold in some cases. *The sensitivity of comparative analyses to topological variation must therefore be examined on a case by case basis, as we have done here, by evaluating the set of relevant trees.*

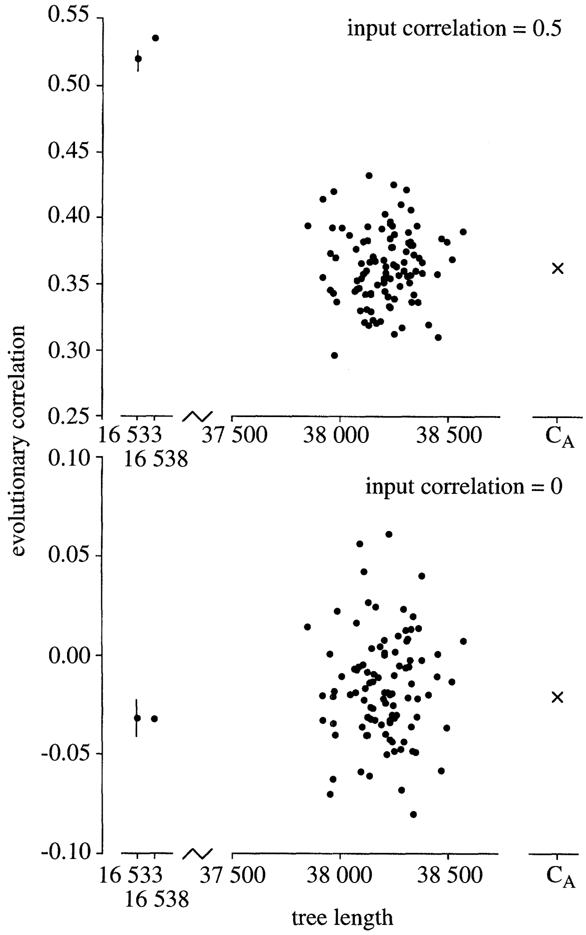

Figure 1. Analyses of simulated character evolution on alternative phylogenetic trees from 500 taxon *rbc*L analyses of seed plants. The reconstructed historical correlation for the two characters is plotted against tree length for 7670 fully resolved *rbc*L trees of 16533 steps, the published tree from Chase *et al.* (1993) of 16538 steps, and 500 random trees generated by MacClade (Maddison & Maddison 1992). On the right is the ahistorical correlation (C_A), calculated using data from the terminal taxa. See text for details.

The historical correlations calculated for the set of 100 random trees differed in two important respects from the results for the *rbc*L trees (figure 1). First, we found extensive variation among the random trees.

This demonstrates that there are alternative phylogenies that will lead to markedly different conclusions. Second, the distribution of correlations calculated on the random trees centres around the ahistorical correlation (see also the *Acer* example below). Thus, the mean of the historical correlations calculated from a set of random trees apparently does not provide a provisional estimate of the historical correlation, as suggested by Martins (1996). At least a preliminary phylogenetic hypothesis is needed for this purpose. Analyses using random trees do, however, set bounds on the possible results of a comparative analysis (Losos 1994).

(*b*) *Uncertainties about rooting*

Uncertainty concerning the exact placement of the root of a tree is common in phylogenetic studies. In part this may be due to the relatively large number of evolutionary changes separating any ingroup taxa and possible outgroups, and the consequent effects of homoplasy, which may render a number of different rootings equally or almost equally parsimonious (e.g. Felsenstein 1978; Maddison *et al.* 1984; Wheeler 1990; Donoghue 1994).

One well known case of uncertainty concerns the position of the root of the angiosperm tree (see Doyle & Donoghue 1993; Crane *et al.* 1995; Taylor & Hickey 1996). Although different datasets are in considerable agreement about a number of major clades within angiosperms (e.g. monocots, eudicots, etc.), different analyses support a woody 'magnoliid' rooting (e.g. Soltis *et al.* 1996), a 'paleoherb' rooting (e.g. Doyle *et al.* 1994), or in the case of the *rbc*L analyses, a *Ceratophyllum* rooting (e.g. Chase *et al.* 1993). Under these circumstances, how should comparative studies proceed? One possibility is to simply explore the consequences of the different plausible topologies for the question of interest. In connection with the simulations described above, we also calculated correlations on four angiosperm rootings designed to mimic viable alternatives to the *Ceratophyllum* rooting found in *rbc*L trees, including woody magnoliid and paleoherb options. These alternative topologies yielded correlations virtually identical to those found for the 'true tree', indicating that root placement has little impact on the method of independent contrasts in this particular case.

This kind of sensitivity analysis has been carried out in other studies involving the angiosperm tree. Sanderson & Donoghue (1994) and Weller *et al.* (1995) examined the effect of alternative rootings on, respectively, rates of diversification and self-compatibility, and found no significant differences among these. In contrast, interpretation of the evolution of many other angiosperm characters depends directly on which rooting is chosen (Doyle & Donoghue 1993). For example, whether the first angiosperms are inferred to be have been woody or herbaceous plants depends on the choice between a magnoliid or paleoherb rooting. Similarly, interpretation of a variety of flower characters (e.g. many versus few flower parts, spiral versus whorled arrangement of parts) depends ultimately on resolution of the rooting problem. In general, *the impact of alternative rootings will need to be established on a case by case basis.*

(*c*) *Neighbouring trees*

The above discussion might suggest that sensitivity analyses are advised only when there are alternative equally parsimonious trees from a single analysis, or alternative trees from different analyses of the same problem. However, testing the robustness of results seems wise even when only one optimal phylogenetic hypothesis has been identified. In particular, we suggest examining what happens to a correlation as one backs away from an optimal tree, in order to assess just how strongly the conclusions of a comparative analysis hinge on commitment to the most parsimonious tree. This approach is illustrated by a preliminary study of the evolution of branching architecture in species of *Acer* (D. Ackerly & M. Donoghue, unpublished data).

An analysis of combined morphological and molecular data for seven species of *Acer* yielded a single most parsimonious tree of length 233, as well as a single tree of length 234, three trees of length 235, four of 236, and so on. Of the 10395 possible rooted bifurcating trees for seven taxa, 398 fall within 20 steps of the most parsimonious tree, and these trees were saved for use in comparative analyses. In addition, 500 random trees were generated (using MacClade; Maddison & Maddison 1992) to examine the range of possible results. Using these trees, we tested the historical correlation between the rate of terminal and lateral branch growth, traits that influence sapling regeneration in relation to light environment (cf. Sakai 1987). These were measured in the field on saplings and on the branches of adult trees for all seven species in the phylogenetic analysis.

Terminal and lateral branch growth have a strong negative historical correlation, but a much weaker ahistorical correlation ($C_R = -0.747$; $C_A = -0.352$; figure 2). The negative correlation inferred from the most parsimonious tree is generally upheld on trees up to ten steps longer, at which point correlations near zero were observed for some trees. The results for 500 random trees (shown as open symbols in figure 2) illustrate the magnitude of variation among other conceivable trees. As in the *rbc*L example above, the mean of the results for the random trees is almost identical to the ahistorical correlation.

Inspection of the trees revealed that the abrupt changes that occur in trees longer than 267 steps is due to the loss of two clades, which contributed strongly to the negative correlation among contrasts (figure 3). Changes of this sort are not limited to analyses of small numbers of taxa, as can be seen in the *rbc*L case above. This observation highlights the desirability of investigating the strength of support for clades (as judged, for example, by bootstrap or decay analyses) that may be especially significant from the standpoint of the comparative analysis. In studies involving many taxa it may not be feasible to examine all trees in the neighbourhood of the optimal trees, but it may be

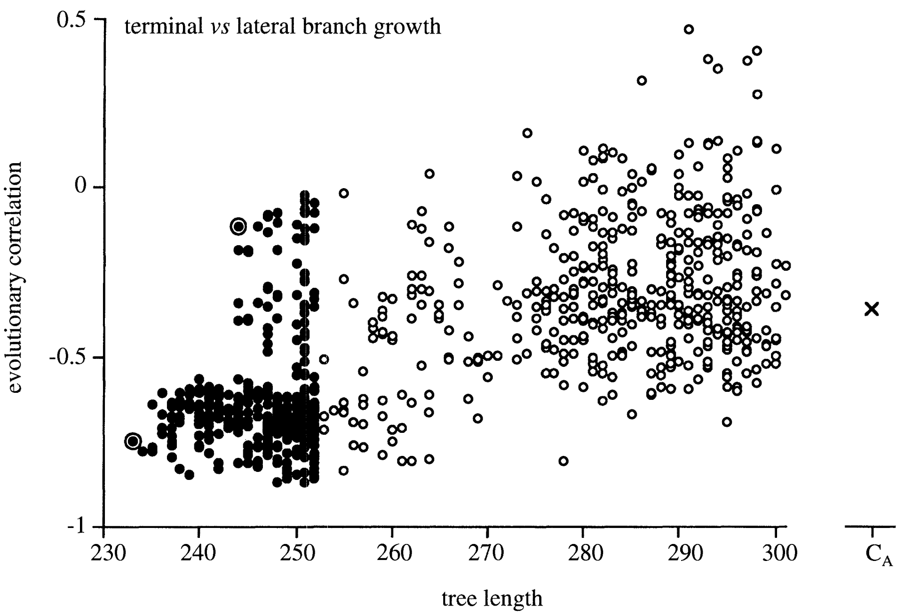

Figure 2. Reconstructed historical correlation between rates of terminal and lateral branch growth, plotted against tree length for all trees within 20 steps of the most parsimonious tree (filled symbols), and for 500 random trees generated in MacClade (open symbols). See text for details. The two circled points represent the trees illustrated in figure 3.

possible to design sensitivity tests that focus on clades that are identified in the original phylogenetic analysis as being especially weakly supported.

(*d*) *Uncertainties in scoring*

A variety of uncertainties tend to arise in designing comparative analyses owing to mismatches between the data available on the characters of interest and the available phylogenetic trees. So far, such practical issues have attracted very little attention. Here we merely highlight several such problems and possible sensitivity tests.

Perhaps the most common difficulty is the lack of relevant character information for taxa included in the available phylogenies (e.g. missing information on mode of dispersal in fossil taxa; Donoghue 1989). These taxa can be coded as 'missing' in character optimizations, contrast analyses, etc., but one wonders how greatly the results of an analysis might change if character information became available. A related problem concerns variation or polymorphism within terminal taxa, which may be more common in characters investigated by evolutionary ecologists (as opposed to systematists interested in higher level relationships), especially as extensive population-level information may have been gathered. In addition, a single species or other terminal taxon in a tree may represent a larger clade, and it may be tempting to score it as polymorphic as a means of expressing the understanding that relatives differ in state from the taxon that happened to be included in the phylogenetic analysis. Again, one wonders what would happen if polymorphisms were resolved in one way or another.

It is important to recognize that the problems posed by missing and polymorphic characters are somewhat different in a comparative analysis than in a phylogenetic analysis (see Nixon & Davis 1991; Platnick *et al.* 1991; Maddison & Maddison 1992; Donoghue 1994), and some solutions are more appealing in the context of a comparative study. In particular, it may be desirable to split up terminal taxa into two or more attached branches on the basis of the character data at hand (cf. Pagel 1992; Purvis & Rambaut 1995). It must then be appreciated, however, that comparative tests are biased conservatively by virtue of having minimized evolutionary change within terminal taxa (i.e. there may have been more changes in the true phylogeny).

A variety of these character coding issues were encountered in the Weller *et al.* (1995) study of self-incompatibility. Alternative resolutions of missing and polymorphic data were explored on a wide range of angiosperm trees. In most cases self-compatibility was optimized as ancestral for angiosperms, and even in those few cases where self-incompatibility was ancestral it was always most parsimonious to suppose that not all self-incompatibility systems were homologous (retained from the ancestral condition); instead several independent losses and originations were required. A similar approach has been applied in a study of the evolution of dioecy in monocotyledons (G. Weiblen & M. Donoghue, in prep.). In this case several codings of a three-state breeding system character (hermaphroditic flowers, dioecy, monoecy) were investigated, including consistently scoring all polymorphisms in favour of each one of the three states. Based on each scoring, the number of transitions between states was then determined on a composite phylogeny of monocots as a means of placing bounds on the number of changes in each direction. Despite considerable polymorphism, the range in the number of inferred changes was not great and clear patterns of transition bias emerged. For example, changes from dioecy to any other state were found to be exceptionally rare regardless of how polymorphisms were resolved.

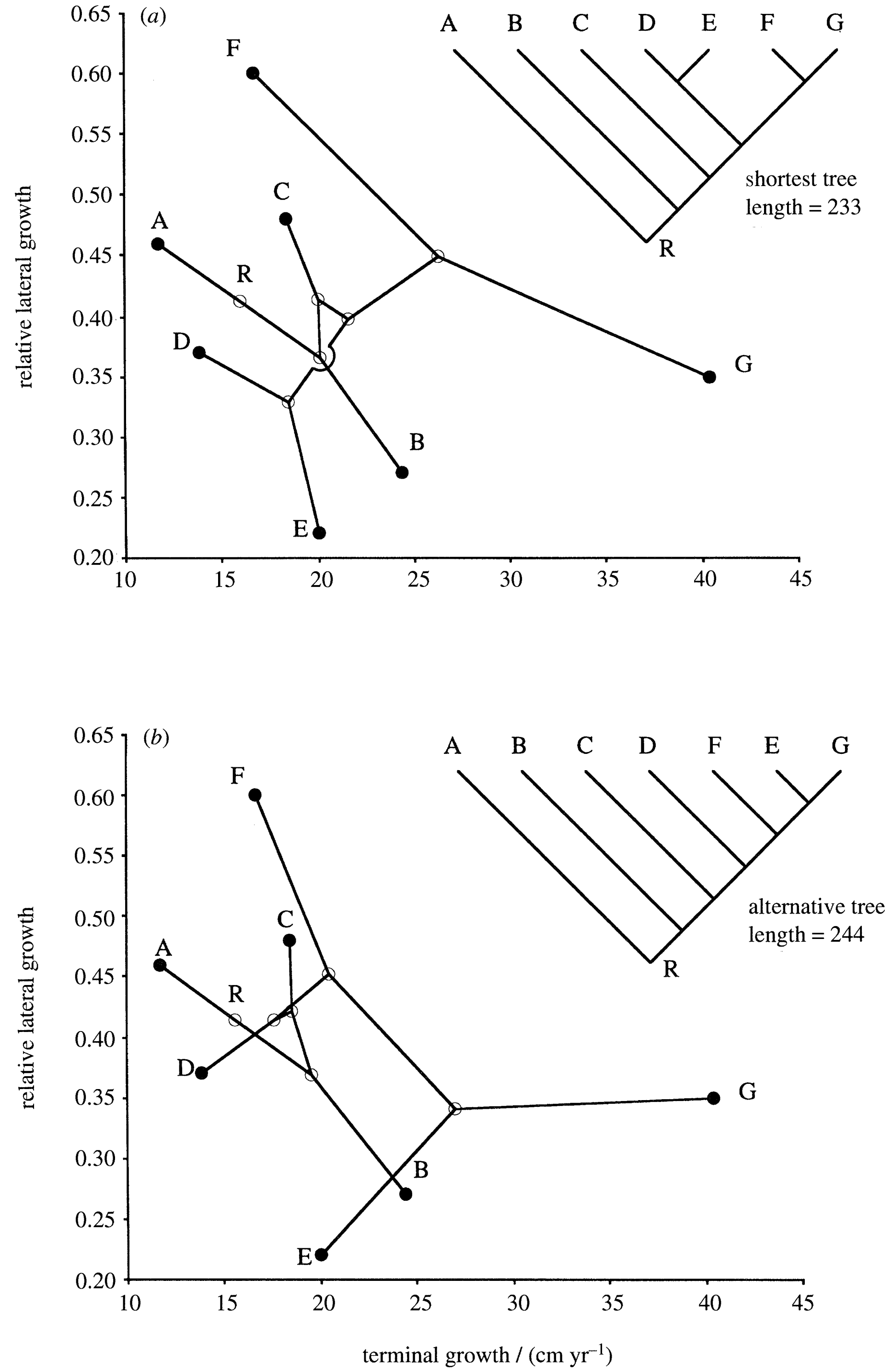

Figure 3. Scatterplot of terminal versus lateral branch growth for seven species of maple (filled symbols), and reconstructed values for ancestral taxa based on squared change parsimony (open symbols). The phylogeny, as shown on the right, is mapped onto the character space. (*a*) The most parsimonious tree, which results in an evolutionary correlation between the two traits of −0.75. (*b*) A tree 11 steps longer with a different pattern of character change and smaller evolutionary correlation of −0.11. The change in the correlation is due to changed relationships among species D, E, F and G; in (*a*) clades DE and FG contributed large negative assocations to the overall correlation, while in (*b*) clades DF and EG contribute positive associations.

It should be noted that character coding experiments such as those described above do not explore all possible permutations and combinations of possible scorings, and as such may not provide an accurate indication of the bounds on the possible outcome. Nevertheless, such exercises may provide a useful expedient when the number of possible resolutions of missing and polymorphic scores is very large. Another possibility would be to repeatedly assign single states at random to those taxa scored as missing or polymorphic, and generate a range of correlations from these alternative scorings (similar approaches apply for continuous variables).

Another critical element of character analysis concerns the weighting of character state changes. In practice, transitions between states (or shifts in different directions in the case of continuous variables) are generally treated as though they are equally likely to occur. That is, a transition from state A to B entails the same cost as from B to A. It would be useful to explore the sensitivity of comparative results to changes in this standard assumption. This can be done using a series of step matrices (e.g. in MacClade; Maddison & Maddison 1992; Maddison 1994) designed to reflect possible inequalities in transitions among states. In each case, the effect of differential weighting on a particular optimization or correlation could be recorded so as to determine the range of weights over which a particular outcome holds. In our laboratory (MJD), this approach has been used in pilot studies of the evolution of fruit types and zygomorphic flowers in Asteridae (unpublished data). In both cases, confidence in the outcome based on equal weighting of state changes is bolstered by the observation that many of the same results are obtained over a reasonably large range of alternative weighting schemes.

(*e*) *Uncertainties in adding taxa*

Another common source of uncertainty in comparative analyses concerns the addition of taxa that were not included in the underlying phylogenetic analyses. In some cases this may involve one or a few species for which comparative data are available, and in other cases it may be tempting to add entire clades. Clearly, such procedures entail significant risks, and the robustness of the conclusions to changes in assumed relationships could be tested in a variety of ways. Most obviously, comparative results can be conducted with and without the additional taxa to determine whether a particular result hinges on the added information. Second, the position of an added species can be shifted to a variety of alternative positions in the tree, to see whether this makes a difference in the outcome. This procedure may require considerable understanding of previous taxonomic treatments, all of which may be misguided about relationships.

The addition of whole trees obtained from separate analyses is appealing from the standpoint of increased sample size, and has become a common practice (e.g. Donoghue 1989; Sillen-Tullberg 1993; Hoglund & Sillen-Tullberg 1994). However, it must be appreciated that this may result in a composite phylogeny that is not a globally parsimonious solution. That is, a simultaneous analysis of all of the taxa could yield a significantly different topology. In effect, the efficacy of the process of piecing together separately derived trees rests on the strength of support for assumptions about the monophyly of particular groups. For example, in an analysis of the historical relationship between dioecy and fleshy propagules, Donoghue (1989) assembled a composite phylogeny by adding an angiosperm tree and a conifer tree to an underlying analysis of relationships among major lines of seed plants. This assumes that both angiosperms and conifers are monophyletic groups, that the position of these clades and other major lines of seed plants would not shift in a simultaneous analysis, and that relationships within each group would not be altered in a global phylogenetic study. The strength of such assumptions is clearly variable. Thus, whereas angiosperm monophyly has been amply confirmed (Doyle & Donoghue 1993; Crane *et al.* 1995), conifer monophyly is less certain (Chase *et al.* 1993; Rothwell & Serbet 1994).

The obvious solution to these problems is to carry out a global phylogenetic analysis, but this will often not be possible. On the one hand, the data from different studies may not be readily combinable into one matrix, and on the other hand, this might create the computational difficulties associated with large datasets. In this case we can only suggest that the sensitivity of comparative analyses might be checked by consideration of a set of plausible alternative composite phylogenies. Steps may also need to be taken to avoid biases in comparative analyses that may result from joining together a possibly biased selection of phylogenies (O'Hara 1992; Sillen-Tullberg 1993; Hoglund & Sillen-Tullberg 1994).

4. TreeBASE

We have emphasized the desirability of taking into account alternative phylogenetic hypotheses. However, we certainly appreciate that this tends to be easier said than done, in part because phylogenetic information has not been readily accessible. The need to develop tools to improve access to phylogenetic knowledge has been recognized (e.g. Sanderson *et al.* 1993; Blake *et al.* 1994; Donoghue 1994), and a prototype relational database of phylogenetic information – TreeBASE – has been developed with this end in mind (Sanderson *et al.* 1994; http://phylogeny.harvard.edu/treebase).

The prototype versions of TreeBASE now contain 155 phylogenetic studies of green plants (*ca.* 3500 taxa, 410 trees), and data matrices are present for about 90% of these studies. TreeBASE allows for browsing and searching on authors, key words, and taxonomic names, and provides tools for downloading datasets, as well as a submission form for receipt of additional studies. The goal is to include phylogenetic data on all groups of organisms, and we anticipate that journals will eventually require electronic submission of trees and data matrices as a corequisite of publication.

5. CONCLUSIONS

Phylogenetic studies of plant life history characteristics are certainly promising, but if such studies are going to be convincing it will be necessary to deal with a variety of phylogenetic uncertainties. Our focus has been on cases in which a set of plausible phylogenetic hypotheses exist at the outset of a study, as is often the case in angiosperms. Tree-based methods have been developed for cases in which nothing is known about relationships for all or part of a tree (Losos 1994; Martins 1996). Judging by polychotomies in published trees this may seem commonplace, but these do not necessarily signify that all possible resolutions are equally well supported (see Maddison 1989), and it may be best to consider each of the equally supported alternative topologies rather than resort to random trees. Even using the characters under study to help constrain a phylogenetic hypothesis might be justified (cf. Pagel 1992; Purvis & Rambaut 1995), either from the standpoint of consciously providing a conservative test (de Queiroz 1996) or providing a better estimate of the historical correlation than the mean of a set of random trees (see above).

At this early stage in the development of our understanding of plant relationships (Donoghue 1994), it is clearly not wise to become narrowly focused on a few phylogenetic hypotheses. While it is evidently tempting to rely on a single phylogenetic analysis, such as the study of *rbc*L sequences presented by Chase *et al.* (1993), we have already achieved a richer understanding of the phylogeny of most groups than is portrayed in such broad analyses, and the more detailed hypotheses for particular groups may be most appropriate for many comparative studies. We appreciate the theoretical difficulties in piecing together more detailed phylogenetic trees, but the benefits may be great and the risks involved might be less than reliance on a single (perhaps suboptimal) tree. Broad phylogenetic analyses also tend to include a limited and biased sample of taxa, and the intercalation of additional taxa for purposes of a comparative analysis entails its own set of risks.

It would also be unfortunate to focus on just a few comparative methods. In particular, despite the popularity and power of phylogenetically independent contrasts (Felsenstein 1985; Harvey & Pagel 1991), there is much to be learned from the analysis of discrete characters (Maddison 1990), or mixtures of discrete and continuous variables (cf. Purvis & Rambaut 1995). Furthermore, independent contrast methods, as these are usually implemented, are unable to keep track of the order of evolutionary events (but see McPeek 1995). Inasmuch as establishing such sequences is often critical in testing causal theories about character evolution (e.g. O'Hara 1988; Donoghue 1989), this may be a very significant limitation. Finally, as emphasized above, deep understanding of the causes of evolutionary change and ecological patterns is most likely to emerge from the integration of 'homology' and 'convergence' approaches, and it seems counterproductive to restrict the purview of 'comparative biology' to one or the other.

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