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Polytomies and Phylogenetically Independent Contrasts: Examination of the Bounded Degrees of Freedom Approach

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Abstract.—We examined the effect of soft polytomies on the performance (Type I error rate and bias) of Felsenstein's (1985; Am. Nat. 125:1–15) method of phylogenetically independent contrasts for estimating a bivariate correlation. We specifically tested the adequacy of bounding degrees of freedom, as suggested by Purvis and Garland (1993; Syst. Biol. 42:569–575). We simulated bivariate character evolution under Brownian motion (assumed by independent contrasts) and eight other models on five phylogenetic trees. For non-Brownian motion simulations, the adequacy of branch-length standardization was checked with a simple diagnostic (Garland et al., 1992; Syst. Biol. 41: 18–32), and transformations were applied as indicated. Surprisingly, soft polytomies tended to have negligible effects on Type I error rates when models other than Brownian motion were used. Overall, and irrespective of evolutionary model, degrees of freedom were appropriately bounded for hypothesis testing, and unbiased estimates of the correlation coefficient were obtained. Our results, along with those of previous simulation studies, suggest that independent contrasts can reliably be applied to real data, even with phylogenetic uncertainty. [Comparative method; computer simulation; hypothesis testing; polytomies.]

Many available phylogenetic trees include polytomies. Hard polytomies represent lack of available evidence to resolve branching order and may indicate true simultaneous speciation events. Soft polytomies represent contradictory results from conflicting data and lack of information about the true bifurcating pattern of speciation (Maddison, 1989); they are also known as unrecognized phylogeny (Grafen, 1989).

When conducting a comparative study, biologists will often be faced with phylogenies that contain polytomies. In the presence of hard polytomies, Felsenstein's (1985) independent contrasts method gives the correct estimates of slopes, correlations, and *P* values. Hard polytomies are represented as a series of bifurcations with branch lengths of 0, and analyses proceed in the standard way (see Purvis and Garland, 1993). The way in which the branches within a hard polytomy are joined is arbitrary and has no effect on the usual calculations done with phylogenetically independent contrasts (Garland et al., 1992).

For soft polytomies, five methods have been proposed. One of them involves using simulated phylogenies (Losos, 1994). For a set of tip data, we generate a random sample of bifurcating phylogenies consistent with what is known (assumed to be true) about the phylogenetic relationships of that set of species. The correlation coefficient (or other statistic of interest) is computed by independent contrasts for each of these phylogenies. If all phylogenies yield the same result, then we may conclude that the obtained result is reliable, regardless of the true phylogeny. However, if not all of the hypothetical phylogenies yield the same result, then it is necessary to examine which phylogenies cause divergent results; conclusions will depend on the number and likelihood of the phylogenies that yield contradictory results (see details in Losos, 1994: 121). A similar proposal, but using completely random trees (Martins, 1996), has been shown to produce estimates that are equivalent to a non-phylogenetic analysis and to yield hypothesis tests that are overly conservative (Abouheif, 1998).

The other three methods do not involve use of random trees. Instead, adjustments are made in the number of contrasts com-

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puted or in the number of degrees of freedom used. Grafen (1989, 1992) and Pagel and Harvey (1989, 1992; Pagel, 1992; Harvey and Pagel, 1991) compute only 1 contrast for each multifurcating node in the polytomous phylogeny. These two methods are arbitrary in the sense that they use information from tip data (the traits under analysis) to indicate how to compute a single contrast for each polytomy. Specifically, Pagel and Harvey suggest "resolving" the tree based on phenotypic similarity; Grafen uses the residuals from the regression of y on x to obtain linear contrasts. Hence, for both methods, the way the single contrast is constructed will vary, depending on the tip data.

Alternatively, following Felsenstein's (1985:10) original suggestion, Purvis and Garland (1993) suggested computing $m - 1$ contrasts for each multifurcating node with m simultaneously radiating branches. This procedure is similar to that used with hard polytomies: the soft polytomy is also represented as a series of bifurcations of length 0, and $m - 1$ contrasts are computed. (To our knowledge, Konarzewski [1995] is the first empirical example to follow Felsenstein's [1985] original suggestion.) For testing hypotheses about bivariate character correlations, Purvis and Garland (1993) suggested bounding the degrees of freedom. If the polytomy were really a hard polytomy, then total degrees of freedom would be $N - 2$, where N is the total number of species (and $N - 1$ the total number of independent contrasts); this constitutes the maximum degrees of freedom. The lower bound on degrees of freedom for a polytomous phylogeny is $p - 1$, where p is the number of real nodes (note that $p - 1 = N - 2 - z$, where z is the number of branches that are set to 0 to represent the polytomies; i.e., $p = N - 1 - z$ is equal to the number of contrasts that do not proceed from 0-length bifurcations). When using the lower-bound degrees of freedom then, although the total number of contrasts is still $N - 1$, critical values are established with $p - 1$ df (instead of $N - 2$ df).

According to Purvis and Garland (1993), their procedure is simple and less arbitrary than the proposals of Grafen (1989, 1992) or Pagel and Harvey (1989, 1992). The method for computing contrasts is the same, regardless of whether polytomies are hard, soft, or firm (firm polytomies are those in which a node is fully dichotomous in reality but resembles a hard polytomy because it is the result of near-simultaneous radiations; see Purvis and Garland, 1993:573); the only needed modification is an adjustment to degrees of freedom. Use of the lower bound on the degrees of freedom should protect against inflated Type I error rates, while at the same time improving estimation by using a full set of $N - 1$ contrasts (see Purvis and Garland, 1993:573). However, the performance of Purvis and Garland's approach has never been examined by computer simulations (Stamps et al. [1997] presented an empirical example comparing this method with that of Losos [1994].) So far, only Grafen (1989) and Purvis et al. (1994) have examined the effects of polytomies on the performance of independent contrasts. The former examined the performance of independent contrasts and the phylogenetic regression (Grafen, 1989) in the presence of unrecognized phylogeny; the latter examined the performance of Pagel's (1992) procedure.

Here, we use computer simulations to examine whether Purvis and Garland's (1993) procedure effectively protects against inflated Type I error rates and whether it produces unbiased estimates of correlation coefficients. We analyze simultaneously the effects of polytomies and misspecification of the model of bivariate character evolution because deviations from a Brownian motion model can have large adverse effects on the performance of independent contrasts (Martins and Garland, 1991; Díaz-Uriarte and Garland, 1996, 1998). We show that the procedure of Purvis and Garland effectively protects against inflated Type I error rates and results in unbiased estimates.

Methods

We simulated independent evolution of two phenotypic traits along a bifurcating phylogeny, using Brownian motion and, in most cases, other evolutionary models. In all simulations the true evolutionary correlation was 0. After the data were simulated,

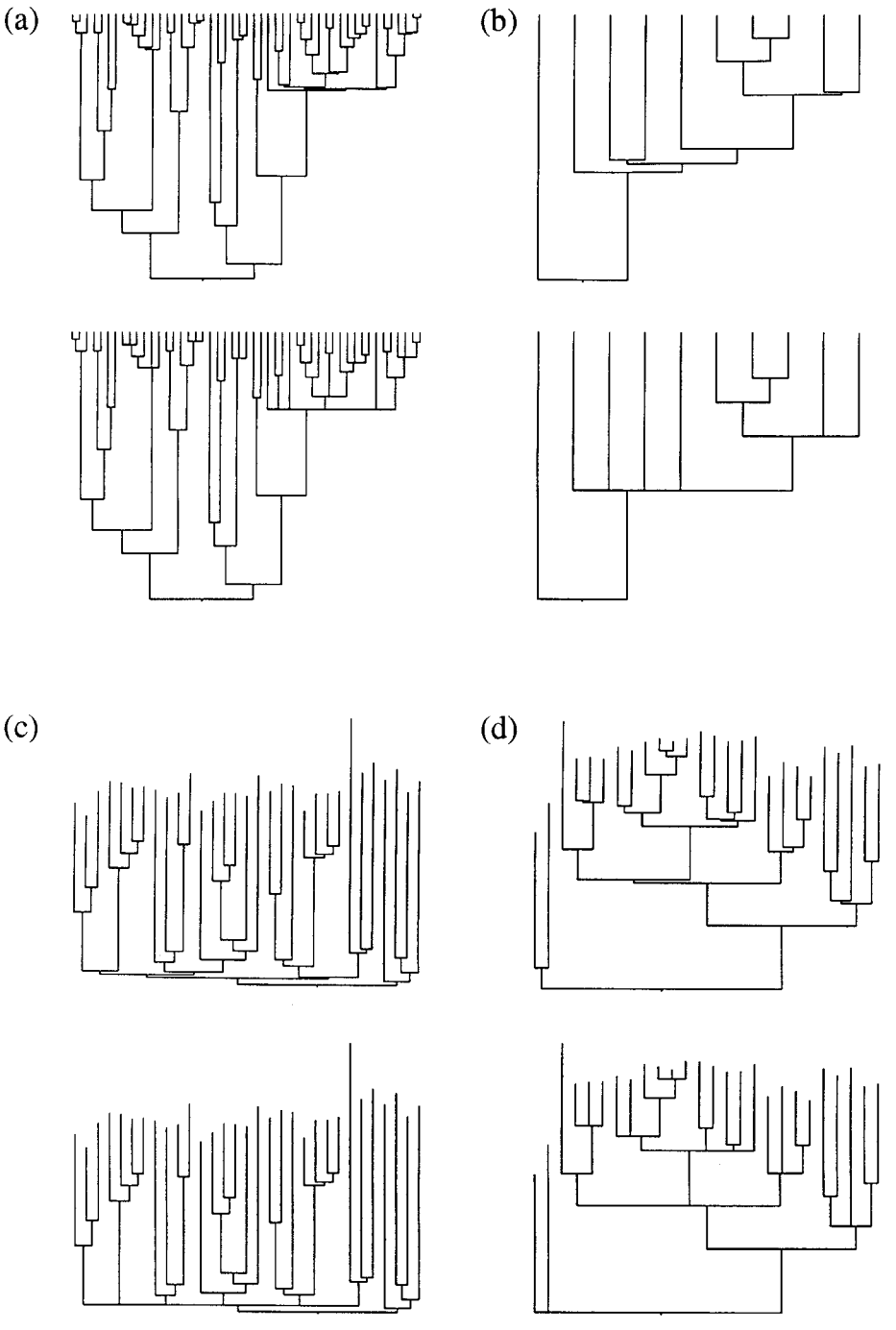

FIGURE 1. Real phylogenies used in simulations. In each case, the original, fully bifurcating tree is shown on top and the version with polytomies is shown on the bottom. (a) Forty-nine species of mammals (Carnivora and ungulates) from Garland et al. (1993); 11 branches set to 0. (b) Ten species of passerine birds from Dutenhoffer and Swanson (1996); four branches set to 0. (c) Thirty-one species of bats from Kirsch et al. (1998); four branches set to 0. (d) Twenty-six species of hummingbirds from Bleiweiss et al. (1997); nine branches set to 0.

we modified the phylogeny by creating some soft polytomies (unresolved nodes) and used these altered phylogenies to estimate correlation coefficients by independent contrasts. This mimics the situation typically faced by comparative biologists: evolution is thought to have occurred along a fully bifurcating phylogeny, but the phylogenetic tree available for analysis contains some soft polytomies. For every combination of phylogeny and model of evolution, we used 5,000 simulated data sets.

Phylogenies Used

We used five different phylogenies. The first (Fig. 1) two have been used in comparative studies (mammalian phylogeny: Garland et al., 1993; passerine birds: Dutenhoffer and Swanson, 1996); the next two are from recent phylogenetic studies (bats: Kirsch et al., 1998; hummingbirds: Bleiweiss et al., 1997). All of these phylogenies were originally bifurcating and were the ones that we used to simulate the tip data. In these phylogenies, polytomies were created by collapsing certain branch lengths to 0. For the mammalian phylogeny, we collapsed all branches less than one million years long as shown in Figure 1 of Garland et al. (1993); this resulted in a total of 11 branches being set to 0 for a phylogeny with 49 species. For the passerine phylogeny, we made 0 all branch segments of length less than 1.5 in Figure 1 of Dutenhoffer and Swanson (1996); this resulted in four branches being set to 0 in a phylogeny of 10 species. The mammalian and passerine phylogeny originally showed all tip species at the same height. To preserve this feature in the polytomous version, after collapsing the specified branches we lengthened the required tip branches until all were lined up at the same height (therefore, the total height of the tree remained constant). For the bat phylogeny, we used the consensus tree in Figure 5 of Kirsch et al. (1998), after excluding the outgroup, and we set to 0 all branches that were unstable in their analyses; this resulted in four branches of 0 length in a phylogeny of 31 species. For the hummingbird phylogeny, we used Figure 3 in Bleiweiss et al. (1997), after excluding the outgroup, and set to 0 all branches that did not show 100% bootstrap support; this resulted in nine branches equal to 0 in a 26-species phylogeny.

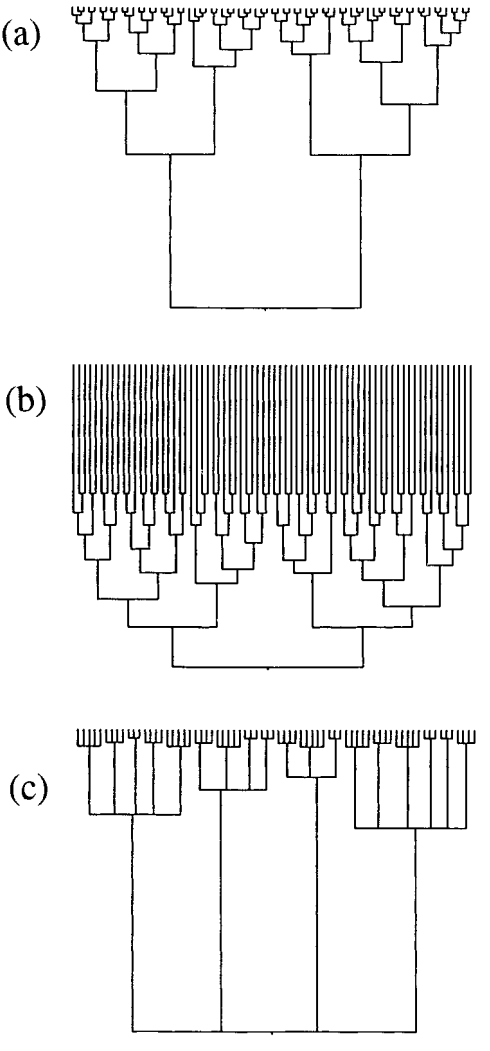

FIGURE 2. Hypothetical, highly unresolved phylogeny for 72 species, as might be derived from taxonomic information only (from Grafen, 1989:Fig. 9). (a) Fully bifurcating, compatible refinement, with arbitrary branch lengths as suggested by Grafen (1989). This tree was used for simulation of data. (b) Same as in (a), but with branch lengths transformed as Grafen's $\rho = 0.2$. This tree was also used for simulation of data. (c) Unresolved version, with Grafen's arbitrary branch lengths; 48 branches set to 0.

Note that in these four real phylogenies (Fig. 1), the branches collapsed were usually the shortest ones in the phylogeny; this seems appropriate, based on the results of many phylogenetic studies (R. E. Bleiweiss, pers. comm.; J. A. W. Kirsch, pers. comm.; R. Olmstead, pers. comm.). An alternative approach would have been to collapse randomly chosen branches, but this is probably less realistic. However, by using different models of evolution, we were also able to examine the effects of collapsing branches that are of the same length as the rest of the branches in the phylogeny (see explanation of BMSpeciat under "Models of Evolution" below).

We also included as a fifth phylogeny (Fig. 2) that used by Grafen (1989: Fig. 9). This "phylogeny" was designed to mimic what a comparative biologist might use if only taxonomic information were available. Accordingly, it contains a very large number of polytomies (Fig. 2c: 48 branch lengths are equal to 0 in a phylogeny of 72 species) and thus also serves as a worst-case scenario. We obtained a bifurcating compatible refinement (Fig. 2a), following the method of Grafen (1989:134). As in his procedure (Grafen, 1989:136), we then assigned branch lengths by using his arbitrary method (Grafen, 1989: Fig. 2). This phylogeny was used to simulate data. A second version of this phylogeny was obtained by use of our PDTREE program (Garland et al., 1999) to apply Grafen's ρ with a value of 0.2 to alter relative branch lengths. This phylogeny (see Fig. 2b) was also used to simulate data. Note that with these phylogenies, the collapsed branches are not the shortest ones (in fact, two of the collapsed branches correspond to the longest branches in Fig. 2a).

At the time Grafen (1989) wrote his paper, biologists were frequently forced to use poorly resolved phylogenies, or even taxonomies, to analyze comparative data (see Harvey and Pagel, 1991). At present, many comparative studies use phylogenies that contain far fewer polytomies. Therefore, the first four phylogenies we used (Fig. 1) are probably more representative of the current application of phylogenetically based statistical methods.

The height (distance from root node to tallest tip) of all phylogenies (except the mammalian one) was re-scaled to allow for branch-length transformations that include the use of base-10 logarithms. (The mammalian phylogeny was already in units that allowed for logarithmic branch-length transformations.)

Models of Evolution

We performed simulations both with Brownian motion (one assumption of independent contrasts) and other models of character evolution (program PDSIMUL; Garland et al., 1993). For each of the phylogenies, we simulated Brownian motion evolution along the bifurcating phylogenies with the original set of branches (termed BM in Fig. 3) and then analyzed the data with independent contrasts, using the phylogeny with polytomies. We also simulated Brownian motion along bifurcating phylogenies that had all branches set equal to 1 (BMSpeciat in Fig. 3) and then analyzed the data with independent contrasts, using the polytomous phylogeny with all branches (except 0-length branches) equal to 1. The main difference between these two cases is that in the BM case the collapsed branches are the shortest branches in the phylogeny (except for Grafen's phylogeny, shown in Fig. 2), whereas in the BMSpeciat case the branches that are collapsed are of the same length as all other branches in the phylogeny. In the original phylogenies (Fig. 1) , the longest branch collapsed was $\approx$9% of the mean (non-collapsed) branch length in the mammalian phylogeny, 16% in the passerine phylogeny, 4% in the bat phylogeny, and 35% in the hummingbird phylogeny. With BMSpeciat, however, the collapsed branches have exactly the same length as the non-collapsed ones. Therefore, with BMSpeciat the effect of polytomies should be exacerbated. Note that polytomies in Grafen's taxonomy also result from collapsing long branches (Fig. 2).

The way we conducted simulations along Grafen's (1989) phylogeny (Fig. 2) is not identical to the procedure he employed. For every one of the 1,000 simulations, he obtained a new, random, compatible refinement to simulate the tip data set. In our case,

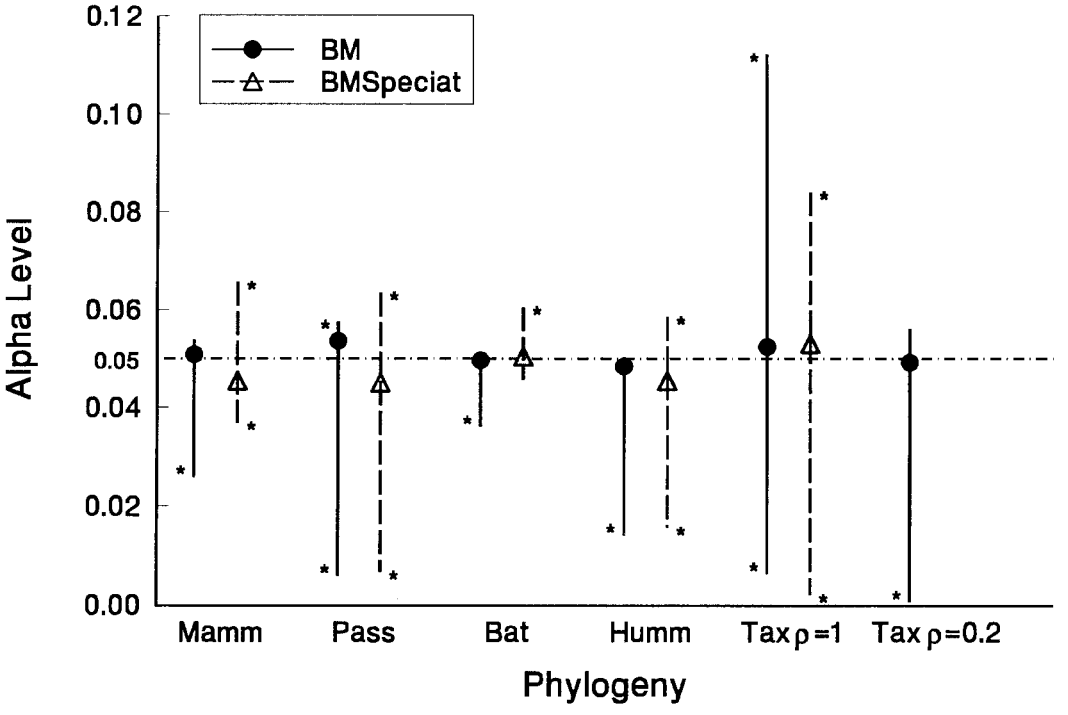

FIGURE 3. Type I error rates of phylogenetically independent contrasts for testing the significance of a bivariate evolutionary correlation in the presence of polytomies, at nominal $\alpha = 0.05$. For each of the phylogenies, we show the results of using as models of evolution Brownian motion (BM) and BMSpeciat (BM, but with all branches set equal to 1). Symbols indicate the Type I error rate ($N - 2$ df) when data are analyzed by using the original, bifurcating phylogeny along which data were simulated. Upper limit of the vertical line is Type I error rate for phylogenies with polytomies when critical value is set by using the maximum $N - 2$ df; lower limit is Type I error rate with the minimum $N - 2 - z$ degrees freedom, where z is the number of branches that are set equal to zero length. The segments themselves connect these points: if the method proposed by Purvis and Garland (1993) works, then these vertical lines should bracket 0.05. If the whole segment lies below the 0.05 level, then the results for both the $N - 2$ and $N - 2 - z$ are too conservative. Asterisks signify that the Type I error rate (with either the maximum or the minimum degrees of freedom) deviated significantly from the nominal one (0.05), according to a binomial test. (For the taxonomy phylogeny, only one set of data exists for BMSpeciat. The phylogeny that results from setting all branches equal to 1 is the same for the two bifurcating phylogenies [Figs. 2a and 2b]: Their topology is the same, and the only difference is branch lengths, because one was subject to a transformation of $\rho = 0.2$.) Figures 3–5, abbreviations for phylogenies (see Figs. 1, 2): Mamm = mammalian, Pass = passerine, Humm = hummingbird, Tax = Grafen's (1989: Fig. 9) phylogeny.

we generated all 5,000 data sets by using a single compatible refinement (Fig. 2a).

For the first four phylogenies (Fig. 1), we also employed another eight models of evolution (following Díaz-Uriarte and Garland, 1996, 1998): Brownian motion with replace and truncate limits (BMR and BMT, respectively), Ornstein-Uhlenbeck (OU), OU with replace and truncate limits (OUR and OUT, respectively), speciational Brownian motion (SP), and SP with replace and truncate limits (SR and ST, respectively). (The difference between this SP and BMSpeciat is that in the former, the phylogeny used for the analyses has branches in the original units, so on top of the effect of polytomies, we are adding a misspecification of evolutionary model. With BMSpeciat, we are isolating the effect of polytomies, as the phylogenies used for analyses all have branches equal to 1, except for the 0-length branches.) Parameters of simulations (limits and starting values) were as in Díaz-Uriarte and Garland (1996, 1998). Decay constants in the OU process

were twice the inverse of the height of the tree. Variances-Tip are shown in Table 1.

Analysis of Simulated Data Sets

After the tip data were simulated, they were analyzed with independent contrasts, by using the phylogenies with polytomies, with the PDERROR program. Simulations with BM or BMSpeciat were analyzed with ordinary independent contrasts (same as IC in Díaz-Uriarte and Garland, 1996, 1998). These simulations examined exclusively the effects of polytomies on independent contrasts. In simulations that used other evolutionary models, we analyzed the data with independent contrasts after checking for adequate branch-length standardization (as described by Garland et al., 1992) and excluding simulated data sets that did not achieve standardization (ICblte; see Díaz-Uriarte and Garland, 1996, 1998; numbers of simulations that were considered adequately standardized are shown in Fig. 5). These simulations combine the effect of polytomies with misspecifications of the evolutionary model. To allow easy comparisons of the effects of polytomies, each simulated data set was also analyzed with the original, fully bifurcating phylogeny used in the simulations.

For each of the simulated data sets, we estimated Type I error rates and bias for testing and estimating a bivariate character correlation. To examine Type I error rates, we computed the observed frequency of correlation coefficients for nominal α levels of 0.1, 0.05, 0.01, and 0.001 (Zar, 1984: Table B.16); in other words, we determined the number of correlation coefficients exceeding the critical value for $\alpha = 0.1$, 0.05, 0.01, and 0.001 in a two-tailed test. When the method of analysis was independent contrasts, we used critical values from a distribution of correlation coefficients with $N - 2$ df. When the method of analysis was ICblte, we used $N - 4$ df, as suggested by Díaz-Uriarte and Garland (1996, 1998): this reduces the degrees of freedom because we have used the data to estimate the transformation of branch lengths (independently for each trait). To obtain the lower-bound degrees of freedom, we used $N - 2 - z$ (IC) or $N - 4 - z$ (ICblte), where z is still the number of branches that were set to 0 length. We then used a binomial test (normal approximation) to obtain the probability that each observed frequency deviated from the expected—the nominal α level—(for example, if the proportion of correlation coefficients larger than critical value at $\alpha = 0.05$ was significantly different from 0.05). This binomial test computes the probability of obtaining as extreme a deviation (in any direction) as observed. In other words, this test will be sensitive to both inflated and deflated Type I error rates. The results from this test are shown with asterisks in Figs. 3 to 5.

To examine bias, we computed a 95% confidence interval (CI) for the mean of the correlation coefficients; if no bias exists, then the 95% CI should include 0. We also performed a sign test, to determine if the numbers of positive and negative correlation coefficients were equal (for more details see Martins and Garland, 1991; Díaz-Uriarte and Garland, 1996, 1998). The 95% CI and the sign test gave very similar results.

Results

Type I Error Rates

Results for the simulations with BM or BMSpeciat are shown in Figure 3. If the

TABLE 1. Variances-Tip parameter used in PDSIMUL program to generate the simulated data sets.

		Model of evolution								
Phylogeny	Fig. 1	BM	BMR	BMT	OU	OUR	OUT	SP	SR	ST
Mammalian	A	100	10,000	10,000	100	40,000	40,000	100	60,000	60,000
Passerine	B	100	70,000	70,000	100	80,000	80,000	100	50,000	50,000
Bat	C	100	20,000	20,000	100	55,000	55,000	100	60,000	60,000
Hummingbird	D	100	30,000	30,000	100	65,000	65,000	100	55,000	55,000

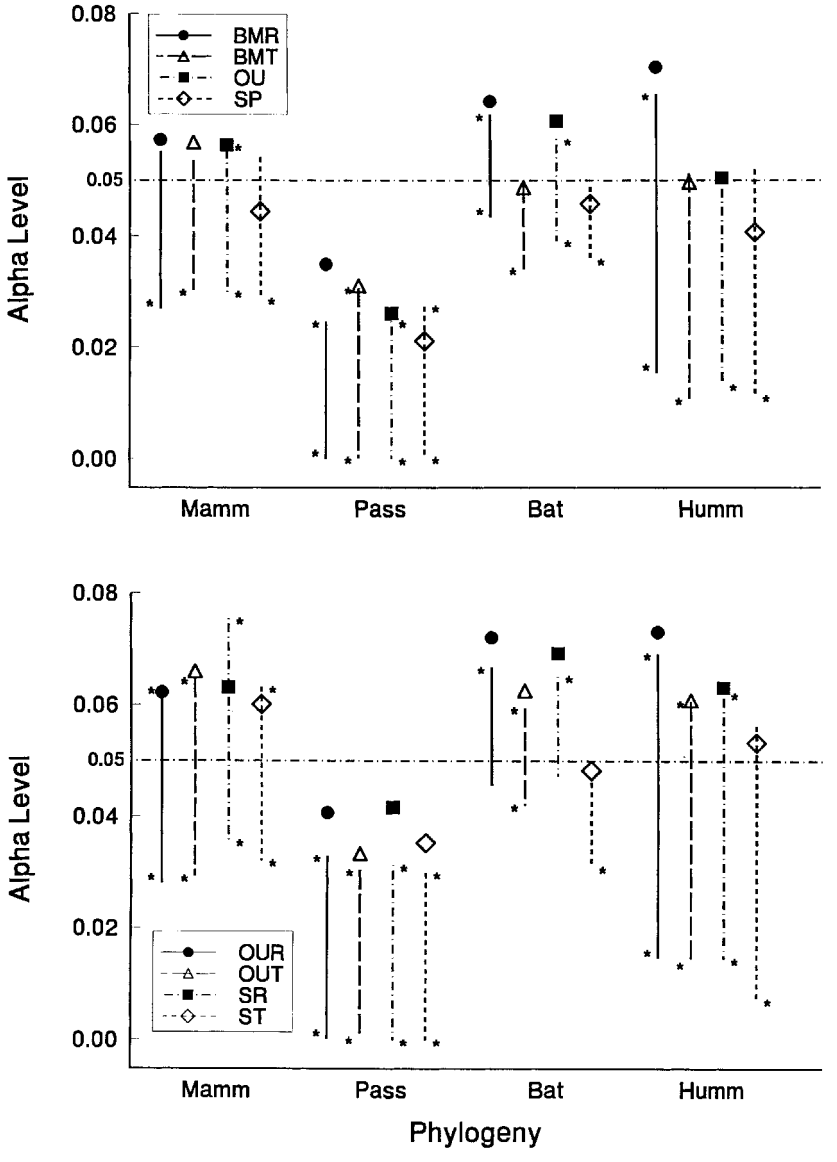

FIGURE 4. Non-Brownian motion models of evolution. As in Figure 3 but with correlation coefficient computed after checking for appropriate standardization (following Garland et al., 1992), transforming branch lengths if necessary, and excluding those simulations that did not achieve appropriate standardization (ICblte; see text for details and Fig. 5 for numbers of simulations retained). Symbols indicate Type I error rate when data are analyzed by using the original, bifurcating phylogeny with $N - 4$ df. Upper limit of vertical line is Type I error rate when the critical value is set by using $N - 4$ df as suggested in Díaz-Uriarte and Garland (1996, 1998) to account for branch-length transformations. Lower limit is Type I error rate with the $N - 4 - z$ df (indicating polytomies). Asterisks signify that the Type I error rate deviated significantly from the nominal one (0.05), according to a binomial test.

method proposed by Purvis and Garland works appropriately, then the vertical segments should bracket 0.05. As expected, when the data are simulated and analyzed on the fully bifurcating trees (symbols in Fig. 3), Type I error rates are always very

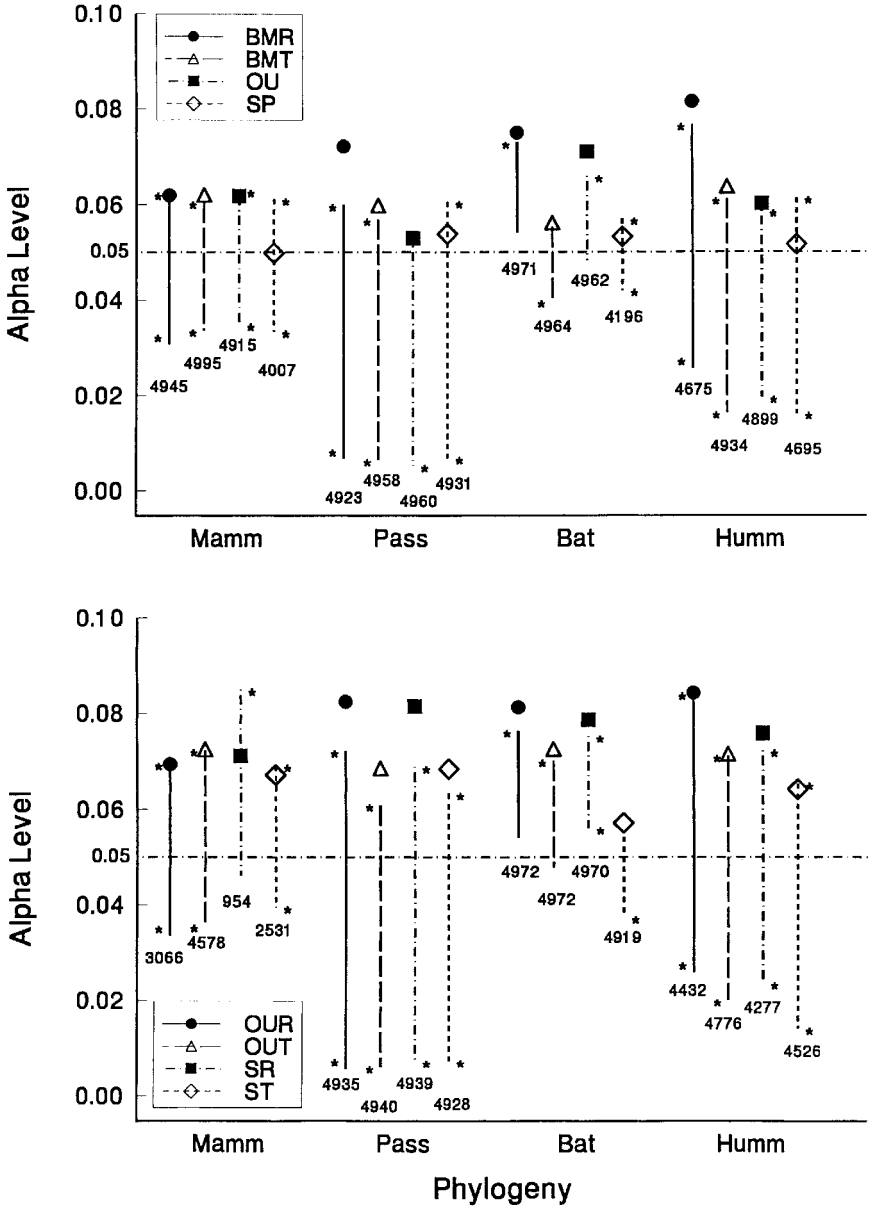

FIGURE 5. As in Figure 4, but without subtracting two df for branch-length transformations (see Díaz-Uriarte and Garland, 1996, 1998). Results are similar to Figure 4, but in one case on the bat phylogeny, the lower bounding does exhibit a significantly inflated Type I error rate. The numbers under each segment indicate how many simulations were considered appropriately standardized (from a total of 5,000 simulations).

close to the nominal 5%. With polytomies, when the degrees of freedom are adjusted downward, as suggested by Purvis and Garland (1993), even the most extreme cases (the highly unresolved phylogenies) do not show inflated Type I error rates. (This holds in every case, even those that came from phylogenies where large [BMSpeciat] or the

largest [Grafen's taxonomy] branches were collapsed.) In fact, the lower bound for degrees of freedom results in very conservative critical values and hence deflated Type I error rates. Figure 3 indicates that using the maximum degrees of freedom ($N - 2$) in the phylogenies with polytomies seldom produces a large inflation of Type I error rates, except for some of the most extreme cases. Similar results were obtained for $\alpha = 0.1$, $\alpha = 0.01$, and $\alpha = 0.001$. Note that the results shown here for Grafen's (1989) phylogeny are comparable with those shown in his Table 3.

In addition to the tests reported on Figure 3, we performed a paired *t*-test to compare the Type I error rate of the data analyzed with the original, bifurcating phylogeny (11 symbols in Fig. 3) versus the Type I error rate of the data analyzed with the phylogeny with polytomies (with the maximum $N - 2$ df; tops of vertical lines in Fig. 3). This test showed that the Type I error rate is indeed significantly larger ($t = 2.902$, df = 10, $P = 0.0158$) in the polytomous case.

Results for simulations with other models of evolution are shown in Figure 4 (see Fig. 5 for number of simulations that were appropriately standardized). As before, the method of Purvis and Garland (1993) appropriately bounds the degrees of freedom. Moreover, even using the maximum $N-4$ df (two df subtracted for branch-length transformation; see Díaz-Uriarte and Garland, 1996, 1998) never results in large inflation of Type I error rates. In fact, the effect of polytomies on Type I error rates is negligible: Paired *t*-tests failed to show any significant increase ($t = -0.844$, df = 31, $P = 0.4051$) in Type I error rates when data were analyzed using the original, bifurcating phylogeny versus using the phylogeny with polytomies (with either $N-4$ [Fig. 4] or $N-2$ df [Fig. 5]). Irrespective of polytomies, no case showed a true Type I error rate larger than twice the nominal level (this result is consistent with those of Díaz-Uriarte and Garland, 1996, 1998). Similar results were obtained for $\alpha = 0.1$ and $\alpha = 0.01$. At $\alpha = 0.001$, the true Type I error rate was ≥ 0.002 in 4 cases (0.0024, 0.0020, 0.0026, 0.0029), but in all of these the Type I error rate of the data set analyzed without polytomies was also > 0.002.

Figure 5 repeats the results of Figure 4, but without subtracting two df for branch-length transformations (i.e., maximum df = $N-2$; see Díaz-Uriarte and Garland, 1996, 1998). Again, Type I error rates are successfully bounded with rare exception.

Bias

Only two cases (passerine phylogeny with BMSpeciat and mammalian phylogeny with OUR) showed any significant ($P < 0.05$) bias. In other words, of the 43 cases examined, in only two did the estimated correlation coefficient deviate from the true value of 0. Given the large number of tests carried out, these results are likely to be attributable to chance: the test-wise significance level for declaring a result biased was 0.05, which means that out of 100 tests we should expect ≈ 5 to result in significant results even if no bias existed. In addition, the magnitude of apparent bias in these two cases was very small (the means of all correlation coefficients were –0.010 and –0.004, respectively).

Discussion

Our results indicate that Purvis and Garland's (1993) suggestion for the analysis of soft polytomies with independent contrasts provides unbiased estimates of the correlation coefficient and that degrees of freedom can be appropriately bounded for hypothesis testing. In the cases we examined, the presence of polytomies had small effects on Type I error rates. These effects are noticeable when the model of evolution is BM (Fig. 3), but they become undetectable when we deviate from BM (in simulations that use limits to character evolution or other evolutionary models: Figs. 4, 5). Therefore, in most cases, using branch-length transformations as indicated in Garland et al. (1992) and the maximum degrees of freedom ($N - 4$ when branch-length transformations are employed; see Díaz-Uriarte and Garland, 1996, 1998) will produce acceptable Type I error rates even with soft polytomies. In the presence of firm polytomies (Purvis and Garland, 1993:573), claiming the max-

imum degrees of freedom should not produce any important increase in Type I error rates. Nevertheless, in the presence of a very large number of polytomies (e.g., Fig. 2), it would be prudent to decrease degrees of freedom.

Note, however, that our results apply to the presence of soft polytomies and not to outright errors in the topology. Soft polytomies represent lack of knowledge about the true branching pattern *within* a clade, whereas a topological error would depict as sister taxa some groups that in reality are not, and would result in contrasts being computed for the wrong taxa. As indicated by Purvis and Garland (1993:569), topological errors could affect performance of independent contrasts (or any other method), to an unknown and potentially serious degree. If competing phylogenetic hypotheses differ substantially, then results should be compared with the different trees (e.g., Bauwens and Díaz-Uriarte, 1997), or the approach of Losos (1994) could be used (e.g., Stamps et al., 1997).

A second caveat relates to the use of branch-length checks and transformations in the presence of polytomies, both soft and hard. Soft and hard polytomies are represented by assigning a length of 0 to some branches; therefore, we can start with two different bifurcating topologies and, by collapsing certain branch lengths, arrive at the same polytomous topology. A problem arises because the statistic we have used in the process of branch-length checking (correlation between absolute values of standardized contrasts and their standard deviations; Garland et al., 1992) is sensitive to the way the polytomy is created. (But note that the actual correlation coefficient estimated by independent contrast IC does not depend on the way the polytomy is created.) This can lead to ambiguous results, because even when the polytomous topology is exactly the same, in some cases the statistic might suggest that a transformation of branch lengths be carried out, and in other cases not. However, we have performed limited simulations (unpublished results) that suggest that, in the long run, the way the polytomy is created has no effect on the distribution of correlation coefficients when branch-length transformations are employed (i.e., the distribution of correlation coefficients is the same regardless of how the polytomy is created). With real data sets, it would be prudent to examine whether the way the polytomy is created results in large differences in the diagnostics that suggest transformation of branch lengths. Nevertheless, the use of branch-length checks and transformations in the presence of polytomies deserves further study.

Two other studies have examined the performance of other methods for dealing with soft polytomies when using independent contrasts to compute bivariate character correlations. Purvis et al. (1994) examined the performance of Pagel's (1992) method, and Grafen (1989) examined the performance of his "phylogenetic regression." Both methods showed acceptable Type I error rates and unbiased estimates in the presence of polytomies. With these methods, only one contrast is computed for each polytomy, and so the degrees of freedom used for testing are the same as the minimum (lower bound) suggested by Purvis and Garland (1993). But as our results indicate, when using the approach of Purvis and Garland (1993), we can often claim close to the maximum degrees of freedom without inflating Type I error rates, which should result in increased power of the test.

Direct comparison of power among the three methods of treating polytomies is, however, not straightforward. Grafen (1989) and Purvis et al. (1994) measured power in a different way (also differently from the method used by Martins and Garland, 1991). Additionally, the suggestion of Purvis and Garland (1993) involves bracketing the degrees of freedom (not just using the minimum), so power cannot be compared among methods in any simple way.

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