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Journal

Molecular biology and evolution, 13(6)

ISSN

0737-4038

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Publication Date

1996-07-01

DOI

10.1093/oxfordjournals.molbev.a025644

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Recognizing the Forest for the Trees: Testing Temporal Patterns of Cladogenesis Using a Null Model of Stochastic Diversification

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Computer simulations are developed and employed to examine the expected temporal distributions of nodes under a null model of stochastic lineage bifurcation and extinction. These Markovian models of phylogenetic process were constructed so as to permit direct comparisons against empirical phylogenetic trees generated from molecular or other information available solely from extant species. For replicate simulated phylads with n extant species, cumulative distribution functions (cdf's) of branching times were calculated, and compared (using the Kolmogorov-Smirnov test statistic D) to those from three published empirical trees. Molecular phylogenies for columbine plants and avian cranes showed statistically significant departures from the null expectations, in directions indicating recent and ancient species' radiations, respectively, whereas a molecular phylogeny for the *Drosophila virilis* species group showed no apparent historical clustering of branching events. Effects of outgroup choice and phylogenetic frame of reference were investigated for the columbines and found to have a predictable influence on the types of conclusions to be drawn from such analyses. To enable other investigators to statistically test for nonrandomness in temporal cladogenetic pattern in empirical trees generated from data on extant species, we present tables of mean cdf's and associated probabilities under the null model for expected branching times in phylads of varying size. The approaches developed in this report complement and extend those of other recent methods for employing null models to assess the statistical significance of pattern in evolutionary trees.

Introduction

To illuminate evolutionary histories and processes in the light of phylogeny, evolutionary biologists often formulate causal hypotheses to explain perceived patterns of branching in reconstructed phylogenies. For example, clusters of lineage bifurcations within a relatively short span of phylogeny are often interpreted as evidence of adaptive radiations. Minimally, however, the patterns upon which such interpretations are based should be evaluated critically against a null model (Nitecki and Hoffman 1987, p. 6; Slowinski and Guyer 1989a).

The first attempts to analyze patterns of evolutionary diversity against a null model of stochastic diversification were made in the early 1970s by paleontologists meeting at the Marine Biological Lab (MBL) at Woods Hole, Mass. Members of the MBL group created a computer model to generate phylogenies based on a random process of lineage branching and extinction (Raup et al. 1973). They then compared temporal changes in biotic diversity profiles from the computer-based phylogenies against observed patterns of diversity for particular clades as recreated from time-series sampling of the fossil record. Of course, such applications may be limited by the often incomplete nature of fossil evidence.

An alternative approach involves development of null models of evolution appropriate for assessing branching patterns as estimated from data on extant taxa. For example, behavioral, physiological, and molecular genetic data normally are available only from living species (except in rare cases). However, because

they lack direct information about extinct lineages, such data have their own limitations as a source of inference about phylogenetic branching pattern. When extinct taxa are unavailable for assay, phylogenetic reconstructions tend to produce trees with the appearance of growth over time, when in fact true species diversity may fluctuate temporally. Corrections for this characteristic must be incorporated into appropriate null models.

Previous applications of null models to phylogenies derived from extant species have been limited to assessment of clade symmetry (Slowinski and Guyer 1989b; Kirkpatrick and Slatkin 1993; Rogers 1994; Sanderson and Donoghue 1994; Losos and Adler 1995) or to changes in levels of diversity through time (Nee, Mooers, and Harvey 1992; Harvey, May, and Nee 1994; Nee, May, and Harvey 1994; Kubo and Isawa 1995). In tests of symmetry, the diversity of one clade is compared to that of a sister clade and the differences are evaluated using an analytical method such as maximum likelihood (Sanderson and Donoghue 1994) or Markov process statistics (Slowinski and Guyer 1989b). In tests of temporal changes in diversity levels, investigators have focused on the effects of variable rates of cladogenesis on phylogenetic pattern.

One question not addressed in the work cited above is whether the temporal distributions of phylogenetic nodes as inferred from contemporary data depart significantly from those expected when cladogenesis is temporally stochastic and lineage-independent. To investigate this question, we construct and evaluate a null model of cladogenetic pattern. Our model is stochastic in the sense that equivalent probabilities of bifurcation and extinction are applied independently to each lineage. Then, for particular empirical phylogenies derived from molecular data on extant species, we evaluated the model using a statistical framework to derive probabilities of observing an empirical phylogenetic pattern under the assumptions of the null model. As in previous work

Key words: speciation, extinction, null model, adaptive radiation, molecular phylogeny, evolution.

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Mol. Biol. Evol. 13(6):833-849, 1996

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dealing with the interpretation of molecular phylogenies, this latter analysis assumes that the empirical phylogenies being evaluated are accurate.

Materials and Methods

The Null Computer Simulations

A computer program (RANDOMTREE) was written to generate phylogenetic trees using a discrete-time process of Markovian bifurcation and extinction (MB&E) (Savage 1983; Slowinski and Guyer 1989b; Losos and Adler 1995). These phylogenies conform to a "molecular" format such that only lineages leading to extant taxa were retained in the final representation of any tree. A set of simulations consisted of all generated MB&E trees that displayed a given user-specified number (n) of extant taxa. For example, one such analysis set consisted of all simulated trees that resulted in $n = 17$ extant lineages.

Each tree simulation was initialized with one lineage. The simulation then ran for 250 program cycles with equivalent probabilities of cladogenesis and extinction. The equivalence of these last two parameters assured that the processes governing expansion or diminution of trees were stochastic with respect to time. We chose to allow the simulation to run for a specified length of time as this should be a realistic representation of what has occurred in history up to the point when extant taxa are sampled. Organismal diversity may have fluctuated above and below the current state, but the present is a fixed elapsed time since the ancestral species of the clade first split. The number of program cycles used was chosen in a rather arbitrary manner. However, because of the scaling of the speciation and extinction parameter with this value (see below), use of a different number of program cycles is not expected to change the outcome of these analyses (nor did it do so in preliminary computer trials). Values initially employed for lineage bifurcation or extinction probabilities were those which maximized the probability of a phylogeny having n terminal taxa at time T (equation 33 in Kendall 1948). This probability is maximized when $bT = (n - 1)/2$, where b is the probability of lineage bifurcation (equal to the probability of lineage extinction) per cycle and T is the duration of the tree (250 cycles). In this way we hoped to circumvent the danger of circularity in estimating b from the empirical tree being analyzed.

During each cycle, each lineage was evaluated for the occurrence of extinction or bifurcation (in that order). Each remaining lineage was extended by one unit. This process continued until the specified number of cycles was reached, all lineages became extinct, or the number of lineages exceeded an upper limit (50 lineages, or $n + 20$ lineages, whichever was larger). This latter limitation was imposed upon the program to prevent data from being written beyond the end of data arrays, which would corrupt the data for that individual tree. (This proactive solution was implemented because the large volume of data generated by these simulations made it impractical to retrospectively check each tree to ensure that the array had not been exceeded.) To remove

the "kinks" from the phylogeny that occur when a lineage goes extinct, the parental lineage and that of the sister clade were combined by removing from consideration the node leading to the extinct lineage. At the end of 250 cycles, if the MB&E tree matched the user-specified number of terminal taxa, the array of branching times for this phylogeny was retained for further processing in that analysis. Although there may exist more efficient algorithms for generating a set of branching times, this MB&E algorithm proved to be unbiased and easy to implement, without the analytical complexities that a coalescent algorithm (for example) might require.

For the MB&E trees of specified extant diversity, all branching times were normalized between zero (the time of the first branching event) and one (the present). Normalization of all branching times forces all trees to be of unit length, and allows comparisons to be based solely on relative temporal patterns of branching. The array of normalized branching times (nodes) in each tree then was converted to a cumulative frequency distribution of branching times.

The Empirical Trees

A brief search of the empirical literature uncovered three molecular-based phylogenies whose properties at face value appeared (and were interpreted by the respective authors) to correspond to three conceivable categories of departure (or lack thereof) from expectations of the null hypothesis of cladogenesis that we wished to test. These three categories are: (a) a recent clustering of lineage branching events, (b) an ancient clustering of branching events, and (c) no apparent clustering of branching events. To diminish any bias resulting from undersampling of taxa (Harvey, May, and Nee 1994; Mooers 1995), we attempted to find trees consisting of all extant species in the taxon under consideration. We were successful for categories b and c, and, for reasons described below, we do not believe that the undersampling of taxa in the "recent clustering" tree (category a) would appreciably change the results of our analysis. The three empirical trees as presented by the original authors are shown in figures 1A, 2A, and 3A. We wished to determine whether these qualitative suggestions about apparent cladogenetic pattern were statistically supportable under the framework provided by the null hypothesis of stochastic temporal variation in the occurrence of speciation and extinction events. Of course, our models cannot test the validity of a phylogeny (however it may have been generated). Rather, they merely utilize whatever information about temporal nodes may be present in a proposed phylogeny to ask whether that temporal pattern is statistically consistent with an MB&E process.

The "recent-clustering" tree was based on nuclear ITS (internal transcribed spacer) sequences for the 5.8S rRNA gene in columbine plants (Hodges and Arnold 1995; an alignment of the ITS DNA sequences was provided by Scott Hodges). For present purposes, the *Aquilegia* clade, *Semiaquilegia adoxoides*, and one species of *Isopyrum* were included. A recent adaptive radiation in the columbines was hypothesized to have occurred in

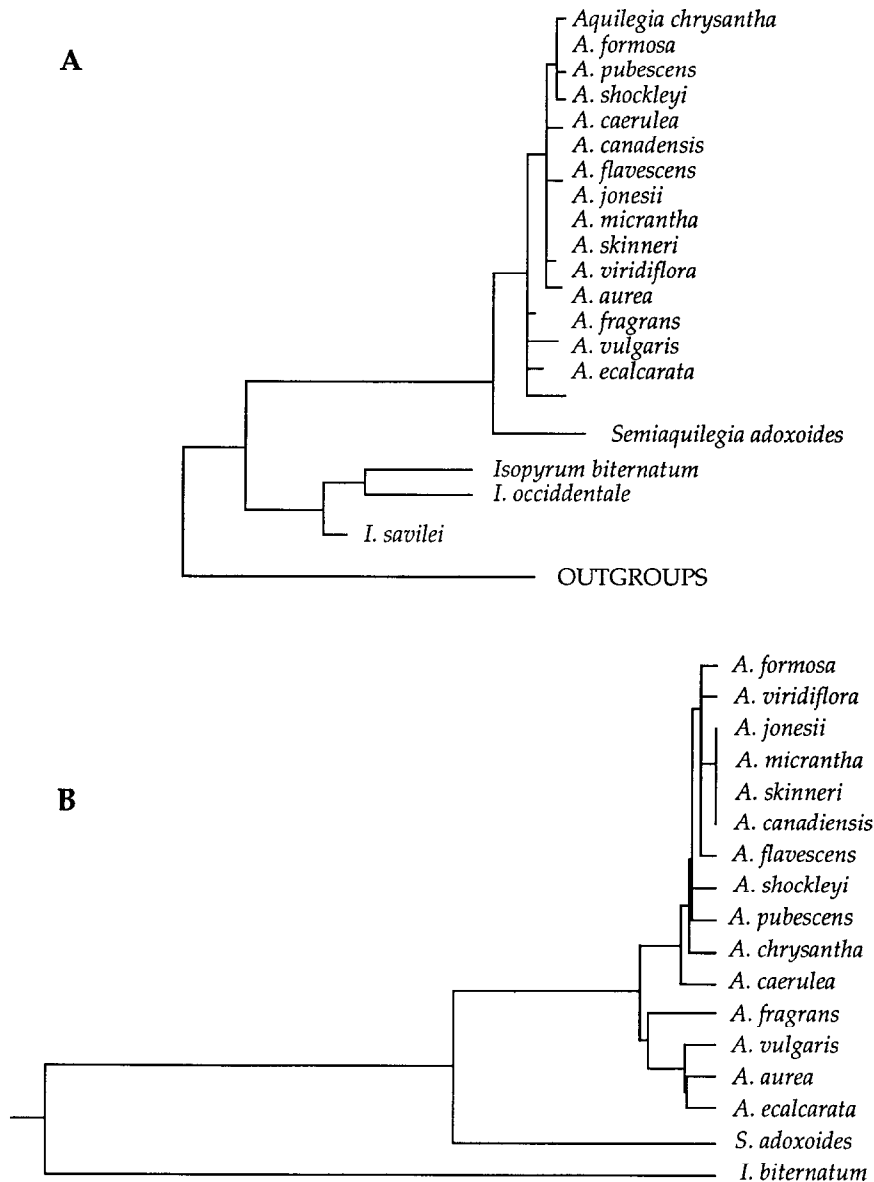


FIG. 1.—Molecular phylogenies of the columbines. (A) Single most-parsimonious phylogeny of *Aquilegia*, *Semiaquilegia*, and *Isopyrum* based on nucleotide sequences of the nuclear ITS region and chloroplast *rbcL* and *atpB* intergenic spacer regions (from Hodges and Arnold 1995). (B) KITSCH phylogeny of *Aquilegia*, *Semiaquilegia*, and *Isopyrum* based on nucleotide sequences of the nuclear ITS region.

the *Aquilegia* + *Semiaquilegia* clade, to which *Isopyrum* is the sister taxon in the analysis. A matrix of maximum-likelihood distances was generated from the alignment of 17 nucleotide sequences using the program DNADIST in PHYLIP 3.55 (Felsenstein 1993).

The example of a putative “ancient-clustering” tree was derived from mitochondrial cytochrome *b* DNA sequences for cranes (Krajewski and Fetzner 1994). For present purposes, we arbitrarily chose one sequence from each species for analysis. An alignment of mitochondrial cytochrome *b* sequences was provided by Carey Krajewski. A matrix of maximum-likelihood distances was generated from this alignment of 16 nucleotide sequences, again using PHYLIP (Felsenstein 1993).

The example of a putative “no apparent clustering” tree was based on analyses of two-dimensional electrophoresis data from the *Drosophila virilis* species group (Spicer 1991). The data employed to construct this tree consisted of a matrix of similarities (Spicer 1991) which themselves were based on comparisons of approximately 200 loci among 13 taxa.

For the sake of consistency, we used the three distance or similarity matrices (one from each empirical study) to generate least-squares-with-contemporaneous-tips trees using the KITSCH program of PHYLIP 3.55 (Felsenstein 1993). The branching patterns seen in the original trees (figs. 1A, 2A, and 3A), which led to the hypotheses of nonrandom temporal patterns of cladogenesis (or lack thereof), generally were recovered in

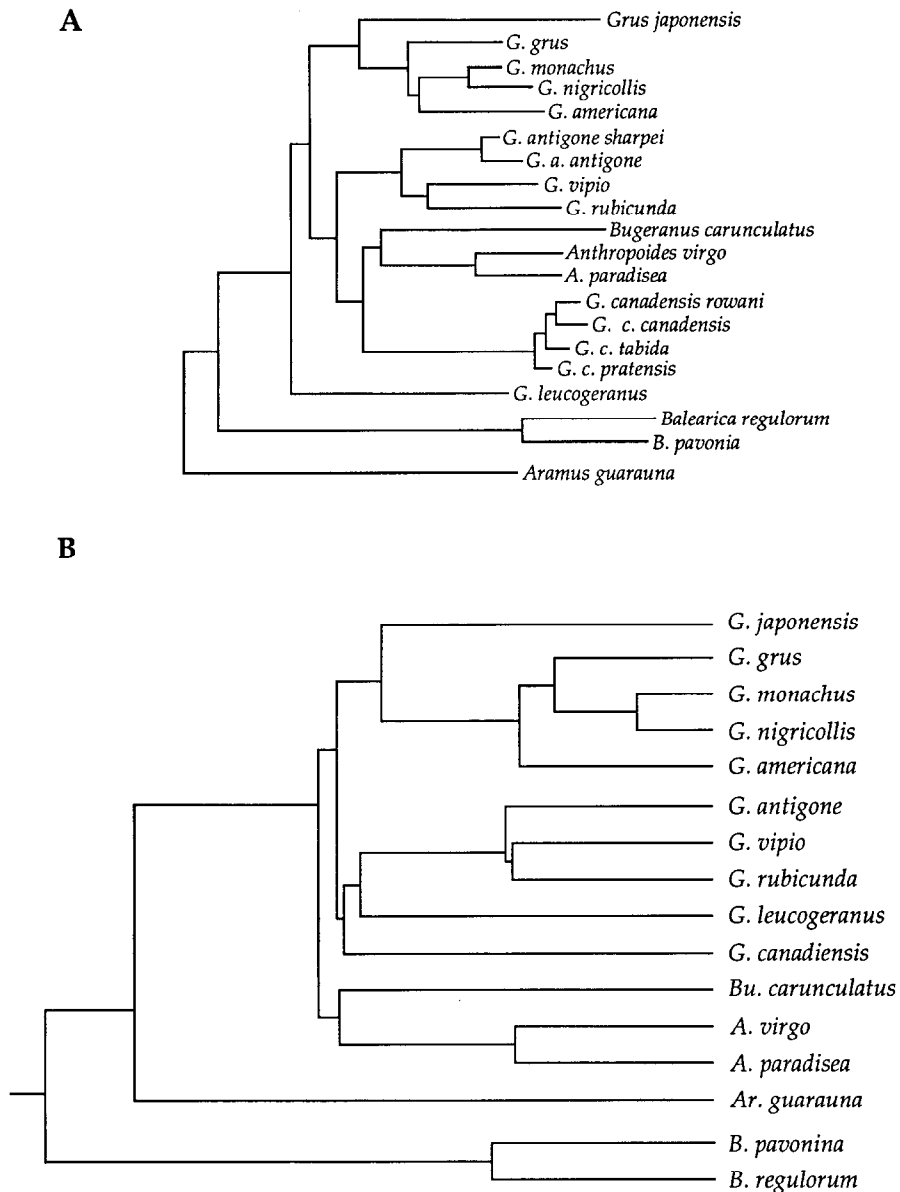


FIG. 2.—Molecular phylogenies of the cranes. (A) Fitch-Margoliash (1967) phylogeny based on mitochondrial cytochrome *b* nucleotide sequences (from Krajewski and Fetzner 1994). (B) KITSCH phylogeny constructed from the same data.

the respective KITSCH trees (figs. 1B, 2B, and 3B). Branching times for the KITSCH trees were normalized, and cumulative frequency distributions of normalized branching times were calculated.

To investigate the effect that phylogenetic frame of reference might have on our results, we reanalyzed the columbine data twice. For each re-analysis, the DNA sequence for the outermost taxon (*Isopyrum* then *Semiaquilegia*) was removed from the overall alignment. The analyses then proceeded as before, with maximum-likelihood distance matrices calculated for each reduced data set, KITSCH phylogenies constructed, branching times normalized, and cumulative frequency distributions of normalized branching times generated.

Comparisons of Empirical Trees Against the Null Expectations

The dissimilarity between one cumulative frequency distribution of branching times and another was quantified using a Kolmogorov-Smirnov (K-S) goodness-of-fit statistic *D* (Sokal and Rohlf 1995, pp. 708–715). We chose this nonparametric goodness-of-fit statistic because of its conservative nature. For this reason, we believe that any of the analyses that refute the null hypothesis also would do so if other statistics were employed. However, a review of the performance of other statistics in this context is beyond the scope of this paper.

For the null expectations, an *average* cumulative distribution of normalized branching times (one for each

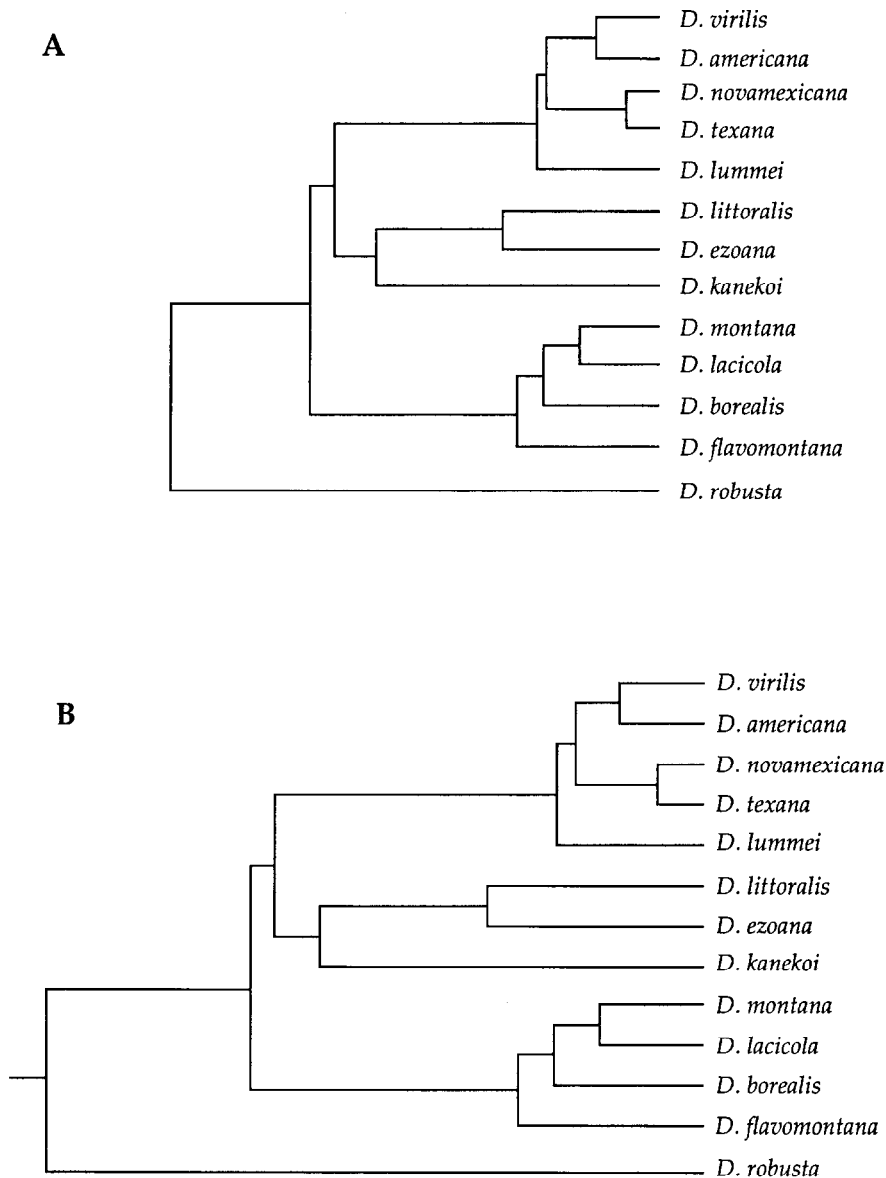


FIG. 3.—Molecular phylogenies of the *D. virilis* species group. (A) UPGMA phenogram based on the $(-\ln)$ -transformed simple matching coefficient of two-dimensional electrophoretic data (Spicer 1991). (B) KITSCH phylogeny constructed from the same distance values.

analysis set of simulations) was calculated from 500 appropriate MB&E trees as generated using RANDOMTREE. Because the assumptions used to generate MB&E trees (lack of complete independence of branching times in historical phylogenies) do not match the assumptions used to generate tables of critical values for the Kolmogorov-Smirnov D , another method was needed to assess the significance of our observed D values. To assess the likelihood that individual MB&E trees were as dissimilar from the *average* normalized branching time cumulative distribution function (cdf) as was the empirical tree, we used a sampled randomization test (Simberloff 1987). For this test, RANDOMTREE was used to generate 5,000 more MB&E trees with the appropriate number of terminal taxa. For each of these 5,000 MB&E trees, a K-S goodness-of-fit statistic was calculated by comparing the cumulative frequency distribution of normalized branch-

ing times for the MB&E tree against the *average* cumulative frequency distribution, and a frequency distribution of the resulting D values was compiled. From this histogram, the distribution of P values for the empirical-to-average D was calculated as the cumulative frequency of all MB&E-to-average D values larger than the empirical-to-average D . In this current application, this should be considered a two-tailed test.

The sample sizes employed for the cdf's and the D value distributions (500 and 5,000, respectively) were decided upon after preliminary trials (not shown) that involved systematic examination of outcomes as sample sizes were increased. The final values utilized were of a magnitude such that increasing them further did not appreciably change the outcome of the analyses.

This P value represents the probability that a phylogeny, generated by a process of Markovian branching

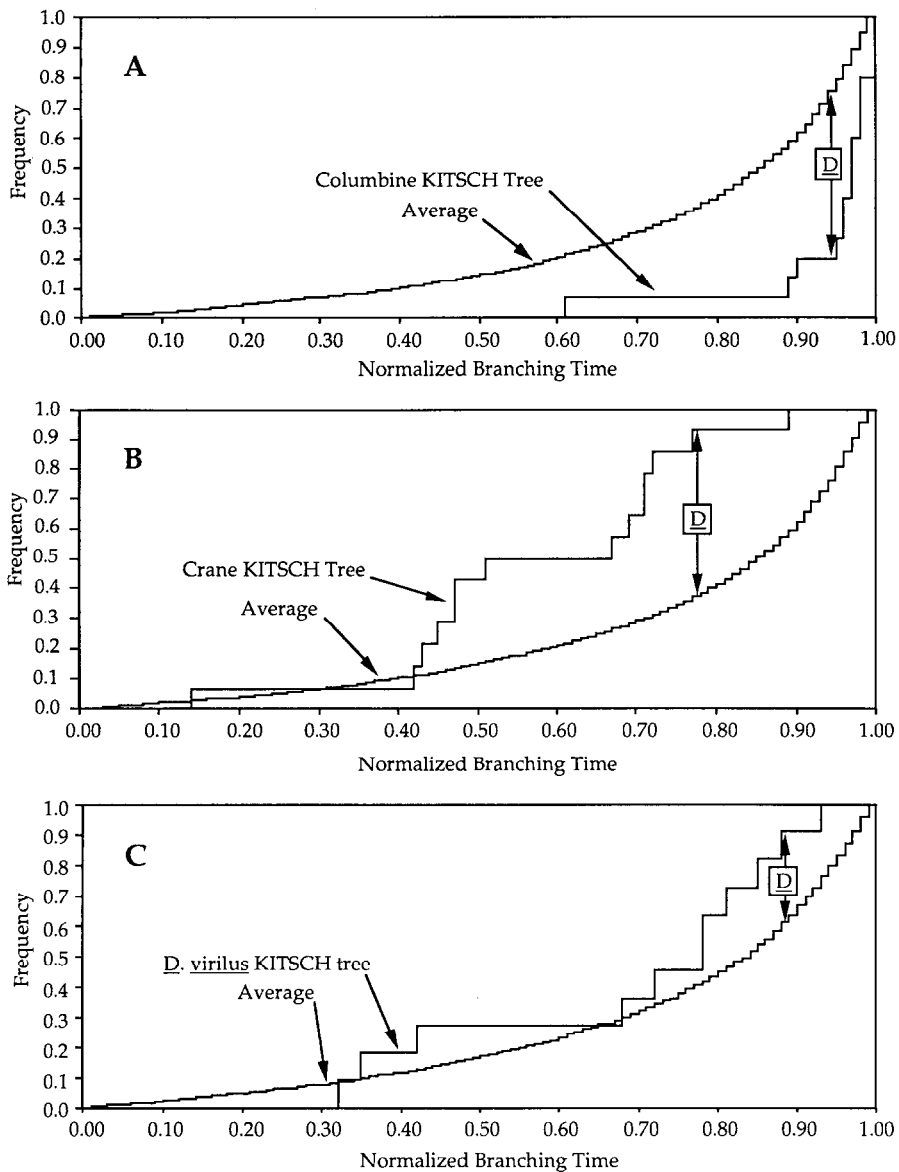


FIG. 4.—Cumulative frequency distributions of normalized branching times. The largest difference D between the empirical cdf and the average cdf is indicated. (A) average of 500 17-taxon MB&E trees, and the KITSCH tree of columbines. (B) average of 500 16-taxon MB&E trees, and the KITSCH tree of cranes. (C) average of 500 13-taxon MB&E trees, and the KITSCH tree of the *D. virilis* species group.

and extinction acting independently on each lineage, had a distribution of branching times more divergent from the *average* than the empirical phylogeny. Thus, for small P values, the null hypothesis would be rejected, lending statistical support to the conclusion that branching patterns in an empirical tree differ from the expectations of stochastic timing of the speciation and extinction events.

To assess how changes in b would affect the outcome of our statistical tests, we also performed sensitivity analyses. Because the curvature of the cdf increases as b increases, it was necessary to analyze only a subset of possible b values for two of the three empirical phylogenies. For the columbines, b values smaller than the optimum value would only increase the empirical D value, which could not change the qualitative results of the analysis. The converse applies to the analysis of the

cranes. Therefore, only b values greater than the optimum were used in the sensitivity analysis of the columbines, whereas only b values less than the optimum were analyzed for the cranes. For the *D. virilis* species group, b values both above and below the optimum were analyzed.

Results

For each of the three empirical phylogenies, comparisons of the cumulative frequency distributions of normalized branching times against the respective average cumulative distributions under the null models are shown in figure 4. For the columbines, the recent clustering of branching events inferred from the ITS DNA sequences shifts the cumulative frequency distribution to the right of the average distribution for 17-species

Table 1
Kolmogorov-Smirnov D Values and Their Associated Probabilities (P) for Comparisons of Three Empirical Molecular-Based Phylogenies Against Null Models of Temporal Stochasticity in Branching Pattern

Empirical Tree	n	b	D	P
Columbines	17	0.032	0.552	0.0004 $> P > 0.0002$
Cranes	16	0.030	0.556	0.0008 $> P > 0.0006$
<i>D. virilis</i> species group	13	0.024	0.297	0.2362 $> P > 0.2354$

NOTE.— n , number of terminal taxa; b , branching and extinction probability.

trees under the null model. For the cranes, the ancient clustering of branching events inferred from the cytochrome b data shifts the cumulative frequency distribution of normalized branching times to the left of the average distribution for 16-taxon trees under the null model. For the two-dimensional electrophoretic phylog-

eny of the *D. virilis* species group, the cumulative frequency distribution (cdf) of normalized branching times more closely matches the average theoretical distribution for 13-species trees under the null model. The D values calculated from the distributions in figure 4 are presented in table 1.

Comparisons of these D values against those for 5,000 MB&E trees of the same terminal diversity (n) are presented in figure 5. The two phylogenies (columbines and cranes) for which a nonrandom pattern of speciation had been hypothesized displayed large D values that fell in the tails of the respective distributions, indicating a small probability (P) that the branching events observed in these phylogenies are temporally random. For the *D. virilis* species group, the D value fell well within the frequency distribution of D values for 5,000 comparable MB&E trees, with a probability greater than 0.23 that the branching pattern seen in this tree was

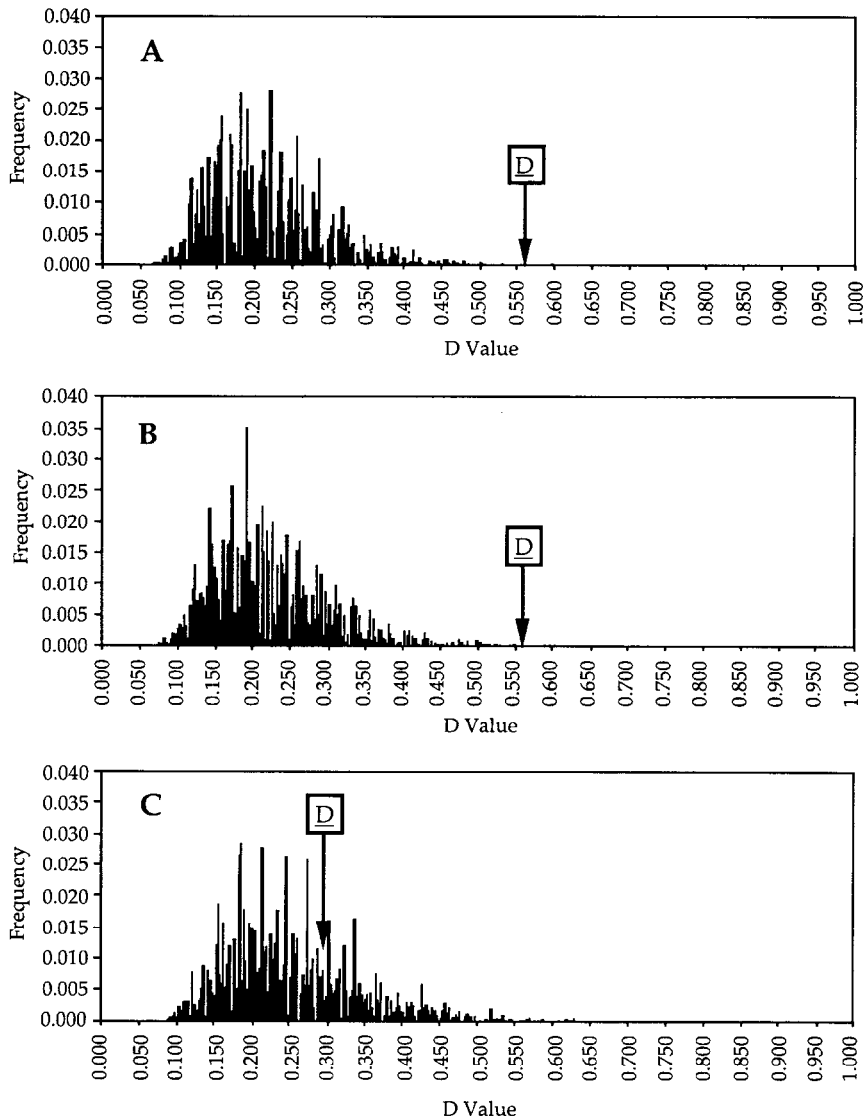


FIG. 5.—Frequency distributions of Kolmogorov-Smirnov D values. (A) 5,000 17-taxon MB&E trees, with the D value for the columbine empirical-to-average comparison indicated. (B) 5,000 16-taxon MB&E trees, with the D value for the crane empirical-to-average comparison indicated. (C) 5,000 13-taxon MB&E trees, with the D value for the *D. virilis* species group empirical-to-average comparison indicated.

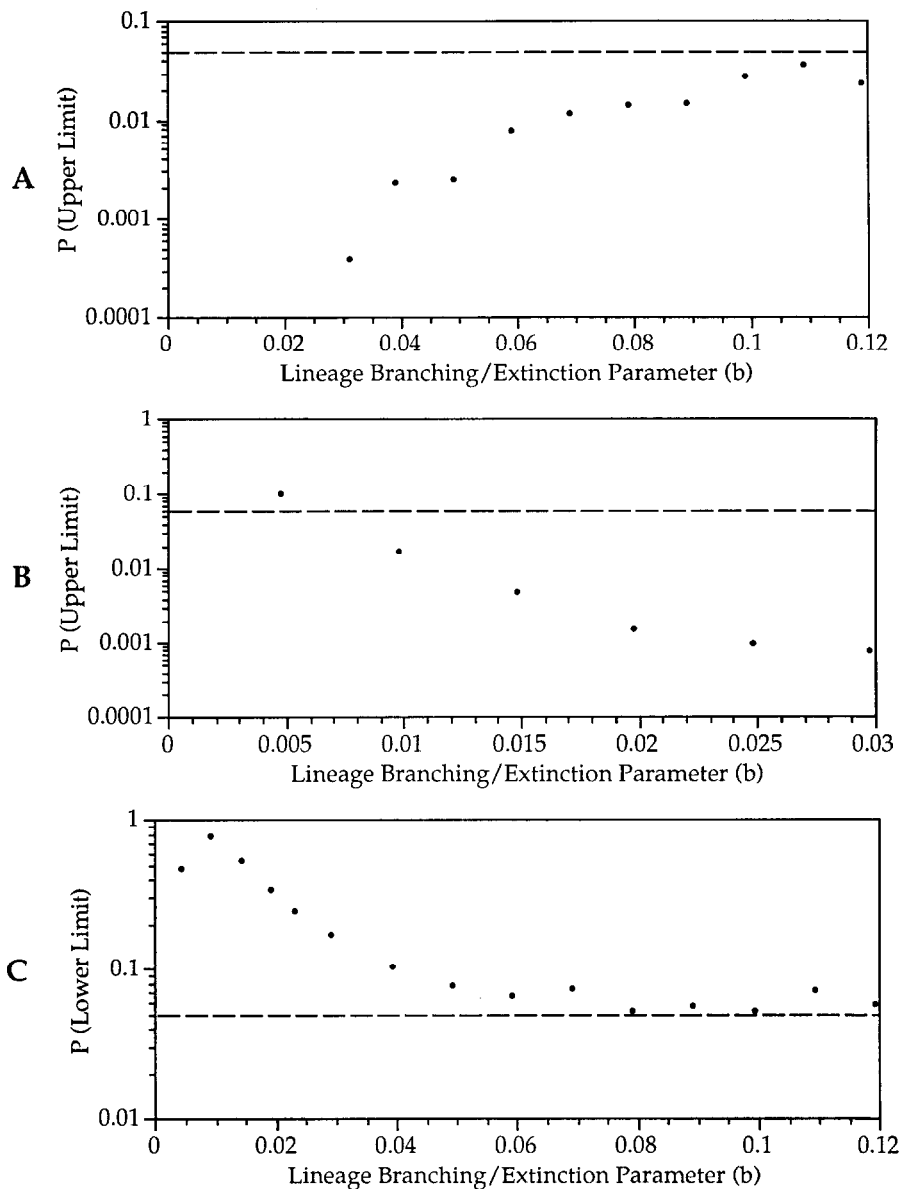


FIG. 6.—Sensitivity analyses for sampled randomization test of D values. Dotted lines indicate $P = 0.05$. (A) Upper bound of P values for the 17-taxon phylogeny of columbines. (B) Upper bound of P values for the 16-taxon phylogeny of cranes. (C) Lower bound of P values for the 13-taxon phylogeny of the *D. virilis* species group.

temporally stochastic. The P values calculated from the frequency distributions in figure 5 are presented in table 1.

Results of sensitivity analyses (variation in P as a function of b) are shown in figure 6. For the columbines, the P values shifted from significant ($P < 0.01$) to marginally significant ($0.05 < P < 0.01$) as b increased. Ten b values were used in this sensitivity analysis: 0.032 (the optimum value), and 0.04, 0.05, . . . , 0.12 (fig. 6A). Results indicate that the placement of nodes in the columbine tree consistently differs with at least marginal statistical significance from that generated by an MB&E process, regardless of the b value assumed. For the cranes, six b values were used: 0.030 (the optimum value), and 0.005, 0.010, . . . , 0.025 (fig. 6B). As b decreased, D decreased also, resulting in an increase in P .

From linear interpolation of the data in figure 6B, the outcome of the statistical test moved from significant ($P < 0.01$) to marginally significant at approximately $b = 0.0125$, and from marginally significant to insignificant ($P > 0.05$) at approximately $b = 0.0055$. For the phylogeny of the *D. virilis* species group, 15 b values were used in the sensitivity analysis: 0.005, 0.010, 0.015, 0.020, 0.024 (the optimum value), and 0.03, 0.04, . . . , 0.12 (fig. 6C). The placement of nodes in this tree did not differ significantly from expectations of the MB&E process regardless of the b value assumed.

To enable researchers to compare other empirical phylogenies against our null models, additional computer simulations were conducted for phylads with 10 to 50 extant species (in increments of five). Tabulated cumulative frequency distributions of branching times,

and associated P values, are presented in appendices 1 and 2, respectively. These appendices describe how to employ these tables to assess the statistical significance of suspected departures in an empirical tree against expectations of the null model of temporally stochastic cladogenesis.

Although we have focused the presentation explicitly on frequency distributions of branching times, in early computer trials the frequency distributions of phylogenetic branch lengths were monitored as well. These proved to yield similar outcomes to those for branching times. Because branch lengths and branch times are partially nonindependent in a given phylogeny, for simplicity only the latter was subsequently employed. Another point about the current models is that they focus exclusively on the temporal positions of nodes in a tree, regardless of where they fall with respect to alternative branches in the tree. In this sense, the current results complement those of previously proposed null tests for symmetry or imbalance in branching structure (Slowinski and Guyer 1989*b*; Kirkpatrick and Slatkin 1993; Rogers 1994; Sanderson and Donoghue 1994; Losos and Adler 1995).

Discussion

In the analysis of phylogenetic trees, evolutionary biologists often formulate causal hypotheses to interpret perceived patterns of lineage branching. Any such conclusions about evolutionary causality must remain merely inferential so long as they are based solely on phylogenetic pattern. Another source of concern, and the one addressed in the current study, is whether a phylogenetic pattern itself can be shown with statistical confidence to depart from expectations of stochasticity in the temporal spacing of speciation and extinction events.

Null Models in Phylogenetic Inference

The computer-based statistical approaches developed here extend the tradition of evolutionary null models introduced more than 2 decades ago by members of the MBL group (Raup et al. 1973). The fundamental assumptions of our null model, shared with those of the MBL modelers, are that speciation and extinction probabilities in an evolutionary phylad are equal, and that speciation and extinction events are temporally stochastic and independent across lineages. One difference of our approach from that of the MBL group is that we restrict our attention to temporal cladogenetic patterns recovered (or inferred) from appraisals of extant taxa. Our reasons for this approach include the following: (1) many data sets employed to estimate phylogeny, including those from DNA and protein comparisons, come solely from extant species; (2) molecular data (in particular) provide a large and growing source of information for phylogenetic reconstruction; and (3) many authors have employed molecular-based phylogenies as a source of inference about evolutionary pattern and process.

The current null models in effect accommodate the branching-pattern biases imposed by molecular data be-

cause the theoretical expectations were derived solely from extant species in the computer simulations. Furthermore, the simulations against which the data sets were evaluated were appropriate in the sense that they equated the numbers of extant species to the particular empirical phylads examined (albeit primarily under specified speciation/extinction rates that maximized the likelihood of given n -taxon trees). For example, the empirical 13-species tree for the *D. virilis* group initially was evaluated against replicate computer simulations that happened to produce 13-species trees (under $b = 0.024$). Of course, this was only a minuscule fraction of the random trees generated under the null model. We interpret this method of comparison as meaning that if the evolutionary play were to be rerun many times independently, the subset of phylogenetic outcomes appropriate as a test for nonrandomness in a given empirical tree would be that which involved the same number of extant taxa. In other words, although there is a low probability that a 13-species tree (or any other) arises during the evolutionary process, given that it has under the speciation/extinction parameter specified, we have addressed the likelihood that the nodes within it are temporally random under the null model.

The current models cannot be expected to capture all aspects of nonrandomness in phylogenetic pattern; other null models and perspectives might be envisioned for related issues. One possibility might be to allow stochastic variation in values of the b parameter used to generate the simulated trees (although this would extend the computational time greatly). Another possibility would be to allow independent but specified variation in speciation and extinction rates; however, such parameter combinations are nearly endless, and each combination would require its own special justification.

When an empirical tree statistically agrees with the current null hypothesis, this does not imply that the underlying evolutionary processes were mechanistically random or chaotic. Every real-life organismal lineage may well have gone extinct, or speciated, for an intelligible causal reason, such as acquisition of a lethal virus or vicariant geographic partitioning, respectively. Rather, when the temporal pattern of branching in an empirical tree agrees with the current null model, this merely implies that the branching pattern could not be statistically differentiated from that expected under a temporally stochastic and lineage-independent process of lineage bifurcation and extinction. Conversely, when an empirical tree statistically departs from the null expectations, this cannot alone be used to determine the particular nature of evolutionary causality underlying a given nonrandom temporal pattern. Rather, statistical significance merely provides an important first step in assessing when additional effort at causal interpretation or hypothesis formulation is justified. For example, columbines display a statistically significant pattern of enhanced recent speciation, but any explanations for this species radiation must come from other sources of information.

The three empirical case histories examined against the null models in the current study illustrate the diver-

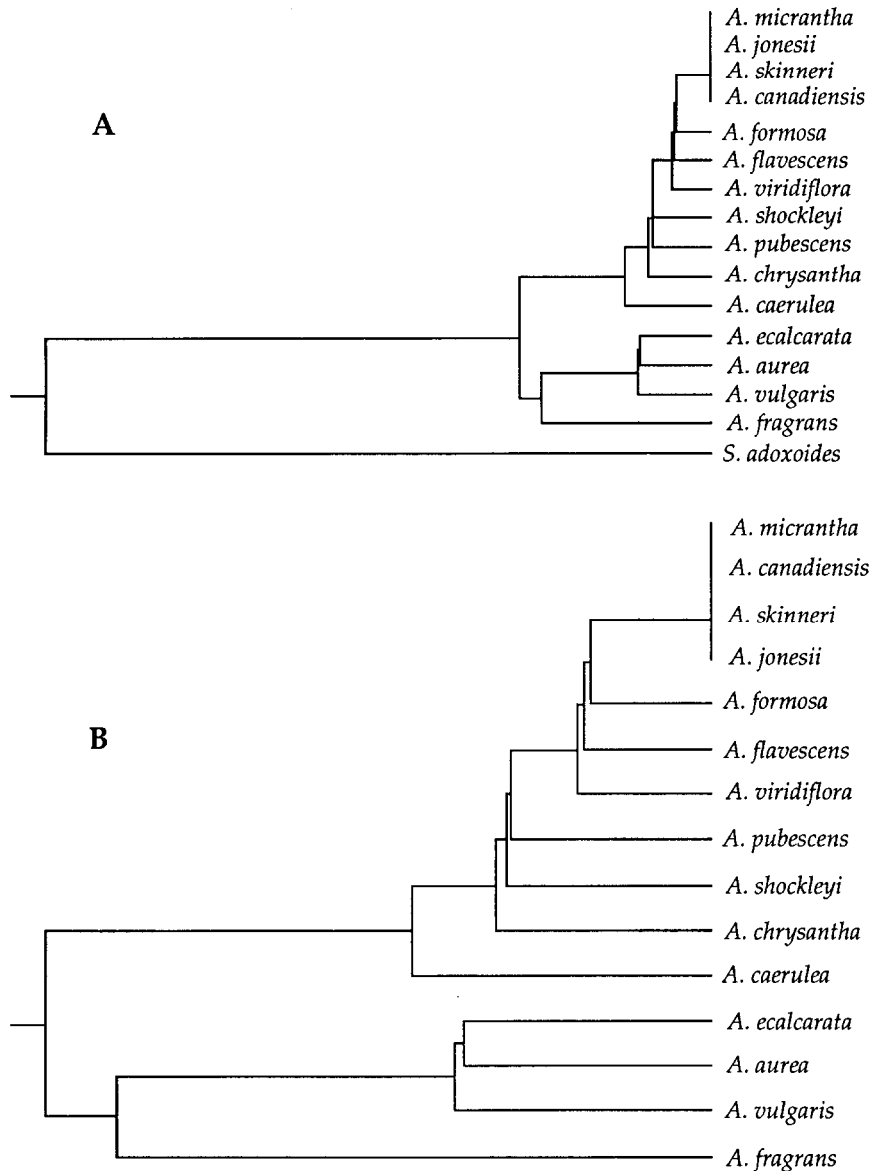


FIG. 7.—KITSCH phylogenies constructed from reduced data sets of nuclear ITS region DNA sequences of columbines. (A) *Aquilegia* species plus *Semiaquilegia adoxoides*. (B) *Aquilegia* species only.

sity of outcomes that might generally be anticipated in statistical tests for temporal nonrandomness of cladogenesis. Generally consistent with the original authors' impressions, the columbines and the cranes do indeed display nonrandom phylogenetic patterns of diversification, with the columbines showing recent and the cranes showing ancient significant clustering of speciation events. These kinds of cladogenetic patterns leave characteristic signatures on the cdf's of branching times that underlie the K-S tests, with early and late radiations shifting the empirical cdf's to the left and right, respectively, of the null cdf's (fig. 4). Furthermore, evident "jumps" in an empirical cdf (e.g., fig. 4B) clearly reveal the temporal positions of clustered nodes that may have contributed to a significant *D* value. Also consistent with the original authors' interpretation were the results for the *D. virilis* species group, where no evidence for tem-

poral nonrandomness in phylad diversification could be identified in statistical evaluations against the null model.

Another point about the current statistical tests is exemplified by the crane phylogeny. The "jump" in the empirical cdf which contributes most strongly to significance of the K-S statistic (region of the *D* value in fig. 4B) stems from the close temporal placement of nodes in four separate lineages of the original phylogeny (fig. 2B). Clearly, this pattern does not conform to what traditionally might be interpreted to reflect an adaptive radiation. By contrast, an earlier "jump" in the crane cdf (fig. 4B) *does* result from close temporal placement of nodes among near descendants of a single lineage, in a pattern more consistent with the conventional meaning of a species radiation. Thus, the current tests merely assess whether nodes are temporally grouped, and ad-

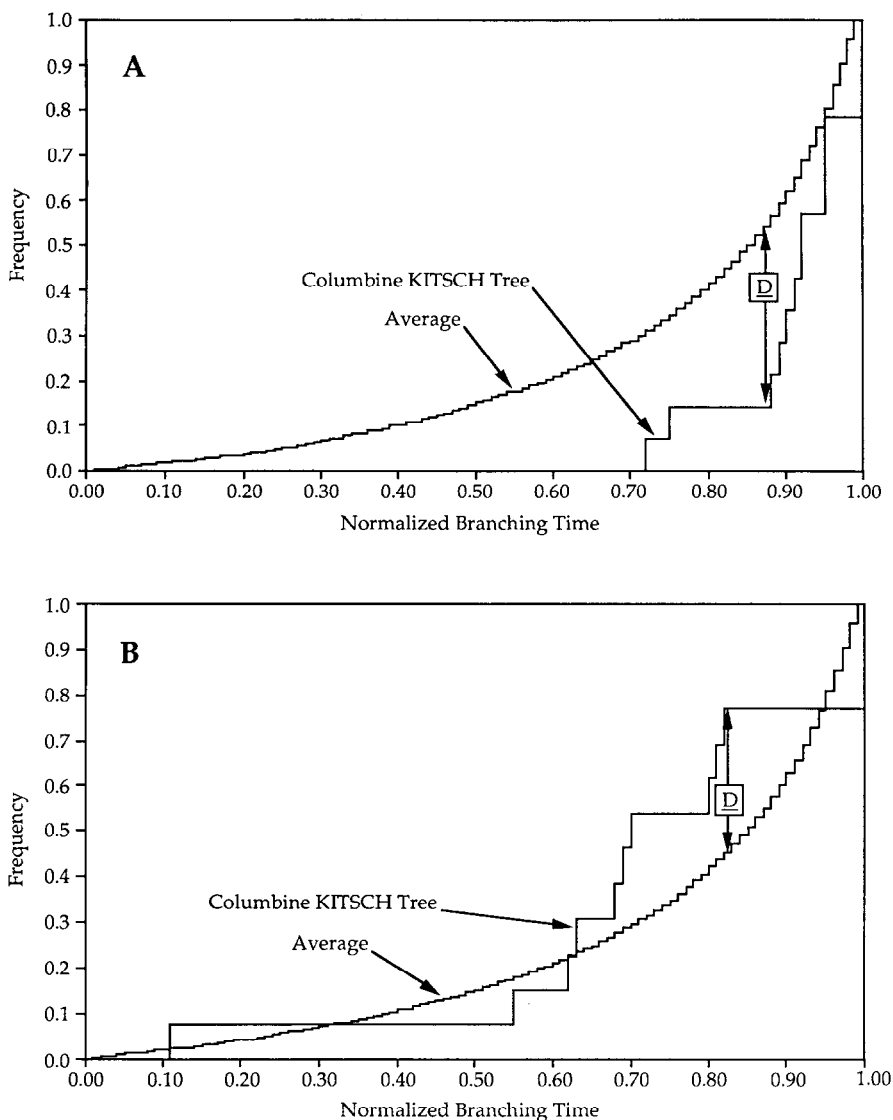


FIG. 8.—Cumulative frequency distributions of normalized branching times for the KITSCH trees of columbine truncated data sets. The largest difference D between the empirical cdf and the average cdf is indicated. (A) *Aquilegia* + *Semiaquilegia*, and for the average of 500 16-taxon MB&E trees. (B) *Aquilegia* species only, and for the average of 500 15-taxon MB&E trees.

ditional inspection is required to determine where these nodes occur among the various lineages of a phylogenetic tree.

Phylogenetic Frames of Reference

As is true also in the use of symmetry criteria for assessing statistical nonrandomness in clades, the phylogenetic frame of reference is crucial. Consider, for example, the columbine case (fig. 1), for which our tests provide statistical support for a relatively recent proliferation of species. However, this conclusion stems in large part from the frame of reference provided by the outgroups *S. adoxoides* and *I. biternatum*, because long branch lengths lead from the ancestral nodes for these taxa to the *Aquilegia* complex. Suppose instead that the question were rephrased as follows: Over the time frame of differentiation within particular subsets of the columbine complex, such as within *Aquilegia* itself, is there

statistical support for temporal nonrandomness in the placement of phylogenetic nodes?

To address this question, we considered phylogenies generated from two truncated columbine data sets that exclude one or both outgroups (fig. 7). Comparison of these phylogenies against the *Aquilegia* + *Semiaquilegia* + *Isopyrum* phylogeny of figure 3B shows how the temporal distribution of branching events in these cases appears to become more uniformly distributed over the tree as the earlier branching points are removed from the phylogeny. For these truncated trees, now rescaled beginning with the new initial branching event, cumulative frequency distributions of branching times were generated (fig. 8), and D values and probabilities (P) were calculated (table 2). The distributions of D values used to calculate these probabilities are shown in figure 9. As the phylogenies were successively truncated, the probability that the temporal pattern of branching

Table 2
Kolmogorov-Smirnov D Values and Their Associated Probabilities (P) for Comparisons of the Two Reduced Columbine Phylogenies Against Null Models of Temporal Stochasticity in Branching Pattern

Empirical Tree	n	b	D	P
<i>Aquilegia</i> + <i>Semiaquilegia</i>	16	0.030	0.400	0.0216
<i>Aquilegia</i> only	15	0.028	0.314	0.1398

NOTE.— n , number of terminal taxa; b , branching and extinction probability.

could have been generated under the relevant null model went from the original highly significant outcome ($P < 0.001$), to marginally significant ($P = 0.02$), to nonsignificant ($P = 0.14$).

Such dependency of statistical outcome on the phylogenetic frame of reference need not be viewed as either a blessing or a curse. With respect to the broader perspective provided by the outgroups, diversification within the columbines can be said to be recent and non-

random in time, but with respect to speciations within *Aquilegia* itself, the data are not yet sufficient to refute the null hypothesis of a stochastic temporal spacing of nodes over the time frame spanning the first *Aquilegia* node to the present. In general, however, one must use caution in interpreting results of statistical tests for phylogenetic nonrandomness, taking into account frame-of-reference considerations. For example, when a distantly related nonsister taxon is used as an outgroup, the temporal pattern of branching events in a taxonomic assemblage of interest will tend to be artificially compressed, and departures from the current null models will tend to be more likely. Conversely, failure to identify important nonrandom patterns in a phylogenetic assemblage can result from too narrow a focus on particular taxa, as was the case for the *Aquilegia* species when considered alone.

Incomplete Sampling of Extant Taxa

It has been demonstrated also that the sampling of terminal taxa can affect the apparent distribution of di-

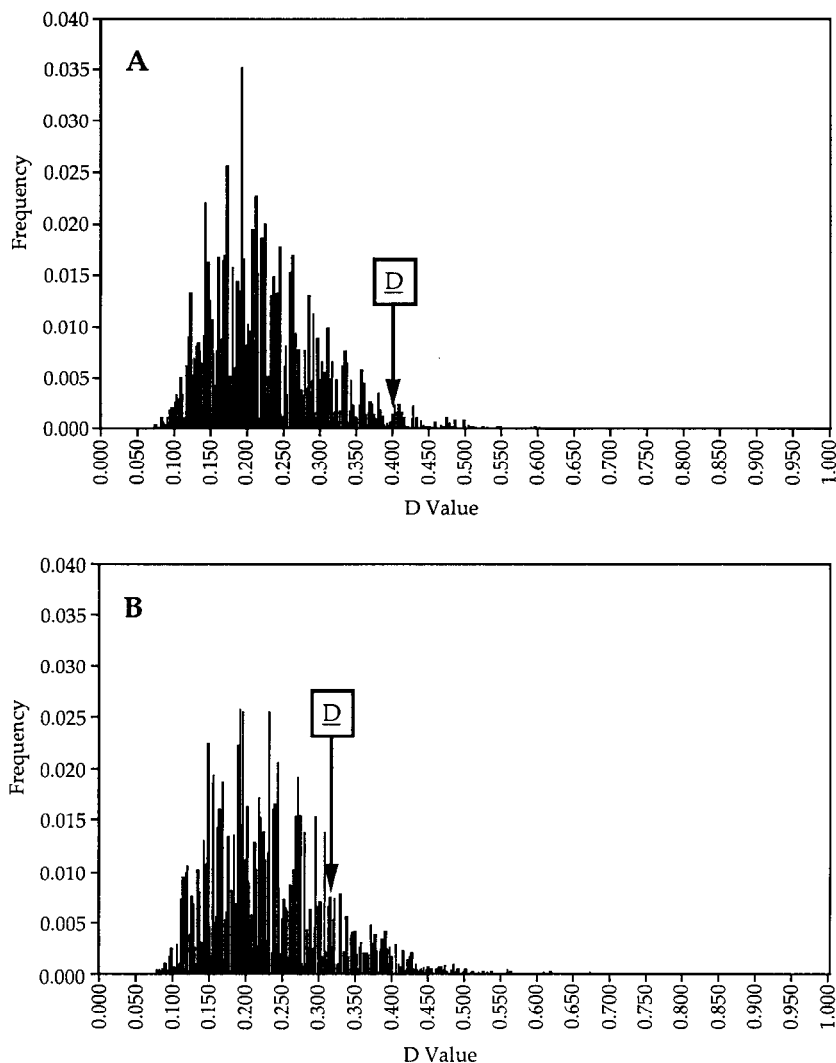


FIG. 9.—Frequency distribution of Kolmogorov-Smirnov D values. (A) 5,000 16-taxon MB&E trees, with the D value for the columbine truncated data set (*Aquilegia* + *Semiaquilegia*) empirical-to-average comparison indicated. (B) 5,000 15-taxon MB&E trees, with the D value for the columbine truncated data set (*Aquilegia* only) empirical-to-average comparison indicated.

versity (Harvey, May, and Nee 1994; Mooers 1995). The effects of incomplete sampling will be expected to bias the outcomes of the statistical tests developed here to the extent that they may bias the distributions of scored nodes along the temporal axis. In the one analysis presented here in which the phylogeny did not include all extant species (the columbines), it is not expected that a more complete sampling of taxa would appreciably change the results. All of the *Aquilegia* species present in this analysis fall into two clades, one containing Eurasian and the other containing North American species (Hodges and Arnold 1995). Assuming that the unsampled species of *Aquilegia* branched off subsequent to the divergence of these two clades, the addition of more terminal taxa will add more short internodes to the recent portion of the tree, a factor that would serve to further skew the distribution of normalized branching times to the right (with reference to the more distant outgroups) and thereby increase the Kolmogorov-Smirnov D calculated for this phylogeny. A larger D means a lower probability that the branching pattern seen in the *Aquilegia* phylogeny was the result of an MB&E process. In general, however, the possible effects of incomplete sampling of extant taxa will have to be considered on a case-by-case basis, and again with frame of reference taken into account.

Sensitivity Analysis

Each b value initially employed was that which led to the highest probability of occurrence for a tree of given diversity, and in this sense can be considered to provide the epitome of the “null” model. While biological history may not have followed the most probable course, for the purposes of testing a null hypothesis some such reasonable parameterization is necessary. However, different b values certainly can be envisioned, and our sensitivity analyses suggest that these may sometimes lead to different statistical outcomes in the simulations and tests. We have found that increases in b result in increases in the curvature of the cdf, and vice versa, such that for the columbines and cranes, increases and decreases, respectively, in b led to increases in the calculated P values. For the *D. virilis* species group, an increase in b led to a decrease in P , which nonetheless did not change the qualitative outcome of the test. Thus, although the exact magnitudes of departure from MB&E models are somewhat labile with regards to the speciation/extinction parameter assumed, these certainly do not invariably alter the statistical outcome. However, any refutation of the null hypothesis under the optimal b value should of course be interpreted only as a provisional indication that an MB&E process has not occurred.

Times of Speciation

Statistical evaluations of empirical trees against the current Markovian models require the availability of absolute or relative temporal placements of nodes as inferred from data on extant species. Molecular data may be especially well suited to this empirical task, particularly if approximate molecular clocks apply to the taxonomic groups under consideration (or if departures

from clocklike behavior are accommodated properly in the phylogeny estimations). However, phylogenetic reconstructions based on other classes of information from extant species might be employed as well, provided that the requisite temporal placements of nodes can be specified. Needless to say, outcomes of our statistical tests can be used to make proper inferences about evolutionary pattern only to the extent that the empirical phylogenetic reconstructions themselves are reliable.

Summary

We have developed a statistical procedure for assessing the probability that an observed phylogenetic tree for extant taxa reflects stochastic, independent variation in the temporal pattern of branching and extinction across lineages. The procedure involves use of a Markovian null model of phylogenetic diversification, employed in conjunction with established statistical methods including the Kolmogorov-Smirnov D statistic and a sampled randomization test. In the future, it may be possible to derive analytical solutions for the expected distributions of branching times and their variances under a null model. Such quantitative, statistical approaches provide an important first step in the consideration of hypotheses about evolutionary process that might be inferred from phylogenetic pattern.

Acknowledgments

Work was supported by an NSF grant and University of Georgia funds to J.C.A. We thank Mike Arnold, Daniel Promislow, and members of the Avise laboratory for constructive comments on the manuscript, Scott Hodges and Carey Krajewski for providing DNA sequences and alignments, and Chris Basten for assistance in computer programming.

APPENDIX 1

Cumulative frequency distributions of normalized branching times. These values are the average of 500 MB&E trees with the specified number of terminal taxa (n). The probability of branching (equal to probability of lineage extinction) used to generate these trees was that which maximized the probability of a tree having n taxa after 250 program cycles.

To assess the statistical significance of suspected departures from random temporal spacing of cladogenetic events in any empirical tree for which nodal times can be estimated, proceed as follows. (1) Standardize the scale of the empirical tree from zero to one. (2) Plot a frequency histogram of the scaled branching times for all nodes in the tree. (3) Convert this frequency distribution to a cumulative frequency distribution (Sokal and Rohlf 1995, pp. 435–436). (4) Compare that cdf with the appropriate cdf in the table below (this may require interpolation when numbers of extant taxa in the empirical tree are other than those heading the columns in the table), using the Kolmogorov-Smirnov test (Sokal and Rohlf 1995, pp. 708–715). (5) Refer to appendix 2 to determine the probability associated with the Kolmogorov-Smirnov test statistic D .

Appendix Table 1

NORMALIZED BRANCHING TIMES	NUMBER OF TAXA								
	10	15	20	25	30	35	40	45	50
0.01	0.0028	0.0038	0.0022	0.0021	0.0019	0.0017	0.0011	0.0008	0.0007
0.02	0.0048	0.0058	0.0033	0.0033	0.0034	0.0024	0.0017	0.0011	0.0016
0.03	0.0070	0.0085	0.0051	0.0043	0.0041	0.0030	0.0022	0.0015	0.0023
0.04	0.0093	0.0105	0.0072	0.0057	0.0046	0.0036	0.0033	0.0020	0.0028
0.05	0.0138	0.0118	0.0087	0.0070	0.0058	0.0045	0.0039	0.0027	0.0033
0.06	0.0165	0.0142	0.0104	0.0083	0.0069	0.0052	0.0048	0.0032	0.0041
0.07	0.0183	0.0162	0.0119	0.0096	0.0079	0.0064	0.0054	0.0040	0.0045
0.08	0.0218	0.0175	0.0127	0.0101	0.0091	0.0075	0.0062	0.0049	0.0049
0.09	0.0258	0.0200	0.0141	0.0120	0.0099	0.0088	0.0070	0.0059	0.0055
0.10	0.0298	0.0215	0.0158	0.0134	0.0111	0.0100	0.0081	0.0069	0.0058
0.11	0.0325	0.0232	0.0171	0.0158	0.0120	0.0110	0.0090	0.0076	0.0069
0.12	0.0353	0.0245	0.0193	0.0177	0.0127	0.0124	0.0097	0.0087	0.0077
0.13	0.0375	0.0269	0.0212	0.0187	0.0139	0.0134	0.0105	0.0096	0.0080
0.14	0.0403	0.0286	0.0227	0.0204	0.0151	0.0145	0.0114	0.0100	0.0085
0.15	0.0450	0.0309	0.0250	0.0217	0.0167	0.0155	0.0129	0.0107	0.0091
0.16	0.0483	0.0331	0.0266	0.0231	0.0181	0.0164	0.0138	0.0115	0.0100
0.17	0.0533	0.0358	0.0288	0.0244	0.0198	0.0179	0.0144	0.0124	0.0107
0.18	0.0575	0.0389	0.0314	0.0262	0.0209	0.0188	0.0157	0.0132	0.0112
0.19	0.0610	0.0409	0.0330	0.0271	0.0219	0.0198	0.0169	0.0137	0.0121
0.20	0.0648	0.0431	0.0349	0.0288	0.0241	0.0210	0.0181	0.0147	0.0135
0.21	0.0685	0.0451	0.0359	0.0298	0.0257	0.0222	0.0191	0.0152	0.0141
0.22	0.0733	0.0482	0.0380	0.0319	0.0275	0.0233	0.0203	0.0157	0.0147
0.23	0.0798	0.0509	0.0400	0.0337	0.0286	0.0248	0.0211	0.0170	0.0154
0.24	0.0850	0.0542	0.0427	0.0353	0.0300	0.0261	0.0218	0.0179	0.0163
0.25	0.0900	0.0572	0.0444	0.0377	0.0311	0.0275	0.0226	0.0187	0.0173
0.26	0.0933	0.0608	0.0473	0.0390	0.0326	0.0288	0.0238	0.0194	0.0183
0.27	0.0963	0.0640	0.0494	0.0409	0.0338	0.0301	0.0251	0.0206	0.0192
0.28	0.1008	0.0665	0.0517	0.0432	0.0356	0.0317	0.0261	0.0216	0.0203
0.29	0.1045	0.0700	0.0538	0.0450	0.0371	0.0332	0.0271	0.0231	0.0211
0.30	0.1083	0.0726	0.0576	0.0465	0.0388	0.0347	0.0286	0.0244	0.0224
0.31	0.1125	0.0765	0.0599	0.0491	0.0412	0.0361	0.0298	0.0256	0.0240
0.32	0.1163	0.0797	0.0623	0.0505	0.0431	0.0376	0.0318	0.0267	0.0248
0.33	0.1203	0.0823	0.0643	0.0525	0.0450	0.0387	0.0332	0.0279	0.0263
0.34	0.1253	0.0852	0.0669	0.0548	0.0475	0.0408	0.0346	0.0288	0.0273
0.35	0.1283	0.0888	0.0706	0.0573	0.0497	0.0421	0.0365	0.0301	0.0289
0.36	0.1333	0.0925	0.0730	0.0590	0.0517	0.0437	0.0385	0.0313	0.0300
0.37	0.1388	0.0955	0.0758	0.0620	0.0532	0.0455	0.0402	0.0327	0.0308
0.38	0.1440	0.1000	0.0778	0.0641	0.0554	0.0469	0.0415	0.0339	0.0323
0.39	0.1493	0.1054	0.0808	0.0658	0.0576	0.0481	0.0429	0.0350	0.0339
0.40	0.1535	0.1094	0.0846	0.0690	0.0598	0.0511	0.0447	0.0369	0.0365
0.41	0.1588	0.1131	0.0878	0.0717	0.0618	0.0532	0.0470	0.0383	0.0383
0.42	0.1658	0.1172	0.0911	0.0748	0.0646	0.0552	0.0489	0.0399	0.0406
0.43	0.1720	0.1222	0.0951	0.0773	0.0677	0.0570	0.0502	0.0420	0.0425
0.44	0.1770	0.1251	0.0983	0.0807	0.0706	0.0589	0.0518	0.0445	0.0438
0.45	0.1840	0.1292	0.1024	0.0834	0.0732	0.0612	0.0537	0.0468	0.0453
0.46	0.1885	0.1335	0.1064	0.0864	0.0762	0.0641	0.0559	0.0486	0.0475
0.47	0.1928	0.1374	0.1104	0.0892	0.0794	0.0662	0.0582	0.0507	0.0500
0.48	0.1988	0.1432	0.1138	0.0923	0.0822	0.0690	0.0604	0.0523	0.0525
0.49	0.2058	0.1466	0.1161	0.0958	0.0855	0.0710	0.0625	0.0539	0.0538
0.50	0.2140	0.1529	0.1214	0.1003	0.0892	0.0738	0.0655	0.0558	0.0560
0.51	0.2210	0.1575	0.1256	0.1047	0.0922	0.0761	0.0679	0.0576	0.0578
0.52	0.2258	0.1634	0.1301	0.1080	0.0955	0.0791	0.0705	0.0602	0.0606
0.53	0.2328	0.1688	0.1339	0.1119	0.0976	0.0813	0.0739	0.0628	0.0636
0.54	0.2398	0.1737	0.1381	0.1165	0.1008	0.0853	0.0765	0.0652	0.0665
0.55	0.2458	0.1805	0.1427	0.1218	0.1039	0.0879	0.0792	0.0684	0.0690
0.56	0.2513	0.1855	0.1479	0.1267	0.1084	0.0910	0.0823	0.0708	0.0723
0.57	0.2605	0.1918	0.1530	0.1324	0.1128	0.0950	0.0849	0.0746	0.0753
0.58	0.2673	0.1991	0.1588	0.1370	0.1168	0.0989	0.0885	0.0769	0.0780
0.59	0.2755	0.2049	0.1646	0.1417	0.1214	0.1031	0.0919	0.0799	0.0805
0.60	0.2843	0.2115	0.1706	0.1470	0.1267	0.1066	0.0958	0.0835	0.0840
0.61	0.2935	0.2212	0.1773	0.1513	0.1326	0.1105	0.0999	0.0866	0.0878
0.62	0.3050	0.2266	0.1829	0.1557	0.1378	0.1152	0.1033	0.0898	0.0927
0.63	0.3165	0.2363	0.1900	0.1608	0.1430	0.1198	0.1075	0.0935	0.0967
0.64	0.3263	0.2437	0.1969	0.1665	0.1499	0.1248	0.1116	0.0977	0.1016
0.65	0.3363	0.2508	0.2031	0.1725	0.1565	0.1298	0.1169	0.1027	0.1053
0.66	0.3453	0.2592	0.2117	0.1784	0.1631	0.1352	0.1218	0.1081	0.1097
0.67	0.3543	0.2678	0.2184	0.1864	0.1699	0.1404	0.1275	0.1126	0.1139
0.68	0.3668	0.2778	0.2280	0.1949	0.1763	0.1482	0.1327	0.1161	0.1189

Appendix Table 1
Continued

NORMALIZED BRANCHING TIMES	NUMBER OF TAXA								
	10	15	20	25	30	35	40	45	50
0.69	0.3793	0.2871	0.2354	0.2021	0.1846	0.1550	0.1382	0.1207	0.1233
0.70	0.3903	0.2952	0.2451	0.2092	0.1913	0.1619	0.1435	0.1264	0.1297
0.71	0.4040	0.3068	0.2543	0.2183	0.1987	0.1688	0.1486	0.1321	0.1356
0.72	0.4148	0.3149	0.2652	0.2279	0.2051	0.1760	0.1566	0.1382	0.1414
0.73	0.4293	0.3252	0.2760	0.2379	0.2151	0.1844	0.1628	0.1438	0.1469
0.74	0.4435	0.3366	0.2852	0.2455	0.2244	0.1908	0.1699	0.1504	0.1528
0.75	0.4590	0.3502	0.2959	0.2564	0.2355	0.1995	0.1785	0.1592	0.1595
0.76	0.4733	0.3642	0.3071	0.2683	0.2459	0.2086	0.1864	0.1674	0.1663
0.77	0.4895	0.3788	0.3203	0.2812	0.2556	0.2170	0.1962	0.1753	0.1745
0.78	0.5048	0.3931	0.3347	0.2929	0.2669	0.2280	0.2069	0.1849	0.1834
0.79	0.5158	0.4063	0.3476	0.3050	0.2786	0.2388	0.2159	0.1931	0.1934
0.80	0.5285	0.4212	0.3600	0.3188	0.2915	0.2526	0.2260	0.2061	0.2053
0.81	0.5415	0.4371	0.3728	0.3327	0.3066	0.2652	0.2387	0.2180	0.2151
0.82	0.5553	0.4552	0.3889	0.3484	0.3202	0.2791	0.2518	0.2310	0.2273
0.83	0.5743	0.4734	0.4078	0.3661	0.3360	0.2941	0.2654	0.2444	0.2411
0.84	0.5920	0.4892	0.4294	0.3837	0.3519	0.3103	0.2796	0.2585	0.2574
0.85	0.6160	0.5089	0.4479	0.4030	0.3700	0.3279	0.2957	0.2745	0.2720
0.86	0.6348	0.5302	0.4689	0.4226	0.3896	0.3468	0.3163	0.2937	0.2897
0.87	0.6558	0.5512	0.4944	0.4436	0.4103	0.3684	0.3372	0.3149	0.3101
0.88	0.6755	0.5748	0.5194	0.4676	0.4321	0.3916	0.3614	0.3386	0.3320
0.89	0.6995	0.6017	0.5439	0.4967	0.4574	0.4168	0.3862	0.3625	0.3542
0.90	0.7260	0.6271	0.5733	0.5269	0.4893	0.4470	0.4139	0.3920	0.3818
0.91	0.7520	0.6578	0.6037	0.5600	0.5203	0.4792	0.4456	0.4244	0.4120
0.92	0.7790	0.6920	0.6416	0.5942	0.5550	0.5145	0.4811	0.4618	0.4507
0.93	0.8025	0.7292	0.6781	0.6336	0.5953	0.5558	0.5205	0.5036	0.4920
0.94	0.8333	0.7682	0.7182	0.6758	0.6442	0.6053	0.5703	0.5519	0.5402
0.95	0.8660	0.8111	0.7682	0.7254	0.6980	0.6644	0.6299	0.6118	0.5992
0.96	0.8998	0.8537	0.8169	0.7825	0.7588	0.7278	0.7027	0.6855	0.6694
0.97	0.9350	0.9049	0.8761	0.8537	0.8356	0.8115	0.7927	0.7803	0.7658
0.98	0.9725	0.9583	0.9438	0.9316	0.9219	0.9097	0.9004	0.8979	0.8872
0.99	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.00	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

APPENDIX 2

Probabilities P of Kolmogorov-Smirnov D values for MB&E phylogenies. These values are based on the frequency distribution of D values for 5,000 MB&E phylogenies.

To assess the probability P that the temporal spacings of cladogenetic events in an empirical tree are more

divergent from the average than those of a phylogeny generated by a Markovian branching and extinction process, proceed as follows. (1) Look up the D value calculated using appendix 1 in the table above. (2) Read across the table to find the P value for the appropriate diversity of the empirical tree (this may require interpolation when numbers of extant taxa in the empirical tree are other than those heading the columns of the table).

Appendix Table 2

D VALUE	NUMBER OF TAXA								
	10	15	20	25	30	35	40	45	50
0.01	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.02	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.03	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.04	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.05	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9998	0.9996	1.0000
0.06	1.0000	1.0000	1.0000	1.0000	0.9960	0.9996	0.9962	0.9978	0.9916
0.07	1.0000	1.0000	0.9998	0.9962	0.9860	0.9954	0.9818	0.9796	0.9736
0.08	1.0000	0.9996	0.9996	0.9830	0.9678	0.9604	0.9574	0.9338	0.9124
0.09	1.0000	0.9986	0.9936	0.9558	0.9206	0.9364	0.8992	0.8872	0.8632
0.10	1.0000	0.9930	0.9860	0.9332	0.8622	0.8872	0.8108	0.7674	0.8042
0.11	1.0000	0.9878	0.9642	0.8670	0.7916	0.7662	0.7450	0.7232	0.7420
0.12	0.9994	0.9598	0.9320	0.8034	0.7364	0.7312	0.6548	0.6152	0.6484
0.13	0.9980	0.9308	0.8946	0.7420	0.6390	0.6514	0.5754	0.5632	0.5644
0.14	0.9924	0.9060	0.8274	0.6752	0.5746	0.5342	0.4786	0.4612	0.4652
0.15	0.9838	0.8500	0.7798	0.6058	0.5120	0.4996	0.3984	0.3988	0.4098

Appendix Table 2
Continued

<i>D</i> VALUE	NUMBER OF TAXA								
	10	15	20	25	30	35	40	45	50
0.84	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.85	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.86	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.87	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.88	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.89	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.90	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.91	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.92	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.93	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.94	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.95	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.96	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.97	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.98	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.99	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

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- FRED W. ALLENDORF, reviewing editor

Accepted April 8, 1996