Evolution, 53(3), 1999, pp. 732–744

PHYLOGENETIC ANALYSIS OF TRAIT EVOLUTION AND SPECIES DIVERSITY VARIATION AMONG ANGIOSPERM FAMILIES

MICHAEL E. DODD,[1] JONATHAN SILVERTOWN,[1,2] AND MARK W. CHASE[3]
[1]*Ecology and Conservation Research Group, Biology Department, Open University, Milton Keynes MK7 6AA, United Kingdom*
[3]*Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, United Kingdom*

Abstract.—Angiosperm families differ greatly from one another in species richness (*S*). Previous studies have attributed significant components of this variation to the influence of pollination mode (biotic/abiotic) and growth form (herbaceous/woody) on speciation rate, but these results suffer difficulties of interpretation because all the studies ignored the phylogenetic relationships among families. We use a molecular phylogeny of the angiosperm families to reanalyse correlations between *S* and family-level traits and use reconstructions of trait evolution to interpret the results. We confirm that pollination mode and growth form are correlated with *S* and show that the majority of changes in pollination mode involved a change from biotic to abiotic pollination with an associated fall in speciation rate. The majority of growth form changes involved the evolution of herbaceousness from woodiness with a correlated rise in speciation rate. We test the hypothesis of Ricklefs and Renner (1994) that "evolutionary flexibility" rather than other trait changes triggered increased speciation rates in some families, but find little support for the hypothesis.

Key words.—Angiosperm phylogeny, dispersal, growth form, key innovations, pollination, species diversity, trait evolution.

Received July 31, 1998. Accepted January 25, 1999.

Traits evolve and themselves influence the rate of evolution. From this reciprocal relationship arises the idea that certain "key innovations" in the evolution of life have unlocked new adaptive zones in which species have proliferated at an increased rate (Maynard Smith and Szathmáry 1995). If this model of the evolutionary process is correct, then among extant taxa one would expect to find that certain clades are much more species rich than others and that this richness correlates with the presence of traits that influence speciation and extinction. Among plants, the huge difference in species richness between the angiosperms (> 250,000 spp.) and the gymnosperms (~ 758 spp.) has long been attributed to the key innovation represented by the evolution of the flower (Stebbins 1981). Within the angiosperms there is great variation in species richness between different clades and there has been considerable recent debate as to which traits are responsible for such differences, both within families (Berenbaum 1983; Herrera 1989; Eriksson and Bremer 1991; Farrell et al. 1991; Hodges and Arnold 1995; Sanderson and Wojciechowski 1996) and between them (Herrera 1989; Fleming 1990; Midgley and Bond 1991; Eriksson and Bremer 1992; Ricklefs and Renner 1994; Tiffney and Mazer 1995).

Studies attempting to account for between-family variation in plant species richness (*S*) or, in the case of Erikson and Bremer (1992), diversification rate ($R = \ln S/t$, where t is the age of the family) have concentrated on three traits as explanatory variables: seed dispersal mode (biotic vs. abiotic), pollination mode (biotic vs. abiotic), and growth form (e.g., herbs vs. woody plants). Making the assumption that allopatric speciation is predominant, it has been suggested that biotic pollination and biotic seed dispersal both promote the reproductive isolation necessary for allopatric speciation to occur because animal pollinators can be species specific in pollen transfer and because animal dispersers can deposit seeds in remote locations (for a review, see Ricklefs and Renner 1994). The herbaceous growth form is expected to be associated with lower generation times and more ephemeral populations, both of which could increase speciation rate (Eriksson and Bremer 1992).

In a sample of 147 angiosperm families Eriksson and Bremer (1992) found a positive relationship between both animal pollination and herbaceous growth form and *R*, but no relationship with animal dispersal. Herrera (1989) and Fleming (1991) also obtained negative results for family-level correlations between *S* and animal dispersal. Tiffney and Mazer (1995) divided a dataset of 383 families into herbaceous and woody subsets and found that although there was no relationship between dispersal mode and *S* for the families overall, there were significant and opposing relationships between these variables among woody and herbaceous families. Animal dispersal was positively correlated with *S* among woody dicots, but showed the reverse relationship among herbaceous families. Using a modification of Eriksson and Bremer's (1992) dataset as well as a much larger dataset of their own, Ricklefs and Renner (1994) confirmed the significant relationships of *S* (and *R*) with animal pollination and herbaceousness and the lack of a relationship with animal dispersal. Although significant, they found the pollination-S relationship to be weak and reported that the families with the greatest diversities of all were those containing both biotic and abiotic dispersal modes or both woody and herbaceous growth forms. They concluded that animal pollination, animal dispersal, and herbaceous growth form were *not* primarily responsible for high values of familial species richness, but that "evolutionary flexibility" giving rise to within-family variation in these traits was the main determinant of S. If true, the presence of these traits could be a consequence of high species richness rather than a cause of it. As stated, this hypothesis is untestable because there is no independent measure of "evolutionary flexibility." We propose an indirect test below.

Although any comparative study of species diversities

[2] Corresponding author. E-mail: j.silvertown@open.ac.uk.

among families should be based upon a phylogeny for the taxa as a whole (Sanderson and Donoghue 1996), none of the family-level analyses so far published have used this approach. There are four main problems with the nonphylogenetic approach that make the results reported difficult to interpret: (1) families (or indeed any other taxa) cannot be treated as though they are statistically independent of one another and assuming they *are* independent gives rise to inflated degrees of freedom in any statistical test (Harvey and Pagel 1991); (2) family species richness is correlated with family age (Erikson and Bremer 1992), but this confounding of variables cannot be solved by using R in place of S because accurate information on the age of angiosperm families is lacking (Ricklefs and Renner 1994); the problem can be solved by comparing sister taxa, which are by definition of equal age; (3) family circumscriptions are artificial, so correlations among variables may be based on values at arbitrarily chosen nodes in the phylogeny; for example, by examining the phylogeny of *Astragalus*, Sanderson and Wojciechowski (1996) found that the very high species diversity of this genus is not unique, but rather a characteristic of a larger clade of which *Astragalus* is a member; and (4) without the historical reconstruction of trait evolution that phylogeny provides, it is impossible to distinguish likely cause and effect among traits. This is particularly a problem in any study of the traits associated with species richness because more species-rich clades are inherently likely to display a greater range of traits than species-poor ones. Thus, without any historical reconstruction of trait evolution, the relationship between traits and species richness is at best ambiguous and at worst may appear circular, and it is impossible to test whether the evolution of certain traits or trait combinations are prerequisites of diversification, as suggested by Eriksson and Bremer (1992) and others, or consequences of it, as suggested by the hypothesis of Ricklefs and Renner (1994).

Although many of the authors of the nonphylogenetic studies cited were aware of some of these problems and took steps to test for the effect of some of them, only the use of a phylogeny can obviate the problems. In this paper we apply a molecular phylogeny of the angiosperm families to the analysis of the dataset compiled by Ricklefs and Renner (1994) to reexamine the relationships they proposed between the three plant traits and familial species richness. Specifically, we ask: (1) Is species richness significantly higher in predominantly animal-pollinated clades than in sister clades lacking animal pollination? (2) Is species richness significantly higher in predominantly animal-dispersed clades than in sister clades lacking animal dispersal? (Because Tiffney and Mazer [1995] found opposing effects among herbs and woody families, we performed our test for the whole phylogeny and also for herbaceous and woody clades separately.) (3) Is species richness significantly higher in predominantly herbaceous clades than in sister clades in which herbs are absent? (4) Is species richness significantly higher in families containing two modes of pollination or seed dispersal or two growth forms than in sister families containing only a biotic mode or only herbs?

By comparing contrasting, sister clades rather than all families to test these four hypotheses, we remove the problem of statistical nonindependence (Felsentein 1985), we compare only groups of equal age, and we reduce the influence of the arbitrary circumscription of taxa. Test 4 provides a phylogenetically controlled check on Ricklefs and Renner's (1994) finding that higher species diversity is associated with multiple growth forms or multiple modes of dispersal within a family. Their suggestion that this association was due to inherent flexibility in some families cannot be tested directly, but an inference from this hypothesis can be tested. The argument is as follows. Because of the way growth forms and the biotic syndromes are defined (see Ricklefs and Renner 1994), it is possible for families but not species to have more than one growth form or syndrome. Therefore, any monophyletic family containing woody and herb species or species with biotic and species with abiotic syndromes of pollination or dispersal must have had a single ancestor with one of the syndromes and not the other. For the same reason, two sister families that diverged from a single common ancestor must also have started out with only a single growth form or syndrome. If either family evolved a second growth form or syndrome, this must be a derived condition in the family. If both sisters evolved a second syndrome, this must be independently derived in each case. According to this reasoning, mixed growth forms or syndromes of pollination or dispersal should not occur in sister families except by chance, *unless* (as Ricklefs and Renner propose) there is some inherent tendency toward evolutionary flexibility in the common ancestors of those families. If that is the case, then one would expect an excess of sister families in which both exhibit two growth forms or both syndromes. This may easily be tested given a phylogeny for the families so that sisters can be identified. Therefore, we ask, "Is there an excess of cases in which both families in a sister pair exhibit two growth forms or two syndromes of dispersal or pollination?"

Correlations between a trait affecting speciation rate and species richness can arise in two ways, either because the evolution of a new mode decreases the net speciation rate by comparison with the effect of the ancestral state (as might occur when abiotic pollination evolves from biotic pollination) or because the new mode increases net speciation rate (as might occur when herbs evolve from woody plants). Thus, in the final part of our analysis, we deduce which of these alternatives is responsible for trait/species richness relationships in each particular case by tracing traits onto the phylogenetic tree and counting changes in each direction from a reconstruction of trait evolution.

Methods

Family Traits

We used the family character dataset assembled by Ricklefs and Renner (1994), who took the number of species from Mabberley (1987) and estimated pollination and dispersal modes from family descriptions in a variety of sources. They coded pollination and dispersal modes each as: (1) abiotic; (2) biotic; or (3) both modes present. Growth forms for each family were tabulated by Ricklefs and Renner (1994) as presence or absence of herbs, shrubs, woody climbers, or lianas. The dataset contains 365 families of flowering plants, which is virtually all of the families recognized by the major classification schemes of Cronquist (1988), Brummitt (1992), and

Thorne (1992). For cases in which there were differences in circumscription, Ricklefs and Renner (1994) chose the wider treatment.

Phylogeny of the Angiosperms

The large-scale structure of our phylogenetic tree was based on one of the nine equally parsimonious trees produced by Nandi et al. (1998: fig 2A) from their analysis based upon angiosperm *rbc*L sequence variation. This phylogeny was the most comprehensive available for the angiosperms, but did not resolve relationships among families in certain groups that were considered monophyletic. Therefore, we had to use other sources to resolve the phylogenetic relationships among families in these groups, as follows: Chase et al. (1995; monocots), Chase et al. (1993; whole phylogeny), Gadek et al. (1996; Sapindales *s.l.* including Rutales), Xiang et al. (1993; Cornaceae group), Downie et al. (1997; Caryophyllales). Individual clades from these molecular phylogenies were grafted onto the overall tree at positions indicated by Nandi et al (1998). If there were any conflicts in the location of families, we then used the position in the Nandi et al. (1998) tree or the most recent of the molecular phylogenies.

Ecological traits were assigned at the family level, and it was therefore essential that all the families used in our analysis should be located unambiguously within the phylogeny. The monocots proved to be a major problem in this regard, with many nonmonophyletic families shown in the Chase et al. (1995) *rbc*L analysis and more recent, unpublished work (M. Chase). These nonmonophyletic families were therefore removed from the phylogeny. There were also a few non monophyletic or unresolved parts of the dicot tree, and families from these areas were also removed from the phylogeny, thus leaving 299 families in our tree. This included 266 of the 365 families in Ricklefs and Renner's (1994) dataset. The only dicot families in the dataset containing more than 50 species that we had to remove from the analysis were Grossulariaceae, Loasaceae, Loranthaceae, Orobanchaceae, and Stylidiaceae. The phylogeny is shown in Figure 1 with one trait (pollination mode) mapped on to it using MacClade's standard parsimony (Maddison and Maddison 1992).

Tests 1–4: Trait/Diversity Relationships

Pollination and dispersal modes and the presence or absence of the herbaceous growth form were each traced on the tree using MacClade's standard parsimony method and unordered transformation type (Maddison and Maddison 1992). Sister clades with contrasting traits were identified and the method of Slowinski and Guyer (1993) was used to compare the number of species belonging to each sister clade against the null hypothesis of equal speciation rates. Each trait of interest was analyzed separately. The method calculates deviations from the null hypothesis for each sister group comparison individually and then calculates a cumulative probability across all comparisons using Fisher's combined probability test. Fisher's test tests the null hypothesis that all the individual null hypotheses are true, using the weights of evidence against the null hypothesis that each individual test provides. Thus, if the individual tests are two-tailed, Fisher's test does not distinguish between those cases that support the alternate hypothesis (e.g., S in clades with herbs present, $>$ S in clades with herbs absent) and those that are significant in the wrong direction (S in clades with herbs present, $<$ S in clades with herbs absent). To avoid this problem of interpretation we calculated the combined probabilities in each direction separately to detect significant exceptions to results that otherwise confirmed our hypotheses.

Slowinski and Guyer's (1993) method does not permit trait reversals within clades, of which there are many for certain traits in a phylogeny as large as that of the angiosperms. Excluding clades containing reversals confines the analysis to a small proportion of the available data. As an alternative method that does not have this drawback, we used the method of independent contrasts using the computer package CAIC, with branch lengths equal (Purvis and Rambaut 1995). This compares the species richness of sister families or the average species richnesses of families belonging to sister clades. It therefore tests our hypotheses using speciation rates below the family level only. As such, this method corrects for the problems of inflated degrees of freedom (see introduction), but not for unequal ages of families or artificiality of family circumscription. Values of S were $\log_{10}$ transformed before analysis.

Test 5: A Test for "Evolutionary Flexibility"

Counts of sister family pairs falling into the three categories: (1) both families had two modes; (2) one family had two modes; and (3) neither family had two modes, were analyzed by G-test with one degree of freedom (Sokal and Rohlf 1995). Our test for evolutionary flexibility has inflated Type I error because we can not reconstruct the phylogeny of "flexibility." However, this is not a problem whenever the test indicates $P \geq 0.05$.

The Direction of Trait Evolution

The trait reconstructions referred to above were used, but the standard parsimony method left several ambiguities in the character tracing. To give an idea of the total possible number of state changes, most of these ambiguities were subsequently resolved using ACCTRAN and DELTRAN options in MacClade. DELTRAN is a parsimony method that delays state changes away from the root (i.e., it permits parallelisms) whereas ACCTRAN accelerates changes and so maximises early gains with subsequent reversals.

RESULTS

Tests 1–4: Trait/Diversity Relationships

All tests based on the analysis of independent contrasts at the family level had considerably more degrees of freedom than the equivalent tests based upon the method of Slowinski and Guyer (1993), although results were generally similar (Tables 1, 2). A complete list of contrasts is given in the Appendix. Species richness varied with pollination and growth form in the predicted directions in both kinds of analysis (Tables 1, 2; tests 1 and 3). The stronger effect in terms of the relative species richness of contrasting clades was a positive effect of growth form; branches with herbs had on average 4.15 times the species of sisters with no herbs (Table

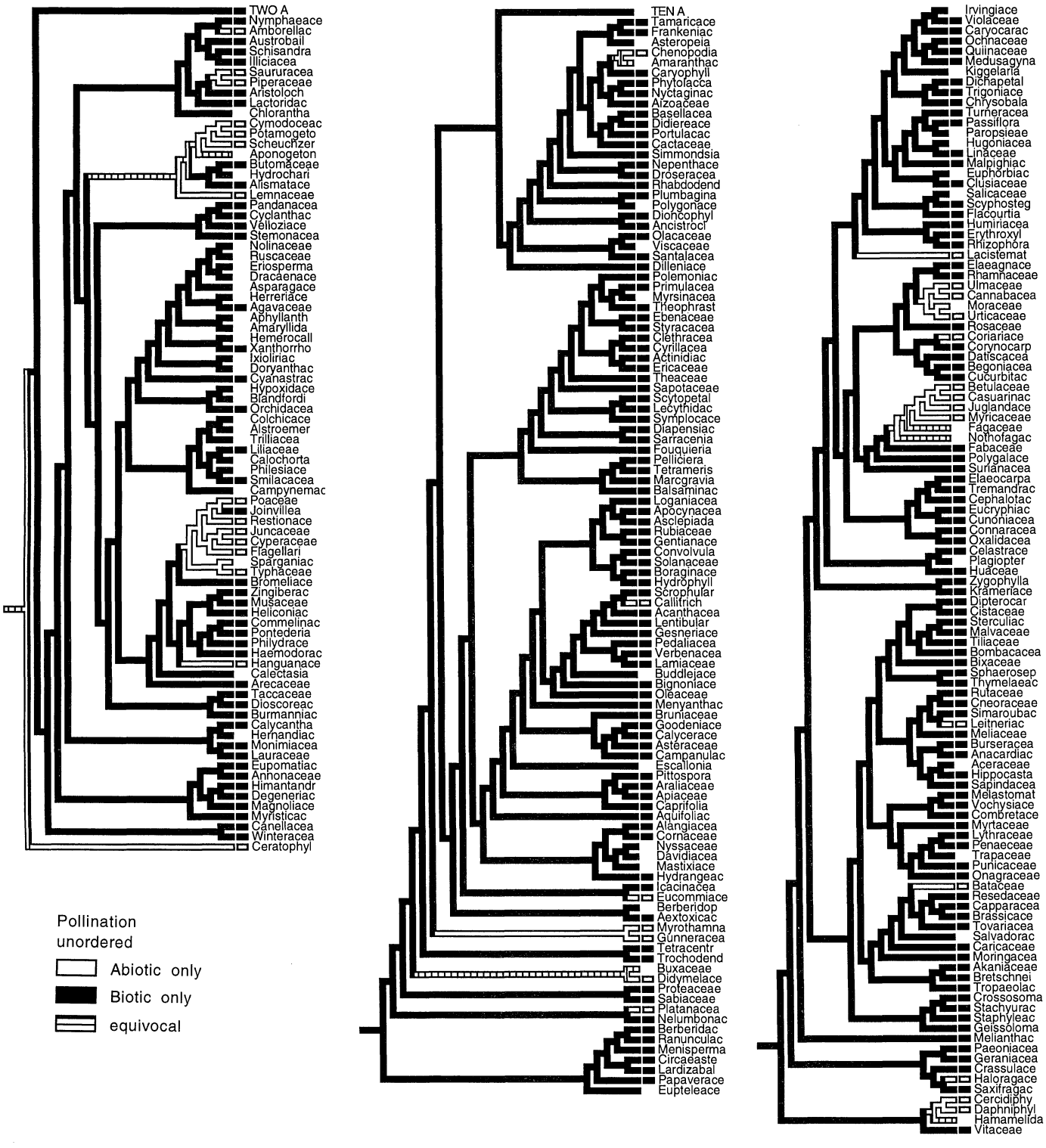

FIG. 1. A composite phylogeny of angiosperm families. Data on pollination mode (biotic/abiotic) from Ricklef and Renner's (1994) dataset have been mapped on to the tree and trait evolution reconstructed using MacClade's standard parsimony (Maddison and Maddison 1992). Biotically pollinated families are indicated by a filled rectangle beside the name and abiotically pollinated ones by an open rectangle. Families with both pollination systems, or where there was no data available, have no rectangle. Terminals 'TWO A' and 'TEN A' indicate where the clade to the right fits into the overall tree.

TABLE 1. Results of tests 1–4 using the method of Slowinski and Guyer (1993). *n*, number of sister clades (proportion of comparisons in the expected direction); *P*, significance of a χ^2 test with $2n$ degrees of freedom. Relative species richness is the ratio of species numbers (*S*) in sister groups with alternative traits, summed first for families within a trait group (e.g., biotic/abiotic: $\Sigma S_{biotic}/\Sigma S_{abiotic}$).

Test	Trait	Hypothesis	Confirmed/ rejected	*n*	Relative species richness	χ^2	*P*	Significant exceptions?
1.	Pollination	biotic > abiotic	confirmed	11 (0.64)	1.19	76.43	< 0.0001	No
2a.	Dispersal	biotic > abiotic	borderline	19 (0.53)	0.32	54.22	0.043	Yes
2b.	Dispersal	biotic < abiotic, among herbs only	confirmed	3 (1.00)	0.08	24.4	< 0.001	No
2c.	Dispersal	biotic > abiotic, among woody plants only	rejected	8 (0.50)	0.64	16.8	0.397	Yes
3.	Growth form	herbs only > no herbs	confirmed	14 (0.64)	4.15	65.75	< 0.0001	No
4a.	Pollination	two modes > biotic only	confirmed	11 (0.73)	1.69	40.0	0.011	No
4b.	Dispersal	two modes > biotic only	confirmed	20 (0.75)	2.58	86.51	< 0.0001	No
4c.	Growth form	two forms > herbs only	borderline	13 (0.62)	1.00	39.53	0.043	No

1). However, the result of the pollination test (test 1, Table 1) was heavily influenced by a single comparison between Joinvilleaceae and Poaceae in the "wrong" direction. When this comparison was removed, the average ratio of species in biotic/abiotically pollinated clades rose from 1.19 to 5.56.

Among family-level contrasts (Table 2) the differential between growth forms was weaker (1.65) and pollination mode had the greater average effect on species richness with biotically pollinated families containing on average 2.36 times the species of abiotically pollinated families. Dispersal mode was significantly associated with *S* in two of the six tests (tests 2a and 2b, Table 1), both made using the method of Slowinski and Guyer (1993). Test 2b contained only three sister clade comparisons, with each of these clades containing only one family (Appendix). The equivalent test using independent contrasts contained eight contrasts and rejected the hypothesis (test 2b, Table 2). Although none of tests 2a,b,c done using independent contrasts confirmed a significant role for dispersal mode in diversification (Table 2), there was a trend for biotic dispersal to be negatively correlated with *S* in herbs and positively correlated with *S* in woody plants as observed by Tiffney and Mazer (1995).

Comparisons of the species richness of families with two modes of pollination, dispersal or growth form with sisters containing only one mode (test 4, Table 1) clearly confirmed Ricklefs and Renner's (1994) finding for dispersal, gave a borderline result for growth form ($P = 0.043$, even though relative species richness = 1.00), and found the pattern for pollination also. Using independent contrasts, only the dispersal pattern was clearly confirmed (test 4b, Table 2).

Test 5: A Test for "Evolutionary Flexibility"

For dispersal, test 5 showed that sister families were no more likely to both contain two dispersal modes than expected by chance (*G*-test, 1 df, $G = 0.65$, $P = 0.419$) and for growth form the result was marginal (*G*-test, 1 df, $G = 3.74$, $P = 0.053$). Test 5 could not be carried out for pollination because there were too few contrasting families and no cases of sister families that both contained two pollination modes.

The Direction of Trait Evolution

The relative numbers of losses and gains for each trait formed a clear pattern that was qualitatively unaffected by which of the three methods was used to trace their evolution (Table 3). Virtually all changes in pollination involved a loss of the biotic mode, changes in dispersal were equally divided between gains and losses of the biotic mode, and a majority (75–84%) of changes in growth form involved a gain for the herbaceous mode.

DISCUSSION

Our results confirm that evolutionary changes in pollination and growth form are both correlated with the contemporary species richness (*S*) of angiosperm families and that the evolution of dispersal mode is not consistently correlated with *S*. Because these correlations were obtained by comparison of sister groups, we can now exclude the possibility, left open by earlier studies, that they are the result of just a

TABLE 2. Results of tests 1–4 for the correlation of species diversity with three traits using independent contrasts. *n*, number of contrasts; *P*, significance of a two-tailed *t*-test. Relative species richness is the ratio of species numbers in sister groups with alternative traits (as in Table 1) calculated by backtransformation of mean contrasts.

Test	Trait	Hypothesis	Confirmed/ rejected	*n*	Relative species richness	*t*	*P*
1.	Pollination	biotic > abiotic	confirmed	22	2.36	2.701	0.013
2a.	Dispersal	biotic > abiotic	rejected	55	0.98	0.099	0.922
2b.	Dispersal	biotic < abiotic, among herbs only	rejected	8	0.39	1.833	0.109
2c.	Dispersal	biotic > abiotic, among woody plants only	rejected	30	1.40	1.820	0.079
3.	Growth form	herbs only > no herbs	confirmed	33	1.65	2.193	0.036
4a.	Pollination	two modes > biotic only	rejected	19	1.36	1.171	0.257
4b.	Dispersal	two modes > biotic only	confirmed	45	2.07	4.297	< 0.001
4c.	Life form	two forms > herbs only	borderline	35	1.48	2.029	0.050

TABLE 3. Losses and gains, excluding taxa with two modes, for unambiguous changes in traits traced on the tree using: (a) standard parsimony; (b) ACCTRAN; (c) DELTRAN options in MacClade.

	(a)		(b)		(c)	
Trait/mode	Gains	Losses	Gains	Losses	Gains	Losses
Pollination/biotic	1	14	2	19	1	20
Dispersal/biotic	9	10	28	27	28	27
Growth form/herb	18	4	24	8	27	5

few evolutionary events affecting many related families (problem 1 of the Introduction). Changes in pollination mode and growth form have both consistently altered diversification rates many times during angiosperm evolution. The usual interpretation of the correlation between biotic pollination and family species richness has been that this trait has enhanced net speciation rate, but our examination of the direction of trait evolution (Table 3) shows that almost all transitions in pollination mode have involved the loss, rather than the gain, of biotic pollination. Therefore, in evolutionary terms a more accurate interpretation would be that during the radiation of the angiosperms abiotic pollination has caused a lowering of the net speciation rate in those families in which it has evolved. Although this is the case on average, an important exception is the Poaceae, a large, wind-pollinated family that is much more species rich than its biotically pollinated sister family, Joinvilleaceae, which contains only two species. Joinvilleaceae appears to be a case of biotic pollination arising from abiotically pollinated ancestors. The majority of evolutionary transitions affecting growth form involved a change from woodiness to herbaceousness, and in this case there appear to be no major exceptions to the rule that this change is correlated with elevated species richness. These conclusions are based upon the reconstruction of trait evolution using parsimony, but we do not believe that they would be altered by the use of maximum- likelihood reconstruction because a comparison of the two methods by Schluter et al. (1997) found little difference between them when changes in character states were rare. Changes in pollination mode and growth form in our phylogeny were certainly rare and, relative to the size of the phylogenetic tree, so too were changes in dispersal mode, although we draw no conclusions about dispersal based on internal nodes of the tree. Any future analysis of species richness in a tree resolved to below family level, where traits become more evolutionarily labile, would benefit from a comparison of alternative methods of trait reconstruction.

Like Ricklefs and Renner (1994), we found that taxa with two modes of dispersal had higher species richness than those with only biotic dispersal, but our test for "evolutionary flexibility" allows us to clearly reject Ricklefs and Renner's (1994) hypothesis that the prior evolution of some general tendency toward flexibility was responsible for this pattern. We conclude that the evidence favors the simpler, alternative hypothesis that more species-rich families tend to have two modes of dispersal due to a sampling effect. Because dispersal is an evolutionarily labile trait (e.g., Janson 1992, Kubitzki and Ziburski 1994), it is statistically more likely to have more modes if there are more species. The lability of dispersal is apparent in the transitions between modes (Table 3) which, unlike in the other two traits examined, involved equal numbers of losses and gains. Ricklefs and Renner (1994, p. 1634) considered the possibility that a sampling effect caused more species-rich families to be more diverse in their traits, but dismissed the idea on the grounds that too many species-rich families had only one trait state. However, they admitted that this pattern could be due to "phylogenetic conservatism," and their calculation of the expected number of monomorphic families ignored phylogeny and treated each family as a statistically independent sampling unit. Our tests for the effect of dual growth forms on S and for the influence of evolutionary flexibility as an explanation of this were both on the borderline of statistical significance, so we cannot reject Ricklefs and Renner's (1994) flexibility hypothesis in this instance. However, we note that our test of evolutionary flexibility probably has an inflated tendency to reject the null hypothesis (Type I error, see Methods), so we consider our result to be inconclusive rather than in support of the flexibility hypothesis in this case.

As a further test of the hypothesis that biotic pollination is directly responsible for increased speciation rate, Ricklefs and Renner (1994) examined species:genus ratios to test the prediction that within-genus species diversity should be higher in animal pollinated families than in wind-pollinated ones. The test proved negative, and they concluded that it failed to support the hypothesis. Because genera are quite arbitrary constructs (as Ricklefs and Renner acknowledge), we believe that species:genus ratios cannot be expected to tell us anything about speciation rates and that Ricklefs and Renner's result simply confirms this.

The present-day species diversity of any extant higher taxon is determined by the balance between past rates of extinction and speciation. Athough both rates are likely to be influenced by species' traits, only the effect on diversification of traits influencing speciation has been examined in any detail. This is not only because extinction rates are intrinsically difficult to estimate (but see Humphries and Fisher 1994; Nee et al 1994), but also because ecological studies suggest that rarity (in its various aspects) is the best predictor of extinction, and it has proved notoriously difficult to find more measurable traits that correlate with rarity that may act as markers for it (Kunin and Gaston 1993).

Our study has no direct bearing on the controversy over the origin of the angiosperms and the nature of the trigger to diversification that followed (Crane et al 1995; Crepet 1996), but it has one important indirect implication. Our finding that a significant number of evolutionary losses of biotic pollination in the angiosperms have been accompanied by a subsequent fall in diversification is the nearest thing to an experimental test we are ever likely to have of the idea that it was the early evolution of biotic pollination that triggered diversification in the first place. The test proved positive.

Our analysis has made a significant advance on previous studies of angiosperm family diversity by incorporating a phylogenetic perspective, but four limitations of our approach need to be considered. First, although Slowinski and Guyer's (1993) method is the best currently available for our purpose, it assumes a Markovian model of evolution, which may be incorrect (Cunningham 1995). This problem is not unique to

our analysis, but in this instance we have shown that the results of using this method are similar to those obtained using independent contrasts. Second, we used only one phylogenetic hypothesis. Third, our tree contains grafts from other trees, so we cannot claim it is globally parsimonious. Finally, we have been unable to look at interactions among traits.

Ideally, we would like to test the robustness of our results against many alternative, globally parsimonious trees as advocated by Donoghue and Ackerly (1997), but because not even one such tree has yet been computed for all the families in our analysis, this ideal is currently unobtainable. In fact, not having the shortest tree has little significance for the investigations carried out here. Estimates of internal support do not require production of trees at all, so these estimates are robust regardless of search strategy. For *rbc*L, it is the terminal groups that have high bootstrap percentages (Nandi et al., 1998; Chase and Albert, in press), and it is clades like these that are the main foci of the sister group comparisons in this study. Recently performed studies on combined datasets of tree genes (two plastid and one nuclear) that have been sampled extensively among angiosperms (Soltis et al. 1997; Chase and Cox 1998; D. E. Soltis, P. S. Soltis, M. W. Chase, M. E. Mort, D. Albach, M. Zanis, V. Savolainen, W. H. Hahn, S. B. Hoot, M. Axtell, S. M. Swensen, K. Nixon, and J. S. Farris, unpubl. ms.) have demonstrated congruence with the Chase et al. (1993) and Nandi et al. (1998) *rbc*L trees. These large datasets are clearly recovering highly congruent and, in the combined trees, robustly supported patterns of relationships. We cannot guarantee that the tree topologies for all the comparisons made in this study are accurate, but such a small percentage of them could be erroneous that this effect is negligible.

We were unable to look at interactions between traits or to calculate the variance in family diversity associated with variation in different traits, as Ricklefs and Renner (1994) were able to do in their nonphylogenetic analysis, because satisfactory comparative techniques for doing this with more than one binary variable at a time do not yet exist. However, this does not compromise our conclusion (contra Ricklefs and Renner 1994) that evolutionary changes in pollination mode and growth form are implicated as direct causes (doubtless among others) of present-day variation in the species-richness of angiosperm plant families.

Acknowledgments

We are very grateful to R. E. Ricklefs and S. Renner for sharing their data with us and for comments on the manuscript. We also thank O. Eriksson and J. Ollerton for comments and K. McConway for statistical advice. The work was supported in part by the Open University Research Development Committee.

Literature Cited

Berenbaum, M. 1983. Coumarins and catterpillars: a case for coevolution. Evolution 37:163–179.

Brummitt, R. K. 1992. Vascular plant families and genera. Royal Botanic Gardens Kew, Kew, U.K.

Chase, M. W., and V. A. Albert. 1998. A perspective on the contribution of plastid *rbc*L DNA sequences to angiosperm phylogenetics. Pp. 488–507 *in* P. S. Soltis, D. E. Soltis and J. J. Doyle, eds. Molecular systematics of plants II. Chapman and Hall, London.

Chase, M. W., and A. V. Cox. 1998. Gene sequences, collaboration, and analysis of large data sets. Austr. Syst. Bot. 11:215–229.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y. L. Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedren, B. S. Gaut, R. K. Jansen, K. J. Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q. Y. Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, S. W. Graham, S. C. H. Barrett, S. Dayanandan, and V. A. Albert. 1993. Phylogenetics of seed plants—an analysis of nucleotide-sequences from the plastid gene rbcl. Ann. MO Bot. Gard. 80:528–580.

Chase, M. W., M. R. Duvall, H. G. Hills, J. G. Conran, A. V. Cox, L. E. Eguiarte, J. Hartwell, M. F. Fay, L. R. Caddick, K. M. Cameron, and S. Hoot. 1995. Molecular phylogenetics of Lilianae. Pp. 109–137 *in* P. J. Rudall, P. J. Cribb, D. F. Cutler and C. J. Humphries, eds. Monocotyledons: systematics and evolution. Royal Botanic Gardens Kew, Kew, U.K.

Crane, P. R., E. M. Friis, and K. R. Pedersen. 1995. The origin and early diversification of angiosperms. Nature 374:27–33.

Crepet, W. L. 1996. Timing in the evolution of derived floral characters—upper cretaceous (turonian) taxa with tricolpate and tricolpate-derived pollen. Rev. Palaeobot. Palynol. 90:339–359.

Cronquist, A. 1981. An integrated system of classification of flowering plants. Columbia Univ. Press, New York.

Cunningham, S. A. 1995. Problems with null models in the study of phylogenetic radiation. Evolution 49:1292–1294.

Donoghue, M. J., and D. D. Ackerly. 1997. Phylogenetic uncertainties and sensitivity anlayses in comparative biology. Pp. 17–35 *in* J. Silvertown, M. Franco, and J. L. Harper, eds. Plant life histories. Cambridge Univ. Press, Cambridge. U.K.

Downie, S. R., and D. S. Katz-Downie. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae—evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot. 83:234–251.

Downie, S. R., D. S. Katz-Downie, and K. J. Cho. 1997. Relationships in the Caryophyllales as suggested by phylogenetic analyses of partial chloroplastDNA ORF2280 homolog sequences. Am. J. Bot. 84:253–273.

Eriksson, O., and B. Bremer. 1991. Fruit characteristics, life forms, and species richness in the plant family Rubiaceae. Am. Nat. 138:751–761.

———. 1992. Pollination systems, dispersal modes, life forms, and diversification rates in angiosperm families. Evolution 46: 258-266.

Farrell, B., C. Mitter, and D. Dussord. 1991. Macroevolution of plant defense: do latex/resin secretory canals spur diversification. Am. Nat. 138:881–900.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Fleming, T. H. 1990. Fruiting plant-frugivore mutualism: the evolutionary theater and the ecological play. Pp. 119–144 *in* P. W. Price, T. M. Lewisohn, G. W. Fernandes, and W. W. Benson, eds. Plant-animal interactions: evolutionary ecology in tropical and temperate regions. J.Wiley and Sons, New York.

Gadek, P. A., P. G. Wilson, and C. J. Quinn. 1996. Phylogenetic reconstruction in myrtaceae using matK, with particular reference to the position of Psiloxylon and Heteropyxis. Austr. Syst. Bot. 9:283–290.

Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford Univ. Press, Oxford. U.K.

Herrera, C. M. 1989. Seed dispersal by animals: a role in angiosperm diversification? Am. Nat. 133:309–322.

Hodges, S. A., and M. L. Arnold. 1995. Spurring plant diversification—are floral nectar spurs a key innovation. Proc. R. Soc. Lond. B Biol. Sci. 262:343–348.

HUMPHRIES, C. J., AND C. T. FISHER. 1994. The loss of Banks's legacy. Phil. Trans. R. Soc. Lond. B Biol. Sci. 344:3–8.
JANSON, C. H. 1992. Measuring evolutionary constraints: a Markov model for phylogenetic transitions among seed dispersal syndromes. Evolution 46:136–158.
KUBITZKI, K., AND A. ZIBURSKI. 1994. Seed dispersal in flood plain forests of Amazonia. Biotropica 26:30–43.
KUNIN, W. E., AND K. J. GASTON. 1993. The biology of rarity—patterns, causes and consequences. Trends Ecol. Evol. 8:298–301.
MABBERLEY, D. J. 1987. The plant book. Cambridge Univ. Press, Cambridge, U.K.
MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution. Vers. 3. Sinauer, Sunderland, MA.
MAYNARD SMITH, J., AND E. SZATHMÁRY. 1995. The major transitions in evolution. Freeman, Oxford, U.K.
MIDGLEY, J. J., AND W. J. BOND. 1991. Ecological aspects of the rise of angiosperms: a challenge to the reproductive superiority hypothesis. Biol. J. Linn. Soc. 44:81–92.
NANDI, O. I., M. W. CHASE, AND P. K. ENDRESS. 1998. A combined cladistic analysis of angiosperms using *rbc*L and non-molecular data sets. Ann. MO Bot. Gard. 85:137–212.
NEE, S., E. C. HOLMES, R. M. MAY, AND P. H. HARVEY. 1994. Extinction rates can be estimated from molecular phylogenies. Phil. Trans. R. Soc. Lond. B Biol. Sci. 344:77–82.
PURVIS, A., AND A. RAMBAUT. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. Computer Appl. Biosci. 11:247–251.
RICKLEFS, R. E., AND S. S. RENNER. 1994. Species richness within families of flowering plants. Evolution 48:1619–1636.
SANDERSON, M. J., AND M. J. DONOGHUE. 1996. Reconstructing shifts in diversification rates on phylogenetic trees. Trends Ecol. Evol.11:15–20.
SANDERSON, M. J., AND M. F. WOJCIECHOWSKI. 1996. Diversification rates in a temperate legume clade: are there "so many species" of Astragalus (Fabaceae)? Am. J. Bot. 83:1488–1502.
SCHLUTER, D., T. PRICE, A. Ø. MOOERS, AND D. LUDWIG. 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51:1699–1711.
SLOWINSKI, J. B., AND C. GUYER. 1993. Testing whether certain traits have caused amplified diversification: an improved method based on a model of random speciation and extinction. Am. Nat. 142:1019–1024.
SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry. W. H. Freeman, San Francisco, CA.
SOLTIS, D. E., P. S. SOLTIS, D. L. NICKRENT, L. A. JOHNSON, W. J. HAHN, S. B. HOOT, J. A. SWEERE, R. K. KUZOFF, K. A. KRON, M. W. CHASE, S. M. SWENSEN, E. A. ZIMMER, S. M. CHAW, L. J. GILLESPIE, W. J. KRESS, AND K. J. SYTSMA. 1997. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. Ann. MO Bot. Gard. 84:1–49.
STEBBINS, G. L. 1981. Why are there so many species of flowering plants? Bioscience 31:573–577.
THORNE, R. F. 1992. Classification and geography of the flowering plants. Bot. Rev. 58:225–348.
TIFFNEY, B. H., AND S. J. MAZER. 1995. Angiosperm growth habit, dispersal and diversification reconsidered. Evol; Ecol. 9:93–117.
XIANG, Q. Y., D. E. SOLTIS, D. R. MORGAN, AND P. S. SOLTIS. 1993. Phylogenetic-relationships of *Cornus*-L *sensu-lato* and putative relatives inferred from *rbc*L sequence data. Ann. MO Bot. Gard. 80:723–734.

Corresponding Editor: A. Sakai

APPENDIX

This appendix lists the locations, in the phylogeny shown in Figure 1, of the nodes from which branch the sister clades used in the tests shown in Tables 1 and 2. To locate any node shown in the table, read its location code from left to right and, starting at the root of the tree, move up the page one node for each A and down the page for each B. The number of leading As is represented by a number that also corresponds to the numbering of nodes on the phylogeny, for example 3AB represents AAAB.

All nodes listed here were used in the CAIC analysis (results given in Table 2), but only a subset were suitable for the method of Slowinski and Guyer (1993; results given in Table 1). Clades belonging to the subset are identified by family names. For brevity, suffixes have been omitted from family names. Clades used only in the CAIC analysis have not been named because they have a complex delimitation (see text). For any particular test, each family was used only once in the CAIC analysis, so to identify exactly which families were involved in contrasts at deeper-level nodes it is necessary to first identify and exclude any families involved in contrasts near the tips of the tree.

The Contrast column gives the standardized contrasts of $\log_{10} S$ calculated by CAIC for the tests in Table 2.

APPENDIX. Continued.

Test 1: Pollination Mode

Node No.	Location	Contrast	Clades: biotic vs. abiotic
1	17A	0.37758	
2	16ABAAAA	0.09776	
3	16ABAABA	−0.04845	Corynocarp vs. Coriari
4	16ABABAA	0.75359	
5	13ABAAABAAB	1.1152	Simaroub vs. Leitneri
6	13ABABAAAA	0.35069	Resed, Cappar, Brassic, Tovari vs. Bataceae
7	11ABBB	0.29875	Saxifrag vs. Halorag
8	10AB	0.90829	
9	9ABAAAAAAABAA	0.08251	
10	8ABAABAAAAAABAAAAAAA	1.22225	Scrophulari vs. Callitrich
11	8ABAB	1.25255	Icacin vs. Eucommi
12	7A	0.2008	
13	5A	−0.10033	
14	3AB	−0.2386	Nelumbon vs. Platan
15	ABAAAAAAA	0.8891	Nymphae vs. Amborell
16	ABAAAAABA	0.24138	Aristolochi vs. Saurur, Piper
17	ABAAABAA	0.23616	
18	ABAAAB	0.24994	
19	ABAAABBBABAAAAAAA	−1.7997	Joinvilleaceae vs. Poaceae
20	ABAAABBBABAAA	0.66763	
21	ABAAABBBABAAB	0.80628	Zingiber, Mus, Heliconi, Commelin, Pontederi, Philydr, Haemodor vs. Hanguan
22	@Root	0.658	

Test 2a: Dispersal Mode

Node No.	Location	Contrast	Clades: biotic vs. abiotic
1	23AB	0.67096	
2	21ABA	0.42015	Dichapetal vs. Trigoni
3	20A	−0.00674	
4	19ABA	−1.2625	Scypostegi vs. Salic
5	17A	0.29231	
6	16AB	0.35037	
7	16ABAAAAB	0.91998	
8	16ABAAB	0.01831	
9	16ABAABBB	−0.04395	Cucurbit vs. Begoni
10	16ABABAAAAAA	−0.03029	
11	13ABAAA	−0.35381	
12	13ABAAAAAABAA	−0.0071	Sterculi vs. Malv
13	13ABAAABB	0.27711	
14	13ABAABBBA	−0.53579	Punic vs. Lythr, Penae, Trap
15	13ABABA	0.20061	
16	13ABABAAA	0.30227	
17	13ABABAAAAB	−1.12292	
18	13ABB	0.30277	
19	11ABA	−0.6659	Paeoni vs. Gerani
20	10AB	0.38739	
21	10ABAA	0.5	Daphniphyll vs. Cercidiphyll
22	9ABA	0.39918	
23	9ABAAAA	−0.30361	
24	9ABAAAAAAAB	−0.01019	
25	9ABAAAAAAABAB	−0.64058	
26	9ABAAAAAAABBA	−0.63715	
27	8ABAAA	0.3137	
28	8ABAAAAA	0.59516	
29	8ABAAAAAAAAA	0.25389	
30	8ABAAAAAAAAAAA	−0.19804	
31	8ABAAAAAAAAAAABA	0.0969	Myrsin vs. Primul
32	8ABAAAAAABA	0.5524	Lecythid vs. Scytopetal
33	8ABAAABAA	0.1505	Tetramerist vs. Pellicier
34	8ABAABA	0.14876	
35	8ABAABAABAB	−0.29415	Arai vs. Api
36	8ABAB	1.25255	Icacin vs. Eucommi
37	8A	−0.37710	
38	7AB	0.65055	Gunner vs. Myrothamn

Test 2a: Dispersal Mode Continued.

Node No.	Location	Contrast	Clades: biotic vs. abiotic
39	5A	0.10033	
40	3A	0.38053	
41	2ABA	−0.2544	
42	A	0.48172	
43	ABAAA	0.10032	
44	ABAAAAA	0	
45	ABAAAAAAA	−0.8891	Amborell vs. Nymphae
46	ABAAAAAABB	0.02445	Schisandr vs. Illici
47	ABAAAAABA	0.28096	
48	ABAAABB	0.55489	
49	ABAAABBAA	0.06827	Pandan, Cyclanth vs. Vellozi
50	ABAAABBBAA	−0.04627	
51	ABAAABBBABAA	−0.57251	
52	ABAAABBBABAAAAAAA	−1.79970	Joinvilleaceae vs. Poaceae
53	ABAAABBBABAAAAAB	−1.08762	Flagellari vs. Junc, Cyper
54	ABAAABBBABAABA	0.12120	
55	ABAABA	−0.43915	Calycanth vs. Hernandi

Test 2b: Dispersal Mode, Herbs Only

Node No.	Location	Contrast	Clades: biotic vs. abiotic
1	16ABAABBB	−0.04395	Cucurbit vs. Begoni
2	11ABA	−0.66590	Paeoni vs. Gerani
3	9ABAAAAAAAB	−0.04649	
4	7A	−0.20517	
5	2A	0	
6	ABAAABBBABAAAAAAA	−1.79970	Joinvilleaceae vs. Poaceae
7	ABAAABBBABAA	−0.64609	
8	ABAAABBBABAABA	0.12120	

Test 2c: Dispersal Mode, Woody Plants Only

Node No.	Location	Contrast	Clades: biotic vs. abiotic
1	23AB	0.67096	
2	21ABA	0.42015	Dichapetal vs. Trigoni
3	19ABA	−1.26250	Scypostegi vs. Salic
4	17A	0.34537	
5	16AB	−0.04583	
6	16ABABAAAAAA	−0.03029	
7	13ABAAA	−0.34270	
8	13ABAAAAAAB	0.27514	
9	13ABAAABB	0.27711	
10	13ABAABBBA	−0.36105	
11	13ABABA	0.01464	
12	13ABABAAAA	0	
13	13ABB	0.30277	
14	10ABAA	0.5	Daphniphyll vs. Cercidiphyll
15	9ABA	0.53726	
16	9ABAAAA	0.16868	
17	9ABAAAAAAA	−0.22453	
18	8A	−0.16619	
19	8ABAAAA	0.59335	
20	8ABAAAAAAAAA	0.22738	
21	8ABAAAAAABA	0.55240	Lecythid vs. Scytopetal
22	8ABAAABAA	0.15050	Tetramerist vs. Pellicier
23	8ABAABAA	0.25700	
24	8ABAB	1.25255	Icacin vs. Eucommi
25	5A	0.07095	
26	3A	0.28555	
27	2AB	0.32293	
28	ABAAAAA	0	
29	ABAAAAAABB	0.02445	Schisandr vs. Illici
30	ABAABA	−0.43915	Calycanth vs. Hernandi

Test 3: Growth Form

No.	Node Location	Contrast	Clades: herbs vs. no herbs
1	16ABAAAABA	0.83450	Cannab vs. Ulm
2	16ABAAB	−0.79892	Cephalot vs. Elaeocarp, Tremandr
3	16ABBAA	0.88904	
4	13ABAABBB	−1.02548	
5	13ABAABBBAA	0.05965	
6	13ABABAAAAB	−0.91448	Resed, Cappar, Brassic vs. Tovari
7	13ABABB	−0.86961	Tropaeol vs. Akani, Bretschneider
8	11A	−0.53250	
9	9ABAAA	−0.89020	
10	9ABAAAAA	−0.71331	
11	9ABAAAAAAA	−0.41375	
12	9ABAAAAAAABBAA	−0.06735	Basell vs. Didiere
13	8ABAAAAA	0.49185	
14	8ABAAAAAAAAAAA	−0.19804	
15	8ABAAAAAAAAAAABA	0.09690	Primul vs. Myrsin
16	8ABAAAB	−0.72320	Balsamin vs. Pellicier, Tetrameris, Marcgravi
17	8ABAABAA	0.26719	
18	8ABAABAAAAAA	0.00879	
19	8ABAABAAAAAAAAAB	−0.07010	Asclepiad vs. Apocyn
20	8ABAABAAAAAABA	0.05163	
21	8ABAABAAAAB	−0.43813	
22	8ABAABAABAB	−0.29415	Api vs. Arali
23	7AB	−0.65055	Gunner vs. Myrothamn
24	3AB	0.23860	Nelumbon vs. Platan
25	2AB	−0.84928	
26	2ABAAB	0.51060	Circaeaster vs. Lardizabal
27	ABAAA	−0.28400	
28	ABAAAAAAA	−0.88910	Nymphae vs. Amborell
29	ABAAAAAB	−0.29879	
30	ABAAABBAA	0.17469	
31	ABAAABBBAB	0.60685	
32	ABAAABBBABAAAAAB	−1.08762	Poaceae, Joinville, Restion, Junc, Cyper vs. Flagellari
33	@Root	0.57385	

Test 4a: Pollination, Two Modes versus Biotic Only

No.	Node Location	Contrast	Clades: two modes vs. biotic only
1	20ABB	0.3795	Euphorbi vs. Clusi
2	19ABA	1.2625	Salic vs. Scyphostegi
3	16ABAAAA	0.26053	
4	16ABABAA	−0.36483	
5	13ABAAABBBA	0.4385	Acer vs. Hippocastan
6	13ABAABBBAA	−0.3876	Trap vs. Lythr, Penae
7	13ABABAAA	−0.2293	
8	10AB	−0.38739	
9	9ABAAAAAAABAA	−0.16857	
10	9ABAAAB	0.18885	Polygon vs. Plumbagin
11	9ABABA	0.1761	Visc vs. Olac
12	8ABAAAAAAAAAAABA	0.0969	Myrsin vs. Primul
13	8ABAABBA	−0.22973	Nyss vs. Alangi, Corn
14	5A	0.43999	
15	2AB	−0.79607	Euptele vs. Berberid, Ranuncul, Menisperm, Circaeaster, Lardizabal, Papaver
16	ABAAAA	0.3158	
17	ABAAABAA	−0.11978	
18	ABAAABAABA	1	Hydrocharit vs. Butom
19	ABAABA	0.43915	Hernandi vs. Calycanth

Test 4b: Dispersal, Two Modes versus Biotic Only

No.	Node Location	Contrast	Clades: two modes vs. biotic only
1	24A	0.64842	Viol vs. Caryocar
2	23ABA	0.50965	Ochn vs. Quiin
3	20A	0.24745	
4	19AB	1.20107	
5	18ABB	−0.15055	Rhizophor vs. Erythroxyl
6	16AB	0.34780	
7	16ABAAA	−0.42542	
8	16ABAAAAA	0.64440	Rhamn vs. Elaeagn
9	16ABAAAABB	−0.02900	Urtic vs. Mor
10	16ABAB	1.15365	
11	16ABABAAAA	0.53979	
12	16ABABAAAAAA	0.16542	
13	15A	0.35372	
14	13ABA	0.49899	
15	13ABAAAAAABA	−0.12892	
16	13ABAAABAAA	1.37020	Rut vs. Cneor
17	13ABAAABAAB	1.11520	Simaroub vs. Leitneri
18	13ABAAABBA	−0.0985	Burser vs. Anacardi
19	13ABAABBB	1.02548	
20	13ABABAAAAB	0.81933	
21	13ABBA	0.28190	Staphyle vs. Crossosomat, Stachyur
22	11A	0.21660	
23	9AB	0.18999	
24	9ABAAAAAAAB	−0.09076	
25	9ABAAAAAAABABA	0.36560	Nyctagin vs. Phytolacc
26	9ABAAAAAAABBAA	0.06735	Basell vs. Didiere
27	8ABAAAAAAA	−0.13284	
28	8ABAAAAAAAAA	−0.02152	
29	8ABAAAAAAAAAAB	−0.23410	Styrac vs. Eben
30	8ABAAAB	0.63803	
31	8ABAABA	−0.02153	
32	8ABAABAAAA	0.01150	
33	8ABAABAABA	−0.21347	
34	8ABAABB	0.35429	Hydrange vs. Alangi, Corn, Nyss
35	5AB	0.73860	Bux vs. Didymel
36	4AB	0.72455	Prote vs. Sabi
37	2ABAAAA	0.24355	Ranuncul vs. Berberid
38	ABAAAAABAA	−1.22795	Saurur vs. Piper
39	ABAAABB	−0.13396	
40	ABAAABBBA	0.00464	
41	ABAAABBBAABAB	0.46167	Lili vs. Smil
42	ABAAABBBABAAA	0.98080	
43	ABAAABBBABAABA	0.22875	
44	ABAAABBBABAABAAA	0.72800	Zingiber vs. Mus
45	ABB	0.28705	Winter vs. Canell

Test 4c: Growth Form, Two Forms versus Herbs Only

Node No.	Location	Contrast	Clades: two modes vs. herbs only
1	16ABAAAAB	0.97254	
2	16ABAABB	−1.03224	Datisc vs. Begoni, Cucurbit
3	16A	−0.08089	
4	16ABB	0.87269	
5	13ABA	0.21031	
6	13ABAAB	0.05457	
7	13ABAABBBAA	0.64801	
8	13ABABAA	−0.26741	
9	11A	−0.35872	
10	9ABA	0.04726	
11	9ABAAAAAAA	−0.55189	
12	9ABAAAAAAABAAA	0.1054	Chenopodi vs. Amaranth
13	9ABAAAAAAABAB	−0.53822	Phytolacc, Nyctagin vs. Aizo
14	9ABAAAAAAABBA	0.58216	
15	8A	−0.03058	
16	8ABAAAAAB	−0.0311	Diapensi vs. Sarraceni
17	8ABAABAAAAA	0.25391	
18	8ABAABAAAAAAAAA	−0.27932	
19	8ABAABAAAAAAAAB	0.47510	Rubi vs. Gentian
20	8ABAABAAAAAAABA	0.09875	Solan vs. Convolvul
21	8ABAABAAAAAAABB	0.47930	Boragin vs. Hydrophyll
22	8ABAABAAAAAABAAAA	0.30298	Gesneri vs. Scrophular, Callitrich, Acanth, Lentibulari
23	8ABAABAAAABB	0.42139	
24	8ABAABAAAABBAA	0.44655	Goodeni vs. Calycer
25	2A	0.51134	
26	2ABAA	0.85532	
27	2ABAAAA	−0.24355	Berberid vs. Ranuncul
28	ABAAA	0.10032	
29	ABAAAAA	0.24093	
30	ABAAAAABAA	1.22795	Piper vs. Saurur
31	ABAAABB	−0.24286	
32	ABAAABBAA	−0.05005	
33	ABAAABBBAAAAAA	0.28440	
34	ABAAABBBAABAB	−0.46167	Smil vs. Lili
35	ABAAABBBBA	0.88910	Dioscore vs. Tacc