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Shifts in Diversification Rate with the Origin of Angiosperms

Michael J. Sanderson* and Michael J. Donoghue

The evolutionary success of flowering plants has been attributed to key innovations that originated at the base of that clade. Maximum likelihood methods were used to assess whether branching rate increases were correlated with the origin of these traits. Four hypotheses for the basal relationships of angiosperms were examined by methods that are robust to uncertainty about the timing of internal branch points. Recent hypotheses based on molecular evidence, or on a combination of molecular and morphological characters, imply that large increases in branching rate did not occur until after the putative key innovations of angiosperms had evolved.

Disparity in species diversity among clades of roughly similar age implies differences in the rates of branching (speciation) and extinction, and therefore shifts in diversification rate during the course of evolution (1). Such shifts have often been attributed to the evolution of "key innovations," morphological novelties that open up new adaptive zones (2) or set the stage for subsequent rapid diversification in the wake of environmental changes. A prime example concerns the apparently elevated rate of diversification of flowering plants as compared to other lines of seed plants, which is usually attributed to one or more angiosperm characteristics, such as the presence of insect pollination, closed carpels, or increased growth rate (3, 4).

Correlation of a change in diversification rate with the evolution of a presumed key character along the same branch of a phylogenetic tree would be consistent with a causal hypothesis connecting the two (a "key innovation hypothesis"), whereas failure to find a historical correlation would argue against a direct causal link. Although quantitative methods for inferring the location of character changes on phylogenetic trees have received considerable attention (5), less attention has been given to the problem of identifying shifts in diversification rate in trees (6, 7). In the absence of quantitative methods, differences in rates commonly have been inferred solely on the basis of levels of diversity among taxa assigned the same Linnaean rank, which can introduce bias into rate calculations (8).

Quantitative studies of diversification rates in phylogenies have generally focused on sister group comparisons (9), which standardize the ages of lineages (10); however, because they are essentially two-taxon phylogenies, they take into account mini-

mal information present in a tree (7, 8, 11). The inclusion of more information on such relationships, even information on just one additional taxon, should permit finer resolution of the timing of changes in diversification rate. This increase in phylogenetic resolution will be informative only to the extent that estimates of the timing of internal branch points are available or if statistics robust to uncertain timing are developed. The first approach has been explored (12) but has relied on debatable assumptions of clock-like molecular evolution (13). Here, we pursue the second approach—the application of a statistical method that holds over the range of plausible branching times—to explore the timing of the radiation of flowering plants in relation to the evolution of their presumed key innovations.

Our analysis was performed in the context of a set of three-taxon phylogenies, each comprising an outgroup and two sister

taxa that form the ingroup. Branching rates were reconstructed within a likelihood framework, assuming an underlying Yule or "pure birth" branching process (7, 12, 14) in which the probability of branching events along a branch follows a Poisson distribution with unknown parameter λ . The likelihood of observing N species in a clade after an interval of time d , given one species initially, is (15)

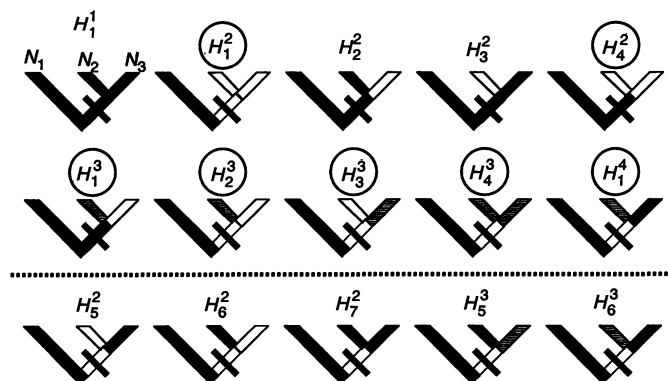
$$e^{-\lambda d}(1 - e^{-\lambda d})^{N-1}$$

The Markov property of the Yule process permits multiplication of terms like this for each branch of the tree, taking into account possibly different rate parameters in different branches corresponding to various alternative models (Fig. 1).

A phylogeny with three taxa and four branches permits models with only one branching rate parameter or as many as four different parameters, one for each branch (Fig. 1). These parameters can be arranged in 15 topologically distinct models: one single-rate model, seven two-rate models, six three-rate models, and one four-rate model (which provides the best possible fit of the model to the data). Our attention was restricted to 10 of these models in which the same rate does not arise twice (Fig. 1). The remaining five unparsimonious models are not needed to explain any of the data described below. A model is denoted by H_j^k , where k , the "class" of the model, is the number of parameters, and j indexes the different arrangements of rate parameters on the tree.

The observations consist of the number of species in the three terminal taxa, $\{N_1,$

Fig. 1. One-, two-, three-, and four-parameter models for the evolution of branching rate in a three-taxon phylogeny. H_j^k refers to the j th model within the class of models that has k parameters. The extant terminal taxa occur at time $t = 0$, the root at time $t = 1$, and the internal node at t with $0 \leq t \leq 1$. The three clades are composed of N_1 , N_2 , and N_3 species, respectively. Only the relationships of the three taxa to each other, not the phylogenies within the three taxa, are assumed to be known. Distinct shading of branches represents the branching rate parameters. For example, a tree with branches having three shadings has three distinct rate parameters in the positions indicated. Models below the dotted line are included for completeness but entail homoplasy in the evolution of rates (more than one transformation to or from the same rate). They are excluded from the analysis as unparsimonious and unnecessary to explain the data. The black bar represents the position of a hypothesized key innovation that unites the two ingroup taxa. Circled models are potentially consistent with a key innovation hypothesis—that is, a correlation between increases in the rate of branching and the evolution of the innovation (23). The other models are inconsistent with a hypothesis of key innovation. Consistent models are not indicated for the five homoplastic models.



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$N_2, N_3\}$ of a given phylogeny, and an upper and lower bound on the possible branching time of the internal node, t_u and t_l , respectively, where time is scaled from 0 (the present) to 1 (the age of the earliest split). The likelihood of the unknown parameters of the model (given the observations), $L(\Lambda|N_1, N_2, N_3, t_u, t_l)$, is proportional to the probability of the observations given the model (16), where Λ is a vector of from one to four parameters depending on the model. The probability can be obtained by integrating over the possible branch times (17), which are constrained to lie between the bounds t_u and t_l :

$$P(N_1, N_2, N_3, t_u, t_l | \Lambda) = \int_{t_l}^{t_u} P(N_1, N_2, N_3, t | \Lambda) dt \quad (1)$$

For example, in model H_2^2 the integrand is $e^{-\lambda_a}(1 - e^{-\lambda_a})^{N_1 - 1} \times e^{-\lambda_a t}(1 - e^{-\lambda_a t})^{N_2 - 1} \times e^{-\lambda_b t}(1 - e^{-\lambda_b t})^{N_3 - 1} \times \lambda_a e^{-\lambda_a(1-t)}$

where λ_a and λ_b are the two branching parameters in the class H^2 . The first three terms stem from the likelihood expression given earlier. The last term is due to the exponentially distributed waiting times between branching events in a Poisson process. For a set of observations on a given phylogeny, the likelihood is obtained as a function of the unknown parameters by numerical integration, and the maximum likelihood estimates of the parameters are then obtained by numerical optimization methods (18). Finally, each model is examined in turn until the "best" is found by means of a strategy that simultaneously minimizes model complexity (the number of param-

eters) but maximizes goodness of fit, β_j^k , relative to the four-parameter model. Values for β_j^k of model H_j^k are measured by its log likelihood ratio relative to the best fit model H_1^1

$$\beta_j^k = \log \left[\frac{\max L(\Lambda_j^k | N_1, N_2, N_3, t_u, t_l)}{\max L(\Lambda_1^1 | N_1, N_2, N_3, t_u, t_l)} \right] \quad k \in \{1, 2, 3\} \quad (2)$$

where Λ_j^k is the vector of unknown parameters associated with H_j^k . First, k was set to 1, and the one-parameter model, H_1^1 , was tested; if it fit as well as the four-parameter model, the procedure terminated and H_1^1 was accepted as an adequate description of the data. Otherwise, k was incremented and each of the two-parameter models was tested. The procedure terminated either (i) upon failure to reject one or more models in a class H^k or (ii) when model H_1^1 was reached by rejecting all simpler models. Significance levels of the log likelihood ratio β were assessed by Monte Carlo simulation (19, 20) using the maximum likelihood rate estimates derived under the relevant null model.

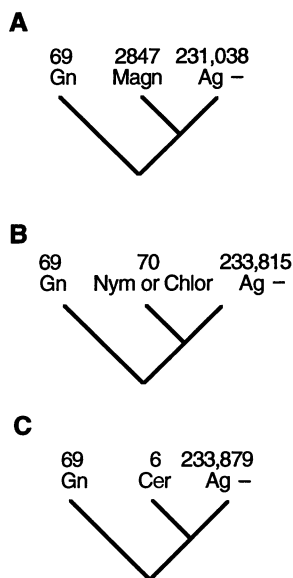
A subset of models is potentially consistent with a key innovation hypothesis (Fig. 1). The other models are inconsistent with a key innovation hypothesis because the innovation occurs on a branch different from the one in which the rate changes. If an inconsistent model is best for a data set, the key innovation hypothesis is unambiguously falsified. However, it can also be falsified in a consistent model under three circumstances: first, if the rate decreased rather than increased after the innovation (this is determined by comparing the maximum likelihood rate estimates on neighboring branches); second, if additional phylogenetic information on one of the three

clades suggests that rates changed within the clade rather than at its base (8, 21); and third, if additional information about diversity from more distant relatives overturns the inferred direction of rate change (11, 22). Moreover, it is difficult to assess whether the origin of a novelty yielded an immediate increase in diversification rate or triggered increased rates only after later character changes or environmental shifts. Thus, rejection of a key innovation hypothesis with the use of the methods described here is potentially straightforward, but acceptance remains more provisional (23).

The methods described above were used to test whether a significant increase in diversification coincided with the origin of the angiosperms and their synapomorphies. Whereas recent phylogenetic analyses agree that Gnetales are the closest living relatives of angiosperms (24–26), there are still disagreements regarding the exact position of the root of the angiosperm phylogeny and hence the order of early branching events. To cope with this ambiguity, we performed tests using four phylogenetic hypotheses regarding the angiosperm root: a magnolialian, Nymphaeaceae, Chloranthaceae, and Ceratophyllaceae rooting (Fig. 2). Estimates of the number of species in each major clade were obtained from Thorne (27) for angiosperms and Mabberly (28) for Gnetales.

Tests were performed with almost the entire range of possible branching times ($t_l = 0.001$; $t_u = 0.999$) and evidently hold asymptotically at the end points (the end points cannot be tested computationally, because they introduce singularities into the likelihood function for one or more models). However, the real branching time lies well within about half that range, which is [0.5, 0.999] (8). On the basis of known stratigraphic ranges and inferred phylogenetic relationships, the split of the "angiosperms" (the line that includes modern angiosperms) from their closest relatives (other anthophytes, including Gnetales and the extinct taxa Bennettitales and *Penoxylon*) must have occurred at least by the Late Triassic, roughly 200 to 210 million years ago (8). There is fossil evidence for five major clades of angiosperms in the Barremian or Early Aptian of the Cretaceous, and some fossils are found in the Hauterivian, approximately 135 to 140 million years ago. This implies that modern angiosperms were diversifying before this time. Estimates of the age of angiosperms on the basis of molecular clocks have varied widely, from the Carboniferous (29), which is inconsistent with the fossil record (30), to the Early Jurassic (31). The time between the origin of angiosperms and the origin of modern angiosperms was then between some small length of time, if an-

Fig. 2. Four recent hypotheses for the basal relationships of angiosperms. (A) Magnolialian rooting based on Donoghue and Doyle's (38) morphological cladistic analysis [figure 3.1 in (38)], which is consistent with traditional views (27, 39) and several other morphological cladistic analyses (40). (B) Nymphaeaceae and Chloranthaceae rootings. The Nymphaeaceae rooting is based on the single tree from Doyle *et al.* (41), a combined analysis of morphological characters and ribosomal RNA sequence data [figure 15 in (41)], which is consistent with trees based on ribosomal evidence alone (25). The Chloranthaceae rooting is based on Taylor and Hickey's (42) morphological cladistic analysis [figure 2 in (42)]. Both phylogenies can be shown as one tree because Nymphaeaceae and Chloranthaceae have the same species diversity. (C) Ceratophyllaceae (*rbcL*) rooting based on nucleotide sequences of the chloroplast gene *rbcL* (26, 43), in which the aquatic plant *Ceratophyllum* is the sister group of the remaining angiosperms [figure 1 in (26)]. Taxa are indicated as follows: Gn, Gnetales; Cer, Ceratophyllaceae; Chlor, Chloranthaceae; Nym, Nymphaeaceae; Magn, Magnoliales; and Ag -, the remainder of angiosperms. Species diversity is indicated for each clade.



giosperms originated soon after the split with Gnetales, and perhaps 70 million years, if the angiosperms originated shortly before the appearance of the first universally accepted angiosperm fossils (32).

Results based on the paleoherb, Chloranthaceae, and Ceratophyllaceae rootings (Fig. 2, B and C, and Table 1) agree that the best model is a two-parameter, non-key innovation model, H_2^2 , in which the ancestral rate of branching persists until after the first split within the angiosperms. Results for the magnolialian rooting (Fig. 2A) are ambiguous (Table 1). Two two-parameter models (H_2^2 and a key innovation model H_1^2) are barely rejected at the 95% level. They are both barely accepted over the interval [0.5, 0.999] supported by the fossil record as described above, but it is perhaps more conservative (especially in view of the binomial sampling variance of the Monte Carlo estimates of significance) to postulate fairly elaborate three-parameter key innovation models to explain the pattern of diversification (23). In any case, only under the magnolialian rooting is there any support for such models. In summary, key innovation models are rejected in some phylogenetic analyses that are based on morphology and in all analyses that are based on molecular data alone or on a combination of molecular and morphological evidence. The preferred model, H_2^2 , suggests that a branching rate increase did not coincide with the evolution of those innovations that are characteristic of angiosperms as a whole; rather, one or more increases occurred after angiosperms had begun to diversify. This analysis does not allow us to localize the rate increases within the clade that contains the bulk of angiosperms, but it does permit us to reject a

close association of angiosperm synapomorphies with increases in rate.

These results must be interpreted cautiously for two reasons. First, basal relationships in angiosperms are still uncertain, and other phylogenies could support a key innovation hypothesis. Second, because a pure birth model effectively equates diversification rate with speciation rate, differential rates of extinction or turnover could conceivably account for the same observations. For example, a decreased extinction rate in the clade that contains the bulk of angiosperms (Fig. 2) could account for the observed diversity patterns, even if the speciation rate remained constant. The robustness of our results can be assessed by "correcting" the observed species diversities upward in each clade in turn. Increasing the diversity in the bulk of angiosperms merely increases support for model H_2^2 . Increasing the diversity in Magnoliales (Fig. 2A) shifts the conclusions in favor of a key innovation model, but there is little fossil evidence that they were much more diverse in the Cretaceous (8). A shift to a key innovation model in the other three angiosperm rootings (Fig. 2, B and C) would require extremely high extinction rates. The fossil record does not provide evidence that these groups were ever exceptionally diverse. Instead, judging by the similarity of fossil and modern plants, it appears that species of Ceratophyllaceae, and possibly of Chloranthaceae and Nymphaeaceae, have persisted for long periods of time with low turnover (33). Finally, however, the fossil record does indicate a high diversity of the gnetalian line in the Mesozoic (34). This raises the possibility that high rates of speciation in angiosperms merely persisted from the common ancestor

of angiosperms and Gnetales. We increased the number of species in Gnetales to 1000, which corresponds to an approximately threefold increase in the gnetalian extinction rate. With these data, model H_2^2 was again preferred for the paleoherb and Chloranthaceae trees and was also preferred for the magnolialian tree. However, three-parameter models are required to explain the unusually low diversity in the Ceratophyllaceae tree (35).

Our results bear on hypotheses concerning the causes of angiosperm diversification. According to arguments developed by Doyle and Donoghue (8), trees rooted among magnolialian groups suggest that the evolution within angiosperms of rhizomatous plants with accelerated life cycles may have triggered the increase in diversity observed during the mid-Cretaceous. In contrast, trees rooted among paleoherbs suggest that angiosperms either originated near the beginning of the Cretaceous or, if they originated earlier, that the rate of evolution was not enhanced until some environmental conditions changed. In our analysis, a magnolialian root is actually most consistent with the view that angiosperm apomorphies triggered increased diversification (36), whereas paleoherb rootings strongly favor a delayed radiation. If a paleoherb rooting is correct, the rhizomatous habit and more rapid reproduction were not by themselves responsible for increased diversification, regardless of when angiosperms originated. Instead, either intrinsic factors, such as the subsequent evolution of other morphological features (perhaps in several lineages independently), or extrinsic factors, such as faunal turnover (37) or global environmental heterogeneity in the Early Cretaceous (8), must have contributed to the eventual rapid radiation of angiosperms.

Table 1. Likelihood ratio tests (β) for various branching models and four phylogenies of basal angiosperm relationships. See Fig. 1 for description of the models; phylogeny A is based on the magnolialian rooting; phylogeny B on the Nymphaeaceae and Chloranthaceae rootings; and phylogeny C on the Ceratophyllaceae rooting (Fig. 2). Constraints on branching times were $t_u = 0.999$ and $t_l = 0.001$. Significance levels (P) were determined from Monte Carlo simulation (20).

Model	Phylogeny					
	A		B		C	
	β	P	β	P	β	P
H_1^1	10.10	1.00*	13.81	1.00*	16.35	1.00*
H_2^1	3.34	0.96*	7.05	1.00*	9.59	1.00*
H_2^2	3.28	0.96*	0.77	0.49†	1.77	0.80†
H_3^2	7.56	1.00*	7.56	1.00*	7.55	1.00*
H_4^2	3.92	0.98*	7.62	1.00*	10.16	1.00*
H_3^3	0.81	0.77†	0.75	0.69	0.73	0.73
H_2^3	0.24	0.43†	0.21	0.42	0.20	0.50
H_3^3	0.42	0.60†	0.69	0.69	1.19	0.84
H_4^3	3.04	0.98*	6.75	1.00*	9.28	1.00*

*Rejection of the model relative to model H_1^1 at the 95% level.

†Preferred models.

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17. Note that integrating this probability over $[0, 1]$ does not yield 1.0 because the data space also includes events in which there are only two taxa—that is, in which no splits occur in the time interval $[0, 1]$.
18. Numerical integration was implemented by Romberg's method. Multivariate numerical maximization of the likelihood used conjugate gradient methods (Powell's method) coded as described [W. H. Press, B. P. Flannery, S. A. Teukolsky, W. T. Vetterling, *Numerical Recipes in C* (Cambridge Univ. Press, New York, 1988)]. Functions were coded in double precision with Symantec's THINK C 5.0 compiler (Cupertino, CA), executed on a Macintosh Quadra 950.
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20. Monte Carlo simulation entailed 500 replicate simulations of the relevant null models. Pseudo-values of rate parameters ($\hat{\lambda}^*$) and likelihood ratios (β^*) were obtained from the simulated values of the observations $\{N_1^*, N_2^*, N_3^*, t^*\}$. Integration over time was unnecessary in the simulations as the values t^* were generated as an outcome of the Yule process. To test the goodness of fit of the model H_k^* , we obtained the maximum likelihood rate estimates under that model, and simulation was used to estimate the distribution of β^* with the use of the parameters estimated on the assumption that H_k^* is true. If the observed β for the observations was in the upper 5% tail of the simulated distribution of β , then H_k^* was rejected. This procedure insures that a model exhibits significantly poor fit before model complexity is increased. Monte Carlo distributions of $-2 \log \beta^*$ were closely approximated by a mean-corrected χ^2 statistic with degrees of freedom of $4 - k$, where k is the class of the model tested, as described in (19).
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23. Certain consistent models must be interpreted as stepwise increases in rate, stepwise increases followed by decreases, or even multiple simultaneous rate changes (as in H_2^*). Corresponding hypotheses in terms of key innovations are still plausible in these cases, but such elaborate scenarios differ from those that are usually envisioned in discussions of angiosperm evolutionary success.
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35. One model, H_2^* , was consistent with a key innovation at the base of angiosperms followed by rapid lowering of rate in the *Ceratophyllum* line; the other two, H_1^* and H_3^* , were not consistent with key innovations because of the magnitude and direction of change of the estimated rates.
36. The conclusions of Doyle and Donoghue (8) can be reconciled with our analysis by noting that they took into account that most diversity in the Magnoliales is confined to one large family, Annonaceae (2300 species), nested within that clade, which appears to have radiated more recently. They therefore concluded that the an-
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G_h : A GTP-Binding Protein with Transglutaminase Activity and Receptor Signaling Function

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The α_1 -adrenergic receptors activate a phospholipase C enzyme by coupling to members of the large molecular size (approximately 74 to 80 kilodaltons) G_{α_h} family of guanosine triphosphate (GTP)-binding proteins. Rat liver G_{α_h} is now shown to be a tissue transglutaminase type II (TGase II). The transglutaminase activity of rat liver TGase II expressed in COS-1 cells was inhibited by the nonhydrolyzable GTP analog guanosine 5'-O-(3-thiotriphosphate) or by α_1 -adrenergic receptor activation. Rat liver TGase II also mediated α_1 -adrenergic receptor stimulation of phospholipase C activity. Thus, G_{α_h} represents a new class of GTP-binding proteins that participate in receptor signaling and may be a component of a complex regulatory network in which receptor-stimulated GTP binding switches the function of G_{α_h} from transglutamination to receptor signaling.

We have shown previously that a GTP-binding protein, termed G_h , copurifies with rat liver α_1 -adrenergic receptors in a ternary complex containing α_1 -agonist, the receptor, and G_h (1). De novo purification of G_h revealed that the 74-kD α subunit (G_{α_h}) is associated with an ~50-kD β subunit (G_{β_h}) (2). G_{β_h} modulates the GTP binding and guanosine triphosphatase (GTPase) activity of G_{α_h} . G_h proteins with α subunits of 74 to 80 kD exist in various species, including humans and cows (G_{h7}); G_{h7}

couple to α_1 -adrenergic receptors and activates a 69-kD phospholipase C (PLC) (2).

Microsequencing of endoproteinase lysine C-generated peptide fragments of G_{α_h} purified from rat liver (3) yielded four sequences—SVXRDXREDITYTYK, YPE-XXPE, SVEVSDPVPAGDXVKXRVLFP, and SVKGYXN (the identity of the residues designated X is uncertain)—all of which are highly similar to sequences contained in guinea pig, mouse, human, and bovine tissue transglutaminase type II (TGase II or TGase C; R-glutamyl-peptide:amino- γ -glutamyltransferase, E.C. 2.3.2.13). These enzymes are Ca^{2+} - and thiol-dependent acyl transferases that catalyze the formation of an amide bond between the γ -carboxamide groups of peptide-bound glutamine residues and the primary amino groups in various

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