Relating Traits to Diversification: A Simple Test

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q1 ABSTRACT: We describe a simple comparative method for determining whether rates of diversification are correlated with continuous traits in species-level phylogenies. This involves comparing traits of species with net speciation rate (number of nodes linking extant species with the root divided by the root to tip evolutionary distance), using a phylogenetically corrected correlation. We use simulations to examine the power of this test. We find that the approach has acceptable power to uncover relationships between speciation and a continuous trait and is robust to background random extinction; however, the power of the approach is reduced when the rate of trait evolution is decreased. The test has low power to relate diversification to traits when extinction rate is correlated with the trait. Clearly, there are inherent limitations in using only data on extant species to infer correlates of extinction; however, this approach q2 is potentially a powerful tool in analyzing correlates of speciation.

Keywords: keyword.

Although it is not entirely inconceivable that species originate at random (Ricklefs 2003, 2005), it does seem very likely that differences in the numbers of species between related clades may relate to differences in the phenotypic traits of those groups (Slowinski and Guyer 1993; Isaac et al. 2003, 2005; Paradis 2005). This may be because rates of speciation or extinction are nonrandom with respect to the traits of species or clades (Dial and Marzaluff 1989). Nonrandom diversification has been hypothesized to account for the evolutionary success of a range of groups as a consequence of their possession of particular characteristics (Mitter et al. 1988; Farrell et al. 1991; Marzaluff and Dial 1991; Barraclough et al. 1995; Parker and Partridge 1998; Owens et al. 1999; de Queiroz 2002; Ree 2005). To some extent, theories for the diversification of groups as a consequence of key innovations can be based on ad hoc arguments and correlations (so-called storytelling; sensu Gould and Lewontin 1979); however, comparative analysis can be used to test such theories more rigorously.

There has been a great deal of work done on measuring diversification rates and how these vary across taxa, using phylogenetic comparative analysis (see Ricklefs 2007). A phylogeny for a group of species contains several layers of information relevant to uncovering patterns of diversification, and a suite of methods for estimating diversification rates has been developed for application to phylogenetic data. First, the shape of the phylogeny may reveal imbalance in the distribution of species through the tree, with some clades possessing more species than others (Slowinski and Guyer 1993; Mooers and Heard 1997) or variation in the tempo of diversification (Purvis et al. 1995; Pybus and Harvey 2000; Rabosky 2006). In the former case, it may be possible to relate such imbalance to species' traits (Phillimore et al. 2006). Second and third, the distributions of branch lengths and node heights specify the geometry of a given tree (Huelsenbeck 1991; Harvey et al. 1994; Pybus and Harvey 2000; Nee 2001; Paradis 2005). Taken together, these can be used to infer much about the relative rates of speciation and extinction (Pybus and Harvey 2000; Nee 2001; Paradis 2005). The recent explosion in the number of groups for which detailed phylogenies have become available has permitted the historical pattern of diversification to be more precisely traced (Slowinski and Guyer 1993; Harvey et al. 1994; Nee 2001), allowing phylogenetic comparative tests to be developed and applied (Agapow and Isaac 2002; Isaac et al. 2003; Paradis q3 2005, forthcoming; Maddison 2006).

In a phylogenetic comparative analysis, the data available are typically traits measured on extant species (although see Hansen 1997; Webster and Purvis 2002), and q4

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the phylogeny is inferred from any of a variety of sources, including molecular, fossil, and hybrid sources (e.g., Purvis 1995; Bininda-Emonds et al. 2007). In a phylogenetic comparative framework, trait data are typically analyzed conditionally on a single phylogeny (e.g., Pagel and Harvey 1988; Grafen 1989; but see Huelsenbeck et al. 2000; Maddison 2006). An assumed model of evolution predicts how traits should vary and co-vary across a given phylogeny (Hansen and Martins 1996; Hansen 1997; Pagel 1997; Freckleton and Harvey 2006).

Recently, two comparative tests have been proposed that link continuous traits to rates of diversification (Isaac et al. 2003; Paradis 2005). The first of these was proposed by Isaac et al. (2003; see also Agapow and Isaac 2002). In the simplest case, it can be used to analyze whether a continuous trait is correlated with species richness, given a phylogeny, and it is based on calculating phylogenetic contrasts (Felsenstein 1973, 1985). The analysis assumes that the continuous trait under consideration has evolved according to a simple Brownian motion process (Felsenstein 1973), where trait variation accumulates at a constant rate, with positive and negative changes in traits being equally as likely (see below). At each node (bifurcation) in the tree, the ancestral state is reconstructed. The ancestral states are then used to calculate phylogenetic contrasts, which are scaled differences in ancestral states between the daughter species subtended from each node in the phylogeny. A measure of diversification is also computed: first, the number of species subtending from each node is calculated, then contrasts in the natural logarithm of the numbers of species subtending from each node are calculated (termed "relative rate differences" by the authors) and correlated with the contrasts in the trait. The computations for calculating contrasts are described in detail by Felsenstein (1985).

The prime aim of the techniques developed by Isaac et al. (2003) is to compare clade richness among higher-level clades. Comparisons among clades cannot include much of the detail available within phylogenies resolved down to the level of individual species, and for that reason, species-level analyses may often be preferred. However, in analyses of species-level phylogenies, to which this method has sometimes been applied (Isaac et al. 2005), the metrics employed by Isaac et al. (2003) do not reliably recover q5 simple relationships between speciation and traits (see appendix in the online edition of the *American Naturalist*). The main issue is that this approach assumes different models for trait evolution and the speciation process: traits are assumed to evolve according to a neutral Brownian process, whereas the speciation process is modeled as a cumulative process, and the variable analyzed is the cumulative number of species subtended at each node, thus increasing from the tips to the root of the phylogeny.

The second test recently proposed is a maximum likelihood approach based on explicit models for trait evolution, speciation, and the link between them (Paradis 2005). This model assumes that species arise according to a Yule process. The Yule process is a pure birth model in which the instantaneous rate of speciation of each lineage is a constant λ. Paradis's method uses per lineage average rates of speciation, directly estimating λ. According to the model by Paradis (2005), a single trait evolves according to a Brownian process, but each lineage in the phylogeny has a lineage specific probability of speciation, λ_i, which is a function of that trait value. Paradis (2005) derived maximum likelihood statistics for measuring this association and explored the power of the test under different models of speciation.

When the assumptions of the Paradis (2005) model were satisfied, the test performed well. However, the test was very sensitive to deviations from the assumptions of the speciation model: specifically, when extinction was introduced into the model, the performance of the test declined enormously. Under even very low rates of extinction, the power of the test declined to only about ~0.2 at most (see fig. 3 in Paradis 2005). Just as seriously, the power of the test was not improved by increasing sample size when extinction was not 0. Thus, the power of the test was the same for a phylogeny of 10 species as for one containing 1,000 or more. These results indicate that the test by Paradis (2005) should be used with caution if nonzero extinction rates are suspected.

If traits determine rates of speciation, then the simplest expectation is that lineages with particular trait values should contain more species. For instance, if some trait were positively linked to the rate of speciation, then those species with large trait values should be more numerous than those with smaller trait values. A simple way to test this association would be, for each root to tip lineage, to calculate the average diversification (bifurcation) rate and to compare this with the value of the trait. If we have n species, there will be n such lineages. For a single lineage, the diversification rate is given by the number of speciation events between the root and the tip (equal to the number of nodes between the root and the tip; termed "node density" or "depth"), divided by the root to tip distance (which is the same for all tips if they are all extant and the tree is measured in units of time).

The problem with this approach is that species are not independent in terms of both traits and diversification owing to shared ancestry; consequently, per lineage diversification rates will be highly phylogenetically dependent across different lineages. Thus, such a test would be invalid owing to phylogenetic nonindependence (Harvey and Pagel 1990; Isaac et al. 2003). Here we propose that both the trait and rate of diversification may be analyzed

using conventional comparative approaches in order to test the correlation between traits and diversification, and we explore this approach using simulations. We simulate phylogenies in which traits determine the rates of speciation and extinction. We show that this approach is powerful in detecting associations between speciation and traits and that the test is relatively robust to varying the rate of random extinction. However, we find that this approach is unsuccessful in uncovering relationships between extinction and traits, a finding that can largely be attributed to the loss of trait variation in extant species. We suggest how such cases may be identified and discuss how the approach we describe may be extended.

Methods

Model of Trait Evolution

The modeling approach taken for evaluating our proposed test closely follows that suggested by Paradis (2005). The model for trait evolution we employ is the Brownian model, which is the model most commonly used in comparative analyses (Felsenstein 1985; Harvey and Rambaut 2000). We consider the evolution of a single trait evolving among n species, the state of which is represented by a vector $\mathbf{x}$. Under the Brownian model, if t is the time over which the trait is evolving, then $\Delta\mathbf{x}$, the change in $\mathbf{x}$, is a multivariate normal (MVN) random deviate:

$$\Delta\mathbf{x} = \mathrm{MVN}(0, \sigma^2\Sigma t). \quad (1)$$

The parameter Σ is an $n \times n$ matrix proportional to the expected variances and covariances for trait changes among the species, which is defined by shared path lengths on the phylogeny (e.g., Hansen and Martins 1996; Martins and Hansen 1997; Pagel 1997). After T units of time (if the elements of Σ are proportional to time), $\mathbf{x}(T)$ is thus a multivariate normally distributed random variate with mean $\mathbf{x}(0)$ and variance-covariance matrix $\sigma^2\Sigma T$.

The parameter σ^2 in equation (1) is the rate of accumulation of variance of traits per unit time. This parameter is important because it determines how widely dispersed trait values will be following a period of evolution. If speciation or extinction is related to the trait, then this parameter will be important in determining the differential in these rates among species by determining how wide the differences in traits among species are. We therefore explored both a low value (0.02) and a high value (0.2) of σ^2. The lower value was used by Paradis (2005); we chose the higher value to explore the effect of increasing σ^2.

From the point of view of modeling trait evolution, the key features of the Brownian model are as follows. (1) Traits evolve constantly; that is, they do not become fixed at an optimum value for each species. (2) Once they have split, the evolution of species is independent, such that potentially several species may adopt similar trait values. (3) The values of traits are effectively unbounded (i.e., the variance in trait values grows linearly with time).

Models of Speciation and Extinction

We used a modeling framework similar to that suggested by Paradis (2005). The instantaneous probability of speciation per lineage per unit time is λ, and the probability of extinction per lineage per unit time is μ. In the first case, we assumed that the probability of speciation is a function of traits. For species i with trait value x_i, the rate of speciation is

$$\lambda_i = \exp(\beta x_i - \alpha). \quad (2)$$

The parameters α and β are constants determining the relationship. We set α at a constant of 3 (this is simply a scaling factor). The parameter β determines the strength of the relationship between traits and speciation; if $\beta = 0$, then there is no relationship, and the value of λ is a constant 0.048. We then varied β between 0 and 4. In the first set of simulations, we varied the extinction rate between 0 and 0.01. These values were chosen on the basis of those used by Paradis (2005); however, compared with that study, we chose to expand the range of extinction rates employed.

In the second set of simulations, we set the rate of speciation at a constant value of 0.048 (i.e., corresponding to values of $\alpha = -3$ and $\beta = 0$ in eq. [2]). The instantaneous rate of extinction of each lineage was then modeled as

$$\mu_i = \mu_{\mathrm{m}} \exp(\beta x_i - \alpha). \quad (3)$$

The parameters α and β, as in equation (1), define the response. The parameter μ_{m} is the rate of extinction at $x = 0$ and was varied between 0.001 and 0.04.

In the final set of simulations, we assumed that the rates of both speciation and extinction were related to trait values. We modeled the rate of speciation using equation (2). Then the rate of extinction was set as a constant proportion of the rate of speciation, varied between 0.1 and 0.9; for example, if the rate of speciation per unit time was 0.2 and the ratio of extinction to speciation was equal to 0.7, then the rate of extinction was set at 0.14.

Phylogenies and traits were evolved using an event-driven model programmed in Java 1.4 using a library of code written by R. P. Freckleton. This code is available on request.

Phylogenetic Analysis

We define node depth for species *i*, N_i, as the number of nodes on the path linking species i to the root of the phylogeny, not including the root. If T_i is the total evolutionary time from the root to the tip containing species *i*, then the mean rate of speciation per unit time for the lineage leading to species i is

$$\lambda_i = \frac{N_i}{T}. \tag{4}$$

In the phylogenies we simulated, all phylogenies were ultrametric; that is, the root to tip distance is the same for all species. Consequently, T_i is the same for all species, and hence for any species *i*, $\lambda_i \propto N_i$. The same should be true for any phylogeny in which all the tips relate to extant species.

Note that because we are dealing with average rates of speciation along single lineages, equation (4) is the correct estimate of the speciation rate, not $\ln(N)/T$. The latter is appropriate when estimating the rate of diversification of a clade of age T that contains N species. This distinction is important if the tree is not ultrametric or if speciation rates are estimated for sublineages.

For a given constant speciation rate $\hat{\lambda}$, for independent lineages, N_i will be Poisson distributed with mean and variance both equal to $\hat{\lambda}T_i$. This is because successive speciation events are assumed to occur at random and at a constant rate. If the mean value of λ varies through time, then if the overall time-averaged rate of speciation (i.e., the average rate of speciation along a single lineage leading from root to tip) is $\bar{\lambda}$, N_i will be Poisson distributed with mean $\bar{\lambda}T_i$. This is because, if λ varies through time, N_i is distributed according to an inhomogeneous Poisson process, which effectively the sum or integral of simple Poisson processes. Under the inhomogeneous Poisson process, N_i is distributed according to a Poisson process (Hogg and Tanis 1996). This is an important property in a phylogeny in which speciation is linked to trait values and the value of λ varies through time and is not fixed. However, in such cases, equation (4) still yields an appropriate estimate of the mean rate of speciation.

Although the time-averaged rate of speciation $\bar{\lambda}$ is an unbiased estimate of the net diversification rate, it is not true that $\bar{\lambda}$ is related to $\bar{x}$, the average state of the trait, through a simple averaging of equation (2) or (3). This is because equations (2) and (3) are nonlinear, and in general for nonlinear functions, $\overline{f(x)} \neq f(\bar{x})$. In a lineage in which x is varying through time, variance in x will lead to a disparity between $\bar{\lambda}$ and the value predicted by equation (2) or (3). As we show in the appendix, for the particular function we use, this has no major consequence for the simulations we report below, although this would not always be expected to be the case, and in the appendix, we give an example.

We analyzed the data using a bivariate comparative model. Although in this case we knew the underlying model (i.e., which of eqq. [2] and [3] generated the data), in reality the model relating traits to speciation would not be known. We therefore analyzed data using simple linear correlations and estimated the Type I errors and statistical power of this test under different conditions. We discuss other possibilities below in the context of uncovering effects of extinction. In particular, analysis of the residuals from the fitted model should be able to reveal whether the linear model is adequate, and we suggest that this may be an important part of the model fitting process in such analyses.

We used a generalized least squares approach (e.g., see Martins and Hansen 1997 and Pagel 1997 for an outline of how this is formulated in a phylogenetic context) in which the trait x was treated as the dependent variable and node depth N as the predictor. The models defined by equations (2) and (3) are linear for log N (we logarithmically transformed N, equivalent to λ, before analysis). In real applications, it would be wise to consider several transformations—for instance, identity, square root, and logarithmic—with a view to ensuring that the assumptions of the test hold.

Note that this is a conventional comparative model that is computationally identical to calculating standardized evolutionary contrasts for x and log N and performing a correlation analysis on these (Felsenstein 1985). This analysis can therefore be implemented using any of a number of existing computer packages for conducting comparative analysis. An example of the analysis is given in figure A2 in the online edition of the *American Naturalist*.

The analysis effectively assumes that the variation in x given log N evolves according to a Brownian process. Note that analyzed this way, the technique models the residual variation in x, conditional on ln N, even though we are ultimately interested in making predictions about the effect of x on ln N. We have framed the analysis in this way because N cannot, strictly speaking, have evolved according to a Brownian process since N is not a continuous trait (one consequence being that sister species share the same value of N). In the analysis outlined below, we assume only that the residual variation in x has evolved in a Brownian manner. We discuss this issue at greater length below; however, in the current analysis, the important point is that, viewed in this way, the phylogenetic distribution of N is irrelevant since it is treated as the predictor.

Another approach to analyzing speciation and comparative data is to test whether the amount of trait change is correlated with branch lengths (Garland et al. 1992; the κ

statistic of Pagel 1997). This is an alternative approach, the main difference being that it is nondirectional in that it assumes that trait change is associated with speciation but not that the trait state is correlated with speciation.

Example Data

We applied the analysis to two data sets, both taken from Isaac et al. (2005). The data sets in question were data on a range of traits in carnivores and primates. We chose these data sets because the phylogenies are large (271 and 233 species, respectively) and the nodes of both phylogenies are dated and comparatively well resolved. The traits analyzed are summarized in table 1. A key assumption in the method described above is that the variation in the traits is well described by the Brownian model. There could be a number of reasons why this would not be the case, including measurement error and phylogenetically independent adaptation. In order to ensure that this assumption was met, we calculated correlations between traits and speciation rates in conjunction with the λ statistic of Pagel (1997). This parameter effectively optimizes the model for the level of phylogenetic dependence observed in the data set. We fitted this model using the method given by Freckleton et al. (2002).

Results

Simulations

To summarize our main conclusions, figure 1 shows examples of phylogenies in which traits are linked to speciation (fig. 1*A*) or to extinction (fig. 1*B*). In figure 1*A*, there is a clear correlation between the trait and node depth, whereas in figure 1*B*, the relationship is not as straightforward. In figure 1*B*, there is some indication that the deeper nodes (i.e., those with low node depth) have higher trait values; however, for the majority of the range of node depth, there is no relationship (when phylogeny has been controlled for) between this and the trait state. The broad result then is that the test we describe is able to detect relationships between traits and node depth in phylogenies in which traits are linked to speciation; however, in the converse case in which extinction is related to traits, the situation is more complex.

Note that in figure 1 many species inevitably share the same node depth. Consequently, in presenting the results of this type of analysis, we have found it useful to present the average trait value (together with error bars) across all species sharing the same node depth in order to more clearly discern patterns (e.g., see figs. 2, A1 [in the online edition of the *American Naturalist*]). The statistical test, however, utilizes the raw data.

Table 1: Correlates of speciation in carnivores and primates

	Carnivores			Primates		
Trait	*r*	*n*	*P*	*r*	*n*	*P*
Body mass	−.024	240	.721	.169	194	**.0185**
Mass dimorphism	.008	101	**.032**	.104	189	.153
Length dimorphism	.015	89	.019	NA	NA	NA
BMR	−.073	24	.112	.267	16	.317
Interbirth interval	−.008	117	.613	.021	112	.821
Geographic range	−.054	236	.299	.119	229	.072
Home range	−.254	99	**.013**	.105	145	.208
Population density	.073	88	.378	.097	134	.263
EPD	.093	70	.294	.119	107	.221
Gestation	−.010	134	.406	.001	120	.988
Litter size	−.003	171	.857	.028	172	.712
Age at sexual maturity	.005	104	.848	.067	96	.514
Group size	−.059	109	.077	.206	187	**.0047**
Diet/trophic level	−.037	189	.052	.173	225	**.0093**

Note: Data are taken from Isaac et al. (2005) and relate to ecological and life-history variables in carnivores and primates. The table shows the correlation of each trait with node depth, together with the sample size and two-tailed *P* value. In order to account for possible deviations from the Brownian model, models were fitted with the λ statistic of Pagel (1997), simultaneously estimated following the method in Freckleton et al. (2002) and set to its maximum likelihood value. Traits were log transformed before analysis. NA = not applicable.

Relationship between Traits and Speciation

Figure 2 shows the relationship between the proportion of significant results obtained (i.e., the power of the test) and the parameter β determining the strength of the speciation effect. The test is most powerful when the Brownian variance is high. However, in both the low- and high-variance simulations, its power increases with both the number of species in the phylogeny. The power of the test also initially increases the value of β and then stabilizes at higher values. The test is also robust to increasing μ, the background stochastic rate of extinction. Finally, for all parameter combinations, the Type I error rate of the test (i.e., the proportion of false significant results) was acceptable and around the 0.05 nominal level.

Relationship between Traits and Extinction

When extinction is correlated with trait values, the test performs poorly (fig. 3). What happens is that, as evolution proceeds, for positive values of β, those species with extreme high values are more likely to become extinct and eventually do. Species with low trait values and hence low probability of extinction are much more likely to survive. This has a triple effect: (1) there is a pruning of the range of trait values, with the consequence that there is only a small proportion of species with a heightened probability of extinction resulting from their trait values; (2) the number of nodes remaining in the tree will be rather less than

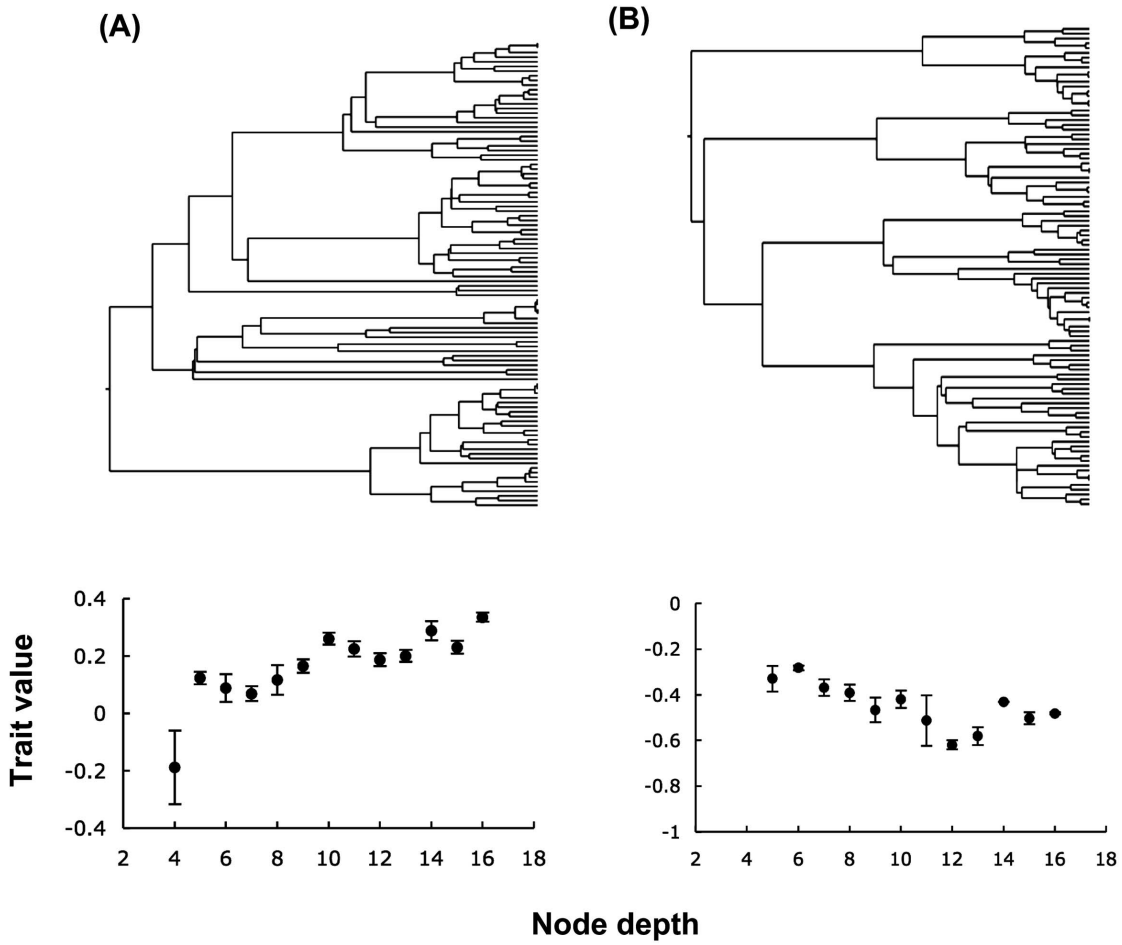

Figure 1: Examples of phylogenies evolved according to trait-dependent speciation (*A*) and trait-dependent extinction (*B*). The phylogenies are shown, together with relationships between trait values and node depth. In both cases, $\beta = 4$ and $\sigma = 0.02$. There was no background extinction in *A*.

the number of nodes that ever existed, thus eradicating much of the information on past diversification, that is, a loss of history; and (3) this loss of history will be more extreme for deeper nodes where trait variation is expected to have been greater. As a consequence, node depth is a poor estimator of extinction rate. It is for these reasons that in figure 1*B*, which shows an example of a phylogeny evolved according to this process, species with a relatively high node depth (i.e., are more recently evolved) appear to have similar trait values. It is only those few species that have very low node depth (and originated further back in time) that still retain some of the historical correlates of extinction.

Association between Traits and Both Speciation and Extinction

Figure 4 shows the performance of the test when both speciation and extinction are related to trait values for the high Brownian variance (i.e., corresponding to fig. 3*e*–3*h*), together with the effects of increasing the ratio of extinction to speciation. The test is capable of identifying relationships; however, as the rate of extinction is increased, the performance is steadily eroded for the reasons discussed above. We do not report simulations in which extinction is correlated with speciation in the opposite way (i.e., species with high rates of speciation have lower extinction). However, much the same result would be expected, since in such cases increasing the relative level of extinction to speciation would progressively erode the signal in the data and hence reduce the power of the analysis.

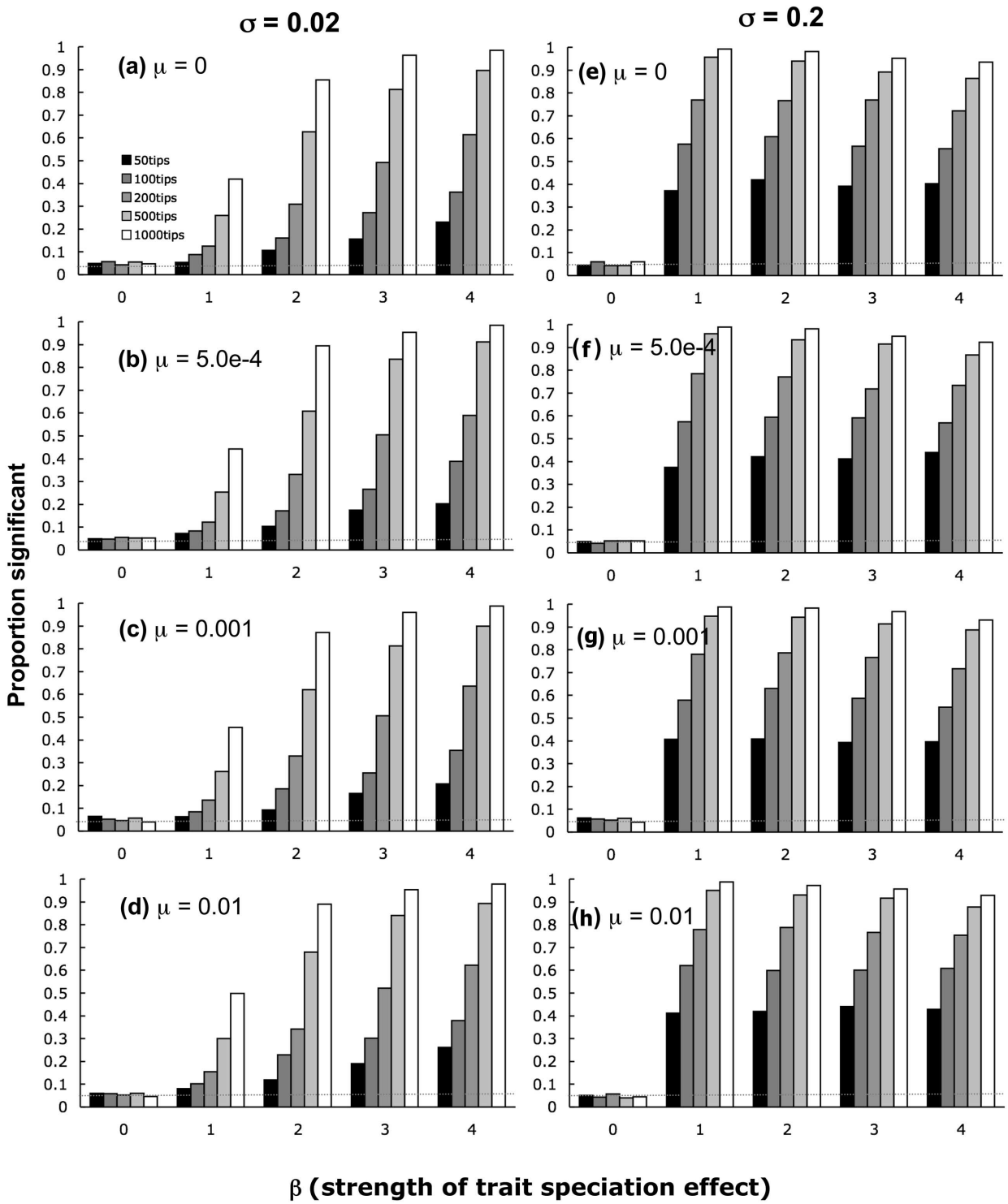

Figure 2: Power of the correlation test when traits are correlated with speciation rates. The parameter β and the number of tips evolved in the phylogeny were varied as shown, along with the extinction rate, μ, as shown. The standard deviation of the rate of change in trait variance was set at a low value (0.02; *a*–*d*) or a high value (0.2; *e*–*h*).

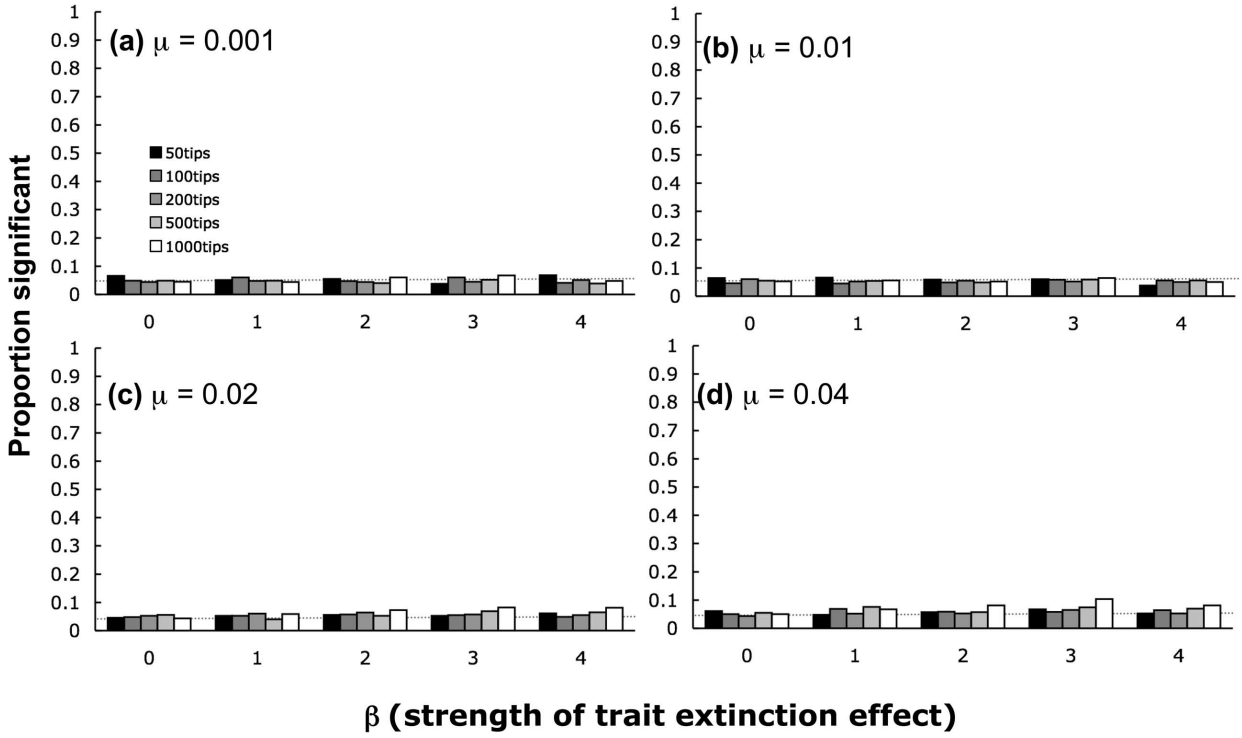

Figure 3: Power of the correlation test when traits are correlated with extinction rates. The parameter β and the number of tips evolved in the phylogeny were varied as shown, along with the extinction rate, μ, as shown. The standard deviation of the rate of change in trait variance was set at a low value (0.02).

Summary

In summary, the test proposed here is capable of correctly identifying associations between speciation rate and traits. However, the test performs poorly in relating traits to extinction or when low rates of trait evolution lead to a reduced variance of speciation rates among taxa. We suggest that these results may well be general for any similar test (e.g., the simulations of Paradis [2005] are in broad accord with this). However, as noted above, careful consideration of the detailed relationship between traits and node depth may yield insights, in particular through employing nonlinear analyses.

Analysis of Example Data

Table 1 summarizes the analysis of the data on carnivores and primates. For the carnivores, we identified only two weakly significant correlates of diversification using phylogenetically corrected correlations of traits with node depth.

In the case of primates, the analysis suggested three statistically significant correlates: body size, group size, and trophic level. Figure 5*a*–5*c* shows the relationships found. In the case of body size, although the correlation found was statistically significant, the relationship was clearly not a simple linear one. Instead, the analysis suggested that most species are large, except for a few species with low node depth (three or four connections from the root).

There is a rather clearer relationship between group size and node density in primates (fig. 5*b*). The relationship between trophic level and node depth in primates is essentially the inverse of that for body size (fig. 5*c*), reflecting a close negative association between trophic level and body size in this data set.

For illustrative purposes, figure 5*d* shows an example of one trait (body size in carnivores) that shows no relationship. It should be noted, however, that in many of the analyses reported in table 1, there are missing data for some species that would be expected to detract from the power of any test to uncover relationships between diversification and traits.

Discussion

Uncovering correlates of speciation and extinction is an important goal in macroevolutionary studies (Dial and

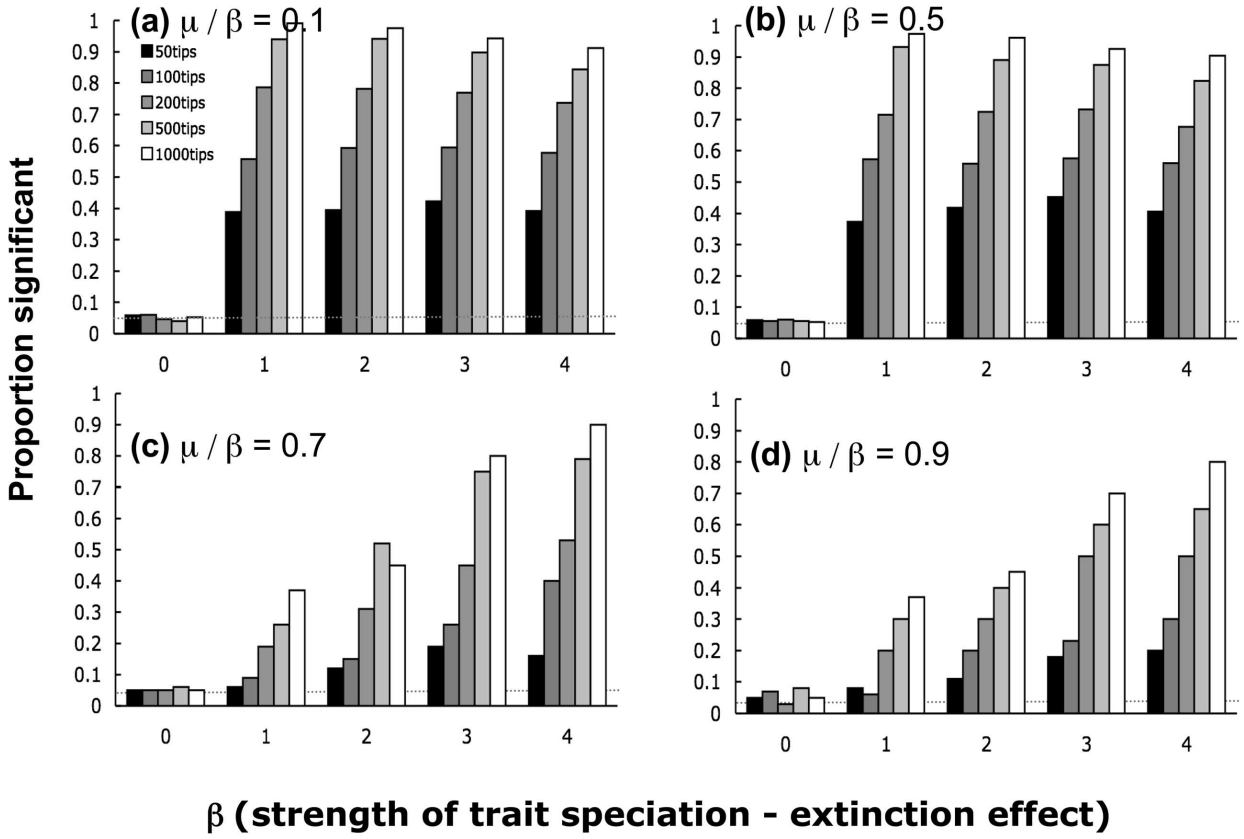

Figure 4: Power of the correlation test when traits are correlated with both speciation and extinction rates. The parameter β and the number of tips evolved in the phylogeny were varied as shown, along with the ratio of extinction rate to speciation rate, as shown. The standard deviation of the rate of change in trait variance was set at a high value (0.2).

Marzaluff 1989; Purvis 1996), and many existing analyses have attempted to do this. Studies performed on ecological timescales (i.e., looking at periods of a number of decades or so) have frequently found close correlates of population declines and extinctions (Duncan et al. 2002; Blackburn et al. 2004; Haugaasen and Peres 2005; Shultz et al. 2005). However, phylogenetic comparative approaches seem to have been less successful in finding strong, clear correlates of diversification (e.g., Isaac et al. 2005; but see Phillimore et al. 2006). In this article, we have illustrated a straightforward test for relating comparative data to diversification rates, and we also explored the limits of this approach. We have shown that when traits are linked to extinction or when evolution of traits is slow, differential extinction and survival of species may erode key signals from comparative data. Moreover, large phylogenies may be required in order to reliably pick up signals from data. As we argue below, it seems likely that these limitations are not specific to the analysis we have looked at but are likely to be general problems in the comparative analysis of diversification patterns.

Models for Diversification

A strength of the approach of Paradis (2005) was that it was centered on an explicit model of diversification as a function of traits. Paradis's model was based on equation (2), in which the instantaneous probability of diversification is a logistic function of the trait state. As a statistical model, the approach we have taken may be justified in the following way: if the phylogeny evolves according to a constant birth process in which the speciation rate is λ and a lineage is of length t, then the expected number of events occurring is a Poisson-distributed random variable with mean λt. As long as the expected number of speciation events (λt) is reasonably large (≥ 5), then the expected distribution of the number of speciation events for a lineage of length t is well approximated by a normal

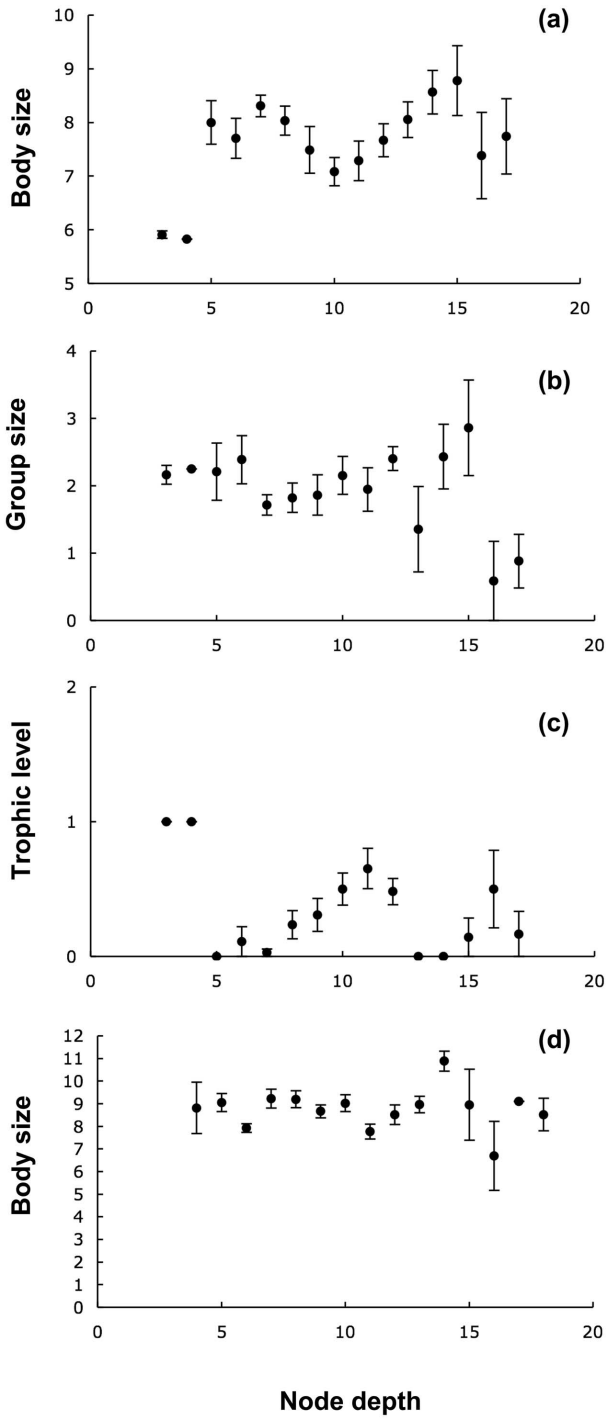

Figure 5: Example of the application of the correlation test to real data. We analyzed data on life-history and ecological variables taken from Isaac et al. (2005; see table 1). The plots show selected correlations; table 1 reports the full analysis. *a*, Relationship between body size and node depth in primates. *b*, Relationship between group size and node depth in primates. *c*, Relationship between diet and node depth in primates. *d*, Relationship between body size and node depth in carnivores.

distribution with variance λt (e.g., Grafen and Hails 2002). For a group of species, the variance and covariance number of speciation events would be expected to be approximately proportional to shared branch length. Thus, the distribution of the number of speciation events shares certain properties with the Brownian model of trait evolution.

We have applied the approach described above in the context of uncovering correlates of speciation and diversification rates in species-level phylogenies. Frequently, analyses of diversification are conducted not on species-level phylogenies but on clade richness within higher-order phylogenies (e.g., Isaac et al. 2003; Phillimore et al. 2006). The approach we have described here has to be modified slightly to be applied to clade richness because, as noted above, for a given speciation rate λ, the expected number of lineages within a clade of age t is $\exp(\lambda t)$. Thus, the correct estimator of the rate of speciation in a clade of S species is $\ln(S)/T$, assuming no extinction. There is no reason, however, why the GLS approach should not be q13
applied in such situations (e.g., see Phillimore et al. 2006). The alternative is the method by Isaac et al. (2003), which differs in the way that the states of continuous variable are calculated at the internal nodes of the phylogeny.

The statistical model we have employed is based on a simple linear correlational model. As illustrated in the appendix, the test we describe is capable of correctly identifying linear relationships when they exist but may sometimes be oversimplistic. For instance, equation (2) may be expected to yield a nonlinear relationship for many parameter combinations, although trait variance will serve to linearize the relationship (fig. A1). As yet, there are no empirical studies that clearly demonstrate what the relationship between diversification and traits might actually look like. In practice, we would suggest that the form of the relationship be examined closely.

The second problem is that relationships between speciation and traits may well be clade specific. For instance, in his analysis of body size and speciation in primates, Paradis (2005) found evidence of significant variation among families. This would be expected to confound broad-brush analyses of the sort described above, and it is probably unrealistic in many cases to expect the same underlying model of diversification to underpin the evolution of major groups over many millions of years.

Multivariable Models

In the simulation analysis, we considered only bivariate correlations, although as pointed out by Paradis (2005), it may often be necessary to consider multiple predictors, since he found that there was a clade-specific relationship between body size and diversification in primates. In bivariate analyses, it is justified to denote the trait as the

dependent variable and treat the number of species as the predictor, even though the aim is to draw inferences about the effect of the trait on the rate of speciation. This is because it would make no difference which way we treat the data, since the estimated correlation and probability level for the test on the correlation will not be affected. However, if we conduct an analysis in which we wish to measure the effects of several predictors simultaneously, then given that it would be statistically not recommended to perform individual bivariate correlations for each potential predictor (e.g., as in our table 1, in which we used bivariate correlations purely for illustrative purposes), it will be necessary to treat speciation rate or number of nodes as the dependent variable.

Treating node depth as the dependent variable creates a potential problem since sister species will share the same node depth, and this would appear to violate the assumption of a multivariate normal distribution of data. However, in a comparative analysis in which the number of speciation events is being modeled as a function of a set of predictors, the comparative analysis actually models the residual variation in traits. Thus, even if two species share the same number of speciation events because they are sister species, if they differ in the values of the predictors used, the residuals for the two species will be different. Consequently, the assumption of a multivariate normal distribution of residuals may well still be justified.

The Problems of Extinction

Understanding the relationship between extinction and species traits is important because it could potentially increase our ability to predict and manage the current extinction crisis (Purvis et al. 2000*a*, 2000*b*; Cardillo et al. 2005*a*, 2005*b*). It is easy to imagine how such relationships might arise, and data based on recent extinctions, population declines, or extinction risk have revealed evidence of such associations (e.g., Purvis et al. 2000*b*; Cardillo et al. 2005*a*, 2005*b*; Haugaasen and Peres 2005; Shultz et al. 2005).

The simulation results indicated that these types of association might be difficult to pick up using phylogenies and trait data on extant species alone. The problem is that when extinction is a function of traits, differential losses of species with trait values with high extinction risk leads to a loss of trait variation. Only species with low probabilities of extinction remain, with the consequence that there is a low power to detect effects (figs. 3, 4). As noted, this problem may perhaps be overcome to some extent by looking in detail at the pattern of trait distribution in relation to node depth (e.g., figs. 1, 5).

We analyzed data on correlates of diversification in carnivores and primates, there being good evidence that traits and diversification are linked in the origins of mammals and other tetrapods (e.g., Ruta et al. 2006). In carnivores, it has previously been suggested that group size is a correlate of extinction (e.g., Munoz-Duran 2002 [although the measure of diversification is different in that article]). We, however, found no correlation: this difference may be explained by the lack of sensitivity of our analysis to correlates of extinction. We found some evidence for a positive correlation of body size with diversification in primates. This may be a function of several factors; for example, larger species tend to have larger range sizes, thus potentially making them more likely to undergo vicariance events. Moreover, the evidence is that mean body size across mammals has been relatively constant through time (e.g., Smith et al. 2004), indicating that either extinction or speciation is not random with respect to size. The results for primates suggest that, for this group at least, nonrandom speciation may be a factor. We should, however, be cautious about inferring too much from the results in table 2, since for most traits, the proportion of species for which trait data are available is low. q14

Extinction risk is frequently used as a surrogate variable for extinction rate in comparative analyses (e.g., Purvis et al. 2000*a*, 2000*b*; Cardillo et al. 2005*a*, 2005*b*). This is one approach to dealing with problems of bias in estimates of the strength of correlates of extinction. This approach obviates the difficulty of estimating rates using only phylogenies of extant species. However, the drawback of this is that this approach is powerful in estimating correlates of current extinction risk; however, current extinction risk may not be correlated with past extinction rates, most notably because of the impact of human activities on current extinction rates (Steadman 2006).

The analysis we describe is more robust to moderate amounts of random extinction than that by Paradis (2005). As suggested previously, the reason why the test by Paradis (2005) is so adversely affected is that it is dependent on the assumption that the distribution of node heights and branch lengths is generated by a Yule model. In a group subject to random extinction, as either phylogeny size or extinction rate is increased, this assumption is progressively less valid. The consequence is that the test appears to be remarkably sensitive to including even very low rates of extinction (e.g., see fig. 3 in Paradis 2005).

Power and Sample Size

The preceding two sections have emphasized that the power of analyses of correlates of speciation may suffer for various reasons relating to the rate of trait evolution and extinction. A further issue that emerged in the simulations was that the size of the phylogeny is important. As a rule of thumb, we would not expect reliable results

from a phylogeny of fewer than 50 species. In practice, around 100–200 species would be desirable to ensure reasonable results, at least on the basis of the results of the model for speciation used here. At face value, this may seem restrictive, although such sample sizes are becoming routine in such analyses (Isaac et al. 2005; Paradis 2005). The problem is that the expected number of speciation events from root to tip does not increase linearly with the number of species in the phylogeny (to an approximation, it would be expected to increase logarithmically, i.e., at a rate proportional to the reciprocal of the size of the phylogeny). Consequently, the relative increase in expected node density of adding 100 species to a phylogeny of 100 existing species is the same as adding 1,000 to 1,000 existing species. However, a tendency to focus on large phylogenies may introduce biases of its own (Ricklefs 2007).

One consequence of this requirement for large sample sizes is that the explanatory power of tests for correlates of diversification will frequently be low. Low explanatory power of such tests has already been noted (e.g., Isaac et al. 2005; Phillimore et al. 2006). In one sense, this should not be surprising. For example, if a phylogeny is evolved according to an equal rates Markov process with a constant rate of diversification, any split is expected to result with equal probability, illustrating that the outcome of the underlying process is enormously stochastic. That this stochasticity should also be manifest in phylogenies evolved when speciation is determined by traits is therefore perhaps not unexpected.

We would expect that these problems would present difficulties for any analysis of speciation rates. For instance, figure 3 in the study by Paradis (2005) indicates that his test does not become reliable until the sample size reaches c. $\exp(5) = 148$ species. The problem is that the probability distribution of speciation events per lineage is inherently very stochastic and phylogenetically constrained. For a given sample size, phylogenies in which there is a great deal of variation in node depth will be much more likely to yield unequivocal results.

In practical terms, one consequence is that poorly resolved trees, or those containing multiple or large polytomies, will be less suitable for analysis of speciation and diversification. The main problem is that in such phylogenies, assuming that polytomies are nodes that cannot be resolved, the speciation rate will be estimated poorly at the tips. If there are i branches subtended from a polytomous node, then in counting the nodes from root to tip, these i speciation events may be counted either as a single polytomous node (i.e., underestimating the speciation rate for all tips) or as i nodes (i.e., overestimating the speciation rate). Whichever course is taken, the net result will be to decrease the power of the test, since several species differing in their trait values will be assigned the same diversification rate. One approach to dealing with phylogenies in which there are a high proportion of polytomous nodes would be to conduct clade-level analyses.

Concluding Remarks

The question of what determines rates of speciation and extinction is a key problem for evolutionary biologists. There now exists an impressive array of analytical tools, trait data, and phylogenetic information with which to address this problem. The approach we have described provides a potentially powerful analytical approach that can be used in analyses of speciation rate. However, in outlining the approach, we have tried to highlight the inherent problems and pitfalls, specifically concerning the effects of extinction and high rates of trait variation. These problems need to be borne in mind and most frequently will result in the researcher failing to detect relationships when they are expected to arise, since the effect is to decrease statistical power. Under such circumstances, the best advice would be to be somewhat circumspect in drawing conclusions, particularly in the absence of correlations.

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