Estimating diversification rates from phylogenetic information

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Patterns of species richness reflect the balance between speciation and extinction over the evolutionary history of life. These processes are influenced by the size and geographical complexity of regions, conditions of the environment, and attributes of individuals and species. Diversity within clades also depends on age and thus the time available for accumulating species. Estimating rates of diversification is key to understanding how these factors have shaped patterns of species richness. Several approaches to calculating both relative and absolute rates of speciation and extinction within clades are based on phylogenetic reconstructions of evolutionary relationships. As the size and quality of phylogenies increases, these approaches will find broader application. However, phylogeny reconstruction fosters a perceptual bias of continual increase in species richness, and the analysis of primarily large clades produces a data selection bias. Recognizing these biases will encourage the development of more realistic models of diversification and the regulation of species richness.

Building diversity

Species richness varies widely over the surface of the Earth, most conspicuously as a decrease in diversity from its peak in the humid tropics towards higher latitudes [1]. The number of species in a region reflects the balance between speciation (see Glossary) and extinction acting over long periods [2]. Biologists have adopted several approaches to evaluate how variation in speciation and extinction rates influences global patterns in species richness [3–5]. Diversity also varies widely among taxonomic groups – for example, among plant families from the monotypic Amborellaceae to the Orchidaceae, with ~25 000 species. Attributes of species, including population size, generation time, mechanisms of pollination and seed dispersal, and strength of sexual selection, are thought to influence rates of speciation and extinction, hence the number of species in a taxon [6,7]. Thus, quantifying speciation and extinction can help us to understand the causes of variation in diversity. A core concept in this endeavor is the clade, which includes all of the species – independently evolving lineages – that have descended from a common ancestor. Here, I review several methods to estimate speciation and extinction rates from reconstructions of phylogenetic relationships within clades.

Comparisons of species richness among sister clades enable one to test hypotheses about the influence of species attributes and environmental conditions, such as climate and landscape heterogeneity, on diversification [5,6,8,9]. Additional methods have been developed to tackle the more difficult task of estimating absolute rates of speciation (lineage splitting) and extinction (lineage termination) [10–12]. As with any set of methods, one must acknowledge certain assumptions, confront potential biases and evaluate confidence in estimated parameters. A limitation of phylogenetic reconstruction based on extant species is that extinct lineages are not represented. As we shall see, this limitation makes estimating speciation and extinction rates problematic.

The random speciation–extinction process

Estimating rates of speciation and extinction from phylogenetic information depends on an underlying model of diversification. The simplest and most widely applied of a variety of models is the random speciation–extinction process [12–15], which resembles random birth–death models used to study stochastic fluctuations in population size and extinction risk in small populations [16,17]. Every clade begins as a single 'stem' lineage that splits to form two descendant lineages. These are the first branches in a phylogenetic tree, which represents lineage relationships within a clade (Figure 1). Each branch in the growing tree has the same potential fate – to terminate or to split. In this

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Glossary

Ancestral lineage: an evolutionary lineage represented by a branch in a phylogenetic tree that has living descendants.
Clade: a group of lineages consisting of a single common ancestor and all of the descendants of that common ancestor.
Extinction: in a phylogenetic context, the termination of a branch in a phylogenetic tree; that is, a branch that has no living descendants and cannot therefore be reconstructed from data obtained from contemporary species.
γ-Statistic: a value describing the distribution of nodes in a phylogeny with respect to time, which has been used to test the hypothesis of time homogeneity in the diversification process.
Lineage: an independently evolving species through time that appears as a branch in a phylogenetic tree.
Lineage-through-time (LTT) plot: the relationship between the logarithm of the number of ancestral lineages and time in a phylogenetic tree, which can be used to estimate speciation and extinction rates.
Phylogeny: the evolutionary relationships among the lineages in a clade, illustrated by the pattern of branching in a phylogenetic tree.
Sister taxa: a pair of species or clades descending from a single common ancestor.
Speciation: in a phylogenetic context, the splitting of an ancestral lineage into two evolutionarily independent descendant lineages.
Time homogeneity: the condition in which rates of speciation and extinction are constant over time.

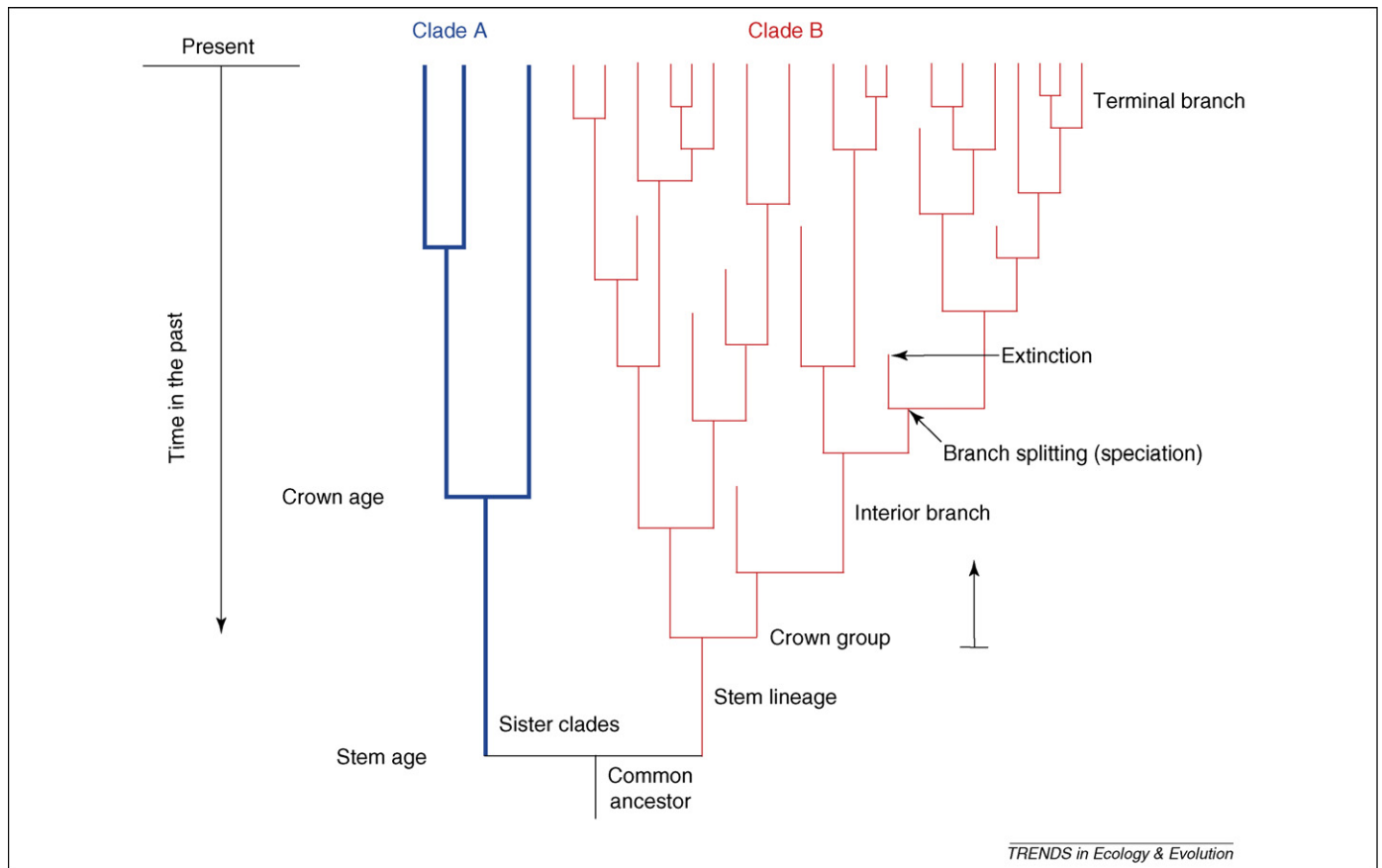*Corresponding author:* Ricklefs, R.E. (ricklefs@umsl.edu).

Figure 1. Terms pertaining to phylogenetic trees. Sister clades A and B have a common ancestor at the base of the phylogeny from which the stem lineage (i.e. common ancestor) of each clade descends. At any one time, a clade consists of two or more independently evolving lineages, which are branches of the tree. The stem age of a clade is measured from its origin. The crown age of a clade is measured from the point of the first branch, which is the stem age for the two daughter lineages (both giving rise to clades that are nested within the next largest clade). In this example, in the present time, clade A includes three species (i.e. terminal branches or lineages) and clade B contains 18 species.

way, a clade can grow over time; it might also dwindle to extinction.

In a random speciation–extinction process, both speciation and extinction have instantaneous probabilities, or rates (λ and μ, respectively; units are 1/time), which determine the probabilities that a clade either splits or terminates within a given time interval. The intervals between the formation of a new lineage by speciation and subsequent lineage splitting or termination are exponentially distributed, with average times $1/\lambda$ and $1/\mu$, respectively. Over time, the probability distribution of clade size changes in a predictable manner (Box 1). In a simple branching process (i.e. speciation without extinction), often called the Yule process [12], the expected (mean) number of lineages in a clade, $E(n)$, increases exponentially with time (t) at rate λ, or $E(n) = \exp(\lambda t)$. No lineages become extinct, the logarithm of the number of lineages increases linearly with speciation rate and time (i.e. $\ln E(n) = \lambda t$), and clade size at any particular time has a geometric probability distribution.

Extinction complicates the model of diversification (Box 1). For example, the relationship between the logarithm of clade size and clade age becomes non-linear – more so as the extinction rate approaches the speciation rate. In addition, different combinations of speciation and extinction rates can produce the same expected clade size. Conversely, differences in the number of species between two

clades can result from differences in rates of speciation, extinction or both, in addition to random variation. Using phylogenetic information to infer the underlying speciation–extinction process depends on several assumptions. Two of these relate to the quality of the phylogenetic data: their completeness and, for estimating absolute rates, the accuracy with which branch lengths are calibrated to time. The third assumption concerns constancy of speciation and extinction rates over the history of a clade, which is crucial to some methods.

The completeness of a phylogeny depends on recognizing and sampling all lineages. Biologists use many definitions of species [18] but agree, at least in the case of allopatric speciation, that lineage splitting entails a substantial period of evolutionary divergence [19]. Consequently, biologists often cannot decide how finely to distinguish lineages. Phylogeographic analyses often reveal cryptic 'species' that are genetically differentiated but have not been recognized and named [20]. Thus, toward the contemporary tips of a phylogenetic tree, the number of lineages and recognition of recent speciation and incipient speciation events become arbitrary. Because this problem has no straightforward solution, some analyses disregard information about recent lineage splitting [21].

Estimates of absolute rates of speciation and extinction depend on a reliable time scale [22]. Phylogenetic trees are

Box 1. The relationship between clade size and time

The random-walk speciation–extinction process developed from the analogous birth–death process in population biology. Rates of speciation (λ) and extinction (μ) quantify the probabilities that a speciation or extinction event will occur within a particular interval of time (t). Models of this process describe the change in the average size of a clade and the variation in size among clades as a function of time. In a simple speciation process [12], the probability that a clade has size n at time t is:

$$P(n|t) = \frac{[E(n) - 1]^{n-1}}{E(n)^n} \qquad \text{(Equation 1.1)}$$

where the average clade size $E(n) = e^{\lambda t}$, and the standard deviation of clade size is:

$$SD(n) = \sqrt{E(n)[E(n) - 1]} \qquad \text{(Equation 1.2)}$$

which is approximately $E(n)$ when the expected number of species per clade is large. Nee [71] describes estimates of the speciation rate under this process.

In a speciation–extinction process, the mean of the probability distribution of clade sizes, including extinct lineages ($n = 0$), changes as $E(n) = \exp[(\lambda - \mu)t]$. When speciation exceeds extinction ($\lambda > \mu$), the distribution of sizes among extant clades ($n > 0$) remains geometrical, as in a pure branching process, with:

$$P(n|t) = (\lambda - \mu) \frac{[\lambda E(n) - \lambda]^{n-1}}{[\lambda E(n) - \mu]^n} \qquad \text{(Equation 1.3) [10,13]}$$

Because many clades become extinct, the average size of extant clades – that is, of clades with $n > 0$, at time t [$N(t)$] – exceeds the average size of all clades, $E(n)$, according to:

$$N(t) = \frac{\lambda E(n) - \mu}{\lambda - \mu} \qquad \text{(Equation 1.4)}$$

The probability that a clade survives ($n > 0$) over period t is: (Figure I)

$$P(n > 0|t) = \frac{(\lambda - \mu)E(n)}{\lambda E(n) - \mu}, \qquad \text{(Equation 1.5)}$$

which is $E(n)/N(t)$. Conversely, the probability that a clade of size N becomes extinct in time t is:

$$P(n = 0|t) = \frac{\mu(N - 1)}{\lambda N} \qquad \text{(Equation 1.6)}$$

which approaches μ/λ for large N.

When speciation and extinction rates are equal, the average number of species in both extinct and extant clades equals the initial clade size; that is, $E(n|t) = n(0)$, and the standard deviation of clade size increases as $SD(n|t) = \sqrt{2n(0)\lambda t}$. The probability of extinction by time t is:

$$P(n = 0|t) = \left(\frac{\lambda t}{\lambda t + 1}\right)^{n(0)} \qquad \text{(Equation 1.7)}$$

and so the average size of extant clades ($n > 0$) at time t is simply $E(n|t)/P(n = 0|t)$ or, for stem lineages ($n(0) = 1$), $N(t) = 1/P(n = 0|t)$.
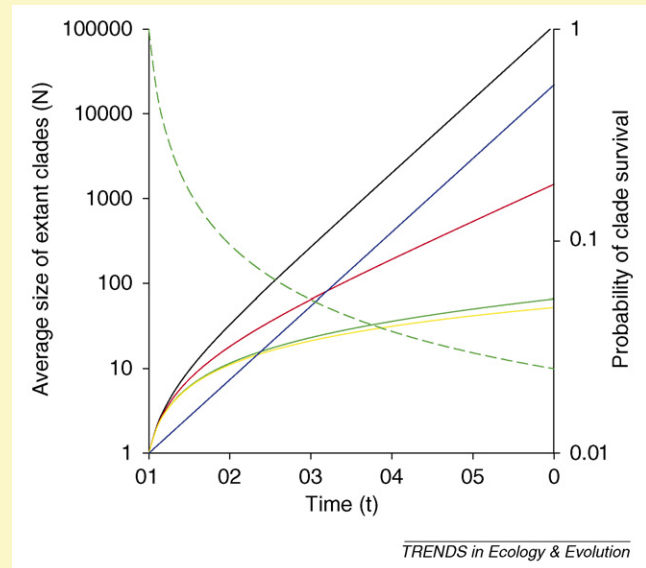

Figure I. Increase in the average number of lineages in extant clades as a function of time in a random speciation–extinction process (Equation 4). The different lines represent extinction rates at 80% (black, $\lambda = 1$, $\mu = 0.8$), 90% (red, 1, 0.9), 99% (green, 1, 0.99) and 99.9% (yellow, 1, 0.999) of the speciation rate. The blue line ($\lambda = 0.20$ and $\mu = 0.00$) represents a pure birth process with the same net proliferation rate as the black line ($\lambda = 1.00$ and $\mu = 0.80$). The descending broken line shows the proportion of the original lineages ($t = 0$) remaining (Equation 5; right-hand scale) for $\lambda = 1.00$ and $\mu = 0.99$.

mostly constructed from information on nucleotide substitutions across contemporary lineages. If nucleotide substitution along a branch in a phylogenetic tree occurred in a clocklike fashion, then time and genetic distance, calculated by a suitable model of nucleotide substitution [23], would bear a linear relationship. However, molecular clocks tick stochastically, rates can vary between lineages and over time [24–27], and their estimation incorporates potential biases that can distort the time scale [28–31]. Although time calibrations are improving, these problems will remain a source of uncertainty in analyses of diversification rates.

The simplest diversification process presupposes that rates of speciation and extinction are the same for all lineages and do not vary over time. This is the assumption of rate homogeneity. Departure from this assumption can bias estimates of speciation and extinction rates. As discussed later, rate homogeneity can be tested by comparing observed and expected distributions of intervals between nodes in a phylogenetic tree [32]. When these differ, more complex models can be substituted. However, as parameters are added to models to account for rate heterogeneity, rates of speciation and extinction have broader confidence limits, if they can be estimated at all.

Testing which environmental and species attributes influence diversification rates

One can determine the influence of a particular environmental condition or species trait on rate of diversification by comparing the number of species in clades that differ in these attributes. Such comparisons use phylogenetic analyses and diversification models in two ways. First, rate estimation depends on knowing the age of a clade, which, in the absence of a fossil record, can only be estimated from a time-calibrated phylogenetic reconstruction. If one chooses to analyze sister lineages, which, by definition, are identical in age, then phylogenetic trees are required to determine the evolutionary relationships by which one recognizes sister pairs. Second, an underlying diversification process provides the quantitative foundation for testing the statistical significance of differences in species richness (Box 2). When comparisons of diversity include clades of different age, diversification models show us that large samples of clades are needed to distinguish the relative contributions of speciation and extinction to differences in species richness. Similarly, absolute rates of speciation and extinction can be estimated only for large time-calibrated phylogenetic trees. Brief discussions of each of these approaches now follow,

Box 2. Likelihood of speciation and extinction rates, given the data

The size of any single clade at a particular time has a probability (its likelihood) associated with any pair of values for the rates of speciation and extinction. One pair has a greater likelihood than all of the others (the maximum likelihood), indicating the best fit of the parameters to the data. In a simple speciation process, only the speciation rate (λ) is estimated. However, although only one value of λ is a maximum likelihood estimate ($\lambda = \ln S/t$), many values of λ will provide reasonable fits to the data. From Equation 1 in Box 1, for a pure speciation process, the logarithm of the likelihood of exactly n species at time t with a speciation rate of λ is:

$$\ln P(n|t) = (n-1)\ln(E(n)-1) - n\ln E(n) \qquad \text{(Equation 2.1)}$$

where $E(n) = \exp(\lambda t)$. Suppose, for example, that $\lambda = 0.03$ and $t = 100$, for which $E(n)$ is close to 20. The probability of obtaining exactly 20 species from this process is $P(20|100) = 0.019$. However, the probability is almost half as much for speciation rates as different as $\lambda = 0.020$ ($P = 0.0085$) and $\lambda = 0.045$ ($P = 0.0090$).

When the data include the sizes of multiple clades diversifying under the same process, the overall likelihood is the product of the individual likelihoods. Thus, for clade i having size n_i ($n_i > 0$) after time t_i, the likelihood $l_i = P(n_i|t_i)$ (Box 1, Equation 5), and the likelihood (L) of k clades having sizes $n_1, n_2, \ldots, n_k$ is the product:

$$L = \prod_{i=1}^{k} P(n_i|t_i) \qquad \text{(Equation 2.2)}$$

Computation is simplified by taking the logarithm of the likelihood, $\ln L = \Sigma \ln P(n_i/t_i)$. The maximum likelihood can be obtained by trial and error, as in Figure I. Bokma [10] has shown that heterogeneity in diversification rates between two sets of clades can be tested by comparing the log-likelihoods of the individual samples and the combined sample: $T = 2(\ln L_1 + \ln L_2 - \ln L_{\text{combined}})$. In this test, T has a χ^2 distribution with two degrees of freedom.
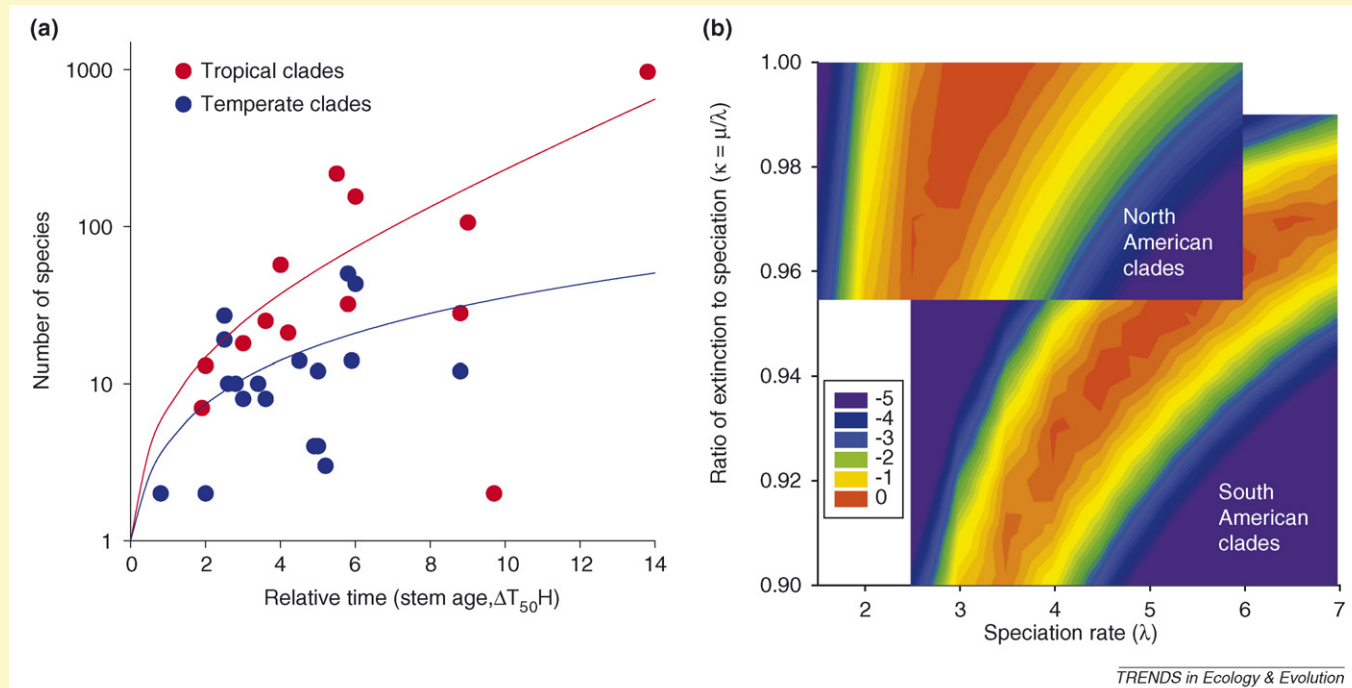

Figure I. **(a)** Maximum likelihood estimates of the relationship between number of species and relative age for South American clades (tropical, red dots) and North American clades (temperate, blue dots) of passerine birds [43] using the method of Bokma [10]. Five additional monotypic North American clades were excluded from the analysis because they are unlikely to have been produced by the same homogeneous process. The stem age of each clade was estimated from the DNA-hybridization phylogeny of Sibley and Ahlquist [72], and is expressed as the melting point difference between homoduplexed and heteroduplexed DNA ($\Delta T_{50}H$, °C). **(b)** Likelihood ratio contours [$\ln L(\lambda,\mu) - \ln L(\max)$] for estimates of λ and μ for the North American and South American clades shown in (a). (a). Statistical inference is uncertain in such comparisons but confidence limits for estimates are thought to lie within the area bounded by likelihood ratios of about −2 or −3. Maximum log-likelihoods (LnL) were −63.5 for the North American clades and −71.3 for the South American clades. For all clades together, maximum $\ln L = -139.4$, and so Bokma's [10] T statistic was 9.28, indicating a significant difference in diversification rates between the regions (two degrees of freedom; $P = 0.010$).

to illustrate their connection to underlying models of diversification.

Whole-tree methods for estimating heterogeneity in diversification rates

Shifts in diversification rate within a clade can lead to 'imbalance' in the number of species in different parts of a phylogenetic tree. An increase in diversification rate along a single branch results in a greater number of descendant lineages, on average, provided that the descendant lineages maintain this higher rate. Such rate heterogeneity can be recognized in various measures of tree imbalance, calculated from the structure of the entire tree and

compared with estimates of these measures based on a homogeneous diversification process [33–36], using software such as SymmeTREE [37] or apTreeshape [38]. Whole-tree approaches can be an important first step in analyses of diversification. If a phylogeny shows significant imbalance, then one can proceed to sister-clade or independent-clade analyses to determine whether rate changes are associated with the evolution of novel phenotypic traits or with areas having particular environmental conditions, such as tropical versus temperate. Absence of significant imbalance suggests that the assumption of rate homogeneity across clades (although not necessarily with respect to time) is reasonable.

Sister clade analysis

Factors that influence rates of diversification can be addressed through comparisons of the number of species in sister clades, where members of each pair differ with respect to the trait of interest [6,39]. Sister comparisons are desirable because both clades have exactly the same age. Although absolute rates are not estimated, differences in species numbers can signify differences in the relative balance of speciation and extinction events in the history of each clade [40]. However, because a single random speciation and extinction process can produce a wide range of outcomes, statistical testing requires multiple comparisons in which the association of a trait with a higher or lower number of species can be evaluated by a sign test or binomial test applied to a sample of clade pairs. For example, Cardillo [7] contrasted sister clades at higher and lower latitudes and found that ten of 11 (passerine birds) and ten of 13 (butterflies) of the more equatorial clades were larger. Given a 'null' expectation of $P = 0.5$ for each comparison, these imbalances were statistically unlikely according to a binomial test ($P < 0.006$ and $P < 0.046$). Various whole-tree methods have been developed to increase the statistical power of sister-clade comparisons [41,42].

Analysis of independent clades

Analysis of variation in species number in a sample of many independently diversifying clades also can identify the effects of species attributes or environmental conditions on diversification [4,10,43]. This approach involves the assignment of clades to groups according to species traits or regional traits of interest, estimating separate speciation and extinction rates (possible with different-aged clades) or relative rates of diversification (same-aged clades) for each group, and comparing these rates between groups. Because clades might share a tendency towards large or small size owing to shared ancestry and evolutionary conservatism, one should determine that clade traits are evolutionarily independent [44,45] or employ a phylogenetic correction to avoid non-independence [46,47]. Isaac *et al.* [41] and Phillimore *et al.* [42] outline approaches to calculating independent contrasts relating clade size and diversification rate to other variables within whole phylogenies.

Rate of diversification (speciation minus extinction) can be estimated from the size and age of a single clade, assuming rate homogeneity (Box 1). For example, in a pure speciation process (extinction rate = 0), where the expected number of species (S) increases exponentially with time (t), the average speciation rate is estimated by $\lambda = \ln(S)/t$. With extinction, different combinations of speciation and extinction rates can result in the same number of species [43]. Accordingly, to estimate the rate of diversification for a single clade, one must fix the extinction rate (μ) as a proportion (κ) of the speciation rate. For example, when Magallón and Sanderson [13] analyzed diversification in orders of flowering plants, they set κ as equal to either 0 or 0.90, which they felt was the highest biologically realistic value.

With a fixed ratio of extinction to speciation, $\lambda = \ln[S(1 - \kappa) + \kappa]/[(1 - \kappa)t]$. However, comparisons of speciation and net diversification [$\lambda - \mu$, or $\lambda(1 - \kappa)$] between clades are valid only with same ratio (κ) of extinction to speciation rate. Regardless of this, confidence limits on these estimates are broad because of stochastic variation in the speciation–extinction process.

Knowing the sizes and ages of multiple clades, one can sometimes estimate both speciation and extinction rates by non-linear regression [43] or maximum likelihood [10] (Box 2). For example, maximum likelihood estimates of λ and κ were 3.16 and 0.995, respectively, for 18 North American (primarily temperate) clades, and 5.32 and 0.954 for 14 South American (primarily tropical) clades of passerine birds (Box 2). These estimates differ significantly. The value of κ close to 1 for North America suggests that extinction balances speciation and that clade size is not increasing in the region.

Estimating rates of speciation and extinction from single phylogenetic trees

Analyses based on clade size and age draw on phylogenetic trees only to identify independent clades and to estimate their ages. The internal structure of the phylogeny – branch lengths indicating intervals between splitting events – is not used. In fact, the distribution of node (splitting event) ages over the duration of a clade, from its stem to the present, provides useful information about species proliferation. In a homogeneous diversification process, the frequency of nodes in a phylogeny increases towards the present as the number of lineages increases. When the assumption of homogeneity over time is violated – that is, when speciation or extinction rates either increase or decrease through the history of a clade – the distribution of node ages changes in predictable ways. Thus, not only can the entire phylogeny be used to estimate rates of speciation and extinction, but in some cases it can also be used to determine whether these rates have changed over time.

Lineage-through-time plots

In a homogeneous diversification process, rates of speciation and extinction can be estimated from the increase over time in number of ancestral lineages (N_A) in a reconstructed phylogeny [48,49] (Box 3). In this usage, 'ancestral lineages' refer to lineages that gave rise to living descendants, beginning with the stem lineage of a clade; extinct lineages are not included in the tally. A graph of the relationship between the logarithm of the number of ancestral lineages and time is referred to as a lineage-through-time (LTT) plot, which has been applied to estimate diversification rates in organisms as diverse as the Hawaiian silverswords [50], the South African Restionaceae [51], aquatic beetles [52], *Plethodon* salamanders [53] and passerine birds [54].

As shown in Box 3, in a time-homogeneous speciation–extinction process, the rate of accumulation of ancestral lineages increases towards the present because more recent lineages have progressively less time to suffer extinction. Thus, the LTT plot curves upward. However, an increase in the speciation rate toward the present in the absence of extinction produces a similar effect, and these scenarios unfortunately cannot be distinguished.

Box 3. The LTT plot

LTT plots portray the number of lineages in a clade that give rise to contemporary species as a function of clade age. These plots are constructed retrospectively from phylogenetic trees by counting the number of ancestral lineages back through time [48,49]. Because extinct lineages are not perceived, the relationship between ancestral lineages (N_A) and time differs from the relationship between lineage number (N) and clade age (t). The total number of lineages (including those that eventually die out and are not apparent in a phylogeny reconstructed from contemporary species), is described by:

$$N(t) = \frac{\lambda e^{(\lambda - \mu)t} - \mu}{\lambda - \mu}$$

(Box 1, Equation 1.4). When extinct lineages are trimmed from a phylogeny of age T (the present), the resulting number of lineages ancestral to extant species (N_A) increases as $N_A = N(T)/N(T - t)$, or

$$N_A(t) = \frac{\lambda e^{(\lambda - \mu)T} - \mu}{\lambda e^{(\lambda - \mu)(T-t)} - \mu} \qquad \text{(Equation 3.1)}$$

When t is small, $N_A(t)$ increases approximately exponentially as $\ln N_A \sim (\lambda - \mu)t$. Towards the present, the slope of $\ln N_A$ approaches the speciation rate λ (Figure I). Thus, the initial slope of $\ln N_A$ with respect to time estimates $(\lambda - \mu)$. As t approaches T, the slope approaches λ because newly formed lineages have progressively less time to suffer extinction. Harvey *et al.* [48] showed that the difference (a) between the linear portions $\ln N(t)$ and $\ln N_A(t)$ curves is equal to $-\ln[(\lambda - \mu)/\lambda]$. Rearranging this expression, we get $\lambda = (\lambda - \mu)/\exp(-a)$. Thus, knowing a from the difference between the present number of taxa and the extrapolated exponential increase from the initial part of the LTT plot and $(\lambda - \mu)$ from the initial slope of the LTT plot, we can estimate λ and, by subtracting $(\lambda - \mu)$ from λ, the value of μ. These values also can be estimated from non-linear regression of $\ln N_A$ as a function of t according to Equation 3.1.
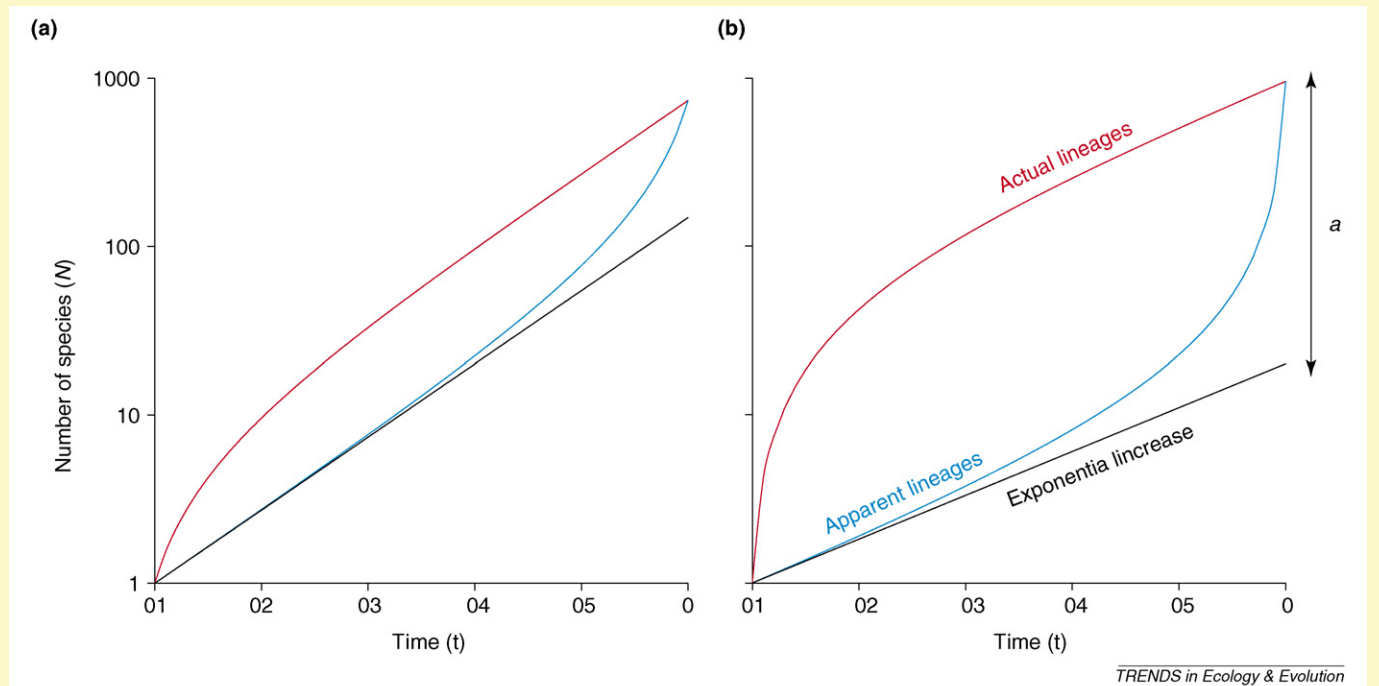

Figure I. LTT plots for the average number of extant (actual) lineages (N) (red lines) and the average number of apparent lineages (i.e. ancestral to living species) (N_A) (blue lines), for two combinations of speciation and extinction rates, (a) $\lambda = 0.50$, $\mu = 0.40$, $\kappa = 0.80$, $N(T) = 738$; and (b) $\lambda = 3.00$, $\mu = 2.94$, $\kappa = 0.99$, $N(T) = 955$. The log-linear curves (black lines) expressing exponential increase have slopes of $\lambda - \mu = 0.10$ (a) and 0.06 (b). The difference between curves for the actual and apparent lineages is indicated by a and can be estimated by the difference between the present (i.e. at time $t = 50$) number of species, $N(T)$, and the extrapolated curve of exponential increase to $t = T = 50$ (See Harvey *et al.* [48]).

Additional problems in LTT analysis arise from poor sampling, as in other phylogeny-based approaches. For example, failure to recognize cryptic diversity can lead to underestimation of speciation and extinction rates because the LTT plot rises too slowly towards the present. Incomplete sampling of lineages that arise early in a clade can similarly underrate diversification. However, when early ancestral lineages are well sampled, net diversification ($\lambda - \mu$) can be estimated accurately, and the present-day number of species can anchor the latter part of the LTT plot, even though not all species are included in the phylogenetic reconstruction [51,54].

In the LTT approach, speciation and extinction rates are estimated from the curvature of the N_A–time relationship under the assumption of rate homogeneity over time. When does the observed pattern depart from this assumption?

Pybus and Harvey [32] introduced the γ-statistic to describe the shape of this curve under a homogeneous process [55,56]. γ is calculated from successive intervals between branch-splitting events (nodes) within a tree, which become shorter towards the present as lineages proliferate. This distribution is well characterized for the homogeneous speciation process (no extinction), for which γ has a mean of 0 and a standard deviation of 1. When γ significantly exceeds 0, internal nodes are concentrated closer to the tips of the tree (present time) than expected from random speciation. However, because random extinction also results in positive values of γ [35], γ > 0 is not informative about rate homogeneity. When sampling is complete [32] and γ is significantly negative (γ < 0), internal nodes are concentrated closer to the root of the tree than expected from a homogeneous speciation process. This can only result from

decelerating diversification caused by a decrease in the rate of speciation.

Pybus and Harvey [32] found $\gamma < 0$ in several clades of birds and mammals, indicating slowing diversification with increasing clade age. Weir [21] reached a similar conclusion from LTT plots for lowland clades of neotropical birds, as did Rabosky [35] for Australian agamid lizards and Ricklefs for Australian corvid birds [57]. The formula for the γ statistic is daunting but several software programs are useful, including GammaStatistic, written by E. M. Griebeler (http://www.oekologie.biologie.uni-mainz.de/people/evi/main.html) [58] and the 'ape' library, written in R language (http://www.pbil.univ-lyon1.fr/R/ape/) [55].

Individual branch lengths in a reconstructed phylogeny estimate the inverse of the diversification rate $[1/(\lambda - \mu)]$, on average, deep in the phylogeny, and the inverse of the speciation rate $(1/\lambda)$ toward the present. Each branch length is statistically independent, and so one can use a sample of branch lengths from one or more phylogenetic trees to estimate rates of diversification. Weir and Schluter [59] applied this principle to the ages of nodes uniting sister species of New World birds and mammals to show that recent speciation and extinction rates at high latitudes exceeded those in the tropics, although net diversification rates were lower. Bininda-Emonds *et al.* [60] used a supertree reconstruction of the phylogenetic relationships of all mammals to characterize diversification rates in successive periods through the early history of the mammalian radiation. They found high rates of diversification associated with the mid-Cretaceous origins of currently recognized orders, little effect of the mass extinction event that ended the Cretaceous era, and a long delay before an Eocene–Oligocene burst of radiation that produced most modern taxa.

Conceptual bias, sample bias and estimation of diversification rates

In spite of recent applications, the use of phylogenetic information to characterize diversification has two important sources of bias that are largely unappreciated. The first is conceptual and derives from our current preoccupation with reconstructing phylogenetic relationships. Every clade begins with a single stem lineage, the descendants of which diversify towards the present. We view a phylogeny from the present, looking back through time to the single ancestor of a clade. From this perspective, diversity seems to increase continually and speciation seems to occur more frequently than extinction.

The second source of bias comes from the idea that larger clades provide better statistics. In most analyses, larger samples (in this case, of nodes or lineages) provide parameter estimates with smaller standard errors. As taxon sampling and phylogenetic methods improve, clades of progressively larger size have become available for analysis. However, these large clades are a non-random sample of diversity. A particular speciation–extinction process produces a geometrical distribution of clade sizes (Box 1): a few clades are species rich but many more are species poor. Thus, the net diversification rate estimated for a large clade exceeds that for a small clade, even though both might have grown under the same random speciation–extinction

process. Thus, focusing on larger clades inevitably inflates estimated diversification rates.

The absence of a relationship between clade size and clade age in several studies is at odds with the perceptual bias that most clades continually increase in size. With a positive rate of diversification, expected clade size increases with age, as it seems to do in the clades of birds analyzed in Box 2. Indeed, only because of this was it possible to estimate speciation and extinction rates for this sample. However, in the case of the flowering plant orders analyzed by Magallón and Sanderson [13], no such relationship exists. This is also true of tribe-to-family level clades of passerine birds [43,61] and the major clades of squamate reptiles [62]. The independence of clade size from age suggests that speciation and extinction are approximately balanced (zero net diversification), even though many clades contain large numbers of species. By implication, diversity changes little over time, barring mass extinctions; rather, lineages are continually replaced, as are individuals through death and birth in a non-growing population. The fossil record reveals such a pattern in several well-sampled groups [3,63,64].

Although a large clade arising from a balanced speciation–extinction process defies the odds, the random nature of speciation and extinction guarantees that this will occur. An LTT plot was recently used to estimate a speciation rate of 0.43–0.86 per million years, depending on the time calibration, and an extinction rate of 82% of the speciation rate for the radiation of endemic suboscine passerine birds in South America, which presently number almost 1000 living species [54]. It is not surprising that the estimated diversification rate $(\lambda - \mu)$ for such a large clade (0.077 to 0.150 per million years) should be so high. However, could this many suboscine passerines have been produced by a balanced speciation–extinction process $(\lambda - \mu = 0)$ from a single clade that existed in South America in the early Tertiary? If so, how many other species were present when this particular clade originated? Might the nearly 1000 living species of suboscine passerines have descended from a single lucky ancestral lineage out of, perhaps, 1000 others? Theory tells us that this is possible, but is a balanced random speciation–extinction process reasonable in this case?

When extinction equals speciation in a random diversification process, the so-called 'critical case', the mean size of all descendant clades (living and extinct) is 1. However, clades that survive are the ones that initially increase rapidly, just by chance, because only these have a reasonable probability of long-term survival. Retrospectively, these clades seem to have had a positive net diversification rate, even though extinction balances speciation in the underlying process. Under such a process, 1000 descendants of a single lineage can replace 999 other lineages. However, as discussed later, the average period required for such a complete turnover is impossibly long.

Balanced speciation and extinction can occur in three ways: (i) a random walk with speciation and extinction events having the same frequency – the type of process discussed up to this point; (ii) regulated (diversity-dependent) speciation and extinction rates that result in stably maintained species numbers [65,66]; and (iii) a 'Moran'

process, in which lineages that die out are replaced by the splitting of a single lineage drawn at random from those available, as in Hubbell's [67] community drift model [12]. In a random walk with the speciation rate equal to the extinction rate (case 1), the total number of lineages increases or decreases at random, regardless of clade size, and every clade eventually goes extinct. In the second case, new lineages are formed and others go extinct at random, but the total number fluctuates around a fixed point below which the net diversification rate is positive and above which it is negative [68]. In the Moran process, lineage number is invariant.

In a balanced random walk, simulations with small clades indicate that the average time required for replacement of N lineages by the descendants of a single one of those lineages is approximately the ratio of the lineage number to the extinction (or speciation) rate (N/μ) [69]. In a Moran process, the average time required for a single lineage to replace $N - 1$ other lineages (i.e. the extinction of all but one of the original lineages) is at least $2N$ generations [70], where a generation is the average time to lineage extinction ($1/\mu$). Turnover of lineages under a regulated diversity process requires a similar period. Thus, $2N/\mu$ provides a rough approximation to the average turnover time.

Returning to the South American suboscine birds, it has been estimated that the time to extinction of an individual lineage ($1/\mu$) is 1.4–2.8 million years, depending on the time calibration [54]. Thus, in a constrained random walk (Moran process), the expected time for 1000 lineages to be replaced by the descendants of a single ancestral lineage would exceed 3–5 billion years. Clearly, the explosion of suboscine species richness, which probably occurred entirely within the Tertiary (i.e. <65 million years), did not come about through balanced, random speciation and extinction in an avifauna that was as diverse as at present throughout the history of the clade. Rather, the suboscines either diversified rapidly in a relative ecological vacuum or were competitively superior to other passerine lineages. In either case, the underlying speciation rate must have exceeded that of extinction, on average, throughout the history of the clade.

Balanced random processes, where extinction equals speciation, are too slow to account for most observed patterns of diversity. This implies that some clades have diversified more rapidly than others, as suggested by many comparative studies [5,42]. Moreover, because clades, even successful ones, cannot diversify indefinitely, the size of each clade is probably regulated at a level that depends on the ecological relationships of its members, attributes of competing clades, and the climate and physiography of the region in which it occurs [43,69].

Conclusions

Estimation of rates of speciation and extinction, and testing hypotheses that address variation in these rates among clades, among regions and over time, presents formidable challenges. Analytical approaches ultimately are limited by the random nature of diversification and the interaction of speciation and extinction in determining species richness. Sister taxon comparisons make no

assumptions concerning rate constancy, and can inform us about the influence of species and region traits on diversification. However, such comparisons cannot distinguish the contributions of speciation and extinction to differences in number of species. Samples of different-aged clades can be used to estimate speciation and extinction rates under the assumption of rate homogeneity over time; however, this assumption is difficult to ascertain with confidence. Complete phylogenetic reconstructions provide the best hope of evaluating changes in diversification rates over time, and of estimating speciation and extinction rates separately when the underlying process seems to be homogeneous over time. However, because the number of lineages in a phylogeny – the lineages that are apparent when viewed retrospectively – continually increases with time, periods of decline in the number of species in a clade remain hidden [48], and it is unlikely that rate homogeneity can be unambiguously supported for any clade. The impact of departures from rate homogeneity on estimates of speciation and extinction rates can be explored, however, by simulation studies [32], by which potential biases can be evaluated, rate estimates refined and outlying clades identified.

A more crucial concern is the bias introduced by the retrospective view of diversity inherent in phylogenetic analysis. The idea that diversity has increased continuously over time applies only to the lineages that have survived to the present. Historical information from paleontology and climate reconstruction provides key contexts of change in diversity and in environmental factors that potentially influence diversification [12]. Where the fossil record is well sampled, the predominant pattern is one of relatively constant diversity, with continual turnover of species and replacement of clades [63,64]. Balanced random processes are too slow to account for this replacement. Instead, it is likely that the rates of diversification, although close to zero, on average, across clades, also vary among clades and within clades over time as the environment changes or lineages acquire adaptations that increase their competitive position within a biota.

As molecular phylogenies are reconstructed for an increasing number of groups with stronger node support and improved time calibration, our ability to estimate rates of speciation and extinction, and to use these estimates to test hypotheses concerning diversification, should improve. However, we should guard against preoccupation with random-walk models of speciation and extinction for continuously increasing diversity just because they generate robust, tractable theory. It remains to be seen how realistically such simple processes represent nature. Future directions should include the integration of paleontological perspectives, further exploration of diversification models that incorporate diversity dependence, and direct investigation of speciation and extinction using population genetic, ecological, and macroecological approaches.

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