

Detecting Trait-Dependent Diversification Under Diversification Slowdowns

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Abstract Testing whether a certain biological trait significantly affects clade diversification is central to macro-evolutionary research. To this end, many scientists use constant-rate estimators (CR estimators) of diversification. However, it has never been examined whether these estimators report meaningful relationships between traits and diversification even when the diversification itself decelerates over time. In this study, I simulate trait-driven diversification concurrently with diversification slowdowns. Then, I test whether CR estimators manage to uncover the simulated relationships. Results suggest that CR estimators are robust against violation of rate constancy and successfully detect trait-dependent diversification in spite of diversification declines. Interestingly, correct results were recovered whether clade age correlated with clade diversity or not. Further comparison of CR estimators with QuaSSE suggested that QuaSSE performs better under constant diversification, but tends to report spuriously significant outcomes when diversification decelerates (=elevated Type I error). Given that diversification

slowdowns have been recently reported for a wide range of taxa, these findings may be of particular relevance for future diversification studies.

Keywords Speciation · Extinction · Evolution · Phylogeny · Ecology · QuaSSE

Introduction

Some clades grow and accumulate diversity faster than others, as the extreme examples of explosive radiations and living fossils illustrate (Sanderson and Donoghue 1996; Ricklefs 2007; Hunt et al. 2007; Jablonski 2008; Glor 2010). To measure the tempo of clade growth, Raup (1985) introduced a series of diversification rate estimators which have later become broadly popular among paleobiologists (Sepkoski 1998; Foote 2000; Alroy 2000) and evolutionary biologists (Magallon and Sanderson 2001; Nee 2006; Ricklefs 2007). These estimators are sometimes termed constant-rate (CR) estimators as they assume that clades diversify at constant rates so that clade diversity grows exponentially over time. The simplest way to calculate diversification rate then is to divide log-transformed clade richness by clade age, $r = \log(N)/t$ (Raup 1985; Magallon and Sanderson 2001).

However, recent studies indicate that the assumption of rate constancy, which CR estimators are built upon, may be unrealistic because diversification decelerates over time in a wide range of taxa (McPeck 2008; Phillimore and Price 2008; Rabosky 2009a; Morlon et al. 2010; Etienne et al. 2012). Due to slowing diversification, clade age and richness may become decoupled; young clades may be more diverse than old clades, and vice versa (Ricklefs 2006; Rabosky 2009b; Vamosi and Vamosi 2010; Rabosky et al.

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2012). Under such circumstances, CR estimators have been supposed to fail (Ricklefs 2007; Rabosky 2009a).

These observations motivated development of novel methods that would be robust against decelerating diversification (Rabosky 2009a; Rabosky and Adams 2012). For example, diversification rates may be more reliably analyzed within likelihood (Rabosky 2006; Maddison et al. 2007; FitzJohn 2010; Morlon et al. 2010; Ingram 2011; Stadler 2011; Etienne et al. 2012) or Bayesian (Bokma 2008a, b; Moore and Donoghue 2009; Silvestro et al. 2011; Wertheim and Sanderson 2011) frameworks which often circumvent the assumption of rate constancy. However, these methods may have their own drawbacks, such as new restrictive assumptions about the input data (e.g. phylogeny is known completely and accurately), trade-off between model complexity and phylogeny size, or high requirements imposed on the computational resources.

Still, it has not been explored to date whether errors, potentially introduced by slowing diversification, are substantial enough to preclude meaningful CR inference. There are many applications of CR estimators but testing which biological traits modulate diversification is arguably among those most relevant. Traits may have a major impact on clade survival and growth over evolutionary time. Floral symmetry, host specificity, geographic range size or sexual coloration are just a few examples of traits that can facilitate or suppress diversification through their effects on speciation, extinction, or both (Jablonski 2008; Glor 2010; Schnitzler et al. 2011). Novel traits, often referred to as key innovations, may trigger explosive radiations that presumably created most of the diversity of life (Hutchinson 1959; Davies et al. 2004; Hunt et al. 2007; Glor 2010; Vamosi and Vamosi 2010). Geographic distributions of speciose taxa then shape the patterns of local and regional richness, including the pervasive latitudinal and altitudinal diversity gradients (Ricklefs 2006; Wiens et al. 2006; Mittelbach et al. 2007; Jansson and Davies 2008; Vamosi and Vamosi 2010; Schnitzler et al. 2011). Trait-diversification inference is therefore fundamental for many biodiversity studies, and it seems important to assess whether it might be compromised by the commonly observed diversification slowdowns. Despite its potentially broad importance, such assessment is currently missing. Biological mechanisms behind the slowdowns themselves also remain unknown, but it has been suggested that niche filling and competition might suppress diversification (as in some classic models of adaptive radiation) (McPeck 2008; Phillimore and Price 2008; Glor 2010).

In this study, I examine whether CR estimators return meaningful conclusions about trait-diversification dependence even under diversification slowdowns. I also compare CR inference with its widely used alternative, the QuaSSE method (FitzJohn 2010), and highlight the

strengths and weaknesses of both approaches under constant and slowing diversification. In the light of the presented findings, I argue that both simple and advanced methods (such as CR and QuaSSE inference) might be powerful under different circumstances, depending on the data and hypotheses tested.

Materials and Methods

To explore whether CR estimators uncover trait-driven diversification in spite of diversification slowdowns, I first simulated a set of phylogenies with trait values at their tips wherein the correlations between diversification rate and traits were known. Then, I examined whether CR estimators can infer these known correlations under gradually increasing strengths of negative diversity dependence. For this analysis, I divided the simulated phylogenies into two groups: (1) phylogenies where clade age and richness were positively correlated and (2) phylogenies where clade age and richness were decoupled. The CR assumption of rate constancy suggests that trait-driven diversification should be easier to detect in the former of the two groups (Magallon and Sanderson 2001; Ricklefs 2007; Rabosky 2009a).

To estimate Type I error, I examined how often CR estimators detect trait-driven diversification even when the simulation process does not include this dependence. Finally, I compared the performance of CR estimators (Raup 1985) and QuaSSE (FitzJohn 2010) under constant and slowing diversification.

The Simulation Procedure

My simulation procedure generally follows the approach of Paradis (2005) and FitzJohn (2010) (Fig. 1). To simulate a series of phylogenies with trait values at their tips, I first specified speciation and extinction functions. Speciation rate varied with trait values whereas extinction rate was simulated as an invariable background process. In concert with phylogeny simulation, I modeled trait evolution using the Brownian motion model (Felsenstein 1985; Paradis 2005; FitzJohn 2010). Each simulation procedure was replicated under different strengths of negative diversity dependence.

Specifically, speciation rate λ_i for lineage i was calculated as a sigmoidal function of that lineage's trait value τ_i so that

$$\lambda_i = \frac{1}{1 + e^{\alpha\tau_i}}$$

This sigmoidal function ensures that speciation rate does not attain negative or extremely large values (Paradis 2005;

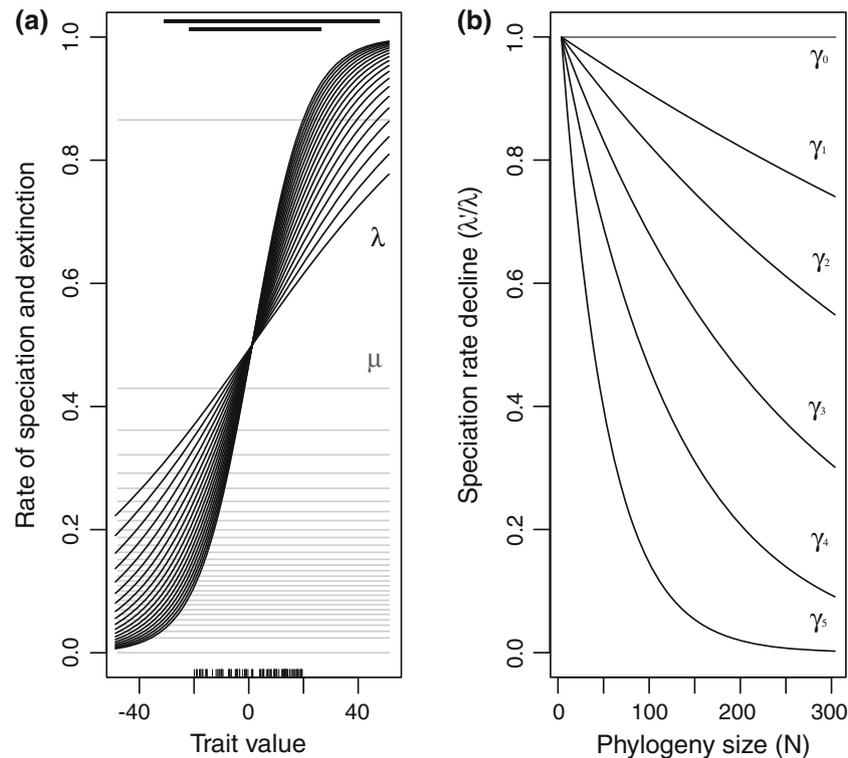


Fig. 1 Outline of the simulation procedure. **a** Speciation rate was modeled as a sigmoidal function of trait values with randomly selected scaling parameter α (black lines). Extinction rate, in contrast, was simulated as an invariable background process with randomly selected intercept (gray lines). Ancestral traits, used as starting values for the simulations, are depicted on the trait axis. The two horizontal segments show the extent of tip values obtained under two disparate

FitzJohn 2010). Rather than using a single sigmoidal function, I used a series of functions with scaling parameters α randomly drawn from the uniform distribution (min = -0.1 , max = -0.025), as illustrated in Fig. 1a.

Extinction was modeled as a constant function of trait values with intercept randomly drawn from the gamma distribution (shape = 2, rate = 12; see Fig. 1a). Parameters of the speciation and extinction functions were chosen at random from the specified distributions so that my simulations would cover a wider range of biological scenarios, allowing more general conclusions.

For each combination of speciation and extinction functions, I randomly selected an ancestral trait value (i.e. root value) from the uniform distribution (min = -20 , max = 20) and started a simulation. Parameters of the uniform distribution were selected so that different ancestral values would yield distinct but still mutually overlapping ranges of tip values (see Fig. 1a). My objective was to assess whether CR estimators yield meaningful results when the assumption of constant diversification is violated. Therefore, each simulation was repeated under six gradually increasing strengths of negative diversity dependence

rates of trait evolution (shorter segment: $\sigma^2 = 0.2$, longer segment: $\sigma^2 = 2.2$). **b** Negative diversity dependence, or the relative decline in speciation rate with phylogeny size (λ'/λ), was modeled at six gradually increasing strengths ($\gamma_0 = 0$, $\gamma_1 = -0.001$, $\gamma_2 = -0.002$, $\gamma_3 = -0.004$, $\gamma_4 = -0.008$, $\gamma_5 = -0.02$). Further details are provided in the text

(Fig. 1b). The diversity dependence function was specified as

$$\lambda'_i = \lambda_i e^{\gamma N}$$

so that

$$\lambda'_i = \frac{e^{\gamma N}}{1 + e^{\alpha \tau_i}}$$

where λ'_i is speciation rate after accounting for diversity dependence, λ_i is speciation rate derived from lineage trait value (see above), and N is the immediate number of species in a phylogeny at the time of speciation (though some of these species may go extinct later). Six scaling coefficients γ were used to simulate negative diversity dependence of increasing strengths ($\gamma_0 = 0$, $\gamma_1 = -0.001$, $\gamma_2 = -0.002$, $\gamma_3 = -0.004$, $\gamma_4 = -0.008$, $\gamma_5 = -0.02$; see Fig. 1b). Low γ values induce steep deceleration in speciation and, consequently, diversification rate. As γ approaches zero, speciation rate becomes independent of the number of extant species and clades diversify without bounds (Fig. 1b). From these steps, I obtained six phylogenies simulated under the same speciation and

extinction functions but with differing strengths of diversification slowdowns.

Clade ages, used for the simulation of individual phylogenies, were taken at random from the avian tribe dataset of Ricklefs (2006), which ensured that simulations included realistic clade age variation. Moreover, this particular dataset is known to have no relationship between clade age and richness, a condition often assumed to be challenging for CR estimators (Ricklefs 2006, 2007; Rabosky 2009a).

The rate of trait evolution (σ^2) is also an important simulation parameter. To inspect two disparate scenarios, I considered a labile trait ($\sigma^2 = 2.2$) and a conservative trait ($\sigma^2 = 0.2$). Simulated values of the labile trait covered a larger range of the speciation function as compared to the conservative trait (see the two horizontal segments in Fig. 1a). Therefore, it should be easier to detect trait-dependent diversification for the labile trait which causes larger speciation rate shifts. Effects of the conservative trait, which evolves 11 times slower, should be more difficult to uncover.

To explore a variety of outcomes, the above described simulation was repeated 1,000 times. First, I generated 1,000 pairs of speciation and extinction functions with randomly chosen parameters. For each pair, I then simulated six sets of 25 phylogenies, each set under a different level of diversity dependence (γ_0 - γ_5). The whole procedure was conducted under two rates of trait evolution (i.e. labile trait and conservative trait). In total, I generated 300,000 phylogenies with trait values at their tips.

The simulated data was then analyzed by methods conventional in empirical studies. Due to the simulation process, we know a priori there is a significant relationship between diversification rate and trait values (see above; Fig. 1a). The major objective of the following analyses was to assess whether these known relationships will be uncovered by CR estimators despite the introduced diversification slowdowns.

Analyzing the Simulated Data

To analyze the simulated dataset, I first estimated diversification rate for each of the 300,000 phylogenies using the simplest CR estimator

$$r = \log(N)/t,$$

which yields the average number of species emerging per unit time (N is clade diversity, t is clade age) (Raup 1985; Magallon and Sanderson 2001). A whole series of supplementary estimators has been defined depending on whether crown-group or stem-group ages are used and whether extinction is considered (Raup 1985; Magallon and Sanderson 2001). Here, I use the simplest CR estimator

but, in an empirical study, the choice may depend on the character of the analyzed data.

To summarize trait values at the tips of each phylogeny, I employed the simplest approach and calculated their arithmetic mean. Therefore, I did not make any assumptions about branch lengths or about the model of trait evolution. If CR estimators return meaningful results even under these crude inferential settings, they will probably perform even better in a refined analysis. For example, for higher accuracy, methods of ancestral reconstruction (Felsenstein 1985; Schluter et al. 1997) can be used instead of simple trait averaging.

Finally, I analyzed correlations between the inferred diversification rates and mean trait values. One thousand correlations were fitted for each of the six strengths of negative diversity dependence. Each correlation was fit for a set of 25 simulated phylogenies. The results were plotted separately for phylogenies (1) where clade age and richness were positively correlated and (2) where clade age and richness were decoupled. The whole analysis was conducted twice: for the labile trait and for the conservative trait. The strength and significance of all the correlations was estimated using non-parametric Spearman's rank test.

Estimating the Rates of Type I Error Under Slowdowns

It is important to establish how often CR estimators report a significant relationship when, in fact, there is none (=Type I error). To estimate Type I error, I set the α parameter of the speciation function to zero and repeated all the above described simulations and analyses. With $\alpha = 0$, speciation rate becomes independent of trait values. Phylogenies and traits are still simulated concurrently, and phylogenetic signal in the simulated traits is preserved. However, CR estimators should not report any trait-diversification relationship under this setup. Significant results may occasionally emerge, but such spurious outcomes should not arise in more than 5 % of the analyzed cases (i.e. the significance threshold of $p = 0.05$).

Comparing CR Estimators with the QuaSSE Approach

Performance of CR estimators can be fully evaluated only in comparison to their alternatives. The simulated data was therefore analyzed by means of QuaSSE as well (Quantitative State Speciation and Extinction modeling) (FitzJohn 2010). Rates of Type I and Type II error were then estimated for both CR estimators and QuaSSE under constant and slowing diversification.

QuaSSE framework (FitzJohn 2010) is statistically far more refined than the CR approach and assumes that diversification of evolutionary lineages follows a birth-death process whereby speciation and extinction rates vary

as the character under study evolves. Variation of speciation and extinction rates can be modeled using different functions which are fitted to the data (i.e. dated tree and trait values) through likelihood optimization. Fits of different speciation and extinction scenarios can then be compared by likelihood ratio tests (FitzJohn 2010).

Since QuaSSE modeling is computationally expensive, analysis of all 300,000 phylogenies would be hardly feasible even using large computer clusters. Moreover, QuaSSE is designed to analyze whole trees rather than clade collections. To obtain a dataset suitable for both CR and QuaSSE analyses, I randomly selected 100 phylogenies with over 1,000 species from the previously simulated data. With this reduced dataset, comparison of CR estimators and QuaSSE became feasible.

To analyze the reduced dataset by QuaSSE, I modeled speciation rate as both constant and sigmoidal functions of trait values. Constant speciation function served as the null model where diversification was independent of trait values. Sigmoidal speciation function was used to model trait-dependent diversification through which the data was simulated (Fig. 1a). Extinction was modeled as an invariable background process. The resultant QuaSSE models were compared by means of likelihood ratio tests.

To analyze the reduced dataset by CR estimators, I split each phylogeny into 10 clades and calculated diversification rates and mean trait values for each clade. Then, the inferred diversification rates were correlated with mean trait values, and the results were recorded. This procedure was repeated for all 100 phylogenies. CR inference was therefore conducted similarly as in my main analyses.

Both methods (QuaSSE and CR estimators) were applied to phylogenies where diversification was controlled by trait values ($\alpha \neq 0$) and where diversification was independent of trait evolution ($\alpha = 0$). Rates of Type I and Type II error under constant and decelerating diversification were then estimated for both methods.

Since CR calculations are relatively fast, I also tested how many clades are necessary to receive reasonable statistical power to detect trait-dependent diversification under diversification slowdowns (high computational requirements preclude similar analyses for QuaSSE). In statistical terms, I examined how steeply Type II error declines with the number of clades analyzed. Randomly selecting from the simulated data, I compiled 15 sets of 100 trees. The trees were then split into isolated clades suitable for CR analyses, whereby the number of clades gradually increased across sets (from 11 to 25 clades). Clade sizes remained comparable across sets (10–30 species) as the original trees had been selected so that their size would increase from one set to another. All tree sets were processed similarly as in the main CR analyses, Type II errors were estimated and plotted against the number of clades

analyzed. The results yielded potentially helpful guidelines on how many clades need to be examined to receive relatively strong CR tests.

Comparing Simulated Phylogenies to Real Phylogenies

In every simulation study, it is necessary to verify whether the simulated data correspond with empirical observations (e.g. whether the simulated phylogenies show realistic slowdowns or imbalance). To this end, I used 245 phylogenies of chordates, mollusks, arthropods and magnoliophytes, compiled by McPeck (2008), and compared topologies and branch-length distributions of these empirical phylogenies with my simulated phylogenies.

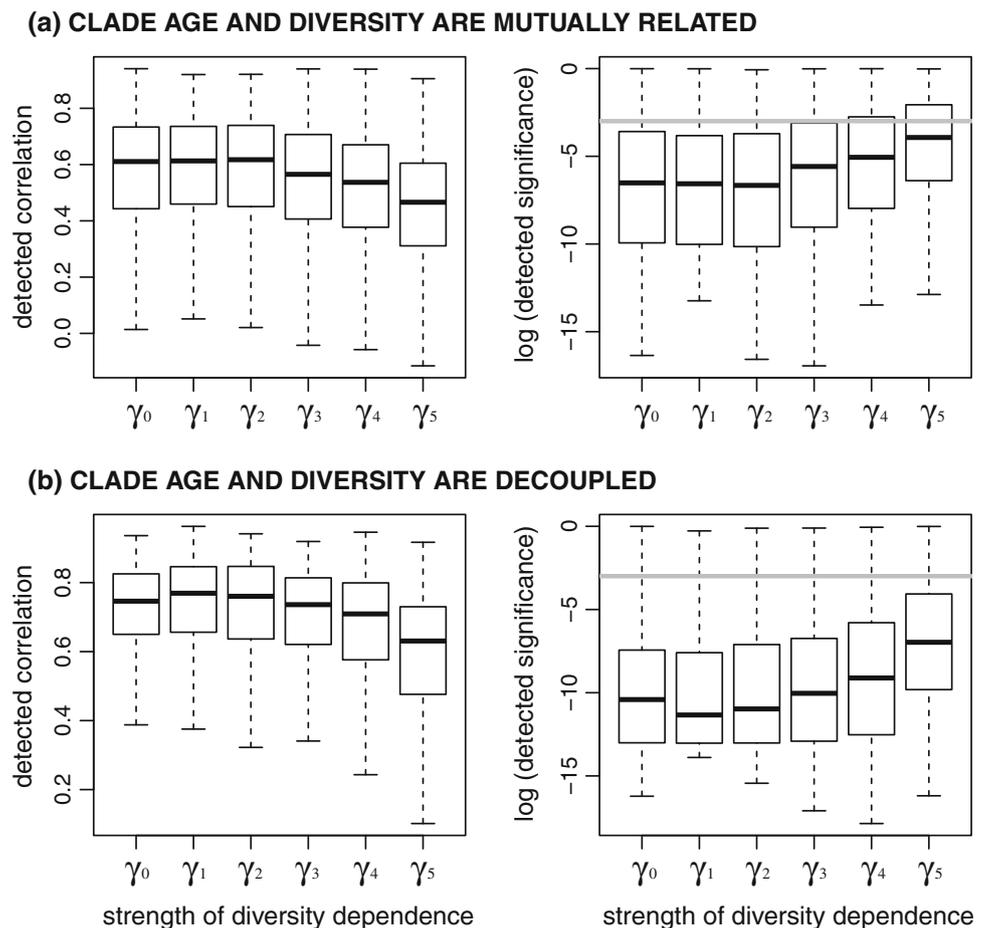
Topology of individual phylogenies was described by means of the gamma statistic (Pybus and Harvey 2000) and the beta parameter (Blum and Francois 2006). The gamma statistic, which is often used to quantify diversification slowdowns, is derived from the distribution of branching times across phylogeny. Negative gamma values indicate that branching times are concentrated near the root, which points to decelerating diversification (Pybus and Harvey 2000). The beta parameter from the beta-splitting model of Blum and Francois (2006) evaluates tree imbalance. Positive beta values indicate that tree topology is more balanced than expected under the Yule model of diversification whereas negative values indicate tree imbalance. Distribution of branch lengths within individual trees was characterized by skewness and kurtosis. These statistics described spread, variation and bias of the branch-length distributions.

I calculated the above described metrics (gamma, beta, skewness, kurtosis) for all the simulated and empirical phylogenies (McPeck 2008). Distributions of all four metrics were then plotted and visually inspected. Since the metrics were not normally distributed, non-parametric Kruskal–Wallis test was used for their comparison. My simulations and analyses were performed using original (most importantly `make.quasse`, `foreach`) and modified (`birthdeath.tree`) functions from the ‘R’ packages `ape`, `apTreeshape`, `diversitree`, `foreach`, and `geiger` (Paradis et al. 2004; Bortolussi et al. 2006; Harmon et al. 2008; FitzJohn 2012; Weston 2013; R Development Core Team 2012).

Results

In sum, CR estimators successfully detected trait-driven diversification in most of the analyses. Significant correlation between diversification rate and trait values was recovered across all the examined strengths of negative diversity dependence (Fig. 2, Fig. S1). Similar conclusions were supported whether clade age correlated with clade

Fig. 2 The detected correlations between inferred diversification rates and mean trait values. Under all the strengths of negative diversity dependence ($\gamma_0 = 0$, $\gamma_1 = -0.001$, $\gamma_2 = -0.002$, $\gamma_3 = -0.004$, $\gamma_4 = -0.008$, $\gamma_5 = -0.02$), CR estimators successfully uncovered the underlying correlations. Results are plotted separately for phylogenies (a) where clade age positively correlates with clade richness (b) where clade age and richness are decoupled. Interestingly, the results are virtually the same in both categories. Threshold of $p = 0.05$ is indicated by the horizontal gray line



diversity or not (Fig. 2, Fig. S1). Moreover, the labile and the conservative trait produced highly consistent outcomes. Results for the labile trait are presented in the main text while those for the conservative trait were placed to the Supplementary material. Strength and significance of the detected correlations tended to diminish with increasing diversity dependence (Fig. 2, Fig. S1).

CR estimators reported spuriously significant results with 4.817 % frequency so that their Type I error was close to the commonly used 5 % threshold (Table 1, Table S1). Acceptable Type I error was further supported by analyses of the reduced dataset (100 trees, each with 10 clades) even though CR estimators often failed to detect trait-driven diversification in this case (high Type II error) (Table 2, Table S2). Type II error decreased with the number of clades analyzed with reasonably strong tests emerging for >20 clades (Fig. 3, Fig. S2). In contrast to CR estimators, QuaSSE successfully uncovered present trait-dependent diversification in the reduced dataset (low Type II error) but reported spuriously significant results under declining diversification (high Type I error $\approx 15\%$) (Table 2, Table S2). Taken together, QuaSSE performed better when applied to phylogenies without diversification slowdowns

Table 1 Rates of Type I error calculated for CR estimators

Strength of diversity dependence	γ parameter	Type I error
γ_0	0.000	0.050
γ_1	-0.001	0.051
γ_2	-0.002	0.050
γ_3	-0.004	0.042
γ_4	-0.008	0.046
γ_5	-0.020	0.049

These results are based on the full simulated dataset (300,000 phylogenies) and indicate that CR estimators report spuriously significant relationships in $\approx 5\%$ of the analyzed cases. The rate of Type I error is therefore consistent with the commonly used threshold ($p = 0.05$) and, importantly, does not increase even under decelerating diversification (γ_1 - γ_5)

while CR estimators returned more conservative results under decelerating diversification.

Sizes of the simulated phylogenies followed a log-normal distribution with the mean phylogeny size being 61 species (interquartile: 15–82 species). Phylogenies with several hundred species (>200 species) were represented as well but less frequently (8 % of the phylogenies). This generally

Table 2 Comparison of CR estimators with QuaSSE based on the reduced dataset (100 trees)

Strength of diversity dependence	γ parameter	Type I error		Type II error	
		CR estimators	QuaSSE	CR estimators	QuaSSE
γ_0	0.000	0.05	<0.01	0.62	0.13
γ_1	-0.001	0.03	0.09	0.66	0.14
γ_2	-0.002	0.05	0.12	0.67	0.16
γ_3	-0.004	0.06	0.15	0.80	0.25
γ_4	-0.008	0.05	0.18	0.77	0.55
γ_5	-0.020	0.05	0.20	0.84	0.73

Rates of Type I and Type II error were calculated for both CR estimators and QuaSSE under constant diversification (γ_0) and diversification slowdowns of increasing strengths (γ_1 – γ_5). QuaSSE outperforms CR estimators under constant diversification. However, when diversification decelerates, CR estimators yield more conservative results and their Type I error stays at the 5 % threshold

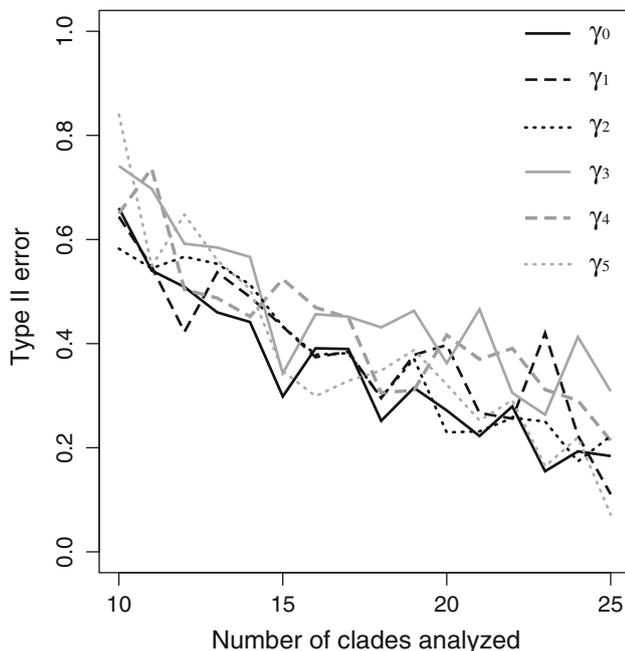


Fig. 3 Type II error associated with CR inference tends to decline with the number of clades analyzed. Similar trends emerge under all the examined strengths of diversification slowdowns ($\gamma_0 = 0$, $\gamma_1 = -0.001$, $\gamma_2 = -0.002$, $\gamma_3 = -0.004$, $\gamma_4 = -0.008$, $\gamma_5 = -0.02$). When the number of clades entering CR analysis is small (<20 clades), statistical power to detect present trait-diversification dependence may be limited. Under such circumstances, CR estimators can detect only strong relationships, thus yielding conservative tests

corresponds to the distribution of sizes of commonly published species-level phylogenies (McPeck 2008; Morlon et al. 2010). Moreover, distributions of the gamma statistic, the beta parameter, skewness, and kurtosis from empirical and simulated phylogenies were highly congruent (Fig. 4, Fig. S3). Kruskal–Wallis test did not detect any significant difference between the distributions of these four metrics (Table 3, Table S3). Therefore, sizes, topologies and branch-length distributions of the simulated phylogenies seem to realistically approximate empirical data.

Discussion

My simulations suggest that CR estimators can assess whether a trait affects diversification even when the diversification itself is negatively diversity dependent. Interestingly, meaningful results were recovered whether clade age correlated with clade diversity or not. In comparison to QuaSSE, CR estimators seem to offer simpler but more robust inference especially when diversification decelerates. Still, their results need to be interpreted cautiously when the number of analyzed clades is small (≤ 20 ; Table 2, Fig. 3, Fig. S2) due to elevated Type II error. Knowing the strengths and weaknesses of available methods, we can now make more informed decisions regarding our data analyses.

My simulation procedure entailed some assumptions that need to be considered. For example, I assumed that extinction rates are invariable and traits evolve following the Brownian motion model. Although this simulation approach is common in diversification studies (Paradis 2005; Bokma 2008b; FitzJohn 2010), shifting extinction rates and complex trait evolution might suppress statistical power of CR inference.

Therefore, CR estimators may be used to test simple hypotheses without complicated trait-diversification dynamics. Such hypotheses may still be highly appealing: Is diversification faster in warmer climates (Ricklefs 2006; Wiens et al. 2006; Jansson and Davies 2008)? Does diversification rate decline from clades with narrow ecological niches to generalist clades, or vice versa (Good-Avila et al. 2006; Nylin and Wahlberg 2008; Schnitzler et al. 2011)? Which quantitative traits accelerate diversification and which suppress it (Davies et al. 2004; Klak et al. 2004; Hunt et al. 2007)? These and many other relevant hypotheses are reviewed and discussed by Jablonski (2008). My results here corroborate that CR estimators offer a simple but robust inferential framework for addressing such questions, even when the analyzed

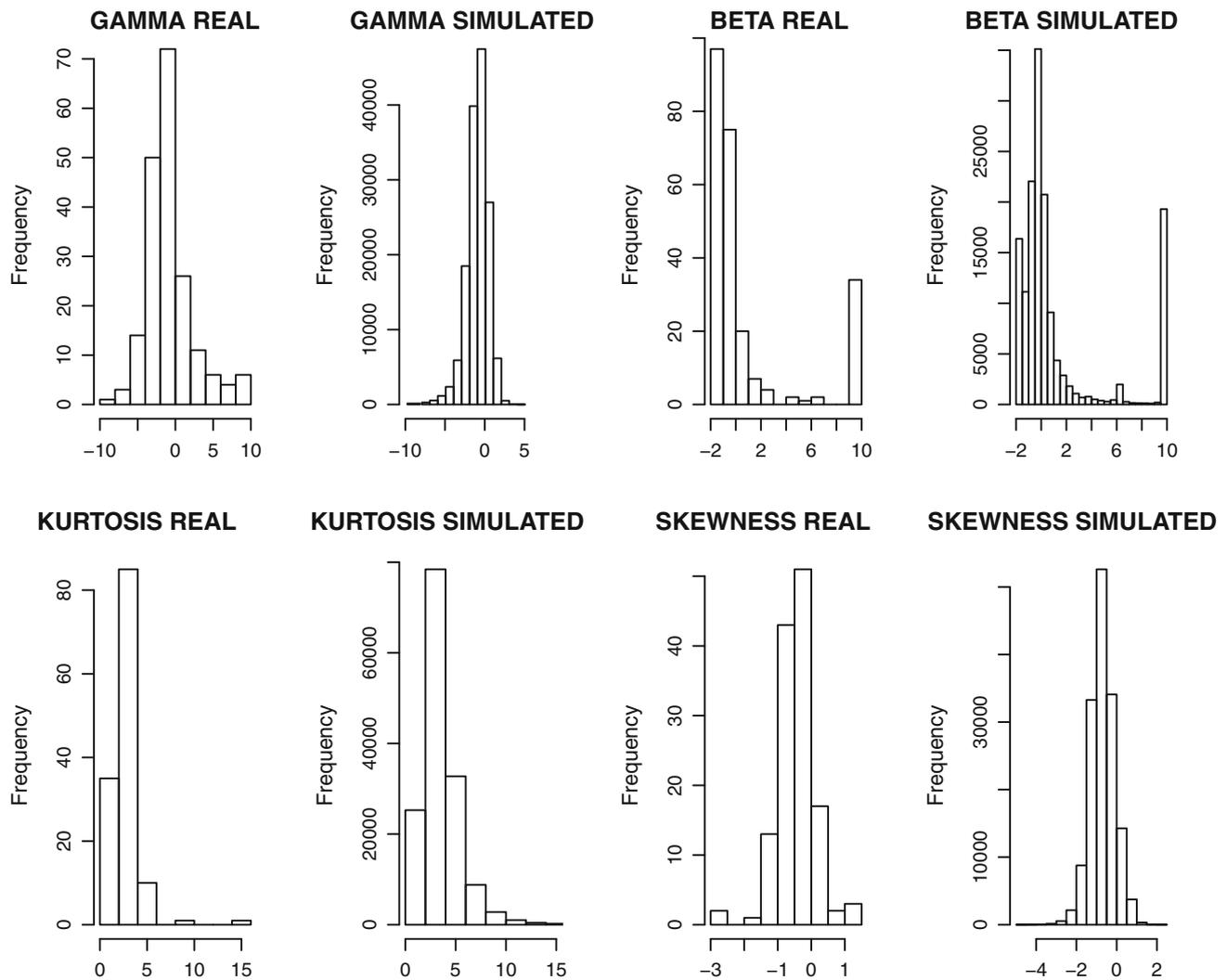


Fig. 4 Topologies and branch-length distributions of simulated and real phylogenies appear highly congruent in terms of gamma, beta, skewness, and kurtosis. My simulations therefore seem to sufficiently

approximate empirical data. For a more quantitative comparison of these distributions, see Table 3

Table 3 Kruskal-Wallis test did not detect any significant difference between the distributions of gamma, beta, skewness, and kurtosis of simulated and empirical phylogenies (see Fig. 4)

Tree statistic	KW Chi squared	df	<i>p</i>
Gamma	2.556	1	0.109
Beta	0.195	1	0.659
Kurtosis	2.909	1	0.088
Skewness	3.473	1	0.062

The simulated phylogenies thus appear realistic in terms of their slowdowns, imbalance and branch-length distributions

phylogeny shows decelerating diversification (Fig. 2, Fig. S1). CR estimators may prove convenient for analyses of accelerating diversification as well. Being a mirror image to slowdowns, accelerations are too defined by

biased branching times; the bias merely pulls toward the tips of a phylogeny instead of its root. Since CR estimators proved robust toward branching time biases, they may be suitable not only for taxa showing diversification declines but also accelerations or frequent shifts between the two (Davies et al. 2004; Hunt et al. 2007).

For inference more refined than CR estimators can supply, many other methods have been developed. The simple birth–death model (Nee et al. 1994) was extended to incorporate variation of speciation and extinction rates in time (Rabosky 2006; Rabosky and Lovette 2008; Morlon et al. 2010; Silvestro et al. 2011; Stadler 2011) and between clades (Alfaro et al. 2009; Moore and Donoghue 2009; Rabosky 2010a). Some of the methods allow correcting for incomplete taxon sampling as well (Bokma 2008a; Stadler 2009, 2011; Morlon et al. 2010; Hohna et al. 2011).

Many of these extensions, however, come at the price of additional assumptions or inference limitations (Ricklefs 2007; Bokma 2008a; Silvestro et al. 2011). For example, correcting for missing species is possible only under the questionable assumption of random sampling (Paradis 2004; Ricklefs 2007; Moore and Donoghue 2009; Stadler 2009, 2011; Morlon et al. 2010; Silvestro et al. 2011). Complex diversification models are more realistic but might require much larger phylogenies to return conclusive results (Bokma 2008b; Alfaro et al. 2009; Moore and Donoghue 2009; FitzJohn 2010; Silvestro et al. 2011; Stadler 2011). Moreover, refined inference may be computationally expensive and virtually impossible without supercomputers and advanced programming (e.g. the QuaSSE analyses presented here expended several years of CPU time).

In contrast, CR inference makes minimal assumptions and seems reasonably robust against their violation (e.g. as compared to QuaSSE) (Tables 1, 2; Fig. 2). It imposes few restrictions on the input data so that readily available information on clade age, clade diversity, and traits can be utilized to test basic hypotheses even when a comprehensive phylogeny is missing. Since biological data are often incomplete, such methods seem particularly important for first exploratory analyses. Thanks to their fast calculations, CR analyses are possible even for massive phylogenetic and trait datasets, allowing broader inference. Even though the resultant conclusions will be necessarily tentative, they may still facilitate our understanding of the diversification process and generate targeted hypotheses for future studies. Once detailed complete phylogenies exist, previous conclusions can be further tested and refined using QuaSSE. Even though QuaSSE does not accommodate slowdowns now, it may be extended to account for diversity-dependent diversification in future. Inferential flexibility of QuaSSE allows, for example, to first estimate the gamma statistic (Pybus and Harvey 2000) and then adjust the fitted speciation and extinction functions correspondingly. Very similar conclusions should apply to BiSSE (Maddison et al. 2007), whose inferential structure corresponds to QuaSSE aside from the fact that traits are allowed to vary along a discrete rather than continuous scale. Both simple and advanced methods therefore have their strengths and weaknesses whose relative weights may change with the data and hypotheses at hand.

Importantly, CR inference and QuaSSE seem to respond differently to slowing diversification even though both make similar rate constancy assumptions. Specifically, CR inference may produce higher Type I and Type II errors than QuaSSE when clades diversify at a constant rate. Under decelerating diversification, however, QuaSSE tends to report spuriously significant results, which is not the case with CR inference (Table 2, Table S2). Consequently,

QuaSSE performs better when applied to phylogenies without slowdowns while CR estimators return relatively robust results whether diversification decelerates or not. These differences between QuaSSE and CR inference, which have not been explored before, seem highly important given that slowing diversification has been recently reported for numerous taxa (McPeck 2008; Phillimore and Price 2008; Morlon et al. 2010; Etienne et al. 2012).

Indeed, CR inference may not yield meaningful results under all circumstances. For example, heterogeneous slowdowns or diversity saturation can impair CR analysis (Rabosky 2009a, 2010b; see also Maddison 2006; Paradis 2008). Interestingly, slowdowns may also result when a trait directionally evolves toward values which suppress speciation or promote extinction. Conversely, diversification rates accelerated by directional trait evolution can mask background slowdowns. Under these conditions, separating trait- and diversity-dependence might be challenging even with refined statistical inference. Apart from these special cases, CR estimators seem to recover reasonably reliable results for most species-level phylogenies of common sizes, topologies and branch length distributions (*sensu* McPeck 2008) (Tables 1, 2; Fig. 2). Potential errors might also arise when trait evolution departs from the Brownian motion model. In that case, branch lengths of the analyzed phylogeny may be transformed to mitigate the resultant bias (see Gittleman and Kot 1990; Freckleton et al. 2002; Blomberg et al. 2003; Butler and King 2004).

Some of my secondary, yet curious, findings relate to the correlation between clade age and richness, an issue of considerable interest in current diversification research (Ricklefs 2007; Rabosky 2009a, b; Wiens 2011). It has been suggested that the lack of correlation between clade age and richness might indicate diversification slowdowns caused by ecological limits on clade diversity (Sepkoski 1998; Mittelbach et al. 2007; Rabosky 2009a). This has been shown for many higher taxa (e.g. avian tribes, mammalian orders, teleost fish orders, plant families) (Rabosky 2009b, 2010b; Vamosi and Vamosi 2010), but species-level phylogenies analyzed here (similar to those from McPeck 2008) suggest that correlation between clade age and richness might not necessarily indicate slowing diversification. Specifically, clade age and richness were decoupled in some of the phylogenies simulated without diversity dependence (category γ_0 in Fig. 2b) and, vice versa, clade age and richness correlated in a number of phylogenies where diversification decelerated (categories γ_1 – γ_5 in Fig. 2a; Fig. S1 shows similar results). Contrary to some previously published work (Rabosky 2009a, b; Rabosky et al. 2012, but see Morlon et al. 2010), these findings suggest that information on clade age and richness might not be sufficient to diagnose clade saturation potentially induced by ecological limits.

In summary, CR inference can help us take advantage of the readily available data on clade age, clade diversity, and traits to draw conclusions about the diversification process. The obtained conclusions may be tentative but potentially sufficient to address simple and appealing diversification hypotheses. Refined tests are possible with QuaSSE, but their results need to be interpreted cautiously under slowing diversification. Rather than favoring one method over another, we may use either under those circumstances that play to its particular strengths. While simple methods allow limited but robust inference with incomplete data, refined analyses may provide further necessary details assuming that all their assumptions are satisfied and comprehensive phylogenies are available. Surprisingly, similar differences between competing diversification methods are rarely emphasized or explicitly tested, and many inferential frameworks have never been examined beyond the paper where they were introduced (Pennell et al. 2012). To further the field of diversification analysis, we need to develop new methods but also learn how to efficiently use those that already exist.

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References

- Alfaro, M. E., Santini, F., Brock, C., Alamillo, H., Dornburg, A., Rabosky, D. L., et al. (2009). Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 13410–13414.
- Alroy, J. (2000). New methods for quantifying macroevolutionary patterns and processes. *Paleobiology*, *26*, 707–733.
- Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, *57*, 717–745.
- Blum, M. G. B., & Francois, O. (2006). Which random processes describe the tree of life? A large-scale study of phylogenetic tree imbalance. *Systematic Biology*, *55*, 685–691.
- Bokma, F. (2008a). Bayesian estimation of speciation and extinction probabilities from (in)complete phylogenies. *Evolution*, *62*, 2441–2445.
- Bokma, F. (2008b). Detection of punctuated equilibrium by Bayesian estimation of speciation and extinction rates, ancestral character states, and rates of anagenetic and cladogenetic evolution on a molecular phylogeny. *Evolution*, *62*, 2718–2726.
- Bortolussi, N., Durand, E., Blum, M., & Francois, O. (2006). apTreeshape: Statistical analysis of phylogenetic tree shape. *Bioinformatics*, *22*, 363–364.
- Butler, M. A., & King, A. A. (2004). Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *American Naturalist*, *164*, 683–695.
- Davies, T. J., Barraclough, T. G., Chase, M. W., Soltis, P. S., Soltis, D. E., & Savolainen, V. (2004). Darwin’s abominable mystery: Insights from a supertree of the angiosperms. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 1904–1909.
- Etienne, R. S., Haegeman, B., Stadler, T., Aze, T., Pearson, P. N., Purvis, A., et al. (2012). Diversity-dependence brings molecular phylogenies closer to agreement with the fossil record. *Proceedings of the Royal Society B Biological Sciences*, *279*, 1300–1309.
- Felsenstein, J. (1985). Phylogenies and the comparative method. *American Naturalist*, *125*, 1–15.
- FitzJohn, R. G. (2010). Quantitative traits and diversification. *Systematic Biology*, *59*, 619–633.
- FitzJohn, R. G. (2012). Diversitree: Comparative phylogenetic analyses of diversification in R. *Methods in Ecology and Evolution*, *3*, 1084–1092.
- Foote, M. (2000). Origination and extinction components of taxonomic diversity: General problems. *Paleobiology*, *26*, 74–102.
- Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist*, *160*, 712–726.
- Gittleman, J. L., & Kot, M. (1990). Adaptation—statistics and a null model for estimating phylogenetic effects. *Systematic Zoology*, *39*, 227–241.
- Glor, R. E. (2010). Phylogenetic insights on adaptive radiation. *Annual Review of Ecology Evolution and Systematics*, *41*, 251–270.
- Good-Avila, S. V., Souza, V., Gaut, B. S., & Eguiarte, L. E. (2006). Timing and rate of speciation in Agave (Agavaceae). *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 9124–9129.
- Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E., & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics*, *24*, 129–131.
- Hohna, S., Stadler, T., Ronquist, F., & Britton, T. (2011). Inferring speciation and extinction rates under different sampling schemes. *Molecular Biology and Evolution*, *28*, 2577–2589.
- Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O. S., Wild, R., et al. (2007). A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science*, *318*, 1913–1916.
- Hutchinson, G. E. (1959). Homage to Santa Rosalia or why are there so many kinds of animals. *American Naturalist*, *93*, 145–159.
- Ingram, T. (2011). Speciation along a depth gradient in a marine adaptive radiation. *Proceedings of the Royal Society B Biological Sciences*, *278*, 613–618.
- Jablonski, D. (2008). Species selection: Theory and data. *Annual Review of Ecology Evolution and Systematics*, *39*, 501–524.
- Jansson, R., & Davies, T. J. (2008). Global variation in diversification rates of flowering plants: Energy vs. climate change. *Ecology Letters*, *11*, 173–183.
- Klak, C., Reeves, G., & Hedderson, T. (2004). Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature*, *427*, 63–65.
- Maddison, W. P. (2006). Confounding asymmetries in evolutionary diversification and character change. *Evolution*, *60*, 1743–1746.
- Maddison, W. P., Midford, P. E., & Otto, S. P. (2007). Estimating a binary character’s effect on speciation and extinction. *Systematic Biology*, *56*, 701–710.
- Magallon, S., & Sanderson, M. J. (2001). Absolute diversification rates in angiosperm clades. *Evolution*, *55*, 1762–1780.
- McPeck, M. A. (2008). The ecological dynamics of clade diversification and community assembly. *American Naturalist*, *172*, 270–284.
- Mittelbach, G. G., Schemske, D. W., Cornell, H. V., Allen, A. P., Brown, J. M., Bush, M. B., et al. (2007). Evolution and the latitudinal diversity gradient: Speciation, extinction and biogeography. *Ecology Letters*, *10*, 315–331.

- Moore, B. R., & Donoghue, M. J. (2009). A Bayesian approach for evaluating the impact of historical events on rates of diversification. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 4307–4312.
- Morlon, H., Potts, M. D., & Plotkin, J. B. (2010). Inferring the dynamics of diversification: A coalescent approach. *PLoS Biology*, *8*(9), e1000493.
- Nee, S. (2006). Birth-death models in macroevolution. *Annual Review of Ecology Evolution and Systematics*, *2006*(37), 1–17.
- Nee, S., May, R. M., & Harvey, P. H. (1994). The reconstructed evolutionary process. *Philosophical Transactions of the Royal Society B Biological Sciences*, *344*, 305–311.
- Nylin, S., & Wahlberg, N. (2008). Does plasticity drive speciation? Host-plant shifts and diversification in nymphaline butterflies (Lepidoptera: Nymphalidae) during the tertiary. *Biological Journal of the Linnean Society*, *94*, 115–130.
- Paradis, E. (2004). Can extinction rates be estimated without fossils? *Journal of Theoretical Biology*, *229*, 19–30.
- Paradis, E. (2005). Statistical analysis of diversification with species traits. *Evolution*, *59*, 1–12.
- Paradis, E. (2008). Asymmetries in phylogenetic diversification and character change can be untangled. *Evolution*, *62*, 241–247.
- Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*, 289–290.
- Pennell, M. W., Sarver, B. A. J., & Harmon, L. J. (2012). Trees of unusual size: Biased inference of early bursts from large molecular phylogenies. *PLoS ONE*, *7*, e43348.
- Phillimore, A. B., & Price, T. D. (2008). Density-dependent cladogenesis in birds. *PLoS Biology*, *6*, e0071.
- Pybus, O. G., & Harvey, P. H. (2000). Testing macro-evolutionary models using incomplete molecular phylogenies. *Proceedings of the Royal Society B Biological Sciences*, *267*, 2267–2272.
- R Development Core Team. (2012). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rabosky, D. L. (2006). Likelihood methods for detecting temporal shifts in diversification rates. *Evolution*, *60*, 1152–1164.
- Rabosky, D. L. (2009a). Ecological limits and diversification rate: Alternative paradigms to explain the variation in species richness among clades and regions. *Ecology Letters*, *12*, 735–743.
- Rabosky, D. L. (2009b). Ecological limits on clade diversification in higher taxa. *American Naturalist*, *173*, 662–674.
- Rabosky, D. L. (2010a). Extinction rates should not be estimated from molecular phylogenies. *Evolution*, *64*, 1816–1824.
- Rabosky, D. L. (2010b). Primary controls on species richness in higher taxa. *Systematic Biology*, *59*, 634–645.
- Rabosky, D. L., & Adams, D. C. (2012). Rates of morphological evolution are correlated with species richness in salamanders. *Evolution*, *66*, 1807–1818.
- Rabosky, D. L., & Lovette, I. J. (2008). Density-dependent diversification in North American wood warblers. *Proceedings of the Royal Society B Biological Sciences*, *275*, 2363–2371.
- Rabosky, D. L., Slater, G. J., & Alfaro, M. E. (2012). Clade age and species richness are decoupled across the eukaryotic tree of life. *PLoS Biology*, *10*, e1001381.
- Raup, D. M. (1985). Mathematical models of cladogenesis. *Paleobiology*, *11*, 42–52.
- Ricklefs, R. E. (2006). Global variation in the diversification rate of passerine birds. *Ecology*, *87*, 2468–2478.
- Ricklefs, R. E. (2007). Estimating diversification rates from phylogenetic information. *Trends in Ecology & Evolution*, *22*, 601–610.
- Sanderson, M. J., & Donoghue, M. J. (1996). Reconstructing shifts in diversification rates on phylogenetic trees. *Trends in Ecology & Evolution*, *11*, 15–20.
- Schluter, D., Price, T., Mooers, A. O., & Ludwig, D. (1997). Likelihood of ancestor states in adaptive radiation. *Evolution*, *51*, 1699–1711.
- Schnitzler, J., Barraclough, T. G., Boatwright, J. S., Goldblatt, P., Manning, J. C., Powell, M. P., et al. (2011). Causes of plant diversification in the cape biodiversity hotspot of South Africa. *Systematic Biology*, *60*, 343–357.
- Sepkoski, J. J. (1998). Rates of speciation in the fossil record. *Philosophical Transactions of the Royal Society B Biological Sciences*, *353*, 315–326.
- Silvestro, D., Schnitzler, J., & Zizka, G. (2011). A Bayesian framework to estimate diversification rates and their variation through time and space. *BMC Evolutionary Biology*, *11*, 311.
- Stadler, T. (2009). On incomplete sampling under birth-death models and connections to the sampling-based coalescent. *Journal of Theoretical Biology*, *261*, 58–66.
- Stadler, T. (2011). Mammalian phylogeny reveals recent diversification rate shifts. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 6187–6192.
- Vamosi, J. C., & Vamosi, S. M. (2010). Key innovations within a geographical context in flowering plants: Towards resolving Darwin's abominable mystery. *Ecology Letters*, *13*, 1270–1279.
- Wertheim, J. O., & Sanderson, M. J. (2011). Estimating diversification rates: How useful are divergence times? *Evolution*, *65*, 309–320.
- Weston, S. J. (2013). *FOREACH: R package for parallel computing*. Palo Alto, USA: Revolutionary Analytics.
- Wiens, J. J. (2011). The causes of species richness patterns across space, time, and clades and the role of ecological limits. *The Quarterly Review of Biology*, *86*, 75–96.
- Wiens, J. J., Graham, C. H., Moen, D. S., Smith, S. A., & Reeder, T. W. (2006). Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: Treefrog trees unearth the roots of high tropical diversity. *American Naturalist*, *168*, 579–596.