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What affects power to estimate speciation rate shifts?

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Abstract

The development of methods to estimate rates of speciation and extinction from time-calibrated phylogenies has revolutionized evolutionary biology by allowing researchers to correlate diversification rate shifts with causal ecological factors. A growing number of researchers are interested in testing whether the evolution of a trait or a trait variant has influenced speciation rates, and three modelling methods – BiSSE, MEDUSA and BAMM – have been widely used in such studies. We simulated phylogenies with a single speciation rate shift each, and evaluated the power of the three methods to detect these shifts. We varied the degree of increase in speciation rate (rate asymmetry), the number of tips, the tip-ratio bias (ratio of number of tips with each character state) and the relative age in relation to overall tree age when the rate shift occurred. All methods had good power to detect rate shifts when the rate asymmetry was strong and the sizes of the two lineages with the distinct speciation rates were large. Even when lineage size was small, power was good when rate asymmetry was high. In our simulated scenarios, small lineage sizes appear to affect BAMM most strongly. Tip-ratio influenced the accuracy of speciation rate estimation but did not have a strong effect on power to detect rate shifts. Based on our results, we provide some suggestions to users of these methods.

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Introduction

Much as the advent of methods to infer phylogenies (Edwards & Cavalli-Sforza, 1963; Sokal & Sneath, 1963; Camin & Sokal, 1965; Hennig, 1965) led to a spectacular revolution in evolutionary biology, the arrival of mathematical methods to estimate divergence times from molecular phylogenies has offered unprecedented novel insights into macroevolution. More recently, a seminal innovation has been the development of tools to estimate rates of speciation and extinction from time-calibrated phylogenies (Nee, May & Harvey, 1994). Until such methods became available, our understanding of macroevolutionary patterns and processes of diversification largely relied on the fossil record, which is incomplete for most taxa (Benton, Wills & Hitchin, 2000; Quental & Marshall, 2010) and virtually non-existent for many soft-bodied life forms (Donoghue & Purnell, 2009). However, the possibility of using dated phylogenies of extant taxa to shed light on macroevolutionary history is appealing, and there has been a phenomenal interest in applying such methods to understand fundamental questions such as how the mode and tempo of diversification have been influenced by trait evolution (especially 'key innovations')(e.g. Hunter & Jernvall, 1995; Hodges & Arnold, 1995; Near et al., 2012; Rainford et al., 2014; Peña & Espeland, 2015; Sahoo et al., 2017), biogeography (e.g. Kozak, Weisrock & Larson, 2006; Wahlberg et al., 2009; Dunn et al., 2009; Sanders, Mumpuni & Lee, 2010; Sundue, Testo & Ranker, 2010), climate change (e.g.Jansson & Davies, 2008; Dunn et al., 2009; Arakaki et al., 2011; Ezard et al., 2011; Near et al., 2012; Xiang et al., 2014), etc.

The diversification rate of a lineage is the difference between its speciation rate λ and extinction rate μ. Testing hypothesis of diversification rate variation is underpinned by the ability to decouple and accurately estimate these rates. Estimates of extinction rates from phylogenies of extant taxa appear to be error prone (Rabosky, 2010; Laurent, Robinson-Rechavi & Salamin, 2015; May & Moore, 2016; but see Stadler, 2013; Beaulieu & O'Meara, 2016). On the other hand, speciation rate estimates are generally considered to be more robust. One of the most common themes in macroevolutionary studies over the last decade has been to test whether a trait (or trait variant) has increased speciation rates (e.g. Sundue, Testo & Ranker, 2010; Claramunt et al., 2012; Escudero et al., 2012; Litsios et al., 2012; Horn et al., 2014; Rainford et al., 2014; Xiang et al., 2014; Gubry-Rangin et al., 2015; Igea et al., 2017, Wiens et al., 2017; Sahoo et al., 2017, Seeholtzer et al., 2017), with a suite of analytical tools providing the framework to infer speciation rate variation across the phylogeny.

For long, analyses testing the effect of a trait on diversification relied on comparisons of species richness of sister clades (e.g. Mitter, Farrell & Wiegmann, 1988; Zeh, Zeh & Smith, 1989). This method cannot distinguish between λ and μ , is prone to Type II error (non-detection of significant diversification rate differences), and does not effectively utilize information from clades with mixed character states (Maddison, Midford & Otto, 2007). The most recent methods aim to utilize information in the branching patterns of dated phylogenies to decouple λ from μ (Stadler, 2011). The BiSSE (Binary State Speciation and Extinction) (Maddison, Midford & Otto, 2007) modelling approach has been especially popular for hypothesis testing because it estimates λ and μ associated with character states, i.e state-dependent diversification rates. BISSE specifies a likelihood model where λ and μ can depend on the character state of a lineage at each time point, and the rates of character state change are allowed to vary (Maddison, Midford & Otto, 2007; Fitzjohn 2009). Likelihood scores are estimated simultaneously for character state-dependent λ and μ as well as rates of character change. AIC (Akaike Information Criterion) based model comparison allows the user to infer whether character state-dependent diversification best fits the data (Maddison et al., 2007; FitzJohn et al. 2009; FitzJohn 2010). BISSE requires a completely resolved dated phylogeny with information on character states of tips as input, and can take into account incomplete sampling (Fitzjohn et al., 2009).

While BiSSE only models binary discrete character states (for example presence or absence of a trait; two states of a trait), extensions of BiSSE can handle other types of data. MuSSE (Multiple SSE; FitzJohn, 2012) can deal with multiple discrete character states, while QuaSSE (Quantitative SSE; FitzJohn, 2010) allows testing the effect of quantitative traits. GeoSSE (Geographic SSE; Goldberg, Lancaster & Ree, 2011) tests region-dependent diversification, while BiSSE-ness (BiSSE-node enhanced state shift; Magnuson-Ford & Otto, 2012) and Cladogenetic SSE (ClaSSE; Goldberg & Igić, 2012) integrate cladogenetic and anagenetic trait evolution. Beaulieu & O'Meara (2016) proposed the HiSSE (Hidden States SSE) model, which attempts to account for unmeasured ('hidden') factors impacting diversification rates of a known trait or character state.

In contrast to the BiSSE family of methods, character-independent diversification methods attempt to identify the number and location of rate shifts in speciation and extinction across the tree, without *a priori* information on the mechanism of rate variation. Once the locations of rate shifts are found, the researcher can test for associations with traits of interest. MEDUSA (Modeling Evolutionary Diversification Using Stepwise Akaike Information Criterion; Alfaro et al., 2009), is one such framework that has been very popular. MEDUSA incrementally assigns rate shifts to all branches of the tree, and uses stepwise AIC to determine the number and location(s) of rate shifts that best fit the data. Rate shifts estimates are thus agnostic of the cause of rate variation among lineages.

BAMM (Bayesian Analysis of Macroevolutionary Mixtures; Rabosky, 2014) is the most widely used character-independent diversification modelling method. BAMM assumes that λ and μ are heterogenous across the phylogeny, and that changes in these parameters across branches occur under a compound Poisson process. It uses reversible-jump Markov Chain Monte Carlo to explore models varying in the number of shifts in diversification parameters. Estimates of λ and μ, and inferences on the number of rate shifts are based on posterior distributions. Both BAMM & MEDUSA require a dated phylogeny, and can accommodate incomplete sampling.

Although BiSSE, BAMM and MEDUSA have been very popular, recent critical evaluations of their performance have highlighted potential shortcomings particular to each method. Using empirical datasets, Rabosky & Goldberg (2015) found that BiSSE is prone to high Type I error rates, wherein diversification-neutral traits are often found to be significantly associated with speciation rate. Surprisingly, such false associations appear to be detected even for traits with weak phylogenetic signal (Rabosky & Goldberg, 2015). BiSSE has been shown to be affected by tree size (number of tips) (Davis, Midford & Maddison, 2013; Gamisch, 2016) and tip-ratio bias (i.e. ratio of tips with one character state versus another) (Davis, Midford & Maddison, 2013). May & Moore (2016) used extensive simulations to understand the statistical behavior of MEDUSA, and showed that the method is prone to a very high rate of false inferences of rate shifts (ca. 30% on average), and that the estimated diversification parameters are biased. The probability of rate shift detection in MEDUSA depends on the number of terminals in the tree (Laurent, Robinson-Rechavi & Salamin, 2015). Moore et al. (2016) showed that the accuracy of BAMM is strongly affected by the priors specified, and that the estimates diversification rate parameters are unreliable (although see Rabosky et al., 2017).

Analyses of simulated phylogenies have proved to be a valuable tool to assess the performance of the various modelling approaches. We simulated phylogenies with a single speciation rate shift each, and evaluated the power of of BiSSE, BAMM & MEDUSA to detect these shifts. We varied the degree of increase in speciation rate, the number of tips, the tip-ratio bias and the relative age in relation to tree age when the rate shift occurred. We found that all methods have good power to detect rate shifts under many conditions, but also identified some scenarios under which these methods have low power.

Materials and Methods

(1) Simulation of phylogenetic trees with single diversification rate shifts

(1a) General procedure

The basic workflow of the simulation process is outlined in Figure 1. To obtain a phylogeny with a single shift in speciation rate, we first simulated two trees - a *basetree* and a *subtree* wherein the *subtree* had a greater speciation rate (λ_1) compared to that of the *basetree* (λ_0). A *subtree* of a given age (S_{age}) was grafted onto the *basetree* following pruning of a randomly chosen *basetree* clade with approximately the same age ($S_{age} \pm 2.5\%$) (Figure 1), using a custom written function (available in Figshare). This generated a composite tree with a single speciation rate shift (λ_0 to λ_1). Details of the *subtree* and *basetree* simulations are described in the next section. The relative age of the subtree in relation to that of the overall tree was varied by varying the age of the *subtree*. This procedure was followed to generate trees with fixed overall size, but with different relative *subtree* ages (S_{ages}), tip-ratios (no. of *basetree* tips: no. of *subtree* tips) and speciation rate asymmetry (λ_1/λ_0) (Table 1).

(1b) Tree simulation

We used the package *TESS* (Höhna, May & Moore, 2015) to simulate trees in R (R Core Team 2016). The package implements tree simulation based on a global, time-dependent birth-death process conditioned either on number tips or age of the tree (Höhna, May & Moore, 2015). In our simulations, we generated phylogenetic trees under a constant birth-death process by conditioning on age using the function *tess.sim.age*, which simulates trees given the age (*basetree* age B_{age} & S_{age}) and diversification rate parameters (speciation rate λ and extinction rate μ). As this method produces trees with varying number of tips, we simulated 1000 trees each time and used the function *tess.nTaxa.expected* from the same package to select the best tree out of the 1000 trees. This was done by comparing the expected number of tips for every 1 unit time with the simulated tree using a correlation test. The tree with the maximum correlation coefficient was chosen as the best tree.

(2) Systematic simulation of composite trees

(2a) Simulation Set 1: Rate asymmetry and relative subtree ages

We fixed λ_0 at 0.20, and varied speciation rate asymmetry (λ_1/λ_0) by altering λ_1, while keeping extinction rates constant ($\mu_0=\mu_1$). Thus, higher asymmetry values indicate a greater degree of speciation rate increase in the *subtree*. B_{age} was allowed to vary between 5 and 35 units to obtain the targeted tree sizes. To estimate the influence of tree size, we eliminated all trees that did not fit within the targeted tree size classes (50±10, 100±10, 150±10, 200±10, 300±10 and 500±10). We simulated 50 composite trees each for all combinations of tree size classes and six λ_1/λ_0 ratios ranging from 1.5X to 5.5X This was done for S_{ages} 20%, 40% and 60% relative to *basetree* age (Table 1). Therefore, this simulation set comprised 1500 trees each for three S_{ages}. Simulated trees were subsequently used in the diversification analyses where BAMM, MEDUSA and BiSSE were used to detecte the simulated rate shifts (Section 3).

(2b) Simulation Set 2: Tip-ratio bias and number of tips

To differentiate between the effects of *subtree* size, *basetree* size and tip-ratio, we simulated trees with three tip-ratio values (*basetree*:*subtree* number of tips; 1:9, 9:1 and 1:19), all with a 2X speciation rate asymmetry. We chose 2X because our pilot simulations indicated that power was neither very high nor very low. For each tip-ratio value, we simulated trees with different overall tree sizes (50±10, 100±10, 200±10, 400±10 and 800±10). In order to achieve the target tip numbers and tip-ratios, B_{age} was allowed vary between 5 and 35 units, and S_{age} between 30 and 70%. We were unable to simulate trees with 1:9 ratio for the 100±10 size class, and we therefore used a 1:8 ratio as an approximation.

(3) Estimation of diversification rate parameters and power of modelling approaches

We used BiSSE, MEDUSA and BAMM to estimate diversification rate parameters (λ_0, μ_0, λ_1 and μ_1) of the simulated composite trees and detect rate shifts. Power was calculated as the proportion of trees in which a significant rate shift was detected at the node where the *subtree* was attached to the *basetree*.

(3a) BiSSE

For the BiSSE analysis, a character was assigned to be present in all *subtree* tips, but absent in all *basetree* tips. Therefore, diversification rate estimates of the BiSSE model will reflect the speciation and extinction rate estimates of the *subtree* (i.e. λ_1 and μ_1) and *basetree* (λ_0 and μ_0). We then compared the unrestricted full model ($\lambda_1 \neq \lambda_0$; $\mu_0 \neq \mu_1$; unequal rates of character state transition) to a model with restricted speciation rates ($\lambda_1 = \lambda_0$) using likelihood ratio tests. If $P<0.05$, the rate shift was considered to have been detected, while the rate shift was not considered detected if $P \geq 0.05$.

(3b) MEDUSA

We performed MEDUSA analyses using the function *MEDUSA (*available from the GitHub as an R package https://github.com/josephwb/turboMEDUSA). We specified the model of tree evolution to be a birth-death process to estimate the diversification rate parameters. We recorded the node where the rate shift was detected and the estimated diversification rates.

(3c) BAMM

We performed BAMM analyses with the default parameter settings using the control file available from the BAMM website (http://bamm-project.org/quickstart.html#control-file accessed: August 2017). The priors used to estimate the speciation and extinction rates were generated using the *setBAMMpriors* for each tree using the *BAMMtools* package (Rabosky et al., 2014) in R. We ran the MCMC analysis for 2 million iterations and checked for convergence using ESS metrics (>200). The *bammdata* object was generated using the *getEventData* function from the *BAMMtools* package after discarding the first 10% of samples as burnin. The *bammdata* object was then used to calculate the diversification rates.

We estimated speciation and extinction rates for the subtree (λ_1 and μ_1) as the average rate of the clade using the function getCladeRates from the BAMMtools package (Rabosky et al., 2014). We estimated λ_0 and μ_0 using the same function by specifying the common ancestor node of the composite tree, but excluding the rates of the subtree.

(4) Accuracy of estimated asymmetry and speciation rates

To compare the difference between the expected and estimated asymmetry, we calculated the estimated asymmetry ratio as the median of the estimated *subtree/basetree* speciation rates for a given set of trees. The estimated asymmetry ratio was calculated independently for the various combinations of tree size class, S_{age} and simulated rate asymmetry in Simulation Set 1. Therefore, each estimated asymmetry value was the median of the estimates of 50 trees.

We also calculated the error in estimation of λ_0 and λ_1 for trees in Simulation Set 2. Error was calculated as the median of expected/estimated λ for each combination of tree size class and tip-ratio. Thus, a value of 1 represents no error, values >1 indicate overestimation of λ, and values < 1 indicate underestimation.

Results

Effect of speciation rate asymmetry

In analyses of trees from Simulation Set 1, power increased both with asymmetry and tree size across all three *subtree* ages and for all methods (Figure 2), but the overall effect of asymmetry and tree size depended on *subtree* age (S_{age}). We first discuss results for S_{ages} 20% and 40%, which showed very similar trends (Figure 2A-F). Power was close to zero for an asymmetry of 1.5X, irrespective of tree size, and power was very high (nearly 1) for all tree size classes when asymmetry was 4.5X or 5.5X. The exception was the set of BAMM analyses on trees of S_{age} 40% (Figure 2F), where 4.5X and 5.5X asymmetries had low power (less than 0.5) for very small tree sizes. For intermediate asymmetries (2.5X and 3.5X), power correlated strongly with tree size for all methods.

In the case of Sage 60%, no asymmetry level resulted in uniformly high or low power across all tree sizes. Rather, there was a strong association between power and tree size for all asymmetries (Figure 2G-I).

The number of *basetree* tips, *subtree* tips and the ratio of *basetree*:*subtree* tips (tip-ratio bias) for a given tree size class depended on S_{age} (Supplemental Information S1). Both the number of *basetree* tips and tip-ratio bias decreased, but *subtree* size increased, with increasing S_{age}. The effect of these three parameters was explored further in Simulation 2.

Figure 3 depicts the relationship between expected (i.e. simulated) and estimated asymmetry ratios. The estimated asymmetry ratio was generally correlated with the expected ratios, but there was a trend for the estimated ratio to be smaller than the expected when trees were small. This trend was particularly strong for BAMM. Power tended to be positively correlated with the estimated asymmetry ratio (comparing Figures 2 & 3)

Effect of tip-ratio bias and the number of tips in the basetree/subtree

Figure 4 depicts results from analyses of trees simulated in Set 2, all of which had a 2X asymmetry, overall size ranging from 50 to 800, and one of three tip-ratios, either biased towards the *basetree* (9:1) or the *subtree* (1:9 and 1:19). Panels D-I depict the error in estimation of λ_0 (D-F) and λ_1 (G-I).

a) BiSSE (Figure 4A): Among trees of 1:9 tip ratio (red bars), power ranged from ca. 0.2 to ca. 1 depending on tip number, and from ca. 0.30 to ca. 0.8 among trees with 1:19 tip ratio (green bars). Power always increased when *basetree* size (5, 10, 20, 40 or 80) increased, but did not increase when *basetree* size remained constant and only the *subtree* size increased (comparisons between adjacent green and red bars). When tip-ratio was *basetree* biased (blue bars), power ranged from ca. 0.4 to 1. Power was marginally higher for tip-ratio 9:1 than for 9:1 (comparison of adjacent blue and red bars).

BiSSE had a strong tendency to overestimate λ_0 (error >1) when the ratio was 1:19. Error was relatively low for other ratios (Figure 4D).

b) MEDUSA (Figure 4B)**:** Power was strongly correlated with overall tree size for all three tip-ratios. Power ranged from ca. 0.2 to 1 for the 1:9 tip-ratio (red bars), and from ca. 0.5 to 1 for the 1:19 tip-ratio (green bars). For smaller tree sizes, power increased when *basetree* size remained constant and *subtree* size increased, for e.g. comparison of trees with 5-45 and 5-90 *basetree-subtree* tips, or 20-180 and 20-380. Power ranged from ca. 0.1 to ca. 1 for the *basetree* biased tip-ratio (blue bars), and was overall higher for tip-ratio 1:9 compared to 9:1 (comparison of adjacent blue and red bars).

MEDUSA had a strong tendency to overestimate λ_0 (error >1) for larger trees with 1:19 tip-ratio and to underestimate λ_0 for smaller trees at 1:9 ratio (Figure 4E).

c) BAMM (Figure 4C): Power remained at ca. 0.25 for both *subtree* biased tip-ratios when tree size $\leq$ 200 tips (red and green bars). For trees of the 400 size class, power was ca. ca. 0.4 when there were 20 *subtree* tips, but increased to ca 0.5 when there were 40 *subtree* tips. Power was low (>0.25) for the *basetree* biased tip-ratio irrespective of tree size (blue bars). Power was highest for trees with 800 tips and *subtree* biased tip-ratios (0.75 or 1). Power was overall higher for tip-ratio 1:9 than for 9:1 (comparison of adjacent blue and red bars).

Error in speciation rate estimates

BAMM tended to understimate λ_0 for all tree sizes and tip-ratios, but this effect was strongest at the 1:9 tip-ratio (Figure 4F). All three methods tended to underestimate λ_1 when the tip-ratio was 1:9, and overestimate this for the 9:1 and 1:19 tip-ratios (Figures 4G-I).

Discussion

Previous studies have identified shortcomings specific to particular modelling approaches for estimation of rate shifts in phylogenies (Rabosky, 2010; Davis, Midford & Maddison, 2013; Laurent, Robinson-Rechavi & Salamin, 2015; Rabosky & Goldberg, 2015; Gamisch, 2016; Moore et al., 2016; May & Moore, 2016; Meyer & Wiens, 2017). We simulated large sets of trees where speciation and extinction rates remained constant throughout the tree (*basetree*), apart from an increase in speciation rate at a single node (*subtree*), and analyzed these trees using three widely used modelling approaches. We are therefore able to assess the relative performance of the three methods, and identify issues that are common to these methods. We find that the power to detect speciation rate shifts was strongly influenced by rate asymmetry and tip number for all methods.

Effect of rate asymmetry

Not surprisingly, power increased as the speciation rate asymmetry increased, which has also been reported in other studies (e.g. Davis, Midford & Maddison, 2013; Laurent, Robinson-Rechavi & Salamin, 2015). All methods performed poorly when the *subtree* speciation rate increased by only 50% (1.5X) relative to the *basetree*, suggesting that moderate rate increases are difficult to detect. However, even with a high asymmetry of 5X, a significant proportion of rate shifts were undetected by all methods (Type II error), especially in smaller trees. Davis, Midford & Maddison (2013) and Gamisch (2016) showed that BiSSE analyses on small trees are prone to high Type II error. They simulated complex evolutionary scenarios with multiple increases and decreases in diversification parameters at random points across the tree, and only assessed the effect of overall tree size. Laurent, Robinson-Rechavi & Salamin (2015) tested the performance of MEDUSA for both simple scenarios with a single speciation rate shift and more complex scenarios with multiple shifts and mass extinctions. Interestingly, they found that overall tree size had a positive influence in the complex scenarios (their Figure 5a), but not for the single rate shift scenario (their Figure 3b). In their single rate shift scenario, power increased with the size of the lineage in which the rate shift occurred (i.e., the *subtree*). This suggests that power may be affected not by the overall tip number, but by the number of tips in the *basetree* or the *subtree*, both of which are correlated with overall tip number. We discuss this in more detail in the section *'Effects of lineage tip number and tip-ratio bias*'.

The performance of all three methods was comparable at most asymmetry values. However, BiSSE and MEDUSA appear to perform better than BAMM when asymmetry is not high. When S_{age} was 20%, BAMM rarely detected a rate shift at 2.5X asymmetry even in the largest trees. At 40% Sage, BAMM had lower power than the other two methods at 2.5X asymmetry, irrespective of tree size. Furthermore, BAMM performed worse than the other two when the second set of simulations with 2X asymmetry were analyzed (Figure 4). On the other hand, power was negligible for all methods at the weakest asymmetry (1.5X) in Simulation Set 1. Power tended to be correlated with estimated asymmetry ratios for all methods (Figure 2 & 3). Thus, our results indicate that BiSSE and MEDUSA perform better when speciation rate asymmetry is between ca. 2X to 2.5X, and all methods fail to detect rate shifts when the rate increase is 1.5X or lesser.

Effect of subtree age

The power of all methods was lower at S_{age} 60% compared to S_{ages} 40% and 20%. This is not due to tree size, because the simulated tree sizes were the same for all S_{ages}. However, for a given asymmetry value and overall tree size, *subtree* sizes are necessarily larger for higher S_{ages} (Supplemental Information S1), and therefore *basetree* sizes need to be smaller to accommodate the larger *subtree*s. Thus, *subtree* size, *basetree* size and tip-ratio bias were all influenced by S_{age}, and one or all of these factors may explain why power was compromised at S_{age} 60% compared to the younger S_{ages}.

Effects of lineage tip number and tip-ratio bias

The second set of simulations explicitly attempted to tease apart the effects of overall tree size, *subtree* size, *basetree* size and and tip-ratio bias. We simulated three tip ratios, two of which were *subtree* biased (1:9 and 1:19) and the third, *basetree* biased (9:1). We varied *basetree* and *subtree* size from 5-720, and the overall tree size from 50-800. We were thus able to not only test whether tip-ratio bias affected power, but also compare power for trees with the same *subtree* or *basetree* size, but varying overall tree size. As expected, overall tree size generally correlated positively with power for all methods. However, although both 1:9 and 9:1 tip ratios had the same tip-ratio bias, these ratios differed in power within the same tree size class; this was the case for all methods (Figure 4A-C; comparison of red and blue bars). For BiSSE, there was either no difference in power between these two tip-ratios or power tended to be greater at 9:1, but power was always greater at 1:9 for MEDUSA and BAMM. Furthermore, power varied significantly among tree sizes for any given tip-ratio. This was so even when tree sizes were very large, and therefore when power may be expected to be uniformly high. This indicates that the number of tips in the lineages with distinct speciation rates (i.e. *basetree* and *subtree*) may play a stronger role than tip-ratio bias *per se*. Indeed, both *subtree* and *basetree* sizes had a strong effect on power for all three methods.

However, the methods differed in terms of how influential *subtree* and *basetree* size were. For BiSSE, power did not increase when tree size doubled at the same *basetree* size, for e.g, when *basetree* was 10 and *subtree* size changed to 190 from 90. If lineages on a tree differ in speciation rates, estimates of these rates should be more error prone, because the size of these lineages is smaller. Generally, if lineages differ in diversification rates, the size of such lineages with distinct diversification parameters will be larger in larger trees. This may explain why power increases with tree size, as has been found here and in other studies (e.g. Davis, Midford & Maddison, 2013; Laurent, Robinson-Rechavi & Salamin, 2015; Gamisch et al., 2016). The effect of lineage size also explains results in Davis et al. (2013), where power initially increased with increasing asymmetry but later decreased with further asymmetry increase, and this pattern was consistent for all overall tree sizes (their Figure 1a). They concluded that the positive effect of asymmetry was counteracted by the negative effect of increasing tip-ratio bias as asymmetry increased. However, at higher asymmetries (and thus at stronger tip-ratios bias), the number of tips available for parameter estimation is likely to have been the limiting factor. For e.g., a tree with 500 tips (the largest tree size they simulated) and an asymmetry of 10X had a tip-ratio of 90:1, and thus one of the lineages would have had <6 tips. Therefore, we suggest that their results are better explained by the effect of lineage size rather than tip-ratio *per se* (although we do not argue that tip-ratio does has not effect), and that stronger asymmetries will *always* improve power of detection by BISSE as long as there are enough tips in each lineage with a distinct speciation rate.

For MEDUSA, there was a tendency for increase in power when *subtree* size increased without a change in *basetree* size (Figure 4B; comparison of red and green bars), indicating that overall tree size may partially offset the deleterious effect of small lineage size. This effect was also seen in the BAMM analyses, where the *basetree* remained at 40, but *subtree* size doubled.

Inferences can be drawn on the relative importance of *subtree* and *basetree* sizes by comparing 9:1 and 1:9 tip-ratios (Figure 4; comparison of red and blue bars for the same tree size). For BiSSE, power tended to be higher for 9:1, suggesting small *basetree* size is more detrimental compared to small *subtree* size. This is probably because parameter estimation is more accurate for the monophyletic *subtree* compared to the paraphyletic *basetree*. On the other hand, power was lower at 9:1 for both MEDUSA and BAMM, which suggests that the three methods are differently affected by *basetree* and *subtree* sizes. Surprisingly, BAMM rarely detected rate shifts at this tip-ratio, even in the largest trees, and this maybe related to inaccurate estimates of speciation rates. However, we find no other clear correlations between error and power for Simulation Set 2.

Recommendations

In practice, a researcher intending to analyze diversification rate shifts may only have information about overall tree size, and not the sizes of lineages with distinct rates. In such cases, we agree with recommendations of Davis and colleagues (Davis, Midford & Maddison, 2013) who suggested inferences based on BiSSE analyses of trees with fewer than 300 tips should be made very cautiously. We extend the same recommendation to BAMM and MEDUSA. Thus, if no rate shift is detected when using BiSSE, BAMM or MEDUSA on small phylogenies, users should be careful when concluding that speciation rate has been constant.

When testing hypotheses of character-dependent diversification, users typically have information through ancestral state reconstructions on the size of lineages that potentially have distinct diversification rates. Davis and colleagues (Davis, Midford & Maddison, 2013) recommended cautious interpretation when analyzing datasets where <10% of species are of one character state. We conclude that the number of tips with a particular character state are a better predictor of power, rather than proportion, especially when using BiSSE. For instance, if 5% of the tips have a character state (1:20 when there are only two character states), power of detection may not be compromised as long as there is strong rate asymmetry, there are at least 100 tips with the character state and the character state is not polyphyletic. Generally, we suggest that tip-ratio may not be a big problem when analyzing very large trees (>1000 tips).

Data availability

Data files and codes supporting the article can be accessed from Figshare via https://figshare.com/s/cb85b1db6a64328b36f9

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Table 1: Parameter values of variables in Simulation Set 1

Range of parameter values of variables during simulation of trees for Simulation Set 1

Variables	Variable name	Values
Basetree age	B_{age}	3-35
Subtree age	S_{age}	20,40,60 % of *basetree*
Basetree speciation rate	λ_0	0.20
Asymmetry (λ_1 / λ_0)	λ_1	1.5, 2.5, 3.5, 4.5, 5.5
Basetree extinction rate	μ_0	0.05
Subtree extinction rate	μ_1	0.05
Overall tree size class	T_s	50,100,150,200,300,500

Figures

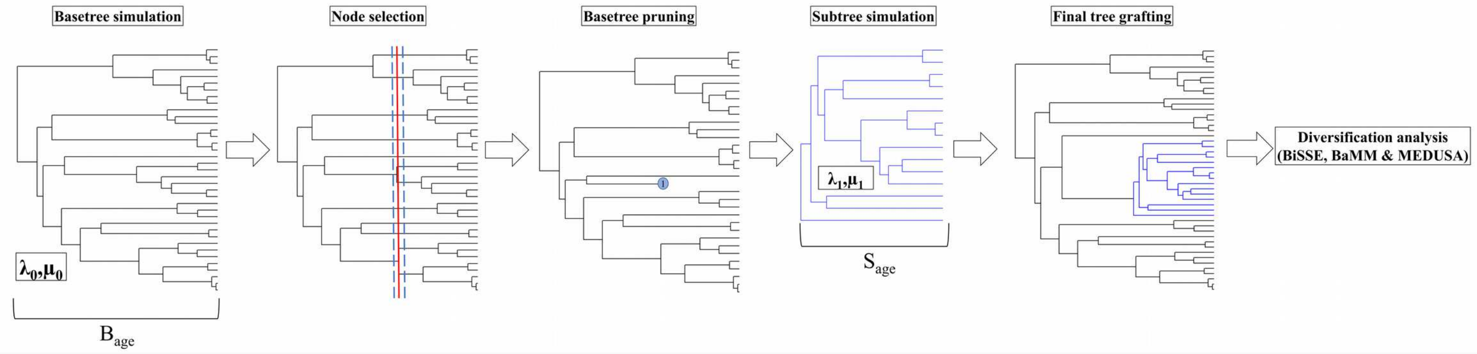

Figure 1: Flowchart of the simulation process

Flowchart of the simulation process used to generate a composite tree with a single speciation rate shift. A *basetree* with a target age (B_{age}) and speciation rate λ_0 was simulated using TESS, and pruned at a target *subtree* age (S_{age}). A *subtree* with speciation rate λ_1 and the target S_{age} was simulated independently and grafted on to the *basetree* at the pruned node.

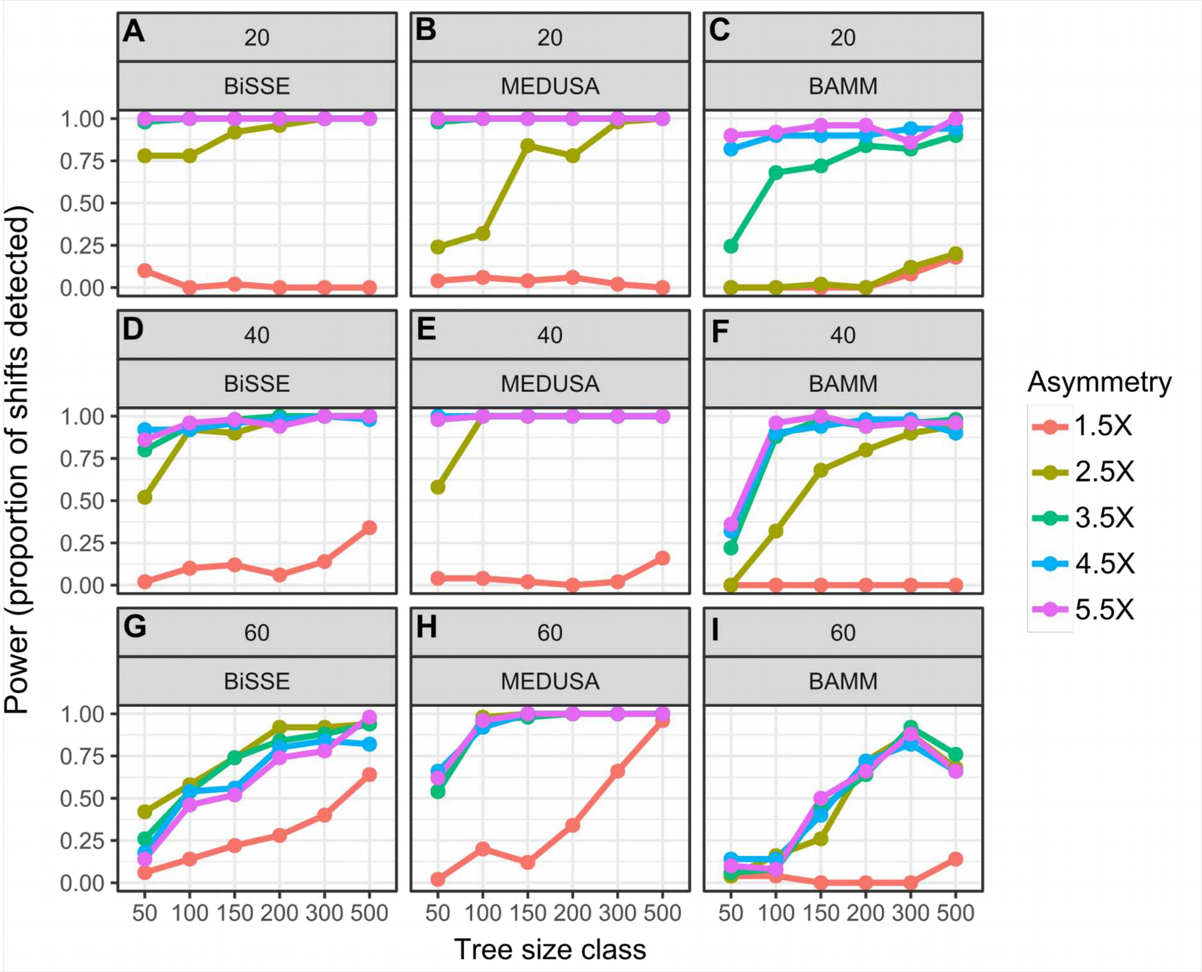

Figure 2: Effect of rate asymmetry and tree size

Power, measured as the proportion of shifts detected, for BiSSE (first column), MEDUSA (second column) and BAMM (third column), in trees from the Simulation Set 1. All trees had the same *basetree* speciation rate of λ_0, but *subtree* speciation rate λ_1, S_{age}, and tree size differed. Trees were of the following size classes: 50±10, 100±10, 150±10, 200±10, 300±10 and 500±10. Speciation rate asymmetry varied from 1X to 5.5X. S_{age} was 20% (Row 1), 40% (Row 2) or 60% (Row 3).

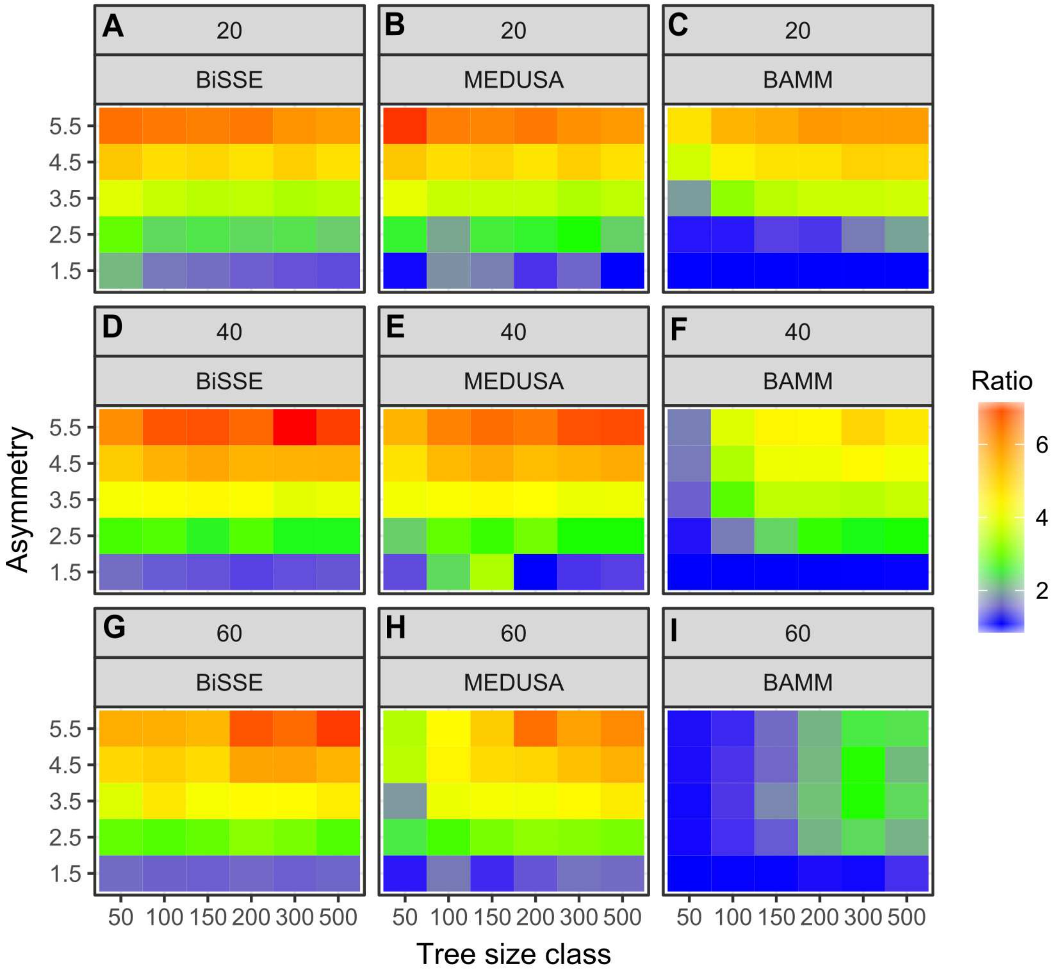

Figure 3: Relationship between expected and estimated speciation rate asymmetry
Relationship between expected and estimated speciation rate asymmetry for trees from Simulation Set 1; BiSSE (first column), MEDUSA (second column) and BAMM (third column). Estimated speciation rate asymmetry was calculated as the median of the estimated ratios of *subtree*/*basetree* speciation rates for a given set of trees.

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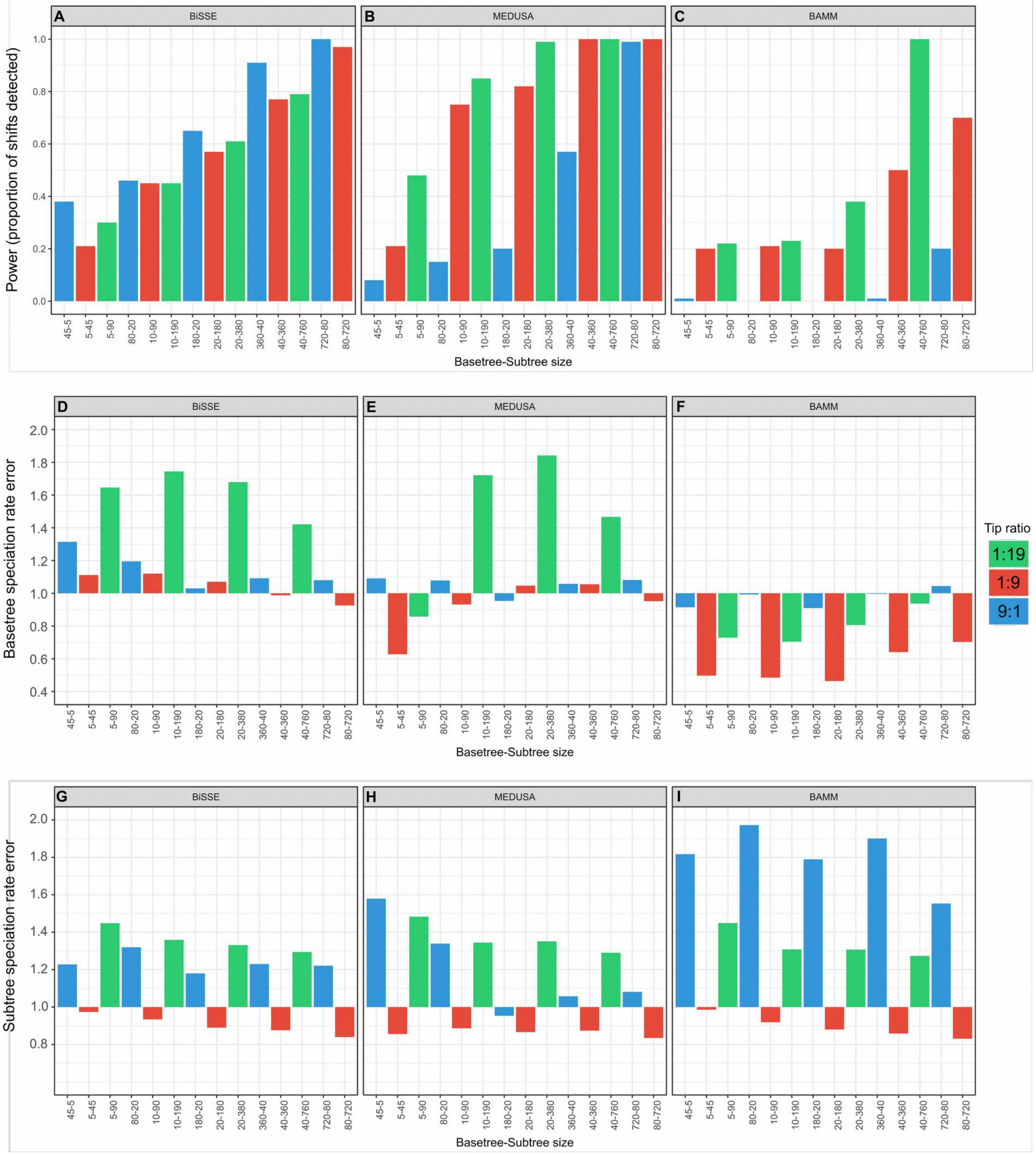

Figure 4: Effect of lineage size, tree size and tip-ratio

Power, measured as the proportion of shifts detected, for BiSSE (A), MEDUSA (B) and BAMM (C), in trees from the Simulation Set 2. All trees had a 2X asymmetry, but belonged to one of five size classes (50±10, 100±10, 200±10, 400±10 and 800±1) and three tip-ratios, i.e., *basetree*:*subtree* tips. Bars are colour coded based on the tip-ratio. X axis labels indicate overall tree size and number of tips in the *basetree* and *subtree* separated by a hyphen. For e.g 45-5 indicates 45 *basetree* and 5 *subtree* tips. Error in estimation of *basetree* speciation rate λ_0 (D-F) and *subtree* speciation rate λ_1 (G-I). A value of 1 represents no error, values >1 indicate overestimation and values < 1 indicate underestimation of λ. Note: We were unable to simulate trees with 1:9 ratio for 100±10 size class, and therefore used a 1:8 ratio as an approximation.