

# Trees of Unusual Size: Sampling Bias Can Influence Inference of Early Bursts from Molecular Phylogenies

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<b>Abstract:</b>	<p>An early burst of speciation followed by a subsequent slowdown in the rate of diversification is commonly inferred from molecular phylogenies. This pattern is consistent with some verbal theory of ecological opportunity and adaptive radiations. One often-overlooked source of bias in these studies is that of sampling at the level of whole clades, as researchers tend to choose large, speciose clades. In this paper, we investigate the performance of common methods across the distribution of clade sizes that can be generated by a constant rate birth-death process. Especially large clades generated under a constant-rate branching process tend to show a pattern of an early burst even when both speciation and extinction rates are constant through time. All methods evaluated were susceptible to detecting this false signature when extinction was low. Under moderate extinction, both the <math>\gamma</math>-statistic and diversity-dependent models did not detect such a slowdown but only because the signature of a slowdown was masked by subsequent extinction. Some models which estimate time-varying speciation rates are able to detect early bursts under higher extinction rates, but are extremely prone to sampling bias. We suggest that examining clades in isolation may result in spurious inferences that rates of diversification have changed through time.</p>
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<b>Suggested Reviewers:</b>	<p>Lee Hsiang Liow University of Oslo l.h.liow@bio.uio.no Dr. Liow is an expert in macroevolutionary studies and has conducted previous simulation studies related to the inference of diversification rates.</p> <p>Natalie Cusimano Ludwig Maximilians Universitat Munchen cusimano@lrz.uni-muenchen.de Dr. Cusimano has previously conducted studies on the influence of sampling on the inference of diversification and her work in this area is closely related to the research we are submitting</p>
<b>Opposed Reviewers:</b>	

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PLoS ONE Editorial Staff

To whom it may concern,

I would like to submit a manuscript titled "Trees of Unusual Size: Sampling Bias Can Influence Inference of Early Bursts from Molecular Phylogenies", for your consideration for publication in *PLoS ONE*.

An early burst of speciation followed by a slowdown in diversification rate is a common observation inferred from empirical phylogenies. Such a pattern is consistent with some theoretical work on adaptive radiations, which postulates that diversification rates will decrease as species fill available niches. Here, we investigate the influence of an overlooked but important source of bias in making these inferences: researchers' tendency to select species rich and interesting clades for study. We show here that the effect of this bias can be severe but it affects different methods in different ways. Furthermore, we make some recommendations on the appropriate use of these methods. We think that this paper will be of interest to both empiricists and theoreticians working in the fields of macroevolution and phylogenetic comparative methods.

We would like to suggest Drs. Keith A Crandall (Brigham Young University) and Robert DeSalle (American Museum of Natural History) as potential Academic Editors for this manuscript.

Thank you for your consideration. Please do not hesitate to contact me if you require any further information.

Sincerely,  
Matthew Pennell,  
Department of Biological Sciences,  
University of Idaho

# Trees of Unusual Size: Sampling Bias Can Influence Inference of Early Bursts from Molecular Phylogenies

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## Abstract

An early burst of speciation followed by a subsequent slowdown in the rate of diversification is commonly inferred from molecular phylogenies. This pattern is consistent with some verbal theory of ecological opportunity and adaptive radiations. One often-overlooked source of bias in these studies is that of sampling at the level of whole clades, as researchers tend to choose large, speciose clades. In this paper, we investigate the performance of common methods across the distribution of clade sizes that can be generated by a constant rate birth-death process. Especially large clades generated under a constant-rate branching process tend to show a pattern of an early burst even when both speciation and extinction rates are constant through time. All methods evaluated were susceptible to detecting this false signature when extinction was low. Under moderate extinction, both the  $\gamma$ -statistic and diversity-dependent models did not detect such a slowdown but only because the signature of a slowdown was masked by subsequent extinction. Some models which estimate time-varying speciation rates are able to detect early bursts under higher extinction rates, but are extremely prone to sampling bias. We suggest that examining clades in isolation may result in spurious inferences that rates of diversification have changed through time.

## Introduction

The branching patterns of reconstructed molecular phylogenies contain information about the tempo and mode of evolution [1], [2]. This insight has been invaluable to our understanding of many evolutionary processes and patterns. Recent studies have identified a common pattern of apparent slowdowns in the rate of diversification through time (reviewed in [3], [4]). Such a pattern is consistent with some theoretical work on adaptive radiations, based on the idea that diversification rates will decrease as species fill available niches [5], [6], [7] (but see [8] for an alternate interpretation). Phylogenies with slowdowns have been inferred for diverse groups of organisms (e.g. lizards [9], birds [10], [11], fish [12], and many groups of plants [13]).

The most commonly used metric for detecting shifts in the rate of diversification is the  $\gamma$ -statistic introduced by Pybus and Harvey [14]. This statistic quantifies how internode distances (i.e. waiting times to speciation) vary through time compared to what one would expect under a pure-birth model of diversification. Under the pure-birth null expectation,  $\gamma$  is distributed according to a standard normal distribution. A  $\gamma$ -value less than -1.645 (one-tailed test) represents a statistically significant slowdown in diversification rate. Positive values of  $\gamma$  can be caused by either speed-ups in diversification rates or species turnover as recently diverged lineages have not been around long enough to have been “pruned” by extinction, creating an overabundance of nodes closer to the present (“pull of the present”; [2]). These two scenarios cannot be distinguished with the  $\gamma$ -statistic, so most studies follow the authors’ [14] original recommendation to disregard significantly positive  $\gamma$ -values.

There has been considerable controversy surrounding the interpretations of slowdowns using the  $\gamma$ -statistic on molecular phylogenies. While a preponderance of studies have inferred significantly negative  $\gamma$  values across a wide range of taxonomic groups, a number of other studies have pointed out that negative  $\gamma$ -values can result from factors other than slowdowns in diversification rates. It has been shown that  $\gamma$  can be biased by under-parameterization of the model of sequence evolution [15] and non-random taxon sampling [16] (see [17] and [18] for potential solutions to this problem). Recent speciation events will have a similar effect; if speciation is modelled as a process rather than as a singular event, this can lead to apparent slowdowns in diversification rates [19], [20].

A recent simulation study, conducted by Liow et al. [21], found that  $\gamma$  tended to be positively biased when trees were simulated under a birth-death process with variable rates of speciation and/or extinction. They found that, even when present, slowdowns in net diversification rates were very difficult to detect using  $\gamma$ , as short branches near the tips of the tree tend to obscure the signal of the slowdown. Under the diversification model of Liow et al. [21], it should be only possible to observe a slowdown for a small subset of values of speciation rate ( $\lambda$ ) and extinction rate ( $\mu$ ) and if lineages are sampled at a particular time in the clades history. It seems unrealistic to propose that these two conditions are met for the majority of phylogenies. Nevertheless slowdowns are commonly inferred from empirical data. The reasons for the discordance between the analysis of empirical data and expectations derived from simulation studies are poorly understood and warrant further investigation.

Several new modeling approaches have been developed to detect non-homogeneous diversification. One is to use a model in which speciation and/or extinction vary through time [22], [22], [23], [24], [25]. Diversity dependence (in which speciation rate varies as function of the number of taxa at a given time) has also been proposed as an explanation for the patterns of species richness [26]. This has recently been modeled in a number of studies (e.g. [27], [28], [29]) to look for signatures of an adaptive radiation. Diversity-dependence is a useful approach as it is seemingly consistent with theory on adaptive radiation, which posits that diversification should slow as niches become filled [6], [7]. There is also some evidence from the fossil record [30] (but see [31] for an opposing perspective) to support this modeling approach although the ecological mechanisms by which diversity dependence would operate on the scale of a clade are not entirely clear [32]. While the mathematics of the various methods differ, they all involve fitting non-homogeneous birth-death models to phylogenies rather than using summary statistics such as the  $\gamma$ -statistic. The behavior and performance of these methods have not been explored as that of the  $\gamma$ -statistic has.

One important consideration that is often overlooked in studies of clade diversification is that our inferences may be biased by the way we choose clades to investigate. There are several types of sampling bias that are likely to be important. Cusimano and Renner [16], Brock et al. [17] and Cusimano et al. [18] investigated systematists' tendency to sample representative taxa, leading to an overabundance of nodes deep in the tree. However, we should also consider that we are only sampling clades that survive to the present day. This means that the observable distribution of surviving trees is only a subset of all possible trees [33], [34]. Another form of bias is that researchers interested in the adaptive radiations are likely to be interested in the speciose clades. These clades likely reside in the 'tails' of the distribution of possible clade sizes and thus give a biased sample for the inference of diversification rates [11]. Inferences of early bursts are particularly problematic because even under a constant pure-birth or birth-death process, large clades are likely to show patterns of an rapid diversification early in their history. This is simply due to the fact that in order to be large, they are more likely to have undergone a stochastically high rate of speciation early in the process and subsequently regressed to the mean speciation rate [35], [11]. Analyzing large clades in isolation may lead to inferring ecological processes (such as adaptive radiations) attributable solely to these stochastic processes.

Phillimore and Price [11] investigated the influence of clade size and age on the  $\gamma$ -statistic though their simulations were limited to a small set of parameter space. In this paper, we follow up on this analysis by exploring parameter space more thoroughly and by investigating the effects of the non-random sampling

of clades using a model selection approach. We show that the effect of this bias can be severe but it affects different methods in different ways, and we make recommendations on the appropriate use of these methods.

## Results and Discussion

For phylogenies generated under all extinction rates, large phylogenies are disproportionately likely to have undergone an initial burst of speciation events [36], [11]. This can be visualized with a lineage-through-time plot (Figure 1). This result has consequences for the inference of diversification rate patterns; as the clades we choose to examine are not a random sample of clades (but often tend to be interesting), are speciose, and, at least for molecular phylogenies, have survived to the present day.

Consistent with previous work [36], [11], larger trees are more likely to harbor a false signal of a slowdown using the  $\gamma$ -statistic when the phylogeny is generated constant process and extinction is low (Figure 2). However this is not the case when background extinction rates are higher as extinction alters the distribution of branch lengths on the reconstructed phylogeny. The resulting distribution effectively obscures any signal of a burst that might have occurred deep in the tree [10], [37], [21]. This is especially true for large phylogenies (Figure 2);  $\gamma$  is a summary statistic for all nodes in the tree and therefore the few early branching events will have an even smaller influence on the calculation of the  $\gamma$ -statistic for large trees with proportionally more nodes.

Our results suggest two things about the use of the  $\gamma$ -statistic. First, the test has very low power to detect changes in speciation rates when species turnover rates are even modestly high [21]. Second, while there is some concern that significantly negative  $\gamma$  values may potentially be misleading [15], [16], [38], bias in sampling large clades does not tend to create false signatures of a slowdown under modest extinction rates. Considering how difficult it is to obtain false positives, it is worth further investigation as to why empiricists so often observe strong signals of a slowdown using this statistic. Pessimistically, we may attribute this to some yet unstudied artifact such as sampling effort. More intensive sampling of lineages would have the effect of increasing the number of nodes close to the present and thus would deteriorate any signal of an early burst detectable by the  $\gamma$ -statistic (though a number of the phylogenies which provide evidence for a slowdown are near complete at the species level [e.g. [11]], though there may still be additional lineages that should actually be recognized as species). It may be the case that if more lineages were included, many of the signatures of early bursts in the literature would no longer be present.

We found that the diversity-dependent models are prone to bias due to sampling in a similar manner as the  $\gamma$ -statistic (Figure 3); when death rates are low, the diversity-dependent model was preferred to a constant birth-death model more frequently for large clades, but with even moderate extinction, the diversity-dependent model was rarely falsely preferred. In fact, for higher extinction rates, the reverse was true, larger clades were less likely to fit a diversity-dependent model than smaller clades (Figure 3). However, it should be noted that the model of diversity dependence we used here did not explicitly model extinction and therefore estimate diversification rate as a function of contemporaneous lineages in the reconstructed phylogeny rather than as a function of contemporaneous lineages in the true (unobserved) phylogeny [39]. Recently Etienne et al. [29] provided a full likelihood solution for diversity-dependence with extinction with uses a Hidden-Markov approach to fit the model to a phylogeny. We justify the use of a diversity-dependent model which does not estimate extinction in our simulation study due to the fact that it is currently the more commonly employed approach. The diversity-dependence model thus has the same drawback as the  $\gamma$ -statistic in that extinction will tend to erode the signal of the early diversification events, especially so for large trees. The results from the time-varying speciation rate model (Figures 4 and 5; discussed below) suggest that this result will not hold if both extinction rates and speciation rates are explicitly modeled. However, it should be noted that there is some concern that inference of extinction can be biased when there is rate variation across clades [40].

A correction that has been employed to increase the power to detect slowdowns for both the  $\gamma$ -statistic

and diversity-dependent models is to collapse some nodes close to the present. Species delimitation is a problem that has recently received a great deal of attention from molecular systematists (e.g. [41], [42], [43]). Subdividing lineages into subspecies will necessary increase the effect of the pull of the present and further obscure evidence of a slowdown. Collapsing these recent nodes will certainly influence our ability to infer slowdowns and this procedure has been done by some researchers (e.g. [11], [44]), by regarding recently-diverged species as not being “good” species. However, there is currently a lack of theory to guide such decision-making and therefore recommend that authors should be cautious in doing so as we do not fully understand how species delimitation affects these methods.

The time-varying speciation models [37], [23] were preferred for data generated under a constant-rate process with increasing frequency as tree size increased. We found this to be true even when extinction was present (Figures 4 and 5), though the effect was dampened as extinction increased. As stated above, large trees generated under a constant rate birth-death process are subject to having undergone stochastic bursts of speciation in order to obtain their current size. The time-varying speciation rate models are sensitive to these bursts, which is both a positive and negative; positive because they have more power to detect changes in diversification through time and negative because these models are more prone to inferring spurious results as a consequence of sampling bias. We took two alternative approaches to compare model fits of a constant-rate birth-death model versus a time-varying speciation model: the full-likelihood approach of Rabosky and Lovette [37] and an approximate approach based on the coalescent process, recently derived by Morlon et al. [23] (see Methods for details). Contrary to our expectations, we found that these two approaches differed substantially in terms of which model was preferred when using Akaike Information Criterion (AIC). For larger trees, the coalescent approach tended to provide support for a time-varying speciation model over a constant-rate model at much higher frequencies than the full likelihood approach, especially when extinction rates were low (Figure 5). The reasons for these differences are not entirely clear. While many of the large trees do show ‘early bursts’ due to stochastically high rates early in the process (see Figure 1 for an example), the proneness of the coalescent-based approach to favor a model more complex than the generating model is worrisome as sampling bias appears to very strongly influence model choice. Both approaches are relatively new and their respective statistical properties have not, to our knowledge, been explored at all beyond the publications in which they were presented [37], [23]. The statistical properties of these and other related models is something that certainly warrants further investigation as researchers are increasingly fitting more complex models of diversification to study diversity dynamics through time and across clades (e.g. [45], [25], [24]).

As researchers, we do not randomly choose clades to study; we are naturally interested in large, unusual clades. This necessarily introduces some sampling bias into our inferences, a fact that is often overlooked (but see [33], [34]). Large clades generated under constant-rate Yule or birth-death processes tend to have stochastic bursts of speciation events early in their history. These stochastic bursts may be falsely inferred to have been caused by ecological processes (e.g. adaptive radiations). If the background extinction is close to zero, then all methods investigated in this paper are susceptible to this false inference. However, when background extinction is relatively high, these stochastically generated slowdowns are not likely to be detected by the  $\gamma$ -statistic as the as subsequent extinction removes the signal. As a summary statistic for the whole phylogeny, the power of the  $\gamma$ -statistic to detect slowdowns (stochastically or ecologically produced) when species turnover is present is very low [21], [46]. It is fair to suggest that while there are some reasons to believe that significantly negative  $\gamma$ -values in the empirical literature are caused by known statistical artifacts [15], [16], [38], the cause of their ubiquity in the empirical literature remains unclear. We suggest that sampling effort may contribute to this. Methods that do not deal directly with extinction and extinct lineages, like the diversity-dependent model [27] used here, show similar patterns of performance to the  $\gamma$ -statistic.

Our findings are also of relevance to studies examining the efficacy of diversification rate models and statistics. There are a multitude of ways to conduct simulations of birth-death models and Stadler [47] has discussed the statistical properties of various conditioning schemes. We caution theoreticians to pay

close attention to these differences as conditioning simultaneously on the number of taxa and the age of the tree can produce phylogenies drawn from the ‘tails’ of the distribution and thus prone to be bottom heavy. Such trees may lead to biased estimation of parameters and misconstrued inferences.

We suggest three future directions to address this issue of sampling bias. First, performing diversification analysis on megaphylogenies, including the clade of interest, may be advisable instead of examining clades in isolation. Investigating large clades while ignoring closely related groups that are less speciose may lead to spurious patterns of slowdowns in diversification. This will require further development of methods that relax the assumption of uniformity of the diversity dynamics across the tree (e.g. [45]). Second, increased attention should be given to how lineages are defined; lineages that are currently denoted as subspecies may soon be considered “good” species [20]. Whether these lineages are included or not will influence the inference of slowdowns. Third, further investigation of the statistical properties of these models may allow researchers to be more confident that the patterns they observe represent truly meaningful variation.

## Methods

Tree simulations were conducted with code modified from Stadler’s [47] `TreeSim` R package. Other methods of tree simulation, such as those implemented in `GEIGER` [48] require that the phylogeny be conditioned either on the number of extant taxa or on the depth (age) of the tree. These two methods have slightly different statistical properties (see [47] for a thorough discussion of these issues). Stadler’s [47] method allows researchers to simultaneously condition on both the number of taxa and the depth of the tree, allowing us to better investigate the distribution of trees resulting from the pure-birth and the birth-death branching processes. We set  $\lambda = 1$ ,  $\tau = 5$  and varied  $\mu$  ( $\mu = \{0, 0.1, 0.25, 0.5, 0.75, 1.0\}$ ). Consider a phylogenetic tree  $\mathcal{T}_N$  where  $N$  denotes the number of extant lineages. Following Foote et al. [49], the probability of observing at least  $n$  taxa surviving to time  $\tau$  is given by the equations:

for  $\lambda = \mu$

$$Pr(N \geq n \mid \lambda, \mu, \tau) = 1 - \frac{\mu(\exp[(\lambda - \mu)\tau] - 1)}{\lambda \exp[(\lambda - \mu)\tau] - \mu} - \sum_{i=1}^{n-1} (1 - \alpha)(1 - \beta)\beta^{i-1} \quad (1)$$

where

$$\alpha = \frac{\mu(\exp[(\lambda - \mu)\tau] - 1)}{\lambda \exp[(\lambda - \mu)\tau] - \mu}$$

and

$$\beta = \frac{\lambda\alpha}{\mu}$$

and for  $\lambda \neq \mu$

$$Pr(N \geq n \mid \lambda, \mu, \tau) = 1 - \frac{\lambda\tau}{(1 + \lambda\tau)} - \sum_{i=1}^{n-1} \frac{(\lambda\tau)^{i-1}}{(1 + \lambda\tau)^{i+1}} \quad (2)$$

Conditioning on the survival at least one lineage to the present day (time  $\tau$ ), we used (1) and (2) to calculate the number of taxa associated with a range of values for the cumulative probability function  $Pr(N \mid \lambda, \mu, \tau) = \{0.01, 0.02, \dots, 0.99\}$ . For each value of  $\mu$  and each value in the cumulative probability function, we simulated 1000 phylogenies under a constant-rate birth-death model (or in the case of  $\mu = 0$ , pure-birth). We also varied  $\lambda$  and  $\tau$  but the results of our analysis did not differ qualitatively. For each simulated phylogeny, we calculated the  $\gamma$ -statistic [14] as implemented in the R package `ape` [50]. We used the R package `laser` [51] to fit a constant-rate birth-death model, a constant rate Yule model and

a diversity dependent model (hereafter DD). The diversity dependent model we used was the forward-based exponential declining model of Rabosky and Lovette [27] where speciation rate is modeled as a function of the number of contemporaneous species in the reconstructed phylogeny. If  $N(\tau)$  the number of contemporary lineages,  $\lambda(0)$  is the initial (background) speciation rate and  $\kappa$  describes the strength of diversity dependence,  $\lambda(\tau)$  can be described as the function

$$\lambda(\tau) = \lambda(0)N(\tau)^{-\kappa}. \quad (3)$$

In the DD model, background extinction rates are assumed to be 0. Thus the diversity dependence is a function of the contemporaneous species in the reconstructed phylogeny and the decline in diversification rates at higher densities is only due to a decrease in speciation rate. To model time-varying speciation, we used the SPVAR model of Rabosky and Lovette [37], implemented in the function `fitSPVAR` in the R package `laser` [51] where speciation rate is a function of time such that

$$\lambda(\tau) = \lambda(0)e^{-x\tau} \quad (4)$$

where  $x$  is a constant describing the relationship. Here, extinction rate  $\mu$  is explicitly modeled but assumed to be constant across the phylogeny.

As an alternative to using the full-likelihood equation for a time-varying speciation model [37], we also employed a recently derived approach [23] for fitting birth-death models, based on the coalescent process from population genetics [52]. Morlon et al. [23] model a population of species evolving under the Wright-Fisher process. Following Morlon et al. [23], the likelihood  $\mathcal{L}$  of observing the internode distances  $g_i$  between node  $i$  and node  $i + 1$  can be written as

$$\mathcal{L}(g_i) = \frac{i(i+1)}{2} \frac{1}{N(u_i)} \exp \left[ -\frac{i(i+1)}{2} \int_{u_i-g_i}^{u_i} \frac{1}{N(\tau)} d\tau \right] \quad (5)$$

where  $u_i$  is the distance between node  $i$  and the present and  $N(\tau)$  is the number of species (population size in population genetics) at time  $t$  in the past. Note that this is an approximation of the likelihood as  $N(\tau)$  is approximated by its expected value  $E[N(\tau)]$  [23]; this was done to make the model analytically tractable. The time-varying speciation model was specified in the same way in the full-likelihood approach described above, such that  $\lambda(\tau) = \lambda(0)e^{-x\tau}$ .

All of our simulations were carried out using constant rates of speciation and extinction through time, so that any detection of a slowdown can be considered a Type-1 error. For the  $\gamma$ -statistic, we evaluated the type-1 error rate in detecting a false signature of a slowdown. For the model-based approaches, we used an AIC approach (sensu [53]) to select the best model using a  $\Delta AIC$  cutoff of 4 required to favor selecting a model other than a constant-rate birth-death model, or, in the case of trees generated under the Yule process, a constant-rate pure-birth process. All analysis were conducted in the R programming environment [54].

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## Figure Legends

**Figure 1. Exemplar Lineage-Through Time Plot.** An example of a lineage-through-time (LTT) plot for a tree (shown on left) drawn from the far right tail of the distribution of tree sizes (5 percent of surviving trees are expected to be this large or larger) for  $\lambda = 1$ ,  $\mu = 0.5$  and  $\tau = 5$ . The dotted line is the expected number of lineages under a constant diversification rate. This LTT plot shows the typical signature of an early burst of speciation yet this signature is not captured by the  $\gamma$ -statistic ( $\gamma = 0.602$ ; not significant) as the burst is masked by later extinction events.

**Figure 2. Type-1 Error Rate for the  $\gamma$ -Statistic.** Results from simulations showing the type-1 error rate for the  $\gamma$  statistic, signifying a false inference of a slowdown (all trees were generated under a constant-rate birth-death process). Extinction rate,  $\epsilon = \mu/\lambda$ , varies across the plots ( $\epsilon = 0, 0.1, 0.25, 0.5$ ); speciation rate,  $\lambda$ , and total tree-depth,  $\tau$  are held constant ( $\lambda = 1$  and  $\tau = 5$ ). All are plotted against the expected number of taxa across the cumulative distribution of probability densities (from 0.99 to 0.01). The dashed vertical line represents the expected value for  $N$  under the simulating conditions. Each point represents 1000 simulations. (Results for  $\epsilon = 0.75$  and  $\epsilon = 1$  not shown.)

**Figure 3. Proportion of Trees Showing Support for Diversity-Dependent Model.** Results from simulations showing the proportion of phylogenies for which a density-dependent (DD) model is preferred over a constant-rate model using an AIC model selection criterion (with a  $\Delta\text{AIC}$  cutoff of 4) when the generating model was a constant-rate birth-death process. Extinction rate,  $\epsilon = \mu/\lambda$ , varies across the plots ( $\epsilon = 0, 0.1, 0.25, 0.5$ ); speciation rate,  $\lambda$ , and total tree-depth,  $\tau$  are held constant ( $\lambda = 1$  and  $\tau = 5$ ). All are plotted against the expected number of taxa across the cumulative distribution of probability densities (from 0.99 to 0.01). The dashed vertical line represents the expected value for  $N$  under the simulating conditions. Each point represents 1000 simulations. (Results for  $\epsilon = 0.75$  and  $\epsilon = 1$  not shown.)

**Figure 4. Proportion of Trees Showing Support for Temporally-Varying Speciation Model.** Results from simulations showing the proportion of phylogenies for which a temporally-varying speciation (TVS) model is preferred over a constant-rate model using an AIC model selection criterion (with a  $\Delta\text{AIC}$  cutoff of 4) when the generating model was a constant-rate birth-death process. Extinction rate,  $\epsilon = \mu/\lambda$ , varies across the plots ( $\epsilon = 0, 0.1, 0.25, 0.5$ ); speciation rate,  $\lambda$ , and total tree-depth,  $\tau$  are held constant ( $\lambda = 1$  and  $\tau = 5$ ). All are plotted against the expected number of taxa across the cumulative distribution of probability densities (from 0.99 to 0.01). The dashed vertical line represents the expected value for  $N$  under the simulating conditions. Each point represents 1000 simulations. (Results for  $\epsilon = 0.75$  and  $\epsilon = 1$  not shown.)

**Figure 5. Proportion of Trees Showing Support for Temporally-Varying Speciation Model Using Coalescent Approximation.** Results from simulations showing the proportion of phylogenies for which a temporally-varying speciation (TVS) model is preferred over a constant-rate model using an AIC model selection criterion (with a  $\Delta\text{AIC}$  cutoff of 4) when the generating model was a constant-rate birth-death process. Extinction rate,  $\epsilon = \mu/\lambda$ , varies across the plots ( $\epsilon = 0, 0.1, 0.25, 0.5$ ); speciation rate,  $\lambda$ , and total tree-depth,  $\tau$  are held constant ( $\lambda = 1$  and  $\tau = 5$ ). All are plotted against the expected number of taxa across the cumulative distribution of probability densities (from 0.99 to 0.01). The dashed vertical line represents the expected value for  $N$  under the simulating conditions. Each point represents 1000 simulations. (Results for  $\epsilon = 0.75$  and  $\epsilon = 1$  not shown.)

Figure  
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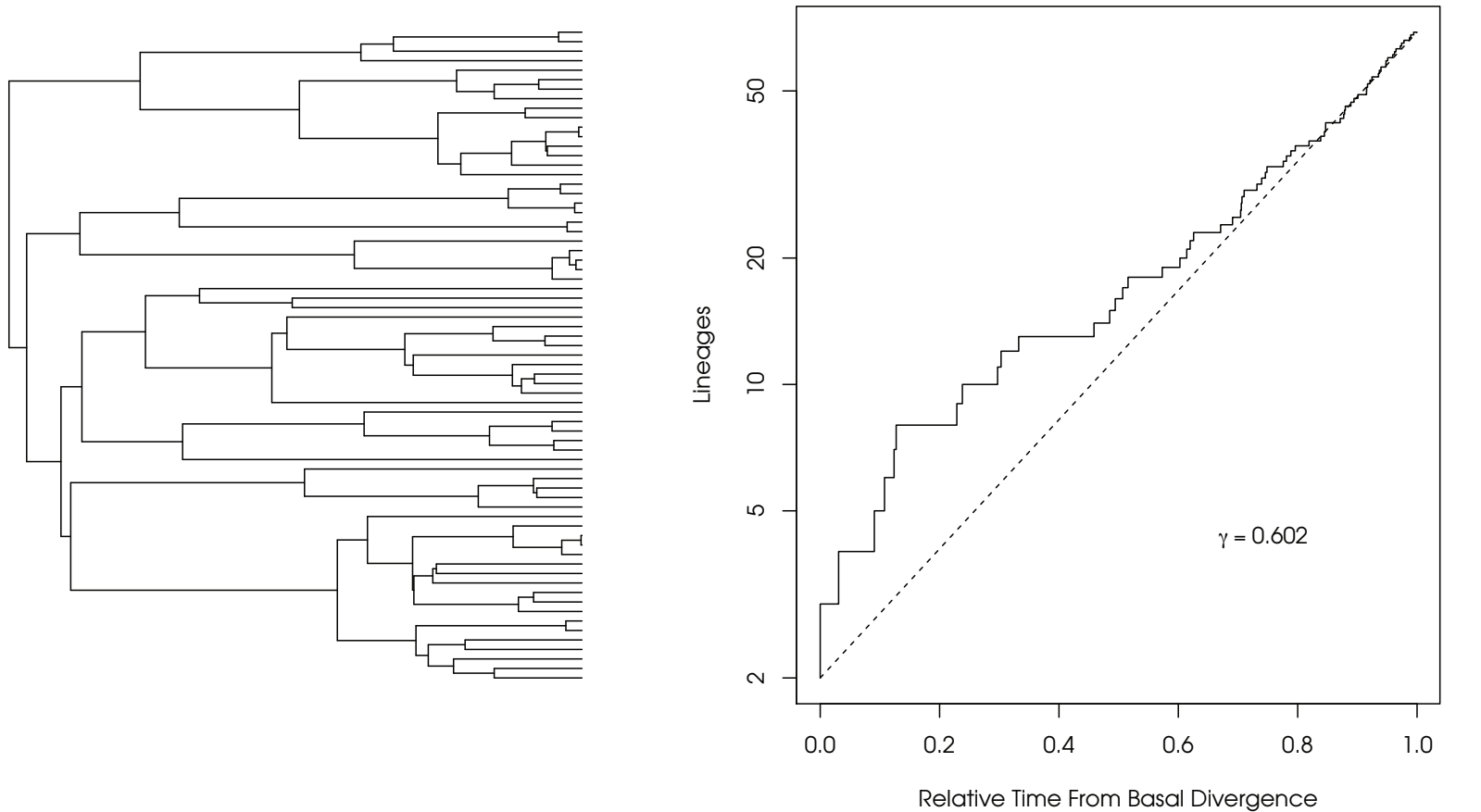


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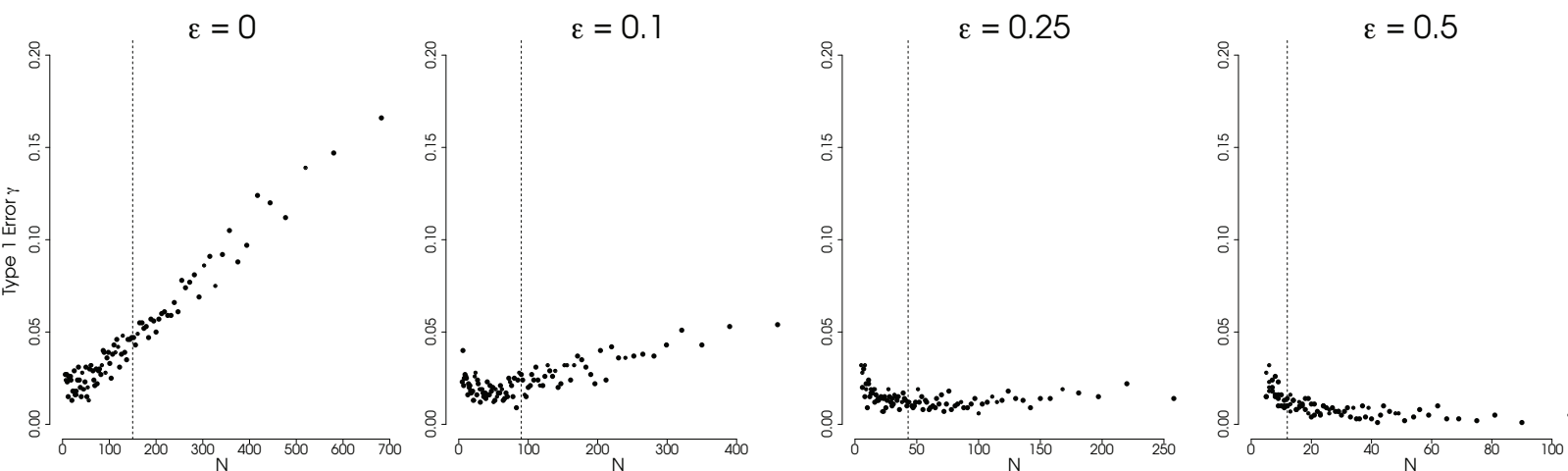


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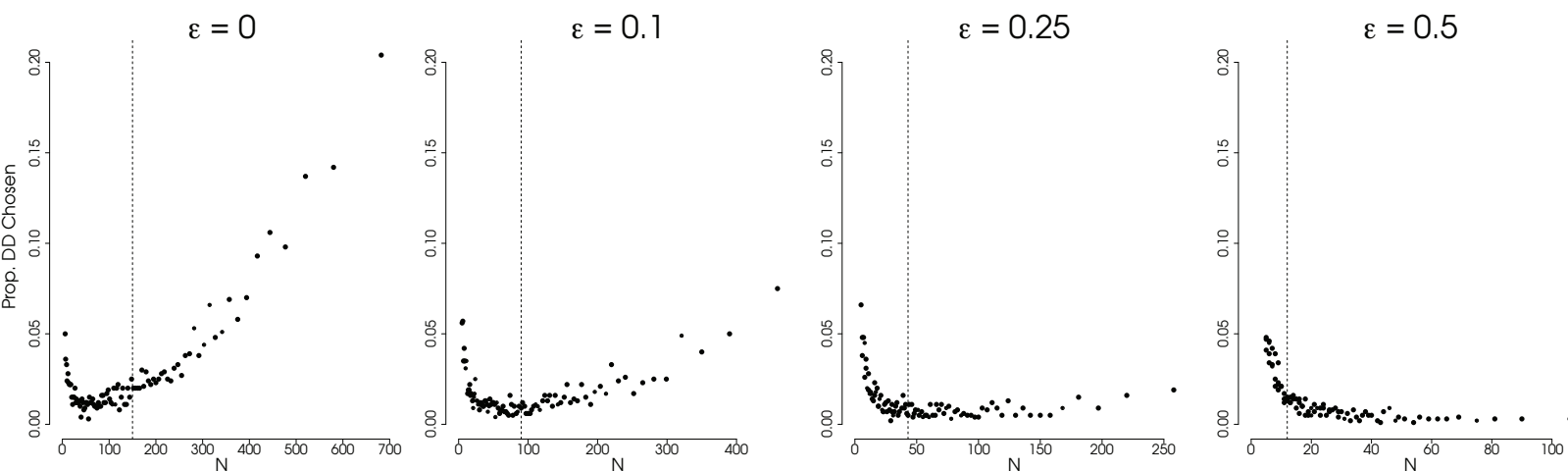


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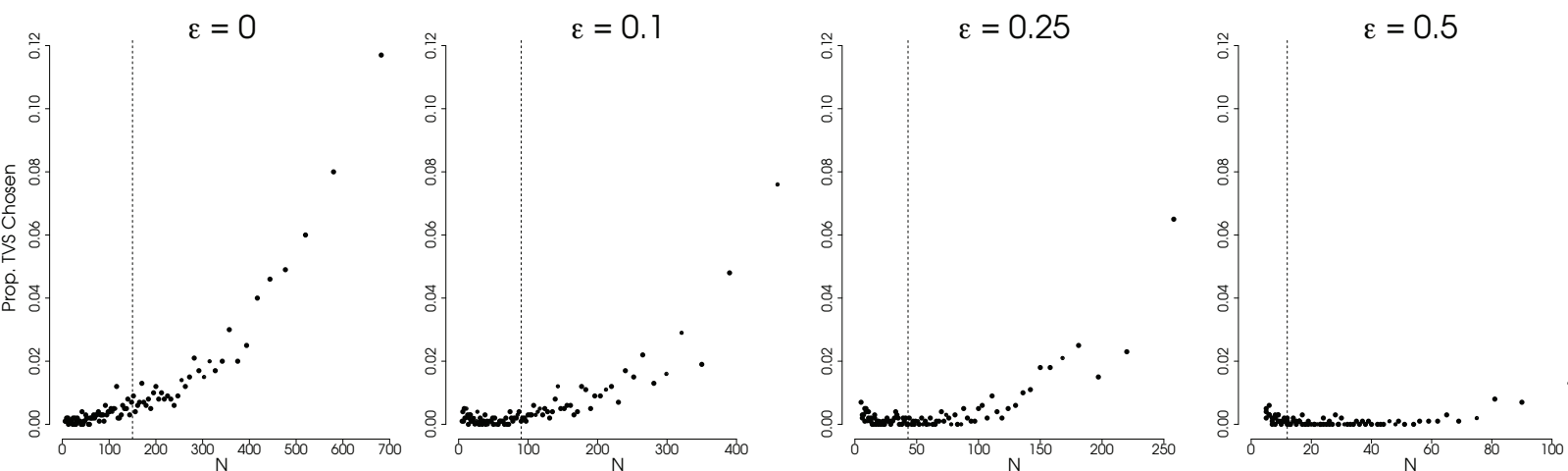




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