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StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates

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December 20, 2016

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Abstract

The multispecies coalescent (MSC) reconstructs species trees from a set of genes, and fully Bayesian MSC methods like *BEAST estimate species trees from multiple sequence alignments. Today thousands of genes can be sequenced for a given study, but using that many genes with *BEAST is intractably slow. One alternative is concatenation, which assumes that the evolutionary history of each gene tree is identical to the species tree. This is an inconsistent estimator of species tree topology, and a worse estimator of divergence times. Concatenation also induces spurious substitution rate variation when incomplete lineage sorting is present. Another alternative is to use summary MSC methods like ASTRAL, but such methods are also unsatisfactory because they infer branch lengths in coalescent units, and so cannot estimate divergence times. To enable fuller use of available data and more accurate inference of species tree topologies, divergence times, and substitution rates, we have developed a new version of *BEAST called StarBEAST2. To improve convergence rates we add analytical integration of population sizes, novel MCMC operators and other optimizations which improved computational performance 13.1× to 13.8× when analyzing empirical data sets, and an average of 33.1× across 30 simulated data sets. To enable accurate estimates of per-species substitution rates we introduce species tree relaxed clocks, and show that StarBEAST2 is a more powerful and robust estimator of rate variation than concatenation. StarBEAST2 is available through the BEAUTi package manager in BEAST 2.4 and above.

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Keywords: Multispecies coalescent, concatenation, phylogenetic methods, incomplete lineage sorting, relaxed clocks, species trees.

1 Introduction

The throughput of sequencing technologies has improved many-fold over the past two decades culminating in next generation sequencing (NGS), and it is now feasible to sequence whole or partial genomes or transcriptomes for phylogenetic studies (Lemmon and Lemmon, 2013). NGS produces hundreds or thousands of phylogenetically useful loci (see for example Blom *et al.*, 2016) with potentially millions of sites spread across a data set of multiple sequence alignments.

While NGS offers hundreds or thousands of loci at relatively low cost, making accurate inferences from the enormous amount of data produced is particularly challenging. In the case of *BEAST, a fully Bayesian method of species tree inference which implements a realistic and robust evolutionary model in the multispecies coalescent (MSC; Degnan and Rosenberg, 2009; Heled and Drummond, 2010), it becomes exponentially slower as the number of loci in an analysis is increased. This scaling behaviour causes *BEAST to become intractably slow after a certain number of loci (the exact number will depend on other parameters of the data set, see Ogilvie *et al.*, 2016). Given the current challenges of using large phylogenomic data sets with *BEAST there have been three broad alternatives available to researchers; concatenate sequences from multiple loci, use alternative MSC methods which are based on

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summary statistics instead of sequence alignments, or choose a tractable subset of loci to use with a fully Bayesian method like *BEAST, BEST (Liu, 2008), or BPP (Yang, 2015).

Using maximum likelihood phylogenetic methods to infer a species tree based on concatenated sequences will return the single tree that best fits the combined sequence alignment according to the phylogenetic likelihood function (Felsenstein, 1981). Popular maximum-likelihood concatenation methods include RAxML, PAML and PhyML (Stamatakis, 2014; Yang, 2007; Guindon *et al.*, 2010). Bayesian methods, such as ExaBayes and BEAST (Aberer *et al.*, 2014; Drummond and Rambaut, 2007), will instead return a distribution of trees which are probable given the combined sequence alignment, a set of priors, and the same likelihood function. Recent results show that likelihood-based concatenation can be counterproductive, producing statistically inconsistent results which assign high confidence to incorrect nodes due to model misspecification (Liu *et al.*, 2015). In the so-called "anomaly zone" of short branch lengths, the most probable gene tree topology will be different from the species tree, and estimated tree topologies will likely differ from the true species tree topologies (Degnan and Rosenberg, 2006; Kubatko and Degnan, 2007).

More recently identified problems with likelihood-based concatenation are systematic errors when estimating branch lengths, including overestimation of divergence times. Because some time is required for genes to coalesce looking backwards from a speciation event, the expected molecular distance between two species is greater than if coalescent events occurred simultaneously when gene flow ceases. This leads concatenation to overestimate the divergence times across a species tree in proportion to effective population size (Arbogast *et al.*,

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2002; Ogilvie *et al.*, 2016).

Incomplete lineage sorting (ILS) also causes systematic errors in estimated branch lengths when using concatenation, because substitutions on a discordant gene tree branch which has no corresponding species tree branch must be explained by multiple substitutions on different species tree branches. Substitutions produced by ILS (SPILS) causes concatenation to overestimate the lengths of specific branches and underestimate the lengths of others, which produces apparent substitution rate variation where none exists (Mendes and Hahn, 2016). For all the above reasons, trees inferred using concatenation are therefore not a reliable approximation of the species tree in terms of branch lengths or topology.

As an alternative to concatenation, MSC methods which use summary statistics instead of sequence alignments have been developed for use with phylogenomic data. Popular summary methods include MP-EST and ASTRAL (Liu *et al.*, 2010; Mirarab *et al.*, 2014), but recent results show that MP-EST should be used with caution as it is sensitive to gene tree errors (Mirarab and Warnow, 2015; Xi *et al.*, 2015). At low levels of ILS, MP-EST is less accurate than likelihood-based or neighbor-joining concatenation at inferring topologies, and even at high levels of ILS it may be no more accurate than concatenation (Ogilvie *et al.*, 2016). While other methods like ASTRAL may be more reliable than MP-EST, summary MSC methods estimate branch lengths in coalescent units instead of substitutions. Molecular-clock informed divergence times therefore cannot be reconstructed using summary methods. If concatenation is used to estimate branch lengths or divergence times for a fixed species tree topology estimated using a summary method, then those estimates will be unreliable

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for the same reasons as pure concatenation.

Another issue with summary methods is when the assumption of no recombination within loci is frequently violated because overly long loci are used (Gatesy and Springer, 2013). To resolve larger and deeper species trees using summary methods, longer and more informative loci may be required to infer more accurate gene trees. However the larger and deeper a tree, the more recombination events will have occurred. The use of longer loci and the higher incidence of recombination will both increase the risk of recombination occurring within loci, which has been dubbed the "recombination ratchet" (Springer and Gatesy, 2016).

As an alternative to increasing locus length, fully Bayesian MSC methods like *BEAST infer more accurate gene trees by sharing information between loci through the species tree (Szöllősi *et al.*, 2015). In this way very accurate species trees can be estimated using only weakly informative loci, which may not be possible using MP-EST (Xu and Yang, 2016). Summary methods can use gene trees estimated by *BEAST for more accurate results and to avoid the recombination ratchet (Zimmermann *et al.*, 2014), but the resulting species trees still cannot be used for molecular dating.

With the aim of improving the computational performance of fully Bayesian MSC inference of species trees, we have developed an upgrade to *BEAST — StarBEAST2 — which is available as a package for BEAST 2 (Bouckaert *et al.*, 2014). By improving computational performance StarBEAST2 enables the use of more loci, which will improve the precision of estimated parameters and provide an alternative to concatenation. We have also developed

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and include in StarBEAST2 new MSC relaxed clock models to enable accurate inference of per-species substitution rates.

2 New Approaches

2.1 Analytical integration of population sizes

Markov Chain Monte Carlo (MCMC) methods like *BEAST jointly integrate over many parameters by proposing small changes at each step to eventually produce a probability distribution for all parameters. From a researcher's perspective, some may be "nuisance" parameters not of scientific interest. For example species tree topology and divergence times may be of interest, but not effective population sizes. For tractable parameters, an analytic solution will integrate over the entire range of values at each MCMC step, and may be faster than MCMC integration. However explicit estimates will not be produced so this approach is suitable only for nuisance parameters. Among-site rate variation is already integrated out at each step; the likelihood of each site is calculated for all possible discrete gamma rates at each step, so individual site rates are not estimated (Yang, 1994).

Analytical integration of constant per-branch population sizes was first implemented as part of BEST (Liu *et al.*, 2008). The analytic solution, which we have added to StarBEAST2, uses an inverse gamma conjugate prior for population sizes. By default StarBEAST2 fixes the shape of the distribution $\alpha = 3$ and only estimates the mean of the distribution μ, which

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is proportional to the scale parameter β:

$$\mu = \frac{\beta}{\alpha - 1} = \frac{\beta}{2} \tag{1}$$

In this special case where $\alpha = 3$, the standard deviation is identical to the mean:

$$\sigma = \sqrt{\frac{\beta^2}{(\alpha-1)^2 \times (\alpha-2)}} = \sqrt{\frac{\beta^2}{2^2}} = \frac{\beta}{2} = \mu \tag{2}$$

The coefficient of variation $c_{\mathrm{v}} = \sigma/\mu$ of the prior distribution for effective population sizes is therefore 1.

2.2 Coordinated tree topology changing operators

One approach to improving the performance of MSC analyses which simultaneously estimate gene and species trees (such as *BEAST) is to develop MCMC operators which propose coordinated changes to both the species tree and the gene trees in the same step. Yang and Rannala (2014) introduced a Metropolis-Hastings (MH; Metropolis *et al.*, 1953; Hastings, 1970) operator which makes nearest-neighbor interchange (NNI) changes to the species tree topology, and simultaneously makes changes to gene tree topologies which preserve compatibility of the gene trees within the proposed species tree. Later, both Jones (2016) and Rannala and Yang (2015) introduced more general coordinated operators which make sub-

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tree prune and regraft (SPR) changes to the species tree. We have reimplemented these coordinated NNI and SPR moves in StarBEAST2 as a single new operator called "CoordinatedExchange". Rannala and Yang (2015) also describe a proposal distribution which favours topological changes on shorter branches as well as less radical changes in topology. StarBEAST2 implements a simpler proposal distribution but still favours less radical changes by applying adjustable proposal probability weights to (less radical) NNI moves and (more radical) SPR moves.

2.3 Coordinated node height changing operators

A novel class of coordinated Metropolis operators was introduced by Jones (2016), which pick at random a non-root non-leaf species tree node S with an existing height of $t(S)$. A new height $t'(S)$ is chosen from a uniform distribution with lower and upper bounds D and U. The height of the species tree node and the heights of subtrees of gene tree nodes (termed "connected components") are all shifted by the amount $\eta = t'(S) - t(S)$.

The D and U bounds limit the minimum and maximum values of η to those which do not require modifying the topology of the gene tree or of the species tree, and the algorithm to determine those bounds is given by Jones (2016). The species tree root node is excluded because there is no natural upper bound in that case. As long as the connected components are chosen with reference only to the topology of the species tree, the topology of the gene trees, and the mapping of sampled individuals to species, operators of this class are

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symmetric.

We have developed a new operator called "CoordinatedUniform" that belongs to this general class but has not been implemented before. Individuals from extant species which descend from a species tree node, or are directly descended from a gene tree node, are referred to here as "descendant individuals". The gene tree nodes s selected by this operator to be shifted in height are all those for which:

1. at least one descendant individual of s is also a descendant individual of the *left* child of S
2. at least one descendant individual of s is also a descendant individual of the *right* child of S
3. all descendent individuals of s are also descendent individuals of S

An example of how gene tree nodes are selected and node heights shifted is given in Supplementary Material.

We have also developed a new adaptive MH (Andrieu and Thoms, 2008) operator called "CoordinatedExponential" which also changes the height of the species tree root node and the height of connected components by an amount η. The gene trees nodes to be shifted are chosen using identical criteria as for CoordinatedUniform. Because this operator changes the height of the root node, a different method must be used to pick η compared to CoordinatedUniform.

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First the lower bound D is identified in the same way as for CoordinatedUniform and as described in Jones (2016). The difference between D and the current root height is referred to as x, and a new random value x' is chosen from an exponential distribution. The value of $x' - x$ is then used for η. The median of the exponential distribution is adaptively modified over the course of an MCMC chain to equal the posterior expectation of x.

Because the proposal distribution for a new species tree root height is independent of the current height, the Hastings ratio which is usually ${}^{q(x',x)}/_{q(x,x')}$ (Hastings, 1970) can be simplified to ${}^{\pi(x)}/_{\pi(x')}$. The natural logarithm of the Hastings ratio may then be derived from the respective probability densities of x and x' drawn from an exponential distribution with the rate λ:

$$\frac{\pi(x)}{\pi(x')} = \frac{\lambda e^{-\lambda x}}{\lambda e^{-\lambda x'}} = \frac{e^{-\lambda x}}{e^{-\lambda x'}} \tag{3}$$

$$\therefore \ln\left(\frac{\pi(x)}{\pi(x')}\right) = \ln\left(e^{-\lambda x}\right) - \ln\left(e^{-\lambda x'}\right) \tag{4}$$

$$= \lambda x' \cdot \ln(e) - \lambda x \cdot \ln(e) \tag{5}$$

$$= \lambda\left(x' - x\right) = \lambda\eta \tag{6}$$

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2.4 Species tree relaxed clocks

The overall rate of evolution occurring at a given locus within a species will be influenced by the nature of the particular gene and also by the natural history of the particular species. For a given gene, the average substitution rate may depend on the effects of selection such as the accelerated molecular evolution of sex-biased genes in *Arabidopsis thaliana* (Gossmann *et al.*, 2014), or on within-genome variation in mutation rate (Baer *et al.*, 2007). For a given species, the average substitution rate is correlated with a multitude of traits including metabolic rate, body size, and fecundity, although causal relationships are difficult to pin down (Bromham, 2011). Unsurprisingly in light of the above, empirical analysis has shown that two major factors contributing to rate variation among gene branches are the per-gene rate and the per-species rate (Rasmussen and Kellis, 2007).

Because variation is expected in the nature of different genes and species, and therefore variation is also expected in the average substitution rate of different genes and species, multispecies coalescent models should take both per-gene and per-species rate variation into account. *BEAST can accommodate both types of rate variation using gene tree relaxed clock models (for examples see Berv and Prum, 2014; Lambert *et al.*, 2015). This involves estimating per-branch substitution rates separately for each branch of each gene tree. While gene tree relaxed clocks may accommodate variation in substitution rates between species, they do not produce estimates of species branch rates. To enable accurate inference of species branch rates, we have developed a new species tree relaxed clock model.

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The challenge of applying a relaxed clock to the species tree is that phylogenetic likelihood calculations require branch rates for each branch of each gene tree. Our clock model computes those rates using the total expected number of substitutions $\Sigma\mathbb{E}(S)$ accumulated along the entire length of a gene tree branch. Substitutions are expected to be accumulated at the mean clock rate of the gene tree c, multiplied by the lengths of time L spent traversing each species tree branch, multiplied by the rates R of the corresponding species tree branches. Typical nuclear substitution rates for mammals are around 10^{-3} substitutions per site per million years (Phillips *et al.*, 2009).

The gene tree branch rates r can then be derived by dividing the total expected number of substitutions by the total length of that branch l. The gene tree branch rates for the illustrated example (Figure 1; Table 1) are therefore:

$$r_a = \frac{\Sigma\mathbb{E}(S_a)}{l_a} = \frac{0.00135}{1.5} = 0.0009 \tag{7}$$

$$r_b = \frac{\Sigma\mathbb{E}(S_b)}{l_b} = \frac{0.00165}{1.5} = 0.0011 \tag{8}$$

The new species tree relaxed clock model is available in StarBEAST2. Branch rate models that can be used with a species tree relaxed clock currently include the well-established uncorrelated log-normal (UCLN) and uncorrelated exponential (UCED) models (Drummond *et al.*, 2006), as well as the newer random local clock model (Drummond and Suchard, 2010).

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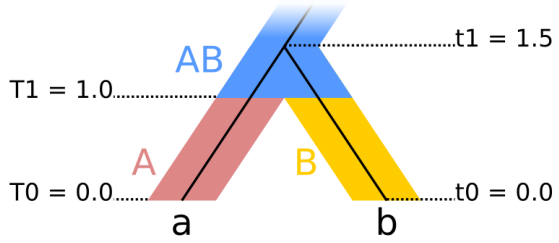

Figure 1: Two-species phylogeny used to illustrate species tree relaxed clocks. There are two extant species "A" and "B", and one ancestral species "AB". Within the species tree there is a single gene tree with extant individuals "a" and "b". The single speciation event occurs at time T1, and the single coalescence event occurs at time t1. Gene tree rates are computed according to Table 1

Table 1: Expected number of substitutions $\Sigma\mathbb{E}(S)$ for gene branches *a, b* under a species tree relaxed clock

Gene branch	Gene rate[1] c	Length[2] L			Species rate[3] R			$\mathbb{E}(S) = c \cdot L \cdot R$			$\Sigma\mathbb{E}(S)$
		A	B	AB	A	B	AB	A	B	AB	
a	0.001	1.0	0.0	0.5	0.7	1.0	1.3	0.00070	0.00000	0.00065	0.00135
b		0.0	1.0	0.5				0.00000	0.00100	0.00065	0.00165

[1] The overall substitution rate at a given locus.
[2] The length of a given gene tree branch within species tree branch A, B or AB.
[3] The substitution rate of species tree branch A, B or AB.

3 Results and Discussion

3.1 StarBEAST2 correctly implements the multispecies coalescent

New methods must be shown to be correct implementations of the target model. One way to accomplish this for MCMC methods is to estimate parameters from a prior distribution using the MCMC kernel, and to also draw independent samples from the same distribution by simulation. The resulting parameter distributions should be identical if the implementation is correct. We used this method to test the correctness of the novel features

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in StarBEAST2; analytical population size integration, coordinated operators, and species tree relaxed clocks. Simulated and StarBEAST2 distributions were identical for species and gene tree topologies (Figure S1,S2), species and gene tree node heights (Figure S3,S4), and for gene tree branch rates (Figure S5,S6). This combination of results supports the correctness of the StarBEAST2 implementation.

3.2 Species tree relaxed clocks prevent SPILS

When using concatenation to infer a species tree, SPILS causes apparent substitution rate variation among predictable species tree branches. However in an ultrametric (time tree) framework like BEAST, branch lengths are constrained so that terminal species begin at time zero. We hypothesized that if a relaxed clock is used with concatenation in an ultrametric framework, SPILS will be absorbed as faster substitution rates for lineages that would be lengthened by SPILS in a non-ultrametric framework.

In an ultrametric framework with a strict clock and no external (e.g. fossil, biogeographical or known clock rate) calibrations, the substitution rate of each branch is set to 1. This ensures that 1 unit of time is equivalent to 1 expected substitution. Using a relaxed clock with no external calibrations the substitution rate of each branch can vary, but the expectation of the mean rate of all branches is 1, preserving the relationship of 1 unit of time = 1 expected substitution. Therefore when SPILS causes the rates of some branches to be faster than 1, the rates of other branches will be slower than 1 to keep the expected mean constant.

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We used BEAST concatenation and StarBEAST2 with a species tree relaxed clock to infer the branch lengths and substitution rates of simulated species trees with the topology ((((A,B),C),D),E), using sequence alignments simulated using a strict clock. Gene tree discordance will increase the estimated length of A, B and C branches for these species trees (Mendes and Hahn, 2016), and as hypothesized substitution rates for A and B branches inferred using concatenation were biased towards being faster than the true rate of 1 (Figure 2). Estimated substitution rates for the C branch were more variable, and could be faster or slower than 1. Substitution rates estimated for the D and E branches were biased towards being slower than 1, presumably to balance the mean rate. Concatenation also overestimated the lengths of tip branches, another known bias when using concatenation to infer a species tree (Ogilvie *et al.*, 2016). No biases were observed for the branch rates or lengths estimated using StarBEAST2 (Figure 2).

A number of estimated branch rates had 95% credible intervals that excluded the true rate of 1 when using concatenation. If a study is testing whether substitution rates vary across a species tree, those branch rates could be erroneously interpreted as faster or slower than average. In our simulations, the clock rate of the D branch would be inferred as slower than average in 37 out of 96 replicates (Figure S7), despite the sequence data being simulated using a strict clock. When applying the same 95% credible intervals to branch lengths, the true simulated length was excluded with just two exceptions for all tip branches across all replicates using concatenation (Figure S8). In contrast, no erroneous results would be inferred for branch rates given the same data using StarBEAST2, and out of the 768 total simulated

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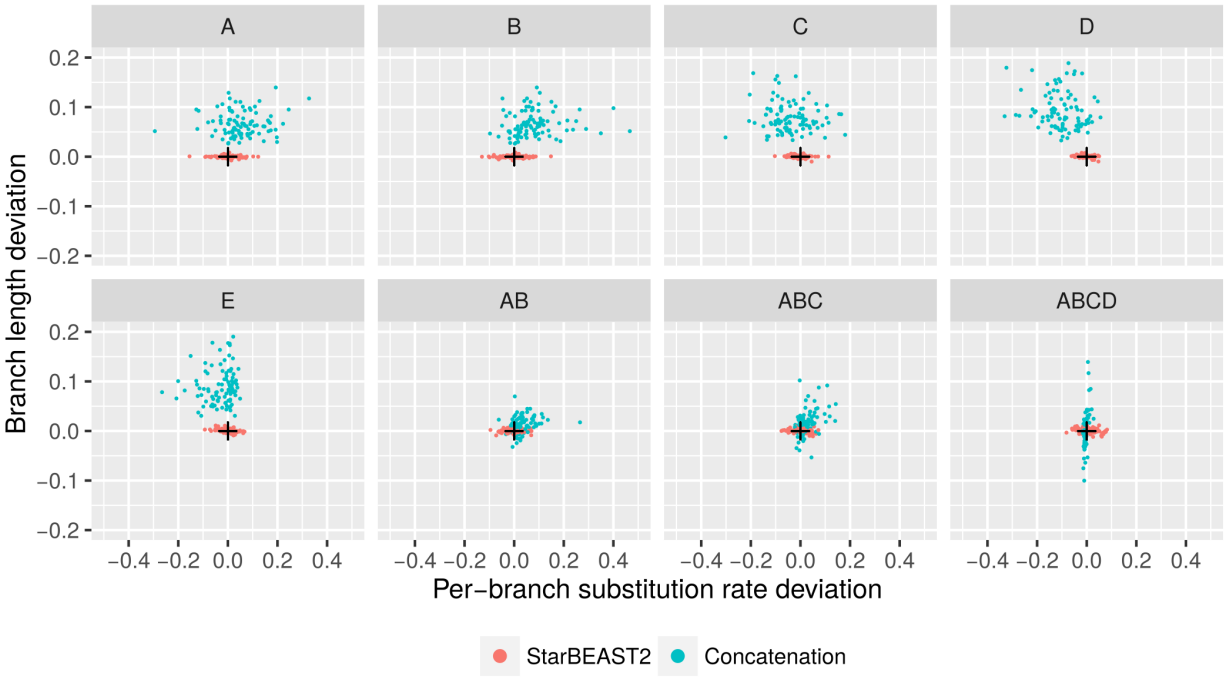

Figure 2: Accuracy of branch substitution rates and lengths inferred by BEAST concatenation and StarBEAST2. Deviation is the difference of each estimated rate and length from the true value. Estimated rates and lengths are the posterior expectation of the overall substitution rate and length for each species tree branch. Black crosses in each panel indicate the point of perfect accuracy. Each panel shows the distributions for the labelled extant or ancestral branch. N = 96.

non-root branch lengths, only five erroneous results would be inferred (Figure S7,S8).

Mendes and Hahn (2016) demonstrated that SPILS causes systematic bias when estimating branch lengths, and we show that this translates into systematic bias when estimating per-branch substitution rates. Because the bias is caused by incomplete lineage sorting which is a function of population sizes and branch lengths, there is no reason to expect that large trees with varying population sizes and branch lengths would be any less biased.

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3.3 *Pseudacris* chorus frogs have intermediate coalescent branch lengths

To characterize the performance of coordinated operators, methods of population size integration and relaxed clocks, we tested StarBEAST2 using empirical and simulated sequence data. The empirical data set used for this analysis is from the North American chorus frog genus *Pseudacris*, and was originally collected and analyzed by Barrow *et al.* (2014). This data set has sequences from 26 nuclear loci across 44 sampled individuals. The individuals belong to 19 extant *Pseudacris* lineages and two outgroup species. Barrow *et al.* (2014) reported phased haplotypes but to avoid wasting computational resources we used a single haplotype per individual.

A key metric of phylogenies that can be used to judge whether it is necessary to employ MSC models is the average branch length in coalescent units $\tau(2N_e)^{-1}$. In this study N_e will always refer to the effective population size of diploid individuals. Given short branch lengths, likelihood-based or neighbor-joining concatenation is unable to infer accurate species trees regardless of the number of loci used, but for long branch lengths, concatenation is approximately as accurate as *BEAST (Ogilvie *et al.*, 2016). Indeed concatenation can be considered a special case of the MSC as the models converge when gene trees are identical to the species tree (Liu *et al.*, 2015). Using StarBEAST2, the average branch length within this genus was estimated to be $2.81\tau(2N_e)^{-1}$. This is an intermediate average length compared to the shallow simulations analyzed by Ogilvie *et al.* (2016) which had a shorter average

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length of $1.08\tau(2N_e)^{-1}$.

Each replicate of each *Pseudacris* empirical analysis used the same sequence data, and the true species tree topology, dates and rates were not known with certainty. For performance results more generally applicable than a single empirical system, and to measure the accuracy of StarBEAST2 inference, we created a simulated data set of 30 replicates. A unique species tree was simulated for each replicate, and gene trees and locus sequences were simulated according to the MSC.

We simulated 26 nuclear loci from 21 extant species with two individual haplotypes per species, very similar to the empirical data set size. The simulation parameters, including the birth rate, death rate and population sizes, were also chosen to be similar to estimated *Pseudacris* parameters. The simulated data set had an average branch length of $2.99\tau(2N_e)^{-1}$, so the relative accuracy of MSC models compared to concatenation should be comparable with empirical systems like *Pseudacris*.

3.4 Coordinated height changing operators and analytical integration improve performance

To determine which configuration of new features would achieve the best performance, we ran StarBEAST2 using different combinations of operators, methods of population size integration and clock models. To measure convergence both effective sample size (ESS) per hour and ESS per million states were computed for each independent chain. ESS per hour

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can be used to calculate the total time required for a converged chain (nominally where ESS equals or exceeds 200), and reflects how effectively operators explore the space of trees and parameters, as well as the computational time required by each operator proposal and likelihood calculation. In contrast, ESS per million states reflects only the exploration of tree and parameter space independently of calculation times. A variety of statistics were recorded for each analysis (Table S2-S7), and for each replicate the statistic with the slowest ESS rate for that particular chain was used when computing the mean and standard deviation of ESS per hour and per million states, and in the rest of the paper.

Multiple linear regressions with log transformed ESS rates as the response variables were used to measure the effect of coordinated topology changing operators, coordinated node height changing operators, and the method of population size integration. Each additional feature was treated as a binary indicator variable so that we could quantify the relative performance as a percentage by exponentiating the coefficient for each addition (Table 2).

Coordinated topology operators consistently and significantly reduced ESS per hour, but had no significant effect on ESS per million states (Table 2), suggesting that coordinated topology operators are no more effective than naïve operators at proposing new states. A decrease in the number of states per hour (Figure S9) shows that they are more computationally expensive than naïve operators, and explains the negative effect on ESS per hour.

Coordinated height changing operators consistently and significantly increased ESS per hour and ESS per million states, however the degree of improvement depended on the clock

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Table 2: Relative performance of operators, population size integration and clock models.

Clock model	ESS rate per	Topology[3]	Height[4]	Analytical[5]
		Pseudacris reanalysis		
Strict	hour	73%***	120%***	130%***
Strict	million states	101%	129%***	143%***
GT-UCLN[1]	hour	72%***	289%***	100%
GT-UCLN	million states	100%	310%***	108%
ST-UCLN[2]	hour	78%**	499%***	154%***
ST-UCLN	million states	95%	484%***	163%***
		Simulated data		
Strict	hour	70%***	137%***	208%***
Strict	million states	100%	148%***	225%***
GT-UCLN	hour	68%***	231%***	228%***
GT-UCLN	million states	98%	248%***	248%***
ST-UCLN	hour	71%*	940%***	136%*
ST-UCLN	million states	86%	920%***	145%**

[1] Gene Tree Uncorrelated Log-Normal relaxed clock
[2] Species Tree Uncorrelated Log-Normal relaxed clock
[3] Coordinated topology changing operators relative to naïve operators
[4] Addition of coordinated height changing operators
[5] Analytical integration of population sizes relative to MCMC integration
Values higher than 100% indicate faster convergence, lower than 100% indicate slower. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. N = 30.

model (Figure 3). For strict clock analyses the increase in ESS per hour was modest at 1.2 times and 1.37 times for empirical and simulated data respectively, whereas for species tree relaxed clocks the increase was 4.99 times and 9.40 times respectively (Table 2). The difference in species tree relaxed clock performance suggests that coordinated height changing operators are necessary for practical implementations of that model.

Analytical population size integration significantly improved ESS per hour performance in all cases, with the exception of gene tree relaxed clocks applied to the *Pseudacris* data set. For the simulated analyses there was a significant improvement to ESS per hour and

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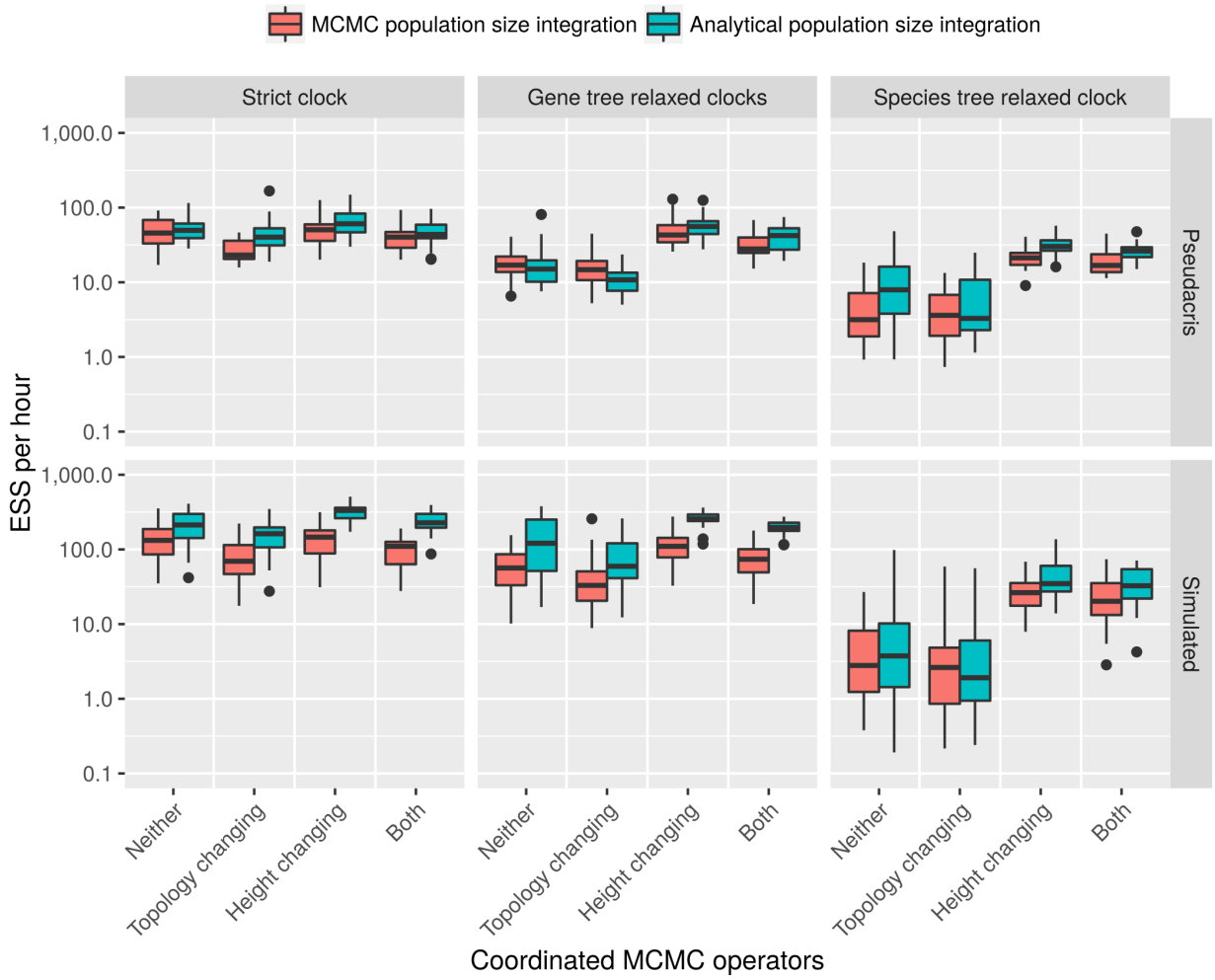

Figure 3: Impact of operators, population size integration and clock models on convergence. The estimated sample size (ESS) per hour for a given replicate used the smallest ESS out of all recorded statistics. Topology refers to the replacement of naïve nearest-neighbor interchange and subtree prune and regraft operators with coordinated operators. Height refers to the addition of operators which make coordinated changes to node heights. Uncorrelated log-normal relaxed clocks were applied to either each gene tree (GT-UCLN) or to the species tree (ST-UCLN). N = 30.

ESS per million states regardless of clock model (Table 2).

Even with new operators and analytical population size integration, the ESS per hour rates for species tree relaxed clocks were slower than for other clock models (Figure 3).

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One reason is that changing a species tree branch rate requires updating the phylogenetic likelihood for all gene trees, so the computational cost is much higher than for strict or gene tree relaxed clocks (Figure S9).

3.5 StarBEAST2 is an order of magnitude faster than *BEAST

StarBEAST2 also optimizes the core multispecies coalescent algorithms by caching intermediate values and by using fast data structures. Operator weights have also been refined by manual iteration for better performance. Building on our results, by default StarBEAST2 enables coordinated height changing operators and analytical population size integration, but keeps naïve topology operators. To measure the combined improvement when StarBEAST2 is applied to *Pseudacris* data we compared the performance of StarBEAST2 with default settings to *BEAST. For the simulation data set, we compare StarBEAST2 with *BEAST and also with concatenation.

NGS data sets may have hundreds or thousands of loci. To gauge the performance of StarBEAST2 applied to these data sets, we tested an empirical NGS data set; ultraconserved element (UCE; Faircloth *et al.*, 2012) sequences from Philippine shrews of the genus *Crocidura* (Giarla and Esselstyn, 2015). This data set consists of 1112 loci sampled from a total of 19 individuals, which belong to 9 extant lineages. Again multiple statistics were recorded to compute the ESS rate means and standard deviations.

Our simulation study confirmed that StarBEAST2 is many times faster than *BEAST

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(Figure 4). For simulated data the average log convergence rate of StarBEAST2 with gene tree relaxed clocks was 5.54 $\ln(^{ESS}/_{hour})$. This compares to 2.04 using *BEAST, an increase in performance of $\exp(5.54 - 2.04) = 33.1$ times (Table S2). In fact, StarBEAST2 was $\exp(4.18 - 2.04) = 8.5$ times faster at analyzing 52 loci than *BEAST was when analyzing 26.

StarBEAST2 was an order of magnitude faster when analyzing either empirical data set. For gene tree relaxed clock reanalyses of *Pseudacris* the difference was $\exp(3.98-1.41) = 13.1$ times. For 50-locus *Crocidura* reanalyses it was $\exp(2.79 - 0.11) = 13.8$ times (Table S4,S6).

The ESS per hour convergence of species tree relaxed clocks was lower than for gene tree relaxed clocks. When applying StarBEAST2 to simulated data, gene tree relaxed clocks were $\exp(5.54 - 3.71) = 6.2$ times faster than using species tree relaxed clocks (Table S2). The difference was much smaller for empirical data; for *Pseudacris* reanalyses gene tree relaxed clocks were $\exp(3.98-3.44) = 1.7$ times faster, and for *Crocidura* they were $\exp(2.79-2.12) = 2.0$ times faster (Table S4,S6). In all three cases species tree relaxed clocks using StarBEAST2 were still faster than gene tree relaxed clocks using *BEAST (Figure 4).

The increased performance of StarBEAST2 will enable researchers to analyze sequence data more quickly and disseminate their findings sooner; a large MCMC analysis which would currently take three months may now be performed in one week. In the case of phylogenomic data which has been subsetted for use with *BEAST, StarBEAST2 can be used to analyze more data for more precise estimates of species trees and other parameters in the same

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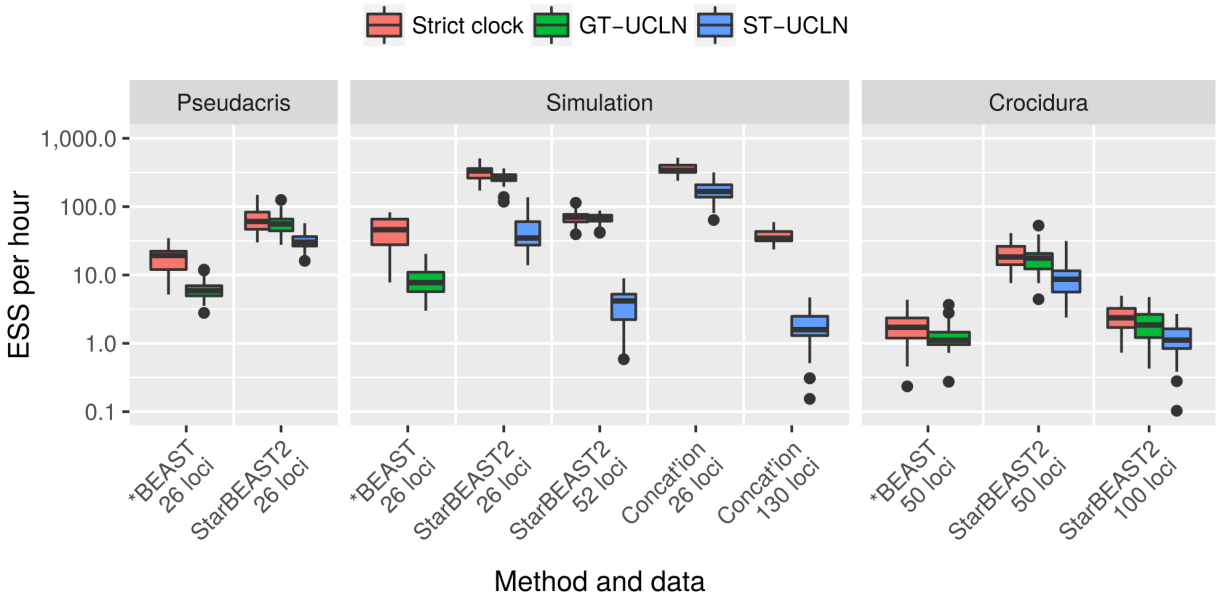

Figure 4: Convergence of different methods applied to simulated and empirical data sets. The estimated sample size (ESS) per hour for a given replicate used the slowest ESS rate out of all recorded statistics. Methods are BEAST concatenation, *BEAST, and StarBEAST2 with uncorrelated log-normal relaxed clocks applied to the gene trees (GT-UCLN) or to the species tree (ST-UCLN). Two *Pseudacris* *BEAST outliers with ESS rates below 0.1 are not shown. N = 30.

amount of time as a more limited *BEAST analysis.

3.6 Concatenation is a worse estimator of branch lengths than topology

The accuracy of StarBEAST2 relative to concatenation varied depending on the type of error and whether equal numbers of loci were used. Relative species tree error measures the accuracy of estimated branch lengths; by that measure StarBEAST2 using just 26 loci outperformed concatenation using 130 loci, and was even more accurate when using 52 loci

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(Figure 5A). Pendant edge bias measures systematic bias in the estimated ages of extant species; regardless of the number of loci StarBEAST2 was much less biased than concatenation which overestimated tip branch lengths by about 250% (Figure 5B). This is less than the 350% reported in Ogilvie *et al.* (2016) because of longer average branch lengths in coalescent units in this study.

Pendant edge bias is important because many published phylogenies show evidence of a slowdown in diversification rate (Moen and Morlon, 2014). If the ages of extant species are overestimated, this will artificially reduce the number of recent speciation events, mimicking a slowdown. We suggest that accurate inference of changing diversification rates requires species trees inferred by fully Bayesian MSC methods like StarBEAST2.

Rooted Robinson-Foulds distance measures the topological accuracy of estimated trees; for that metric concatenation using 130 loci was similar in accuracy to StarBEAST2 using 52 loci (Figure 5C). When the number of loci was the same, StarBEAST2 outperformed concatenation. For no type of error did the choice of gene or species tree relaxed clocks significantly affect the accuracy of StarBEAST2 (Figure 5A,B,C).

Using unphased sequences with ambiguity codes for heterozygous sites improved the accuracy of concatenation by reducing the pendant edge bias from about 250% to about 50% (Figure S10). Ambiguity codes are treated by most phylogenetic methods (including BEAST) as base call uncertainty, indicating the nucleotide at a given site could be one of several possibilities. When used with unphased sequences, they actually indicate the presence

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of two nucleotides simultaneously, which is therefore a model violation. Using concatenation to analyze unlinked loci is also a model violation, but in the region of parameter space investigated by this simulation study the two errors may *partially* cancel out.

3.7 StarBEAST2 is superior at inferring substitution rates

While the convergence of species tree relaxed clock analyses took longer than for gene tree relaxed clocks in StarBEAST2, species tree relaxed clocks enable inference of species branch rates within an MSC framework. To gauge the accuracy of estimated branch rates, we used simple linear regressions with the true rate of each simulated branch as the explanatory variable, and the posterior expectation of the rate of that branch (conditional on the corresponding clade being monophyletic in the posterior samples) as the response variable. If all estimates are equally proportional to the truth, then the R^2 coefficient of determination will equal 1. There are intrinsic limits to our ability to estimate substitution rates, primarily that branch length is confounded with substitution rate (Thorne and Kishino, 2002).

For analyses of simulated data using 26 loci the R^2 using StarBEAST2 was 0.39 and by doubling the number of loci to 52 was increased to 0.43. In contrast the R^2 when using concatenation with 26 loci was 0.26 and even after increasing the number of loci to 130 it was only 0.34, in either case worse than StarBEAST2 using 26 loci (Figure 6). StarBEAST2 is clearly superior to concatenation at inferring branch rates.

Concatenation is an even worse estimator of branch rates when using unphased sequences

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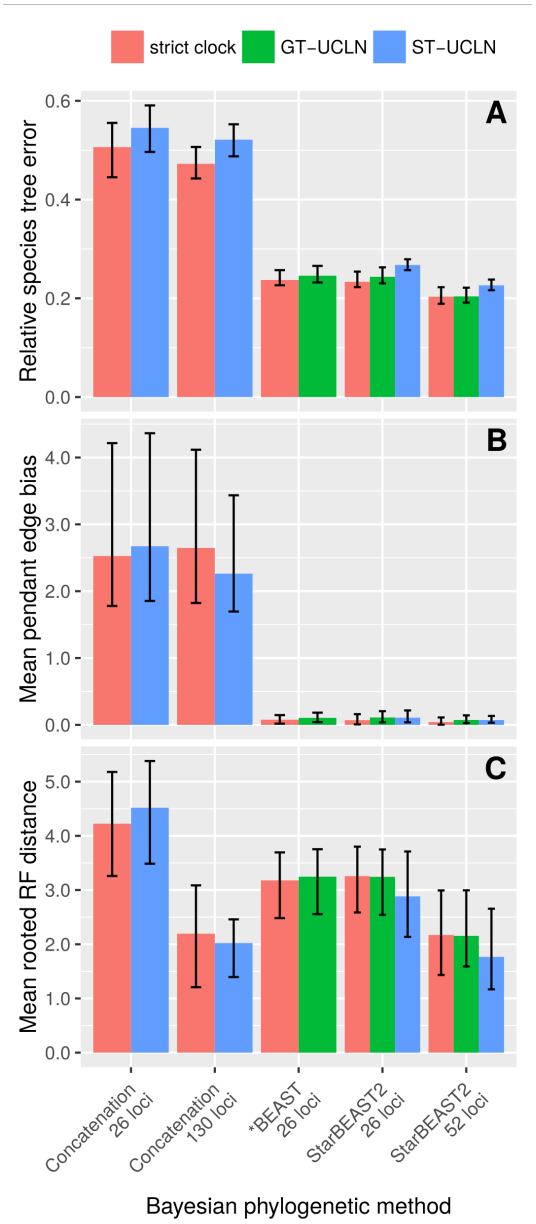

Figure 5: Accuracy of different methods applied to simulated data. Methods are StarBEAST2, *BEAST and BEAST concatenation with uncorrelated log-normal relaxed clocks applied to the gene tree (GT-UCLN) or to the species tree (ST-UCLN). (A) Trimmed mean of relative species tree error, a measure of branch length error. (B) Trimmed mean of mean pendant edge bias, which measures biased estimates of the ages of extant species. (C) Trimmed mean of mean rooted Robinson-Foulds (RF) distances, a measure of topological error. 25% trim was used to reduce the influence of outliers. All error bars are 95% confidence intervals calculated by bootstrapping. N $\geq$ 22.

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with ambiguity codes for heterozygous sites. When applying concatenation to either 26 or 130 loci, R^2 was very weak at 0.12 and 0.13 respectively (Figure S11).

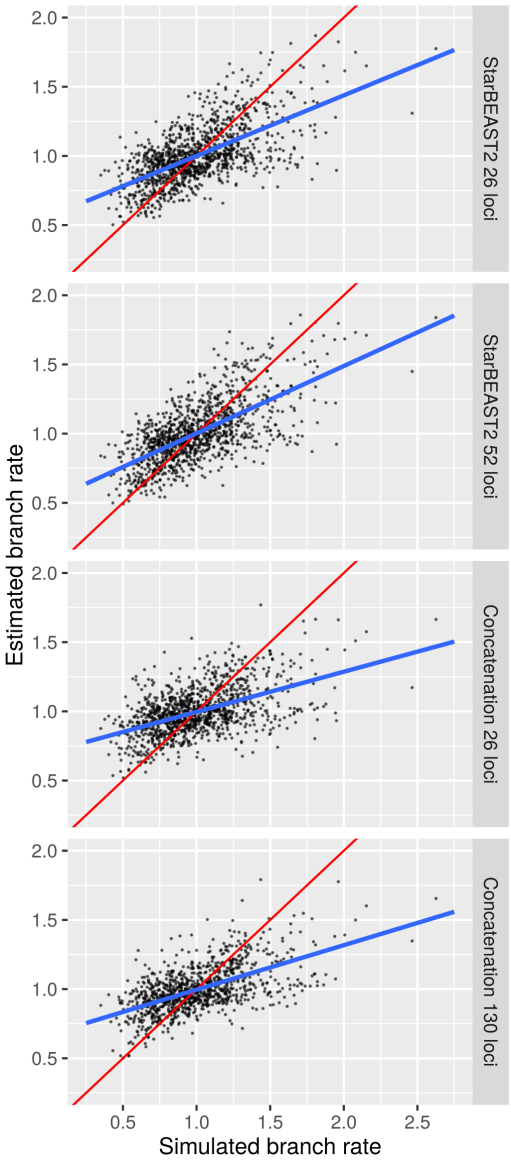

Figure 6: Estimates of species tree branch rates using BEAST concatenation versus StarBEAST2. Estimated rates are the posterior expectations of each branch rate from each replicate. Root branch rates, which were fixed at 1, were excluded. In blue are simple linear regression lines of best fit, and in red are the $y = x$ lines showing a perfect relationship between estimates and truth. N $\geq$ 22.

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4 Conclusions

When estimating divergence dates and substitution rates, the choice is often between using a subset of available loci with a fully Bayesian MSC method, or all available loci with concatenation. Researchers have often opted for the second choice, but we have shown that concatenation may not accurately estimate the ages of extant species or per-species substitution rates, even for trees of intermediate branch lengths. The increased performance of StarBEAST2 should further encourage the adoption of fully Bayesian MSC methods for estimating divergence times, and the new species tree relaxed clock will enable accurate inference of species branch rates despite ILS. StarBEAST2 is free and open source software, and its source code and development history is available through GitHub (`https://github.com/genomescale/starbeast2`).

5 Materials and Methods

For all StarBEAST2, *BEAST and concatenation analyses, the version of BEAST used was 2.4.4. For all simulations, the version of biopy (Heled, 2013) used was 0.1.9.

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5.1 Mathematical correctness of StarBEAST2

Simulated trees were generated using biopy, and trees sampled from a prior distribution were generated using StarBEAST2 with all new features enabled. This included analytical integration of population sizes, coordinated tree topology and node height changing operators, and a species tree relaxed clock. 100,000 species trees were simulated, one gene tree was simulated per species tree with a rate of 0.5, and a second gene tree was simulated per species tree with a rate of 2.0.

100,000 species trees, 100,000 half rate gene trees and 100,000 double rate gene trees were sampled from the prior at a rate of one every 1000 after a 10% burn-in period.

Identical parameters were used for the simulation and for the StarBEAST2 run including the prior distributions. We fixed the number of species at 5 and the number of haplotypes per species at 1. The birth and death rates were fixed at 200 and 100 per substitution respectively. Haploid population sizes followed an inverse gamma distribution with shape $\alpha = 3$ and scale $\beta = 0.004$.

This procedure was repeated for both UCLN and for UCED species branch rates. Branch rates were sampled from a lognormal or exponential distribution, in either case with a mean of 1, discretized into 100 bins.

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5.2 Reanalysis of *Pseudacris* sequence data

Phased and aligned *Pseudacris* sequence data were retrieved from Dryad (`http://dx.doi.org/10.5061/dryad.23rc0`). Replicating the original analysis we applied the HKY nucleotide substitution model (Hasegawa *et al.*, 1985) to 22 out of 26 nuclear loci and the GTR model (Tavaré, 1986) to the remaining 4. For all models we used four discrete Γ categories to accommodate among-site rate variation (Yang, 1994). Transition/transversion rates and ratios and rate variation shape parameters were estimated, and empirical base frequencies used, all separately for each locus. The relative substitution rate of each locus was estimated using a lognormal prior with a mean μ in real space of 1 and a standard deviation σ of 0.6. We used a single haplotype sequence per individual per locus, halving the total number of sampled sequences to avoid wasting computational resources.

For inference of *Pseudacris* trees, we ran 30 independent StarBEAST2 chains for all 24 conditions for a total of 720 chains. The conditions were each possible combination of strict, species tree relaxed or gene tree relaxed clocks, analytical or MCMC population size integration, coordinated or naïve topology changing operators, and the inclusion or exclusion of coordinated height changing operators. Each chain used the same sequence data but was a partially independent estimate of convergence because a different random seed was used to initialize each chain.

A birth-death prior was used for the species tree and both the net diversification and extinction fraction hyperparameters were estimated. A gamma prior was used for MCMC

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estimated population sizes with a shape fixed at 2 and an estimated mean population size hyperparameter, matching the original *BEAST model (Heled and Drummond, 2010). The number of branch rate categories was equal to the number of estimated branch rates (as is the default in BEAST 2), and the standard deviation of the UCLN clock model was fixed at 0.3.

To ensure convergence of all chains, we ran each chain for an initial length of 2^{24} = 16,777,216 states, sampling every 2^{11} = 2,048 states. Initial chain lengths and sampling rates for all other analyses are in Table S8. ESS values were computed for all recorded statistics after discarding 12.5% of state samples as burn-in. Recorded statistics included (1) the posterior probability, (2) the coalescent probabilities of gene trees, (3) the overall prior probability, (4) the birth death prior probability of the species tree, (5) the phylogenetic likelihood, (6) the net diversification rate, (7) the extinction fraction, (8) the mean population size, and (9) the height of the species tree.

If any recorded statistic had an ESS below 200, the chain was resumed until the length of the chain had doubled. ESS values were then re-evaluated, again after discarding 12.5% of state samples. The length of a chain was continually doubled and ESS values re-evaluated until the ESS values of all recorded statistics were above 200. The rate at which trees and statistics were sampled was halved with every chain doubling so that the total number of samples remained constant.

ESS per hour was calculated by dividing the final ESS value for a given statistic by

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87.5% of the total CPU time used by that chain to account for burn-in. Likewise ESS per million states was calculated by dividing the final ESS value by 87.5% of the total number of the states in the chain, then multiplied by one million. For all analyses of computational performance including graphs and linear models, the ESS rate for any given chain was that of the slowest converging statistic for that particular chain.

Average branch length in coalescent units was calculated by concatenating the output (after discarding the first 12.5% of states as burn-in from each chain) of all 30 chains which used the combination of MCMC population size integration, naïve topology operators, co-ordinated node height operators and species tree branch rates. For every sample in the combined posterior distribution, the coalescent length of each branch $\tau(2N_e)^{-1}$ was calculated from its length in substitution units τ and its effective population size N_e. The mean coalescent length of all branches across all samples was taken as the average.

5.3 Testing the effects of SPILS on estimated substitution rates

To test how SPILS affected estimates of per-species branch substitution rates, 96 fully asymmetric species trees were simulated with the topology ((((A,B),C),D),E). All species trees were simulated according to a pure birth Yule process (Yule, 1925) with a speciation rate of 10 per substitution.

Haploid population sizes for each branch were chosen independently from an inverse gamma distribution with a shape of 3 and a scale of 0.2. 100 gene trees with one individual

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per extant species were then simulated for each species tree according to the MSC process using biopy. Finally 1000nt sequence alignments were then simulated for each gene tree according to the Jukes-Cantor substitution model (Jukes and Cantor, 1969), equal base frequencies, no among-site rate variation, a strict molecular clock, and a substitution rate of 1 for each locus. Sequence alignments were simulated using Seq-Gen (Rambaut and Grassly, 1997).

BEAST concatenation and StarBEAST2 were then used to estimate the branch rates and divergence times with the species tree topology fixed to the truth. The same substitution model used for simulating sequences (i.e. Jukes-Cantor, no rate variation among sites or loci) was also used for inference. UCLN relaxed clocks were applied to the tree inferred by concatenation and to the StarBEAST2 species tree.

The same strategy as applied to *Pseudacris* was used to ensure convergence of StarBEAST2, but for concatenation mean population sizes and coalescent probabilities are not part of the model and so were not recorded.

For every converged chain, the posterior expectation and 95% credibility intervals of per-species branch rates were calculated using the TreeAnnotator program supplied with BEAST.

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5.4 Simulations to measure computational performance and statistical accuracy

All simulation parameters were chosen to be broadly similar to those observed in or estimated from the *Pseudacris* data set.

First, 30 species trees were simulated according to a birth-death process (Gernhard, 2008) using biopy with 21 extant species, a speciation rate of 100 and a death rate of 30. This corresponds to a net diversification rate of 70 and an extinction fraction of 0.3. Haploid population sizes for each branch were chosen independently from a gamma distribution with a shape of 2 and a scale of 0.002. For a species with annual generation times, as is the case for at least some *Pseudacris* species (Caldwell, 1987), and a substitution rate of 10^{-9} per year this corresponds to an effective population size N_e of around 2 million individuals per generation. Species branch rates were chosen from a log-normal distribution with a mean in real space of 1 and a standard deviation of 0.3, then scaled so that the mean of the branch rates for a given species tree was exactly 1. This ensured that per-branch rates always reflected relative differences in substitution rates.

For each species tree, 130 gene trees with two sampled haplotype sequences per species were simulated according to the MSC process using biopy. The mean clock rate for each locus was chosen from a log-normal distribution with a mean in real space of 1 and a standard deviation of 0.6.

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For each gene tree, 600nt long sequence alignments were simulated using Seq-Gen (Rambaut and Grassly, 1997). An HKY model was used for all sequence alignments with equal base frequencies, a κ value of 3, and a four rate category discretized gamma model of among-site rate variation with a shape α value of 0.2. Hence all inference based on simulated data applied the HKY+Γ substitution model to all loci.

The same combinations of clock models, population size integration and new operators were explored using the simulated data as for *Pseudacris* to provide more generally applicable results regarding those new techniques. The same number of loci, convergence strategy and calculations of ESS rates were used for both. Both haplotype sequences were used for each species.

5.5 Comparison of StarBEAST2 with *BEAST and concatenation

To compare the performance of StarBEAST2 with *BEAST, we ran 30 strict clock and 30 gene tree relaxed clock replicates of the *Pseudacris* reanalysis using the *BEAST package built into BEAST 2. We also reran each simulation replicate using *BEAST with a strict clock and gene tree relaxed clocks. The same priors, substitution models, and convergence strategies as used for StarBEAST2 were used with *BEAST.

For both data sets we reused the StarBEAST2 results for the combination of analytical population size integration, coordinated height-changing operators and naïve topology operators, which are all enabled by default in StarBEAST2. To demonstrate the scaling of

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StarBEAST2, we also reran each simulation replicate with an additional 26 loci (for a total of 52 loci) for all three clock models.

To compare concatenation with StarBEAST2, we reran each simulation replicate for each combination of either unphased ambiguity coded sequences or a single haplotype sequence per species, either a strict clock or species tree relaxed clock, and either the original 26 loci or with an additional 104 loci (for a total of 130 loci). We estimated the per-locus rates in the same way as for StarBEAST2, and applied the same convergence strategy as for SPILS concatenation. For species tree clock rates we used the same UCLN parameters as StarBEAST2 but applied to the concatenated tree, a model equivalent to that described by Rasmussen and Kellis (2007).

We also generated 30 replicates from a UCE data set of *Crocidura* shrews to show that StarBEAST2 can scale to 100 loci. For 1020 out of 1112 loci, the best fitting substitution model was either HKY or a nested model (Giarla and Esselstyn, 2015). To simplify configuring substitution models, we chose 100 unique loci at random, and separately for each replicate, from the set of loci which best fit HKY or a nested model. For each replicate we ran *BEAST with a strict clock or gene tree relaxed clock and a subset of 50 loci, StarBEAST2 with all three clock models and the same subset, and StarBEAST2 with all three clock models and all 100 loci. The same priors, substitution model and convergence strategies were used as for the simulated data set.

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5.6 Measurements of species tree accuracy

Subsequent analyses of species tree accuracy were limited to fully converged simulated data replicates. At least 22 out of 30 simulated data replicates had fully converged (all ESS values $\geq$ 200) for each tested configuration of *BEAST, StarBEAST2 and BEAST concatenation.

Relative species tree error is based on "rooted branch score" (RBS; Heled and Bouckaert, 2013). Given two trees T_1 and T_2, the sets of monophyletic clades c present in each tree are defined as $\mathbb{C}_1$ and $\mathbb{C}_2$. The length of the parent branch extending from the root of the subtree defined by c is then $b(c)$. To calculate the rooted branch score, sum all absolute differences in $b(c)$ between trees T_1 and T_2:

$$RBS(T_1, T_2) = \sum_{c \in \mathbb{C}_1 \cup \mathbb{C}_2} |b^{(1)}(c) - b^{(2)}(c)| \tag{9}$$

If a clade c is present in only one of T_1 or T_2, then the branch length $b(c)$ from the tree containing c is added to the RBS. Relative species tree error is the mean RBS between an estimated species tree and the true species tree over the posterior distribution of species trees, and is normalized by dividing by the total length of the true species tree (Ogilvie *et al.*, 2016).

Pendant edge bias is defined as $\hat{h}-h/h$, where the estimated age for each extant species is $\hat{h}$ and the true age is h. The mean pendant edge bias is the average pendant edge bias for

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all extant species across all posterior samples.

Rooted Robinson-Foulds distances (Robinson and Foulds, 1981) are defined as the count of clades present only one of T_1 and T_2. In this study, the mean rooted Robinson-Foulds distance is the average of all distances between each estimated tree and the true tree for all posterior samples.

6 Supplementary Material

Supplementary figures and tables will be made available online.

7 Acknowledgments

This work was supported by a Rutherford Discovery Fellowship awarded to A.J.D. by the Royal Society of New Zealand. H.A.O. was supported by an Australian Laureate Fellowship awarded to Craig Moritz by the Australian Research Council (FL110100104). This research was undertaken with the assistance of resources from the National Computational Infrastructure (NCI), which is supported by the Australian Government. We wish to thank Jason Bragg and Renee Catullo for testing StarBEAST2 before its official release, Timothy Vaughan for suggesting the addition of a root height changing operator, Joseph Heled for insight into the multispecies coalescent, and Graham Jones for input regarding operator per-

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formance. We also thank Tanja Stadler for hosting H.A.O. and A.J.D. during part of the development of StarBEAST2, and thank Fábio Mendes, Matthew Hahn, the editor and two anonymous reviewers for suggested improvements to this manuscript.

References

Aberer, A. J., Kobert, K., and Stamatakis, A. 2014. ExaBayes: Massively parallel Bayesian tree inference for the whole-genome era. *Molecular Biology and Evolution*, 31(10): 2553–2556.

Andrieu, C. and Thoms, J. 2008. A tutorial on adaptive MCMC. *Statistics and Computing*, 18(4): 343–373.

Arbogast, B. S., Edwards, S. V., Wakeley, J., Beerli, P., and Slowinski, J. B. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annual Review of Ecology and Systematics*, 33(1): 707–740.

Baer, C. F., Miyamoto, M. M., and Denver, D. R. 2007. Mutation rate variation in multicellular eukaryotes: causes and consequences. *Nature Reviews Genetics*, 8(8): 619–631.

Barrow, L. N., Ralicki, H. F., Emme, S. A., and Lemmon, E. M. 2014. Species tree estimation of North American chorus frogs (Hylidae: *Pseudacris*) with parallel tagged amplicon sequencing. *Molecular Phylogenetics and Evolution*, 75: 78–90.

Berv, J. S. and Prum, R. O. 2014. A comprehensive multilocus phylogeny of the Neotropical

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cotingas (Cotingidae, Aves) with a comparative evolutionary analysis of breeding system and plumage dimorphism and a revised phylogenetic classification. *Molecular Phylogenetics and Evolution*, 81: 120–136.

Blom, M. P. K., Horner, P., and Moritz, C. 2016. Convergence across a continent: adaptive diversification in a recent radiation of Australian lizards. *Proceedings of the Royal Society of London B: Biological Sciences*, 283(1832).

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A., and Drummond, A. J. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 10(4): e1003537.

Bromham, L. 2011. The genome as a life-history character: why rate of molecular evolution varies between mammal species. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 366(1577): 2503–2513.

Caldwell, J. P. 1987. Demography and life history of two species of chorus frogs (Anura: Hylidae) in South Carolina. *Copeia*, 1987(1): 114–127.

Degnan, J. H. and Rosenberg, N. A. 2006. Discordance of species trees with their most likely gene trees. *PLoS Genetics*, 2(5): e68.

Degnan, J. H. and Rosenberg, N. A. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution*, 24(6): 332–340.

Drummond, A. J. and Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7: 214.

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Drummond, A. J. and Suchard, M. A. 2010. Bayesian random local clocks, or one rate to rule them all. *BMC Biology*, 8: 114.

Drummond, A. J., Ho, S. Y. W., Phillips, M. J., and Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology*, 4(5): e88.

Faircloth, B. C., McCormack, J. E., Crawford, N. G., Harvey, M. G., Brumfield, R. T., and Glenn, T. C. 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*, 61(5): 717–726.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, 17(6): 368–376.

Gatesy, J. and Springer, M. S. 2013. Concatenation versus coalescence versus "concatalescence". *Proceedings of the National Academy of Sciences*, 110(13): E1179.

Gernhard, T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology*, 253(4): 769–778.

Giarla, T. C. and Esselstyn, J. A. 2015. The challenges of resolving a rapid, recent radiation: Empirical and simulated phylogenomics of Philippine shrews. *Systematic Biology*, 64(5): 727–740.

Gossmann, T. I., Schmid, M. W., Grossniklaus, U., and Schmid, K. J. 2014. Selection-driven evolution of sex-biased genes is consistent with sexual selection in *Arabidopsis thaliana*. *Molecular Biology and Evolution*, 31(3): 574–583.

bioRxiv preprint doi: https://doi.org/10.1101/070169; this version posted December 19, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., and Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, 59(3): 307–321.

Hasegawa, M., Kishino, H., and Yano, T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22(2): 160–174.

Hastings, W. K. 1970. Monte Carlo sampling methods using Markov chains and their applications. *Biometrika*, 57(1): 97–109.

Heled, J. 2013. biopy — a library for phylogenetic exploration. `https://figshare.com/articles/biopy_a_Library_for_Phylogenetic_Exploration/761224`.

Heled, J. and Bouckaert, R. R. 2013. Looking for trees in the forest: summary tree from posterior samples. *BMC Evolutionary Biology*, 13(1): 1–11.

Heled, J. and Drummond, A. J. 2010. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, 27(3): 570–580.

Jones, G. 2016. Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *Journal of Mathematical Biology*. Advance access.

Jukes, T. H. and Cantor, C. R. 1969. Evolution of protein molecules. In *Mammalian Protein Metabolism*, pages 21–132. Academic Press.

Kubatko, L. S. and Degnan, J. H. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Systematic Biology*, 56(1): 17–24.

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Lambert, S. M., Reeder, T. W., and Wiens, J. J. 2015. When do species-tree and concatenated estimates disagree? An empirical analysis with higher-level scincid lizard phylogeny. *Molecular Phylogenetics and Evolution*, 82, Part A: 146–155.

Lemmon, E. M. and Lemmon, A. R. 2013. High-throughput genomic data in systematics and phylogenetics. *Annual Review of Ecology, Evolution, and Systematics*, 44(1): 99–121.

Liu, L. 2008. BEST: Bayesian estimation of species trees under the coalescent model. *Bioinformatics*, 24(21): 2542–2543.

Liu, L., Pearl, D. K., Brumfield, R. T., and Edwards, S. V. 2008. Estimating species trees using multiple-allele DNA sequence data. *Evolution*, 62(8): 2080–2091.

Liu, L., Yu, L., and Edwards, S. V. 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. *BMC Evolutionary Biology*, 10: 302.

Liu, L., Xi, Z., Wu, S., Davis, C. C., and Edwards, S. V. 2015. Estimating phylogenetic trees from genome-scale data. *Annals of the New York Academy of Sciences*, 1360(1): 36–53.

Mendes, F. K. and Hahn, M. W. 2016. Gene tree discordance causes apparent substitution rate variation. *Systematic Biology*, 65(4): 711–721.

Metropolis, N., Rosenbluth, A. W., Rosenbluth, M. N., Teller, A. H., and Teller, E. 1953. Equation of state calculations by fast computing machines. *The Journal of Chemical Physics*, 21(6): 1087–1092.

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Mirarab, S. and Warnow, T. 2015. ASTRAL-II: coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. *Bioinformatics*, 31(12): i44–i52.

Mirarab, S., Reaz, R., Bayzid, M. S., Zimmermann, T., Swenson, M. S., and Warnow, T. 2014. ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics*, 30(17): i541–i548.

Moen, D. and Morlon, H. 2014. Why does diversification slow down? *Trends in Ecology & Evolution*, 29(4): 190–197.

Ogilvie, H. A., Heled, J., Xie, D., and Drummond, A. J. 2016. Computational performance and statistical accuracy of *BEAST and comparisons with other methods. *Systematic Biology*, 65(3): 381–396.

Phillips, M. J., Bennett, T. H., and Lee, M. S. Y. 2009. Molecules, morphology, and ecology indicate a recent, amphibious ancestry for echidnas. *Proceedings of the National Academy of Sciences*, 106(40): 17089–17094.

Rambaut, A. and Grassly, N. C. 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Computer applications in the biosciences*, 13(3): 235–238.

Rannala, B. and Yang, Z. 2015. Efficient Bayesian species tree inference under the multi-species coalescent. *ArXiv e-prints*.

Rasmussen, M. D. and Kellis, M. 2007. Accurate gene-tree reconstruction by learning gene-

bioRxiv preprint doi: https://doi.org/10.1101/070169; this version posted December 19, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

and species-specific substitution rates across multiple complete genomes. *Genome Research*, 17(12): 1932–1942.

Robinson, D. and Foulds, L. 1981. Comparison of phylogenetic trees. *Mathematical Biosciences*, 53(1–2): 131–147.

Springer, M. S. and Gatesy, J. 2016. The gene tree delusion. *Molecular Phylogenetics and Evolution*, 94, Part A: 1–33.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9): 1312–1313.

Szöllősi, G. J., Tannier, E., Daubin, V., and Boussau, B. 2015. The inference of gene trees with species trees. *Systematic Biology*, 64(1): e42–e62.

Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. In R. Miura, editor, *Some Mathematical Questions in Biology: DNA Sequence Analysis*, volume 17 of *Lectures on mathematics in the life sciences*, pages 57–86. American Mathematical Society, Providence, Rhode Island.

Thorne, J. L. and Kishino, H. 2002. Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, 51(5): 689–702.

Xi, Z., Liu, L., and Davis, C. C. 2015. Genes with minimal phylogenetic information are problematic for coalescent analyses when gene tree estimation is biased. *Molecular Phylogenetics and Evolution*, 92: 63–71.

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Xu, B. and Yang, Z. 2016. Challenges in species tree estimation under the multispecies coalescent model. *Genetics*, 204(4): 1353–1368.

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution*, 39(3): 306–314.

Yang, Z. 2007. PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, 24(8): 1586–1591.

Yang, Z. 2015. The BPP program for species tree estimation and species delimitation. *Current Zoology*, 61(5): 854–865.

Yang, Z. and Rannala, B. 2014. Unguided species delimitation using DNA sequence data from multiple loci. *Molecular Biology and Evolution*, 31(12): 3125–3135.

Yule, G. U. 1925. A mathematical theory of evolution, based on the conclusions of Dr. J. C. Willis, F.R.S. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 213(402-410): 21–87.

Zimmermann, T., Mirarab, S., and Warnow, T. 2014. BBCA: Improving the scalability of *BEAST using random binning. *BMC Genomics*, 15(6): S11.