

## ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Issue: *The Year in Evolutionary Biology***Estimating phylogenetic trees from genome-scale data**Liang Liu,<sup>1,2</sup> Zhenxiang Xi,<sup>3</sup> Shaoyuan Wu,<sup>4</sup> Charles C. Davis,<sup>3</sup> and Scott V. Edwards<sup>3</sup><sup>1</sup>Department of Statistics, University of Georgia, Athens, Georgia. <sup>2</sup>Institute of Bioinformatics, University of Georgia, Athens, Georgia. <sup>3</sup>Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts. <sup>4</sup>Department of Biochemistry and Molecular Biology & Tianjin Key Laboratory of Medical Epigenetics, School of Basic Medical Sciences, Tianjin Medical University, Tianjin, China

Address for correspondence: Scott Edwards, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138. sedwards@fas.harvard.edu

The heterogeneity of signals in the genomes of diverse organisms poses challenges for traditional phylogenetic analysis. Phylogenetic methods known as “species tree” methods have been proposed to directly address one important source of gene tree heterogeneity, namely the incomplete lineage sorting that occurs when evolving lineages radiate rapidly, resulting in a diversity of gene trees from a single underlying species tree. Here we review theory and empirical examples that help clarify conflicts between species tree and concatenation methods, and misconceptions in the literature about the performance of species tree methods. Considering concatenation as a special case of the multispecies coalescent model helps explain differences in the behavior of the two methods on phylogenomic data sets. Recent work suggests that species tree methods are more robust than concatenation approaches to some of the classic challenges of phylogenetic analysis, including rapidly evolving sites in DNA sequences and long-branch attraction. We show that approaches, such as binning, designed to augment the signal in species tree analyses can distort the distribution of gene trees and are inconsistent. Computationally efficient species tree methods incorporating biological realism are a key to phylogenetic analysis of whole-genome data.

**Keywords:** bias–variance dilemma; transcriptome; isochore; anomaly zone; recombination

**Introduction**

The emergence of genome-scale data provides unprecedented opportunities to resolve the history of species and, ultimately, the tree of life. For example, in the last few years, a number of phylogenetic and phylogeographic methods for analyzing the evolutionary history of whole genomes have emerged.<sup>1–4</sup> These new methods are gradually replacing methods that were once the bulwarks of evolutionary genomics and molecular ecology in the polymerase chain reaction era. As a case in point, recently, the classic isolation with migration model of phylogeography, originally introduced by Hey, Nielsen, and others<sup>5–7</sup> and widely used in likelihood and Bayesian formats, has been updated to accommodate whole-genome data.<sup>8,9</sup> Like most methods in phylogeography, these methods model genomic data as a series of unlinked or partially linked loci whose histories are influenced by the underlying demographic history of the species

in question. The coalescent provides a robust and general framework for many of these new genome-scale models in phylogeography.

Like models in phylogeography, genome-scale methods in phylogenomics are undergoing a transition, grappling with the heterogeneity of signals that frequently emerge from genome-scale data.<sup>10,11</sup> Recent research has revealed a surprising array of such heterogeneous signals, including variation among loci in base composition, evolutionary rate, and, perhaps most conspicuously, topological congruence.<sup>12–16</sup> Indeed, mirroring early insights into gene tree heterogeneity discovered in the 1980s in the context of phylogeography, gene tree heterogeneity in phylogenetics has emerged as a ubiquitous element, particularly as the number of loci in phylogenetic studies has increased.<sup>10</sup> Yet, surprisingly, whereas phylogeography has dealt with this heterogeneity by acknowledging and modeling stochasticity—indeed, statistical phylogeography

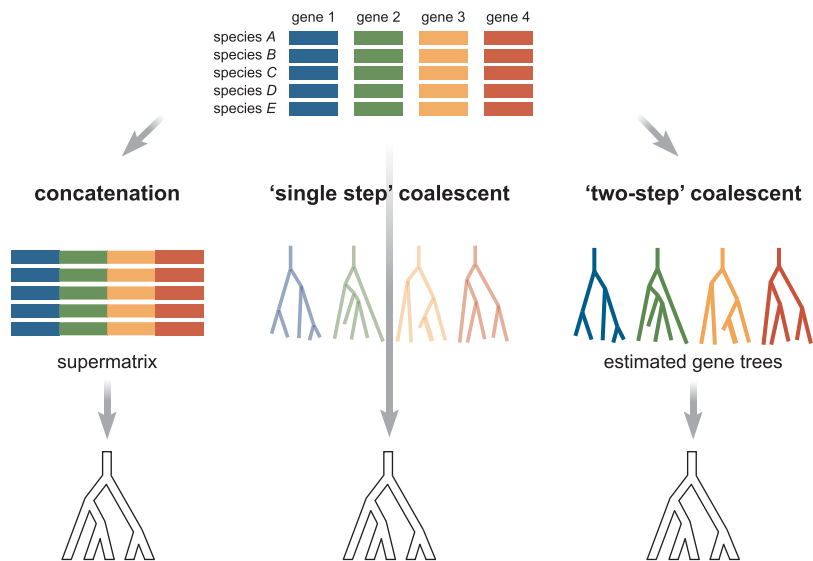
doi: 10.1111/nyas.12747

has not known any other means of modeling such variation—for decades such models made few inroads into phylogenetics. Even as heterogeneity in gene trees was acknowledged as a significant issue in phylogenetics,<sup>17</sup> concatenation or supermatrix methods provided the main paradigm in which phylogenetic models were developed to deal with diverse signals found in multilocus data.<sup>18</sup> Perhaps more intriguing, then, is that, even as such so-called “species tree” or coalescent models have been developed in the context of phylogenetics, they are being embraced by phylogeneticists cautiously,<sup>112</sup> or in some cases openly questioned.<sup>19,20</sup> Such caution and questioning is no doubt healthy, but it also suggests that, for some researchers, the heterogeneity observed in gene trees is either deemed unimportant or inconsequential for phylogenetic analysis, or that the models developed thus far to deal with this heterogeneity are unsatisfying, incomplete, or flawed.

Phylogenomic data have intensified debates over whether concatenation or coalescent methods in phylogenomics are more appropriate for analyzing multilocus sequence data.<sup>10,19,21,22,112</sup> Although recent phylogenomic studies suggest that the majority of relationships yielded by concatenation and coalescent trees are consistent with each other, or differ from each other without high statistical support,<sup>15,16,23,24</sup> recent examples of highly supported conflicting relationships favored by concatenation and coalescent methods have highlighted the details, weaknesses, and assumptions of both sets of methods.<sup>15,25–30</sup> The significant differences in performance of concatenation and coalescent methods in estimating species trees flow directly from the distinct assumptions on which the two methods are based.<sup>17,31,32</sup> Perhaps more importantly, it can be shown that, under certain conditions, the coalescent model reduces to the concatenation model<sup>22</sup> (see below), which can help explain the frequent similarities between concatenation and coalescent trees in empirical data analyses. Finally, in comparing concatenation and coalescent methods, important questions have been raised regarding the performance of coalescent methods under genetic forces such as within-gene recombination and gene flow, or issues with data, such as sampling effort and strategies, missing data, misrooting of gene trees, binning (concatenating) of multiple loci, or errors in gene tree estimation.<sup>19,20,33–37</sup> Whereas population

genetic effects such as gene flow and recombination rarely figured in discussions of phylogenetic models in the supermatrix era, recent discussions of these topics appear to be a direct result of the advent of coalescent methods and mark a growing appreciation by phylogeneticists of the links between population genetics and phylogenetics.<sup>38,39</sup> Overall, the current debates offer a glimpse of a field in transition, grappling with new signals and heterogeneity brought on by phylogenomics.

In this review, we discuss a number of recent trends in the application of coalescent models to phylogenetic analysis and address some recent criticisms of such models. At its simplest, the multispecies coalescent model (MSC) builds on the neutral coalescent model for single, isolated populations, in which the genealogy of sampled alleles follows probability distributions governed by simple demographic models, such as the Wright–Fisher model.<sup>40,41</sup> The MSC treats each branch in a species tree as a single, neutral coalescent population, and, going backwards in time, keeps track of the number of gene lineages entering extant or ancestral populations and exiting those populations as they merge with other ancestral populations in the species tree (Fig. 1). These gene lineages ultimately comprise gene trees, which in turn provide the signal for estimating the overarching species tree that is usually the primary interest (Fig. 1). Most coalescent models in phylogenomics assume simple models of instantaneous speciation, in which no gene flow occurs after species begin to diverge. Moreover, these models assume complete neutrality, no recombination within loci, and free recombination between loci, such that loci can be treated as independent neutral replicates conditional on the phylogenetic history of the lineages under study.<sup>40,41</sup> Several recent papers have shown that violations of these assumptions can have varying effects on the outcome of phylogenetic analysis (Table 1), with some more severe than others. The main criticisms of coalescent methods in phylogenetics—articulated most forcefully in a series of papers and comments by Gatesy and Springer<sup>19,20,25</sup>—focus on features of phylogenomic data sets that are perceived to violate the MSC. Such concerns focus on several issues, including the potential for recombination within loci, particularly for transcriptome data in which exons that might span megabases in the genome yet are concatenated together, either *in silico* or by the



**Figure 1.** Schematic of the concatenation and coalescent paradigms in phylogenetics. At the top is depicted a multilocus data set consisting of five species (A–E) and four genes (1–4). On the left is indicated the classic supermatrix approach, in which all genes are concatenated to produce a single supergene, which is then subjected to phylogenetic analysis by classical or updated traditional algorithms, such as RAxML,<sup>107</sup> ExaBayes,<sup>84</sup> or MrBayes.<sup>108</sup> Although the resulting tree at lower left is in truth a gene tree, it is often called a species tree or phylogeny because it is the result of analysis of a complete data set. In the center is depicted a class of species tree (coalescent) methods in which both gene trees and species trees are estimated concurrently according to multilocus sequence data, priors, and a multispecies coalescent likelihood model. Examples of algorithms estimating species trees in this way include \*BEAST<sup>49</sup> and BEST.<sup>109</sup> On the right are depicted so-called two-step species tree methods, in which gene trees are first estimated using classical approaches and then used as input data to estimate a species tree using algorithms such as MP-EST,<sup>48</sup> STAR,<sup>47</sup> STEM,<sup>87</sup> NJst,<sup>110</sup> STELLS,<sup>88</sup> or ASTRAL.<sup>89</sup> The methods depicted typically used sets of loci each consisting of linked DNA sites.<sup>2</sup> Yet other species tree methods such as SNAPP<sup>90</sup> and quartet inference<sup>92</sup> use unlinked SNPs rather than linked sites, and require different statistical models.

cell during the process of transcription; the claim that different species tree methods yield conflicting results when applied to the same data sets; the suggestion that most gene tree heterogeneity results from effects other than incomplete lineage sorting (ILS); and confusion over the effects and implications of low-resolution gene trees on species tree estimation. This last issue has also motivated the development of add-on methods to species tree estimation, such as naive and statistical binning,<sup>42,43</sup> whose aim is to augment phylogenetic signal when inferring species trees using coalescent methods.

Here, we discuss these various criticisms and concerns in an effort to clarify a host of issues raised about coalescent models in phylogenetics. We also highlight recent empirical studies that evaluate the signal in gene trees and explore their implications for the adoption or rejection of species tree methods. We show that concatenation methods can be considered a special case of the more general MSC. As such,

the more specific concatenation model is expected to yield results that are biased but with a smaller variance (e.g., higher bootstrap support) for the estimates of model parameters than the more general MSC—a perspective that helps explain recent trends in observed phylogenomic data sets. We also suggest that naive binning—a proposal to augment the signal in multilocus data sets by concatenating genes at random into bins—will only work under highly restricted conditions, and that the lower performance of coalescent methods versus concatenation methods claimed in those studies reflects an undue focus on point estimates, a restricted set of simulation conditions, an undervaluation of the variance of those estimates, and the often inflated support of concatenation analyses. Overall, we find that currently proposed species tree methods represent a promising start to the challenge of analyzing phylogenomic data and highlight conceptual and practical challenges for the future.

**Table 1.** Studies evaluating the robustness of species tree phylogenetic methods to various genetic forces and sampling schemes

Topic	Conclusions/comments <sup>a</sup>
General violation of multispecies coalescent model <sup>104</sup>	<ul style="list-style-type: none"><li>• Claims the majority of multilocus sequence data sets are a poor fit to the multispecies coalescent model, although much of the violation stems from fit of substitution model or unknown sources on a minority of genes.</li></ul>
Gene flow <sup>73,105</sup>	<ul style="list-style-type: none"><li>• The coalescent method is robust to low levels of gene flow</li><li>• Concatenation performs poorly relative to the coalescent methods in the presence of gene flow.</li><li>• Gene flow can lead to overestimation of population sizes and underestimation of species divergence times in species trees.</li></ul>
Sampling/mutation <sup>33,74,94</sup>	<ul style="list-style-type: none"><li>• Increased sampling of individuals per species can significantly improve the estimation of shallow species trees.</li><li>• Sampling more individuals does not significantly improve accuracy in estimating deep species trees. Adding more loci can improve the estimation of deep relationships.</li></ul>
Recombination <sup>39,106</sup>	<ul style="list-style-type: none"><li>• Mutational variance is a major source of error in estimates of species trees.</li><li>• Recombination has minor effect on species tree estimation except on extremely short species trees.</li><li>• The negative effects of recombination can be easily overcome by increased sampling of alleles</li></ul>
Missing data <sup>19,63,67</sup>	<ul style="list-style-type: none"><li>• Missing data can decrease the support of species tree estimates</li><li>• Missing data can significantly affect the accuracy of species tree estimation</li><li>• Species tree methods are “remarkably resilient” to missing data<sup>68</sup></li></ul>
Taxon sampling <sup>15</sup>	<ul style="list-style-type: none"><li>• Compared to concatenation, coalescent methods are more robust to poor taxon sampling</li></ul>
Long-branch attraction <sup>29</sup>	<ul style="list-style-type: none"><li>• Species tree methods are more resilient to the effects of long-branch attraction than concatenation methods</li></ul>
Random rooting of gene trees <sup>36,37</sup>	<ul style="list-style-type: none"><li>• Misrooting of gene trees can mimic the coalescent process</li></ul>
Other <sup>80</sup>	<ul style="list-style-type: none"><li>• Anomalous gene trees are unlikely to pose a significant danger to empirical phylogenetic study, in part because species trees in the anomaly zone are likely to be rare.</li></ul>

<sup>a</sup>Conclusions of different papers are presented as simplified bullets. In some cases, conclusions of different papers may conflict, likely due to differences in simulation parameters and assumptions. Comments on papers are the opinion of the authors.

**Concatenation versus coalescent models in phylogenomics**

The coalescent and concatenation models differ in their treatment of individual gene trees. In the coalescent model, which assumes free recombination among genes, the gene trees are treated as conditionally independent random variables  $G = \{g_i, i = 1, \dots, k\}$  given the species tree  $S$ ,<sup>40</sup> where  $k$  is the number of genes. However, independent gene trees may have the same topology, especially when the species tree has long internal branches in coalescent units.<sup>44,45</sup> Under the coalescent model, the

likelihood function of the species tree  $S$  given the multilocus sequence data  $D = (d_1, d_2, \dots, d_k)$  is given by

$$L(S|D) = \int_G f(D|G, \lambda) \times \phi(G|S) dG \quad (1)$$

In Eq. (1),  $f(D|G, \lambda)$  is the probability density function of sequence data  $D$  given gene trees  $G$  and parameters  $\lambda$  in the substitution model, and  $\phi(G|S)$  is the coalescent distribution function of gene trees  $G$  given the species tree  $S$ . The function  $f(D|G, \lambda)$  is the traditional likelihood function used for

building maximum-likelihood (ML) gene trees. When gene trees  $G$  are identical with the species tree  $S$  (i.e.,  $g_1 = g_2 = \dots = g_k = S$ ), the coalescent probability function  $\phi(G|S)$  is 1 for  $G = S$  and 0 otherwise. Therefore, when all gene trees are identical with the species tree  $S$ , the likelihood function of the species tree  $S$  is reduced to

$$L(S|D) = f(D|S, \lambda) \quad (2)$$

As model parameters  $\lambda$  may or may not be linked across genes (i.e., partitions),  $f(D|S, \lambda)$  in Eq. (2) is the likelihood function for the concatenation model with or without partitions. Equation (2) shows that the concatenation model with or without partitions is a special case of the coalescent model. This result has at least two important implications: (1) although concatenation methods may produce inconsistent estimates of species trees under the coalescent model,<sup>32</sup> coalescent methods can consistently produce the true species tree under the concatenation model; and (2) the comparison between the coalescent and concatenation methods falls into the general bias-variance dilemma (i.e., the reduced model (concatenation) in general is biased and has smaller variance for the estimates of model parameters<sup>46</sup>).

As a reduced model, concatenation has a smaller number of parameters, because all gene trees in the concatenation model are treated as the same variable. Thus, the estimates of parameters in the concatenation model tend to have a smaller variance. Since small variance corresponds to high bootstrap support or posterior probability, overestimation of bootstrap support by concatenation methods is a consequence of the fact that it is a reduced model. Moreover, two-step, gene-tree-based coalescent methods—those methods that estimate the species tree using separately estimated gene trees<sup>47,48</sup> (Fig. 1)—can in turn result in larger variance for their estimates of species trees, because these methods estimate gene trees and the species tree separately using summary statistics or a pseudolikelihood function. This is the same as saying that gene-tree-based coalescent methods may sometimes result in bootstrap support that is lower than those coalescent methods using the full coalescent model, such as BEST<sup>31</sup> and \*BEAST.<sup>49</sup> (Indeed, we have observed this empirically for the 30-locus data set from birds<sup>50</sup> analyzed by Liu and Pearl,<sup>31</sup> which yielded a posterior probability of 0.9

when analyzed by BEST but only ~54% bootstrap support when analyzed by the simpler model in STAR<sup>47</sup>). However, the problem of larger variance in gene-tree-based coalescent methods can be greatly alleviated by either improving the efficiency of these methods or by increasing the number of genes. Because phylogenomic data often contain hundreds of genes, species tree analyses using gene-tree-based coalescent methods have produced highly supported species trees for empirical phylogenomic data.<sup>15,24,27,28,51</sup> In contrast, inconsistency of concatenation tends to become severe when there are a large number of genes.<sup>32</sup> Thus, the major concern of phylogenomic data analysis is not the high variance of gene-tree-based coalescent methods,<sup>112</sup> but rather the inconsistency of concatenation methods due to model misspecification,<sup>48</sup> especially in cases where phylogenomic inferences are based on highly supported relationships.

In empirical phylogenomic data analyses, concatenation and coalescent methods often produce similar relationships for the majority of branches in the estimated trees. As discussed above, when the concatenation model applies, we do not expect to encounter highly supported relationships that conflict between concatenation and coalescent trees, because with the assumption of identical gene trees, the coalescent model is reduced to the concatenation model. Thus, highly supported but conflicting relationships in concatenation and coalescent trees, as has recently been observed in several phylogenomic data analyses,<sup>15,28,52</sup> indicate either a high amount of ILS (owing to short internal branches (in coalescent units) in the species tree) that misleads concatenation methods<sup>15</sup> or that the major cause of gene tree variation is not ILS. In the latter case, neither concatenation nor coalescent models can adequately explain the distribution of heterogeneous gene trees.

## Empirical examples of conflict between coalescent and concatenation methods

### *Coalescence versus concatenation in plant phylogenomics*

To date, very few studies have utilized coalescent methods for inferring plant phylogenies.<sup>34,53–55</sup> Of these studies, strongly conflicting relationships involving concatenation versus coalescent analyses have recently been demonstrated for key nodes in land plant phylogeny. (When we write “strongly”

we typically mean supported by a bootstrap support of at least 75% to >90%, depending on context). Despite tremendous effort, relationships between the five main seed plant clades—flowering plants, conifers, cycads, *Ginkgo*, and gnetophytes—have remained uncertain. A first broad coalescent analysis of seed plants by Xi *et al.*<sup>27</sup> incorporated 305 nuclear genes and suggested an explanation for why concatenation methods may result in strong topological incongruence, manifested as phylogenetic “flip-flops” between analyses involving different subsets of data. Unlike most previous analyses using concatenation, which have strongly placed cycads and *Ginkgo* as successive sisters to the remainder of extant gymnosperms, coalescent results instead strongly identified *Ginkgo* and cycads as monophyletic. Suspecting that the rate of nucleotide substitution might be influencing this difference, Xi *et al.*<sup>27</sup> binned sites into fast- and slow-evolving categories and reanalyzed these data. Coalescent analyses continued to support the monophyly of *Ginkgo* and cycads regardless of rate category, but concatenation did not. Instead, fast-evolving sites strongly supported the more traditional placement of cycads and *Ginkgo*, whereas slow-evolving sites supported the placement inferred from coalescent methods. This finding raised the hypothesis that rate variation among sites may explain the striking topological differences observed in concatenated analyses, reflecting problems commonly encountered in, for example, cases of long-branch attraction.<sup>56,57</sup> Furthermore, more recent coalescent analyses using expanded taxon sampling and transcriptome data corroborated the monophyly of *Ginkgo* and cycads.<sup>54</sup>

In a second paper, Xi *et al.*<sup>28</sup> further explored this hypothesis by examining a slightly larger phylogenomic data set including 310 nuclear genes, mostly from flowering plants. Here, phylogenetic relationships were congruent between concatenation and coalescent methods, except for the placement of *Amborella*, which has long been heralded as the sister to all other flowering plants.<sup>58,59</sup> Here, coalescent analyses consistently and strongly support the less traditional placement of *Amborella* as sister to water lilies across all nucleotide-rate partitions. In contrast, concatenation showed the same kind of strongly conflicting results that were observed in the earlier study involving the placement of *Ginkgo* and cycads: slow-evolving sites corroborated the

results from coalescent analyses and fast-evolving sites placed *Amborella* alone as the first lineage of extant plants. An additional assessment of these fast-evolving sites showed particularly strong evidence of saturation, suggesting one explanation for these artifacts. In this case, it appears that distributing saturated sites among many individual gene trees and analyzing each separately, as occurs in coalescent methods, may be more effective at diluting the deleterious effects of such characters. In contrast, when analyzing all such sites simultaneously within a single super matrix, as is done using concatenation, such deleterious effects will be exacerbated. We should note that this question has been reevaluated more recently with coalescent analyses using expanded taxon sampling and transcriptome data.<sup>54</sup> This study strongly supports the more traditional placement of *Amborella* alone. However, one of us (Z.X., unpublished data) has re-analyzed the 455 transcripts common to both *Amborella* and water lilies. Here again, slow-evolving nucleotide and amino acid sites strongly support the placement of *Amborella* and water lilies as monophyletic.

Xi *et al.* investigated this placement of *Amborella* further with simulations. The first of these simulations used the *Amborella* data set. Here, they randomly constrained each gene tree to be consistent with one of the alternative placements of *Amborella*. Branch lengths and substitution model parameters were then estimated on each constrained gene tree from the original data. Data sets were then simulated on these gene trees and the resulting simulated data were binned into fast- and slow-evolving sites. Coalescent analysis of these data reconstructed the constrained topologies as expected, regardless of rate category. Surprisingly, however, despite 60–80% of the gene trees constrained to the *Amborella*-plus-water-lilies placement, concatenation of the fast-evolving sites still resulted in the placement of *Amborella* and water lilies as successive sisters to all remaining flowering plants. The fact that the concatenated data reconstructs the traditional *Amborella*-alone topology, even when the fast-evolving sites come from a tree in which *Amborella* and water lilies are sisters, shows that concatenation of fast-evolving sites likely plays a strong role influencing the misleading placement of *Amborella*.

Additional simulations<sup>28</sup> on species trees with both long and short branches suggested that, when



ILS is high, concatenation methods perform very poorly, suggesting that concatenation may strongly be influenced by the shape of the species tree topology and its interaction with ILS. A related follow-up simulation study<sup>29</sup> suggests that, when long external and short internal branches occur simultaneously with high ILS, concatenation methods can be misled, especially when two of these long branches are sister lineages. In contrast, species tree methods (in this case MP-EST<sup>48</sup> and STAR<sup>47</sup>) are more robust under these circumstances. This result is particularly relevant because many ancient radiations across the tree of life suggest this particular pattern of adjacent long and short branches. Because short internal branches in the species tree can increase the potential for ILS and gene tree discordance, these results indicate that coalescent methods are more likely to infer the correct species tree in cases of rapid, ancient radiations where short internal and long external branches are in close phylogenetic proximity.

#### *Coalescence versus concatenation in mammal phylogenomics*

Despite recent progress in classifying eutherian mammals into four superorders—Afrotheria, Xenarthra, Laurasiatheria, and Euarchontoglires—several key relationships within eutherian mammals remain controversial, including the root of Eutheria and the interordinal relationships within Euarchontoglires, Laurasiatheria, and Afrotheria.<sup>60,61</sup> To date, however, the reconstruction of mammalian phylogeny has relied mostly on concatenation methods, which, as we indicate above, may suffer from systematic bias owing to the unrealistic assumption of gene tree homogeneity across loci. To empirically address the effect of gene tree heterogeneity on estimating deeply diverging phylogenies, Song *et al.*<sup>15</sup> took the approach of subsampling loci and taxa so as to investigate the robustness of concatenation and coalescent methods to different analyses of the same taxa. Using a data set of 447 nuclear genes for 35 mammalian taxa, Song *et al.* demonstrated that concatenation indeed behaves inconsistently across data sets, as evidenced by the conflicting and strongly supported relationships from different subsamples of loci. In contrast, coalescent methods were able to estimate a consistent phylogeny for eutherian mammals from the same subsets of data, and demonstrated a clear positive relationship between

nodal support values and the number of loci. In this regard, the study on mammals was consistent with predictions of a recent simulation study that showed a correlation between number of loci and species tree support for nodes exhibiting high rates of ILS.<sup>35</sup>

Several studies,<sup>63</sup> including the mammal study,<sup>15</sup> have revealed the sometimes striking contrast between support values for trees analyzed by concatenation versus coalescent methods, with the latter often yielding much lower values, even when the tree is largely similar. It is often the case that a tree that appears well resolved by concatenation methods is found to be poorly resolved using coalescent methods. So which set of support values better reflects reality? Such results likely reflect the tendency for concatenation methods, especially Bayesian concatenation methods, to overestimate credibility values in phylogenetic trees.<sup>63,64</sup> They may also partly reflect the tendency of two-step coalescent methods to yield lower confidence levels than full Bayesian coalescent methods when the number of gene trees is small (see above). Differences in the method of bootstrapping may also contribute to these discrepancies; the multilocus bootstrap<sup>65</sup> is generally believed to more accurately capture support in large data sets than the simple bootstrap.<sup>20</sup> In the study by Song *et al.*,<sup>15</sup> the 26 genes that resolved a sample of mammalian taxa using concatenation resulted in a poorly resolved tree when analyzed by MP-EST. They suggested that, in a coalescent framework, approximately 400 genes would be required to resolve the sample of taxa in the particular tree for mammals. In this case, missing data may also play a role, since coalescent methods appear to be more sensitive to missing data than concatenation methods.<sup>62</sup> However, a simulation study<sup>66</sup> recently suggested that species tree methods were “remarkably resilient to the effects missing data” (Table 1). In our view, the sensitivity to missing data displayed by species tree methods reflects the true impact of missing data on phylogenetic analysis—an impact that is obscured by analyses employing concatenation. There are now many examples of well-resolved phylogenies employing concatenation on highly incomplete data sets.<sup>55,67,68</sup> We believe these examples illustrate the power of concatenation to obscure the true support for trees by the collected data (see also Ref. 69).

## Maximizing signal in species tree analysis

Species tree analysis has moved past the stage of uncritical adoption to evaluation of sampling strategies and methods for maximizing phylogenetic signal.<sup>33,38,70–73</sup> Much of our understanding of the behavior of species tree reconstruction comes from simulation studies, although analyses of empirical data have also yielded important insights. An important rule of thumb that has emerged from both simulation and empirical studies is that species trees are only as good as the gene trees on which they are built.<sup>14,38,62,70,74,75</sup> This maxim applies both to two-step species tree methods, in which gene trees are used as input data, and to single-step approaches, such as Bayesian methods, in which gene and species trees are estimated simultaneously. For example, several empirical studies on organisms as varied as turtles, mammals, fish, and flowering plants have shown that species tree estimation can be misled by biased gene tree estimation due to long-branch attraction and base compositional heterogeneity among lineages, a manifestation of substitution model nonstationarity.<sup>12,14,28</sup> Even so, recent work suggests that species tree methods, even those in which gene trees are estimated first and separately from the species tree, may be less susceptible to classic challenges in phylogenetic analysis, such as long-branch attraction (Table 1).<sup>29</sup> This lowered susceptibility of species tree methods may be due to the fact that a typical gene tree is based on at most a few thousand base pairs, which may be small enough such that departures from stationarity may be less visible in the underlying data sets. Although it is valid to criticize the ensemble of constituent approaches that constitute gene tree and species tree analysis, including gene tree and species tree reconstruction, it is unwarranted to criticize species tree methods per se, especially when it is the reconstruction of gene trees that is responsible for misestimation.<sup>20</sup> Thus, a number of authors have suggested improving and maximizing signal in gene tree estimation as a means of improving species tree estimation as a whole. Such improvements in signal take a variety of forms, including binning of subsets of genes, using longer or more informative genomic regions for each locus,<sup>2,15,30,75</sup> minimizing base compositional heterogeneity among lineages, and even choosing genes with specific trends in base composition, for example those genes trending

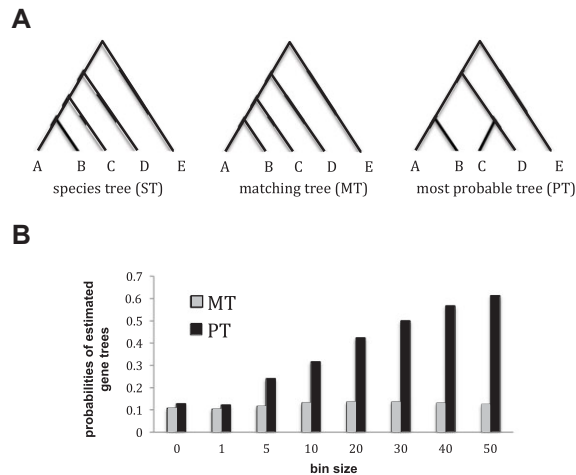
toward AT richness in mammals.<sup>76</sup> In this section, we evaluate a variety of suggestions for signal enhancement in species tree analysis.

### *Naive and statistical binning*

The naive binning technique was proposed to improve the support of coalescent estimates of species trees by reducing the estimation error of gene trees.<sup>43</sup> This technique concatenates DNA sequences across randomly selected genes, regardless of whether the selected genes share the same history. The binned sequences are treated as a *supergene*, and used to estimate supergene trees. As the binned sequences are longer than the original data, the resulted gene trees are often well supported.<sup>43</sup> However, as shown by several groups,<sup>32,77</sup> binning sequences from genes with distinct histories can mislead ML concatenation methods, which consistently produce the wrong estimate of the species tree under broad conditions. This inconsistency problem may also occur for the supergenes in the binning technique. Indeed, although the paper title implied that binning yielded a general improvement for phylogenomic data (“Naive binning improves phylogenomic analyses”), in the paper, the authors were more equivocal (“This paper should not be interpreted as recommending the use of naive binning, but instead as an indication of the potential for binning techniques to improve species tree estimation” (p. 2284)). From first principles, we know that, for the case of a four-taxon species tree (see Fig. 3F in Kubatko and Degnan<sup>32</sup>) when the bin size (BS) is large, all of the concatenated genes (i.e., supergenes) will have the same ML tree, a tree that is incongruent with the species tree. These biased supergene trees can significantly mislead the MP-EST estimate of the species tree. Even when the BS is small (10–15 genes), binning sequences can increase the probability of estimating gene trees that disagree with the species tree. When this probability is greater than a threshold, it will mislead the MP-EST and other methods, causing them to consistently produce the wrong estimate of the species tree.

To demonstrate these phenomena, we simulated gene trees from a five-taxon species tree (labeled species A–E, Fig. 2A) under the coalescent model using the function *sim.coaltree.sp* in the R package Phybase.<sup>78</sup> Because the MP-EST method assumes that gene trees are rooted trees, species E is used as





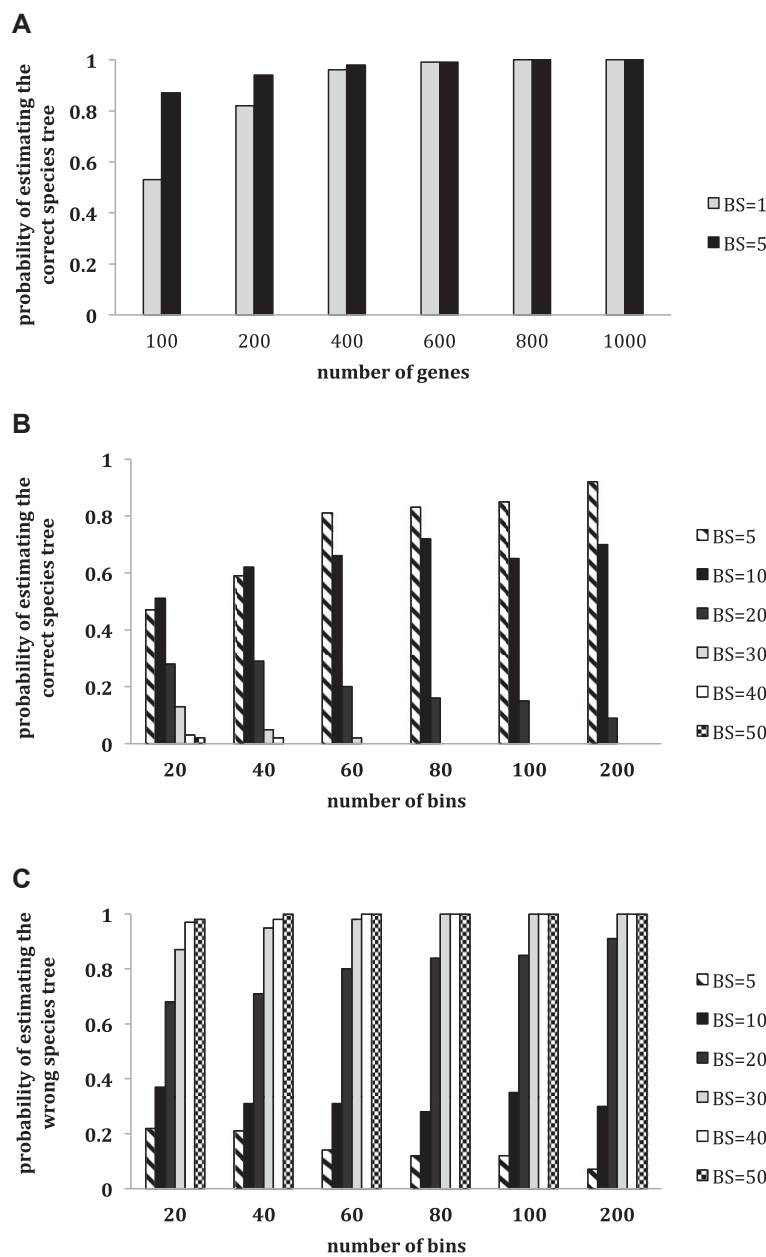
**Figure 2.** Inconsistency of the binning technique. (A) The species tree used for simulating gene trees. In this species tree (((A:0.01, B:0.01):0.005, C:0.015):0.005, D:0.02):0.08, E:0.1), species E is used as the outgroup. To reduce the rooting error, we set a small population size parameter ( $\theta = 0.01$ ) and a long internal branch (length = 0.08) between ingroup species A–D and the outgroup species E. The population size parameter is  $\theta = 0.1$  for other ancestral populations in the species tree. (B) The probabilities of two estimated gene trees for binned genes. We consider only two trees, the matching tree (MT) and the most probable tree (PT). The bars at 0 represent the true (coalescent) probabilities of MT and PT generated from the species tree under the multispecies coalescent model.

the outgroup for rooting the estimated gene trees. To reduce the rooting error, we intentionally set a small population size parameter  $\theta = 0.01$  and a long internal branch (length = 0.08) between the ingroup species and the outgroup species E. Under these conditions, the ingroup species almost always form a monophyletic group in the simulated gene trees. In addition,  $\theta$  is set to 0.1 for other ancestral populations in the species tree. This species tree is in the anomaly zone, because the internal branches for species A, B, C, and D are very short (0.005/0.1 = 0.05 in coalescent unit), and the most probable gene tree (PT) does not match the species tree.<sup>79</sup> Although species trees in the anomaly zone might be considered unrealistic,<sup>80</sup> recent empirical examples seem to suggest otherwise.<sup>52</sup> DNA sequences of length 1000 base pairs (bp) were generated from the simulated gene trees using SeqGen<sup>81</sup> with the Jukes–Cantor (JC69) model.<sup>82</sup> The simulated DNA sequences were binned at random to form supergenes. The BS was set to 10, 20, 30, 40, and 50 genes, respectively. A ML supergene tree was estimated for each bin by PhyML.<sup>83</sup> The estimated supergene trees were then used to estimate species trees using the MP-EST method. Each simulation was repeated 100 times.

When the BS is 1 (i.e., no binning was performed), the probabilities of the estimated gene trees are

similar to the true probabilities of gene trees generated from the species tree (Fig. 2B). However, the probability of the PT significantly increases as the BS increases (Fig. 2B). When the BS is 30, almost half of the estimated supergene trees are PT, which is incongruent with the species tree (Fig. 2B). It is clear from this exercise that binning sequences from genes with distinct histories can bias the distribution of the estimated gene trees, with a high probability of producing the tree that is incongruent with the species tree.

Without binning (i.e., BS = 1), the MP-EST method can consistently estimate the correct species tree as the number of genes increases (Fig. 3A). The proportion of trials yielding the correct species tree appears to increase as the number of genes increases, and reach 1.0 when the number of genes is 1000. When the BS is 5, the probabilities of estimating the correct species tree are greater than those without binning. This result indicates that, when the BS is 5, binning can improve the performance of MP-EST in estimating this particular species tree. However, when the BS is greater than or equal to 10, the probability of estimating the correct species tree is in general less than the probability without binning (Fig. 3B). Moreover, the probability of estimating the correct species tree appears to decrease as the number of bins (i.e., the number of supergenes) increases (Fig. 3B). For BSs of 30, 40, and 50,



**Figure 3.** The probability of estimating the correct and incorrect species trees under different conditions. (A) For bin size (BS) = 1 (no binning) and 5, the probabilities of estimating the correct tree increase to 1 as the number of bins increases. (B) For BS = 10, the probability of estimating the correct tree is stable around 0.6 as the number of bins increases. (C) In contrast, the probabilities of estimating the incorrect species tree for BS = 20, 30, 40, and 50 increase to 1 as the number of bins increases.

the probability of yielding the correct species tree decreases to zero when the number of bins reaches 80 (Fig. 3B). Meanwhile, when the BS is greater than 10, the MP-EST tree based on the binned genes appears to consistently estimate the incorrect species tree as the number of bins increases (Fig. 3C). Interestingly, for the simulation parameters studied here, for a BS of 10, the probability of the correct species tree stabilizes around 0.6, while the probability of an incorrect species tree stabilizes around 0.4. Although this result suggests that when the BS is less than 10, the binning technique can

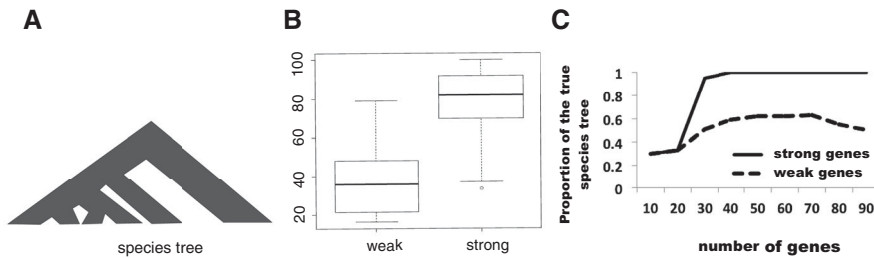
be beneficial for improving the performance of the MP-EST method in estimating species trees, additional simulation results suggest that this may not always be the case (not shown). Further research on naive binning is needed. We can certainly state that, when the BS is greater than 10, binning sequences across genes with distinct histories can significantly bias the distribution of estimated gene trees and result in inconsistent estimates of species trees.

Ideally, loci should be concatenated if no or only a few recombination events occurred between those loci. A model based on biology would suggest that binning should be based on loci that are closely linked in genomes, such as often occurs in transcriptomes, because the chance of recombination is positively related to the physical distance between two loci. Recently, Mirarab *et al.*<sup>42</sup> proposed a statistical binning technique that attempts to bin loci with the same gene tree. In this approach, loci are binned when there are no strongly supported topological conflicts among the estimated gene trees of those loci, for example, when the gene trees do not conflict on branches with >75% bootstrap support. However, the statistical properties of binning have not yet been fully explored. As discussed in the previous section, the assumption of free recombination between genes and no recombination within genes plays a key role in the MSC. Loci are treated as conditionally independent, owing to the assumption of free recombination between genes. Two loci may have the same gene tree even though they are conditionally independent. This may occur, for example, when the species tree has long internal branches (in coalescent units). When the genes have the same history, binning their sequences can improve gene tree estimation, but it also reduces the sample size of independent genes. Thus, from first principles, we can state that binning sequences from genes with the same history may not necessarily improve species tree estimation. In addition, two gene trees with no strongly supported conflicts do not necessarily indicate that they are topologically identical with each other. As we discussed for the naive binning, combining loci with different histories may seriously bias the distribution of the estimated gene trees, which in turn will mislead species tree estimation. In conclusion, further studies are needed to evaluate the performance of statistical binning.

### *Information content of genes for species tree reconstruction*

Despite the recent arrival of genome-scale Bayesian phylogenetic methods for concatenation,<sup>84</sup> the Bayesian or approximate Bayesian coalescent model cannot presently be applied to phylogenomic data owing to excessive computational burden.<sup>31,49,85</sup> Alternative gene tree approaches, including MP-EST,<sup>48</sup> GLASS,<sup>86</sup> Maximum Tree,<sup>111</sup> STAR,<sup>47</sup> STEM,<sup>87</sup> STELLS,<sup>88</sup> ASTRAL,<sup>89</sup> and STEAC,<sup>47</sup> build species trees from estimated gene trees. These approaches have computational advantages that allow them to be used for phylogenomic data analyses<sup>75</sup> and novel molecular markers, such as ultraconserved elements.<sup>2,23</sup> Some of these methods (e.g., Refs. 47, 48, 86, 87, and 88) use biological-informed models, often simplified from the full MSC model; others, such as ASTRAL,<sup>89</sup> are not biologically motivated but rather minimize a specific function. Additionally, recent species tree methods utilizing information from SNPs<sup>90,91</sup> and haplotypes<sup>92</sup> may be scalable to large data sets. However, the species tree estimates given by gene-tree-based approaches often suffer the problem of big variance (i.e., low bootstrap support). As these approaches employ bootstrap techniques to account for errors in estimating gene trees, large estimation error for gene trees can greatly lower the bootstrap support of the species trees estimated by those approaches.<sup>33</sup> Moreover, these approaches estimate species trees based only on the topologies of gene trees, ignoring the branch-length information, which further reduces the efficiency of those approaches. The simulation study by Huang *et al.*<sup>33</sup> suggests that high amounts of gene tree estimation error may be the major cause of low bootstrap support in estimates of species trees using STEM (Table 1). It has been suggested by empirical studies that using highly supported gene trees (average bootstrap support value >0.5) can improve bootstrap support of species trees estimated by gene-tree-based approaches,<sup>15,21</sup> and adding poorly supported gene trees (i.e., weak genes) does not contribute more information regarding the phylogeny of species. We evaluated this hypothesis through a simple simulation analysis.

To evaluate the effect of weak genes on the performance of gene-tree-based approaches in estimating species trees, DNA sequences were simulated from the true species tree (((A:0.002,



**Figure 4.** The effect of noninformative genes on the performance of gene-tree-based approaches in estimating species trees. DNA sequence data were simulated from the true species tree. Sequences of 1000 base pairs were generated for strong genes, and 100 base pairs were generated for weak genes. (A) The species tree used in the simulation. (B) The box plot of the average bootstrap support values for weak and strong genes. (C) The effect of different numbers of strong and weak genes on the performance of MP-EST in estimating species trees. See text for further explanation.

B:0.02):0.002, (C:0.002, D:0.002):0.002):0.002, E:0.006):0.01, F:0.016) with  $\theta = 0.008$  (Fig. 4A). The population size parameter  $\theta$  is constant across branches of the species tree. Specifically, gene trees were generated from the species tree under the MSC, again using Phybase.<sup>78</sup> DNA sequences were generated from the simulated gene trees using SeqGen<sup>81</sup> with the JC69 model.<sup>82</sup> We generated 1000 bp for strong genes, and 100 bp for weak genes. The average bootstrap support values of strong genes range primarily from 70% (first quantile) to 91.75% (third quantile), with median = 81.83% (Fig. 4B), whereas the average bootstrap support values for 100-bp genes are mostly <50% (Fig. 4B). Thus, we selected 100-bp genes with bootstrap values <50% as weak genes. Species trees were reconstructed from 10 to 90 estimated gene trees (in increments of 10) for strong genes using MP-EST. Each simulation was repeated 100 times. The proportion of trials estimating the true species tree was 0.33 for 30 strong genes, and it increased to 1.0 when the number of strong genes was 40 (Fig. 4A). However, when adding weak genes to the set of 30 strong genes, the proportion of trials estimating the true species tree increased slowly to 0.63 and then decreased to 0.50 (Fig. 4B). This result suggests that adding weak genes may not contribute more information to an otherwise strong data set when estimating species trees. When adding weak genes, the distribution of estimated gene trees becomes flat and the coalescent signal contained in the estimated gene trees is significantly reduced. Thus, adding weak genes may actually reduce the performance of species tree estimation methods, negating the old adage that “more data is always better.”

### *Transcriptomes, base composition, and location-aware concatenation*

Analysis of transcriptomes provides an important type of data for estimating species trees. Because of their ease of alignment and characterization, transcriptomes will continue to be an important data type for many kinds of phylogenetic analysis. However, because transcriptomes consist solely of coding regions, they are potential targets of natural selection.<sup>93</sup> Indeed, genome-wide phylogenetic comparisons in primates have shown that the rate of ILS in coding regions is lower than that in non-coding regions.<sup>93</sup> This higher incidence of reciprocal monophyly and lower incidence of ILS in coding regions is likely driven by recurrent bouts of positive selection on coding regions, with surrounding noncoding regions adhering to patterns more consistent with neutrality. Whether or not this type of departure from the neutral coalescent will be problematic for species tree analysis is unclear, because by lowering the rate of ILS, natural selection could help eliminate some of the discordance that is known to decrease phylogenetic signal in species tree analysis.<sup>47,94</sup> However, recent genome-wide analyses of avian phylogeny suggest substantial convergence in protein-coding regions, resulting in species trees that are clearly incongruent with non-coding genomic partitions.<sup>16</sup>

Transcriptomes are also characterized by strong base compositional biases, and it is well known that the third positions of nuclear genes can evolve rapidly and become misleading over long time scales. Departures from base compositional stationarity in phylogenomic data sets have long been known to cause serious problems for

phylogenetic analysis, potentially linking together lineages that are unrelated but share similar base compositions.<sup>16,95–97</sup> Variation in base composition among taxa is known for both transcriptome data and whole-genome data. Additionally, it is well known that different subgenomes can possess different base compositions; for example, coding regions are generally more GC-rich than noncoding regions. Examination of variation in base composition among lineages and genes as well as saturation patterns in different data partitions has shed some light on optimal choice of markers for species tree analysis. For example, Chiari *et al.*<sup>14</sup> showed that species tree analysis of turtle relationships changed dramatically depending on whether amino acid sequence or nucleotide sequence data were used to build gene trees, with amino acid data sets providing more congruent results. Betancur-R *et al.*<sup>12</sup> showed convincingly that substantial apparent gene tree heterogeneity in fish data sets arises from misestimation of gene trees, most likely owing to base compositional heterogeneity among lineages. They found that choosing sets of genes with base compositional homogeneity among lineages substantially reduced the apparent evidence for gene tree heterogeneity and ILS. Although we view this result as significant, we suggest that it does not invalidate the use of species tree methods or necessarily support supermatrix approaches, because species tree methods do not require gene tree heterogeneity to work well. A final example in which base composition plays a role in species tree reconstruction was provided by Romiguier *et al.*,<sup>77</sup> who found that the gene trees in mammals based on GC-rich coding regions were more heterogeneous than those with an AT bias. Moreover, the species tree suggested by AT-rich data sets favored the Afrotheria hypothesis, with Afrotheria as the first branch within placental mammals, whereas those based on the more heterogeneous GC-rich data sets favored the Atlantogenata rooting, in which Xenarthra and Afrotheria are sister groups. These authors suggested that the way forward was not so much to account for ILS using novel phylogenetic methods as to reduce its incidence by using particular sets of markers. Again, although we do not dispute the observation of lower gene tree heterogeneity in AT-rich markers, this does not mean that supermatrix approaches are necessarily favored. So long as different genes are conditionally independent from one another owing

to recombination, we suggest that species tree analysis will still accumulate phylogenomic signal very differently, and we hypothesize more accurately, than will concatenation approaches. Species tree approaches are not invalidated by the absence of ILS; rather, they represent a fundamental recognition of the importance of stochasticity from gene to gene that is unaccounted for by concatenation methods, even when ILS is low or absent.

An important issue in the ongoing discussion of concatenation versus coalescent methods is whether the location-aware concatenation of exons in transcriptome data is reasonable or whether it violates the MSC.<sup>25</sup> Location-aware concatenation occurs when adjacent exons or genomic regions are concatenated to one another. This type of concatenation has biological realism insofar as adjacent regions of the genome are known to be correlated in their historical ancestry, with stretches of chromosomes yielding information suggesting similarity in gene trees as one moves along the chromosome. On the one hand, transcriptome data have been shown in multiple studies to yield phylogenetic trees produced by MSC models that are either congruent with previous results or provide novel hypotheses that are plausible (e.g., turtles, plants). On the other hand, transcriptome data are indeed concatenated by cells when converting pre-mRNAs into the mature mRNAs that are often used in phylogenomic studies. A key difference between the location-aware concatenation performed by cells and the naive binning recommended by Bayzid and Warnow<sup>43</sup> is that, in location-aware concatenation, exons that are adjacent to one another in the genome are concatenated, whereas binning approaches are agnostic as to the location of binned genes in the genome. Still, exons that are concatenated by cells can still occur at varying distances from one another in the genome, and may experience levels of recombination in their history that rivals those experienced by loci located at a distance in the genome. However, as Lanier and Knowles<sup>39</sup> point out, recombination will only be a challenge on extremely short internal branches of the species tree; recombination occurring on long branches will involve sequences closely related within species and will provide discordant signal primarily when taking place in common ancestral species. The empirical effects of varying genomic distance on the variety of observed gene trees need to be studied in

more detail. Using a variety of statistical methods, several studies, particularly in primates and rodents, have found a patchwork of gene tree signals in chunks when moving along a chromosome.<sup>98–100</sup> Methods for delimiting genomic segments that display consistency of phylogenetic signal are emerging, and these may prove extremely useful for delimiting loci for species tree analysis and minimizing the negative effects of recombination. In general, however, when faced with insufficient signal in a species tree analysis, we advocate increasing the size of phylogenomic data sets<sup>2</sup> rather than pseudoconcatenation or naive binning as a means of augmenting signal. Despite the plethora of large-scale phylogenomic studies, empiricists have not yet exhausted phylogenetic information, and until then, data collection, rather than binning uninformed by genomic context, should be the method of choice for data augmentation.

### *Computational trade-offs in species tree analysis*

In addition to concerns about marker choice and the statistical properties of species tree estimation methods, another major concern is computational cost. When phylogenomic data include thousands of genes, the total length of the concatenated sequences will begin to explode, resulting in an extremely high computation burden when using concatenation methods. For such data sets, it is practically impossible to perform bootstrap concatenation analyses (with just 100 replicates), or model-selection analysis for choosing the best substitution model for the data. For some cases, it is even challenging to perform a single ML analysis for the concatenated sequences. Recent analyses of genome-scale data have been unable to complete computation for concatenated data sets, and phylogenetic inferences are often made in the absence of analyses that have reached convergence or have searched tree space substantively (e.g., Refs. 16 and 101). Recent advances in computer architecture of phylogenetic analyses may help alleviate the challenges of analyzing genome-scale supermatrices.<sup>84</sup> On the other hand, Bayesian coalescent models<sup>31,49</sup> have the same computational issues as concatenated analyses when the sequence data involve hundreds of genes. Simpler coalescent methods, such as MP-EST, STAR, and STEAC rely on estimated gene trees to infer species trees, and their computational costs are

manageable, even for thousands of genes and species. Of course, as a price for computational efficiency, gene-tree-based methods suffer low statistical efficiency, in the sense that they often require more loci to produce a highly resolved tree. In practice, it is extremely difficult to estimate the sample size for phylogenetic methods such that they can achieve reasonable bootstrap support for their estimates of the species trees. Clearly, the landscape of phylogenetic methods for genome-scale data, whether for supermatrices or unlinked loci, is rapidly changing.

### **Conclusion**

Phylogenetic analysis of genome-scale data inevitably invites the use of methods that acknowledge the stochasticity of gene histories, and the MSC provides a robust framework for incorporating the information in this stochasticity. Yet newer methods for analyzing genome-scale data, relying on supertrees<sup>102</sup> or alignment graphs,<sup>103</sup> will likely yield further insights. The justification for species tree methods lies not in the ubiquity of gene tree heterogeneity in empirical data sets, although this heterogeneity has certainly spurred the advent of such methods. Rather, the justification lies in their acknowledgement of fundamental genetic processes inherent in all organisms, including recombination along the chromosome, which renders gene histories independent of one another and conditional on the phylogeny; and genetic drift, which generates stochasticity in gene tree topologies and branch lengths. Thus, even when all gene trees are topologically similar, species tree methods will yield results differing from concatenation methods, if not in phylogenetic topology then often in phylogenetic support, because species tree methods better model the accumulation of signal that is accrued with increasingly large data sets. Different methods of species tree inference incorporate different amounts of detail of the MSC process, and there is a trade-off between model accuracy and computational burden. For now, two-stage species tree methods, in which estimated gene trees are used as input data, are useful insofar as they can analyze large-scale genome-wide data sets with ease (Fig. 1). But more complex and computationally efficient models are sorely needed.<sup>11</sup> Although concatenation and species tree approaches often yield similar estimates of phylogeny, an increasing



number of examples of strong conflict between concatenation and coalescent analyses shows that the conditions for conflict among methods occur in empirical data. Additionally, concatenation methods appear more sensitive to classic phylogenetic challenges such as long-branch attraction and rate variation among lineages than are gene-by-gene species tree analyses. Some of the differences in behavior between concatenation and species tree methods can be understood as manifestations of the classic bias–variance problem in statistics, because concatenation is a special case of the more general model used by species tree methods, and therefore can exhibit low variance (such as high bootstrap support) despite being more biased than species tree approaches. Further studies aimed at understanding the connections between concatenation and species tree methods and the types of data that maximize signal under the MSC will allow phylogenetics to take full advantage of the flood of data in the genomics era.

## Acknowledgments

We thank two anonymous reviewers, Douglas Braaten, and anonymous series editors for their comments on the first submission of this manuscript. This work was supported by the United States National Science Foundation (DMS-1222745 to L.L. and DEB-1120243 to C.C.D.) and by the National Natural Science Foundation of China (31470111 to S.W.).

## Conflicts of interest

The authors declare no conflicts of interest.

## References

1. Rannala, B. & Z. Yang. 2008. Phylogenetic inference using whole genomes. *Ann. Rev. Genomics Hum. Genet.* **9**: 217–231.
2. Lemmon, E.M. & A.R. Lemmon. 2013. High-throughput genomic data in systematics and phylogenetics. *Ann. Rev. Ecol. Evol. Syst.* **44**: 99–121.
3. Lachance, J. & S.A. Tishkoff. 2013. Population genomics of human adaptation. *Ann. Rev. Ecol., Evol. Syst.* **44**: 123–143.
4. Leaché, A.D. *et al.* 2014. Species delimitation using genome-wide SNP data. *Syst. Biol.* **63**: 534–542.
5. Hey, J. & R. Nielsen. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proc. Natl. Acad. Sci. U.S.A.* **104**: 2785–2790.
6. Machado, C.A. *et al.* 2002. Inferring the history of speciation from multilocus DNA sequence data: the case of *Drosophila pseudoobscura* and close relatives. *Mol. Biol. Evol.* **19**: 472–488.
7. Wakeley, J. & J. Hey. 1997. Estimating ancestral population parameters. *Genetics* **145**: 847–855.
8. Mailund, T. *et al.* 2012. A new isolation with migration model along complete genomes infers very different divergence processes among closely related great ape species. *PLoS Genet.* **8**: e1003125.
9. Liu, K.J. *et al.* 2014. An HMM-based comparative genomic framework for detecting introgression in eukaryotes. *PLoS Comput. Biol.* **10**: e1003649.
10. Edwards, S.V. 2009. Is a new and general theory of molecular systematics emerging? *Evolution* **63**: 1–19.
11. Szöllősi, G.J. *et al.* 2015. The inference of gene trees with species trees. *Syst. Biol.* **64**: e42–e62.
12. Betancur-R, R. *et al.* 2013. Addressing gene tree discordance and non-stationarity to resolve a multi-locus phylogeny of the flatfishes (Teleostei: Pleuronectiformes). *Syst. Biol.* **62**: 763–785.
13. Betancur-R, R., G.J.P. Naylor & G. Orti. 2014. Conserved genes, sampling error, and phylogenomic inference. *Syst. Biol.* **63**: 257–262.
14. Chiari, Y. *et al.* 2012. Phylogenomic analyses support the position of turtles as the sister group of birds and crocodiles (Archosauria). *BMC Biol.* **10**: 65.
15. Song, S. *et al.* 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. *Proc. Natl. Acad. Sci. U.S.A.* **109**: 14942–14947.
16. Jarvis, E.D. *et al.* 2014. Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science* **346**: 1320–1331.
17. Maddison, W.P. 1997. Gene trees in species trees. *Syst. Biol.* **46**: 523–536.
18. deQueiroz, A. & J. Gatesy. 2007. The supermatrix approach to systematics. *Trends Ecol. Evol.* **22**: 34–41.
19. Gatesy, J. & M.S. Springer. 2014. Phylogenetic analysis at deep timescales: unreliable gene trees, bypassed hidden support, and the coalescence/concatalescence conundrum. *Mol. Phylogenet. Evol.* **80**: 231–266.
20. Springer, M.S. & J. Gatesy. 2014. Land plant origins and coalescence confusion. *Trends Plant Sci.* **19**: 267–269.
21. Zhong, B., L. Liu & D. Penny. 2014. The multispecies coalescent model and land plant origins: a reply to Springer and Gatesy. *Trends Plant Sci.* **19**: 270–272.
22. Liu, L. *et al.* 2009. Coalescent methods for estimating phylogenetic trees. *Mol. Phylogenet. Evol.* **53**: 320–328.
23. McCormack, J.E. *et al.* 2012. Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species tree analysis. *Genome Res.* **22**: 746–754.
24. Edwards, S.V., L. Liu & D.K. Pearl. 2007. High-resolution species trees without concatenation. *Proc. Natl. Acad. Sci. U.S.A.* **104**: 5936–5941.
25. Gatesy, J. & M.S. Springer. 2013. Concatenation versus coalescence versus “concatalescence.” *Proc. Natl. Acad. Sci. U.S.A.* **110**: E1179.

26. Wu, S. *et al.* 2013. Reply to Gatesy and Springer: the multispecies coalescent model can effectively handle recombination and gene tree heterogeneity. *Proc. Natl. Acad. Sci. U.S.A.* **110**: E1180.
27. Xi, Z., J.S. Rest & C.C. Davis. 2013. Phylogenomics and coalescent analyses resolve extant seed plant relationships. *PLoS ONE* **8**: e80870.
28. Xi, Z. *et al.* 2014. Coalescent versus concatenation methods and the placement of *Amborella* as sister to water lilies. *Syst. Biol.* **63**: 919–932.
29. Liu, L., Z. Xi & C.C. Davis. 2015. Coalescent methods are robust to the simultaneous effects of long branches and incomplete lineage sorting. *Mol. Biol. Evol.* **32**: 791–805.
30. Leaché, A.D. *et al.* 2014. A hybrid phylogenetic-phylogenomic approach for species tree estimation in African Agama lizards with applications to biogeography, character evolution, and diversification. *Mol. Phylogenet. Evol.* **79**: 215–230.
31. Liu, L. & D.K. Pearl. 2007. Species trees from gene trees: reconstructing bayesian posterior distributions of a species phylogeny using estimated gene tree distributions. *Syst. Biol.* **56**: 504–514.
32. Kubatko, L.S. & J.H. Degnan. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Syst. Biol.* **56**: 17–24.
33. Huang, H.T. *et al.* 2010. Sources of error inherent in species tree estimation: impact of mutational and coalescent effects on accuracy and implications for choosing among different methods. *Syst. Biol.* **59**: 573–583.
34. Zhong, B. *et al.* 2013. Origin of land plants using the multispecies coalescent model. *Trends Plant Sci.* **18**: 492–495.
35. Lanier, H.C. & L.L. Knowles. 2014. Applying species tree analyses to deep phylogenetic histories: challenges and potential suggested from a survey of empirical phylogenetic studies. *Mol. Phylogenet. Evol.* **83**: 191–199.
36. Tian, Y. & L.S. Kubatko. 2014. Gene tree rooting methods give distributions that mimic the coalescent process. *Mol. Phylogenet. Evol.* **70**: 63–69.
37. Rosenfeld, J.A., A. Payne & R. DeSalle. 2012. Random roots and lineage sorting. *Mol. Phylogenet. Evol.* **64**: 12–20.
38. Knowles, L.L. *et al.* 2012. Full modeling versus summarizing gene tree uncertainty: method choice and species tree accuracy. *Mol. Phylogenet. Evol.* **65**: 501–509.
39. Lanier, H.C. & L.L. Knowles. 2012. Is recombination a problem for species tree analyses? *Syst. Biol.* **61**: 691–701.
40. Rannala, B. & Z. Yang. 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* **164**: 1645–1656.
41. Degnan, J.H. & N.A. Rosenberg. 2009. Gene tree discordance, phylogenetic inference, and the multispecies coalescent. *Trends Ecol. Evol.* **24**: 332–340.
42. Mirarab, S. *et al.* 2014. Statistical binning enables an accurate coalescent-based estimation of the avian tree. *Science* **346**: 1250463.
43. Bayzid, M.S. & T. Warnow. 2013. Naive binning improves phylogenomic analyses. *Bioinformatics* **29**: 2277–2284.
44. Degnan, J.H. & L. Salter. 2005. Gene tree distributions under the coalescent process. *Evolution* **59**: 24–37.
45. Degnan, J.H. & N.A. Rosenberg. 2006. Discordance of species trees with their most likely gene trees. *PLoS Genet.* **2**: 762–768.
46. Kelchner, S.A. & M.A. Thomas. 2007. Model use in phylogenetics: nine key questions. *Trends Ecol. Evol.* **22**: 87–94.
47. Liu, L. *et al.* 2009. Estimating species phylogenies using coalescence times among sequences. *Syst. Biol.* **58**: 468–477.
48. Liu, L., L. Yu & S.V. Edwards. 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. *BMC Evol. Biol.* **10**: 302.
49. Heled, J. & A.J. Drummond. 2010. Bayesian inference of species trees from multilocus data. *Mol. Biol. Evol.* **27**: 570–580.
50. Jennings, W.B. & S.V. Edwards. 2005. Speciation history of Australian grass finches (*Poephila*) inferred from 30 gene trees. *Evolution* **59**: 2033–2047.
51. Faircloth, B.C. *et al.* 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Syst. Biol.* **61**: 717–726.
52. Linkem, C.W., V.N. Minin & A.D. Leaché. 2014. Detecting the anomaly zone in species trees and evidence for a misleading signal in higher-level skink phylogeny (Squamata: Scincidae). doi: <http://dx.doi.org/10.1101/012096>.
53. Zhao, L. *et al.* 2013. Phylogenomic analyses of nuclear genes reveal the evolutionary relationships within the BEP clade and the evidence of positive selection in Poaceae. *PLoS ONE* **8**: e64642.
54. Wickett, N.J. *et al.* 2014. Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proc. Natl. Acad. Sci. U.S.A.* **111**: E4859–E4868.
55. Weitemier, K. *et al.* 2014. Hyb-Seq: combining target enrichment and genome skimming for plant phylogenomics. *Appl. Plant Sci.* **2**: 1400042.
56. Felsenstein, J. 2003. *Inferring Phylogenies*. Sunderland, MA: Sinauer Associates, Inc.
57. Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* **27**: 401–410.
58. *Amborella* Genome Project. 2013. The *Amborella* genome and the evolution of flowering plants. *Science* **342**: 1241089.
59. Soltis, D.E. & P.S. Soltis. 2004. *Amborella* not a “basal angiosperm”? Not so fast. *Am. J. Bot.* **91**: 997–1001.
60. Murphy, W.J. *et al.* 2001. Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* **294**: 2348–2351.
61. Murphy, W.J. *et al.* 2007. Using genomic data to unravel the root of the placental mammal phylogeny. *Genome Res.* **17**: 413–421.
62. Thomson, R.C. *et al.* 2008. Developing markers for multilocus phylogenetics in non-model organisms: a test case with turtles. *Mol. Phylogenet. Evol.* **49**: 514–525.
63. Suzuki, Y., G.V. Glazko & M. Nei. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 16138–16143.

64. Misawa, K. & M. Nei. 2003. Reanalysis of Murphy *et al.*'s data gives various mammalian phylogenies and suggests overcredibility of Bayesian trees. *J. Mol. Evol.* **57**: S290–S296.
65. Seo, T.K. 2008. Calculating bootstrap probabilities of phylogeny using multilocus sequence data. *Mol. Biol. Evol.* **25**: 960–971.
66. Hovmoller, R., L.L. Knowles & L.S. Kubatko. 2013. Effects of missing data on species tree estimation under the coalescent. *Mol. Phylogenet. Evol.* **69**: 1057–1062.
67. Driskell, A.C. *et al.* 2004. Prospects for building the tree of life from large sequence databases. *Science* **306**: 1172–1174.
68. Dunn, C.W. *et al.* 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* **452**: 745–745.
69. Simmons, M.P. & P.A. Goloboff. 2014. Dubious resolution and support from published sparse supermatrices: the importance of thorough tree searches. *Mol. Phylogenet. Evol.* **78**: 334–348.
70. Lanier, H.C., H. Huang & L.L. Knowles. 2014. How low can you go? The effects of mutation rate on the accuracy of species tree estimation. *Mol. Phylogenet. Evol.* **70**: 112–119.
71. Knowles, L.L. & L.S. Kubatko. 2010. "Estimating species trees: an introduction to concepts and models." In *Estimating Species Trees: Practical and Theoretical Aspects*. L.L. Knowles & L.S. Kubatko, Eds.: 1–14. New York: Wiley-Blackwell.
72. Leaché, A.D. *et al.* 2014. The influence of gene flow on species tree estimation: a simulation study. *Syst. Biol.* **63**: 17–30.
73. Leaché, A.D. & B. Rannala. 2011. The accuracy of species tree estimation under simulation: a comparison of methods. *Syst. Biol.* **60**: 126–137.
74. DeGiorgio, M. *et al.* 2014. An empirical evaluation of two-stage species tree inference strategies using a multilocus dataset from North American pines. *BMC Evol. Biol.* **14**: 67.
75. Xi, Z., L. Liu & C.C. Davis. 2015. Genes with minimal phylogenetic information are problematic for coalescent analyses when gene tree estimation is biased. *Mol. Phylogenet. Evol.* In review.
76. Romiguier, J. *et al.* 2013. Less is more in mammalian phylogenomics: AT-rich genes minimize tree conflicts and unravel the root of placental mammals. *Mol. Biol. Evol.* **30**: 2134–2144.
77. Roch, S. & M. Steel. 2015. Likelihood-based tree reconstruction on a concatenation of alignments can be statistically inconsistent. *Theor. Pop. Biol.* **100**: 56–62.
78. Liu, L. & L. Yu. 2010. Phybase: an R package for species tree analysis. *Bioinformatics* **26**: 962–963.
79. Rosenberg, N.A. 2013. Discordance of species trees with their most likely gene trees: a unifying principle. *Mol. Biol. Evol.* **30**: 2709–2713.
80. Huang, H.T. & L.L. Knowles. 2009. What is the danger of the anomaly zone for empirical phylogenetics? *Syst. Biol.* **58**: 527–536.
81. Rambaut, A. & N.C. Grassly. 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Comput. Appl. Biosci.* **13**: 235–238.
82. Jukes, T.H. & C.R. Cantor. 1969. "Evolution of protein molecules." In *Mammalian Protein Metabolism*. H.N. Munro, Ed.: 21–123. New York: Academic Press.
83. Guindon, S. *et al.* 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59**: 307–321.
84. Aberer, A.J., K. Kobert & A. Stamatakis. 2014. ExaBayes: massively parallel Bayesian tree inference for the whole-genome era. *Mol. Biol. Evol.* **31**: 2553–2556.
85. Fan, H.H. & L.S. Kubatko. 2011. Estimating species trees using approximate Bayesian computation. *Mol. Phylogenet. Evol.* **59**: 354–363.
86. Mossel, E. & S. Roch. 2010. Incomplete lineage sorting: consistent phylogeny estimation from multiple loci. *IEEE-ACM Trans. Comput. Biol. Bioinfo.* **7**: 166–171.
87. Kubatko, L.S., B.C. Carstens & L.L. Knowles. 2009. STEM: species tree estimation using maximum likelihood for gene trees under coalescence. *Bioinformatics* **25**: 971–973.
88. Wu, Y. 2012. Coalescent-based species tree inference from gene tree topologies under incomplete lineage sorting by maximum likelihood. *Evolution* **66**: 763–775.
89. Mirarab, S. *et al.* 2014. ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics* **30**: i541–i548.
90. Bryant, D. *et al.* 2012. Inferring species trees directly from biallelic genetic markers: bypassing gene trees in a full coalescent analysis. *Mol. Biol. Evol.* **29**: 1917–1932.
91. Chifman, J. & L. Kubatko. 2014. Quartet inference from SNP data under the coalescent model. *Bioinformatics* **30**: 3317–3324.
92. Wu, Y. 2015. A coalescent-based method for population tree inference with haplotypes. *Bioinformatics* **31**: 691–698.
93. Scally, A. *et al.* 2012. Insights into hominid evolution from the gorilla genome sequence. *Nature* **483**: 169–175.
94. McCormack, J.E., H.T. Huang & L.L. Knowles. 2009. Maximum likelihood estimates of species trees: how accuracy of phylogenetic inference depends upon the divergence history and sampling design. *Syst. Biol.* **58**: 501–508.
95. Hedges, S.B., K.D. Moberg & L.R. Maxson. 1990. Tetrapod phylogeny inferred from 18S and 28S ribosomal RNA sequences and a review of the evidence for amniote relationships. *Mol. Biol. Evol.* **7**: 607–633.
96. Sharma, P.P. *et al.* 2012. Phylogenetic analysis of four nuclear protein-encoding genes largely corroborates the traditional classification of Bivalvia (Mollusca). *Mol. Phylogenet. Evol.* **65**: 64–74.
97. Breinholt, J.W. & A.Y. Kawahara. 2013. Phylotranscriptomics: saturated third codon positions radically influence the estimation of trees based on next-gen data. *Genome Biol. Evol.* **5**: 2082–2092.
98. Dutheil, J.Y. *et al.* 2009. Ancestral population genomics: the coalescent hidden Markov model approach. *Genetics* **183**: 259–274.
99. Hobolth, A. *et al.* 2011. Incomplete lineage sorting patterns among human, chimpanzee, and orangutan suggest recent orangutan speciation and widespread selection. *Genome Res.* **21**: 349–356.

100. White, M.A. *et al.* 2009. Fine-scale phylogenetic discordance across the house mouse genome. *PLoS Genet.* **5**: e1000729.
101. Ryan, J.F. *et al.* 2013. The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* **342**: 1242592.
102. Steel, M. & A. Rodrigo. 2008. Maximum likelihood supertrees. *Syst. Biol.* **57**: 243–250.
103. Smith, S.A., J.W. Brown & C.E. Hinchliff. 2013. Analyzing and synthesizing phylogenies using tree alignment graphs. *PLoS Comput. Biol.* **9**: e1003223.
104. Reid, N.H. *et al.* 2013. Poor fit to the multispecies coalescent is widely detectable in empirical data. *Syst. Biol.* **63**: 322–333.
105. Eckert, A.J. & B.C. Carstens. 2008. Does gene flow destroy phylogenetic signal? The performance of three methods for estimating species phylogenies in the presense of gene flow. *Mol. Phylogenet. Evol.* **49**: 832–842.
106. Castillo-Ramírez, S. *et al.* 2010. “Bayesian estimation of species trees: a practical guide to optimal sampling and analysis.” In *Estimating Species Trees: Practical and Theoretical Aspects*. L.L. Knowles & L.S. Kubatko, Eds.: 15–33. New Jersey: Wiley-Blackwell.
107. Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.
108. Ronquist, F. *et al.* 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**: 539–542.
109. Liu, L. 2008. BEST: Bayesian estimation of species trees under the coalescent model. *Bioinformatics* **24**: 2542–2543.
110. Liu, L. & L. Yu. 2011. Estimating species trees from unrooted gene trees. *Syst. Biol.* **60**: 661–667.
111. Liu, L., L. Yu & D.K. Pearl. 2010. Maximum tree: a consistent estimator of the species tree. *J. Math. Biol.* **60**: 95–106.
112. Tonini, J., A. Moore, D. Stern, *et al.* 2015. Concatenation and species tree methods exhibit statistically indistinguishable accuracy under a range of simulated conditions. *PLoS Currents Tree of Life*. doi: 10.1371/currents.tol.34260cc27551a527b124ec5f6334b6be.