Evaluating fast maximum likelihood-based phylogenetic programs using empirical phylogenomic data sets

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Abstract

Phylogenetics has witnessed dramatic increases in the sizes of data matrices assembled to resolve branches of the tree of life, motivating the development of programs for fast, yet accurate, inference. For example, several different fast programs have been developed in the very popular maximum likelihood framework, including RAxML/ExaML, PhyML, IQ-TREE, and FastTree. Although these four programs are widely used, a systematic evaluation and comparison of their performance using empirical genome-scale data matrices has so far been lacking. To address this question, we evaluated these four programs on 19 empirical phylogenomic data sets from diverse animal, plant, and fungal lineages with respect to likelihood maximization, topological accuracy, and computational speed. For single-gene tree inference, we found that the more exhaustive and slower strategy (ten RAxML searches per alignment) outperformed faster strategies (one tree search per alignment) using RAxML, PhyML, or IQ-TREE. Interestingly, single trees inferred by the three programs yielded comparable coalescent-based species tree estimations. For concatenation–based species tree inference, IQ-TREE consistently achieved the best-observed likelihoods for all data sets, and RAxML/ExaML was a close second. In contrast, PhyML often failed to complete concatenation-based analyses, whereas FastTree was the fastest but exhibited lower likelihood values and topological accuracy in both types of analyses. Finally, data matrix properties, such as the number of taxa and the information content, sometimes substantially influenced the relative performance of the programs. Our results provide real-world gene and species tree phylogenetic inference benchmarks to inform the design and execution of large-scale phylogenomic data analyses.

Introduction

Phylogenetic analysis – that is, the identification of the tree best representing the evolutionary history of the underlying data – is of fundamental importance to many biological disciplines, including but not limited to systematics, molecular evolution, and comparative genomics (Felsenstein 2003; Xia 2013; Hamilton 2014; Yang 2014). However, finding the best tree is an exceptionally difficult task because evaluation of each tree requires a considerable amount of calculations (Bryant, et al. 2005) as well as because the number of candidate strictly bifurcating trees grows very rapidly with the number of sequences (Felsenstein 1978) – for example, there are ~8 × 10^{21} possible rooted topologies for a set of 20 taxa. Therefore, fast programs that employ heuristic algorithms that can efficiently infer the best tree (or nearly as good alternatives) are of pivotal importance to phylogenetic analysis. This is evident by the success of the Neighbour-Joining (NJ) method, a distance-based clustering (instead of tree searching) algorithm (Saitou and Nei 1987) that is the most highly cited phylogenetic method (Van Noorden, et al. 2014). NJ and its variants (e.g. BIONJ which takes the variance of distance estimation into consideration) (Gascuel 1997; Bruno, et al. 2000) were among the few available options for analyzing large data sets until the 2000s, and are still widely used today to quickly produce good starting points for more sophisticated methods (e.g. (Guindon, et al. 2010; Nguyen, et al. 2015)).

It is now generally accepted that statistical methods, such as maximum likelihood (ML) (Felsenstein 1981), produce more reliable results than distance and parsimony methods (Yang and Rannala 2012; Whelan and Morrison 2017). However, ML-based methods are also computationally more expensive, necessitating the use of heuristic search algorithms for searching the enormity of tree space (Chor and Tuller 2005). Heuristic search algorithms typically adopt iterative, "hill-climbing" optimization techniques that involve three steps: (1) generate a quick starting tree (e.g. BIONJ tree, stepwise-addition parsimony tree, etc.); (2) modify the tree using certain topological rearrangement rules and evaluate the resultant trees under the ML criterion; and (3) replace the starting tree and repeat step 2 if the rearrangements identify a better tree, or otherwise terminate the search. The most common rearrangement algorithms for step 2 are Nearest-Neighbor-Interchange (NNI), where the four subtrees connected by a given internal branch are re-arranged to form two new, alternative topologies (Robinson 1971), and Subtree-Pruning-and-Regrafting (SPR), in which a given subtree is detached from the full tree and re-inserted onto each of the remaining branches (Swofford, et al. 1996). SPR is more expansive in searching tree space than NNI since it can evaluate many more trees from one initial topology, but it is also much slower because of the extra tree evaluations.

Four of the most popular fast ML-based phylogenetic programs that differ in their choices or implementations of rearrangement algorithms are PhyML (Guindon and Gascuel 2003; Guindon, et al. 2010), RAxML / ExaML (Stamatakis 2014; Kozlov, et al. 2015), FastTree (Price, et al. 2010), and IQ-TREE (Nguyen, et al. 2015). First introduced in the early 2000s, PhyML has been one of the most widely used programs for ML-based phylogenetic inference (Guindon and Gascuel 2003). The original algorithm was based solely on NNI and achieved comparable performance as other contemporary ML methods but with much lower computational costs. The latest version of PhyML (version 20160530) performs hill-climbing tree searches using SPR rearrangements in early stages and NNI rearrangements in later stages of the tree search (Guindon, et al. 2010). Specifically, during the SPR-based search, candidate re-grafting positions are first filtered based on parsimony scores; the most parsimonious ones are then subject to approximate ML evaluation where branch-lengths are only re-optimized at the branches adjacent to the pruning and re-grafting positions. To accelerate the tree search, the best “up-hill” SPR move for each subtree is accepted immediately, potentially leading to the simultaneous application of multiple SPRs in one round. Once the search has converged to a single topology, the resultant tree is further optimized by NNI-based hill-climbing. Similar to the SPR stage, PhyML evaluates candidate NNIs only approximately by re-optimizing the five relevant branches, and may apply multiple NNI moves simultaneously at each round. The addition of the SPR algorithm in PhyML has significantly improved its accuracy, although at the cost of longer runtimes (Guindon, et al. 2010).

RAxML is another widely used program for fast estimation of ML trees (Stamatakis 2006, 2014). The latest version (8.2.10) implements the standard SPR-based hill-climbing algorithm and employs important heuristics to reduce the amount of unpromising SPR candidates, including: 1) candidate re-grafting positions are limited to only those within a certain distance from the pruning position (known as the “lazy subtree rearrangement”) (Stamatakis, et al. 2005); and 2) if the re-grafting to a candidate position results in substantially worse likelihood, all branches further away from that point will be ignored (Stamatakis, et al. 2007). As in PhyML, the approaches of approximate pre-scoring of SPR candidates and simultaneous SPRs are also used by RAxML to speed up the analysis (Stamatakis, et al. 2005). In addition to RAxML, its sister program ExaML is specifically engineered for large concatenated data sets

(Kozlov, et al. 2015). As RAxML has exhibited excellent performance in both accuracy and speed (Stamatakis 2006), it is considered by many to be the state-of-the-art ML fast phylogenetic program.

Although both PhyML and RAxML represent great advances in developing fast and accurate phylogenetic programs, efforts aimed at improving the speed of ML tree estimation continue. For example, the recently developed FastTree program can be orders of magnitude faster than either PhyML or RAxML / ExaML (Price, et al. 2010). FastTree (latest version 2.1.10) first constructs an approximate NJ starting tree which is then improved under the minimum evolution criterion using both NNI and SPR rearrangements, followed by ML-based NNI rearrangements to search for the final tree. With computational efficiency at the very heart of its design, FastTree makes heavy use of heuristics at all stages to limit the numbers of tree searches and likelihood optimizations. As a tradeoff, FastTree generates less accurate tree estimates than SPR-based ML methods (Price, et al. 2010). The substantial edge of the FastTree program in speed has made it very popular, particularly in analyses of very large phylogenomic data matrices.

An important weakness of pure hill-climbing methods is that they can be easily trapped in local optima. The IQ-TREE program, the most recent of the four fast ML-based phylogenetic programs, was developed aiming to overcome this local optimum problem through the use of stochastic techniques (Nguyen, et al. 2015). Specifically, IQ-TREE (latest version 1.5.4) generates multiple starting trees instead of one and subsequently maintains a pool of candidate trees during the entire analysis. The tree inference proceeds in an iterative manner; at every iteration, IQ-TREE selects a candidate tree randomly from the pool, applies stochastic perturbations (e.g., random NNI moves) onto the tree, and then uses the modified tree to initiate a NNI-based hill-climbing tree search. If a better tree is found, the worst tree in the current pool is replaced and the analysis continues; otherwise, the iteration is considered unsuccessful and the analysis terminates after a certain number of unsuccessful iterations. IQ-TREE takes advantage of successful preexisting heuristics (e.g., lazy subtree rearrangement (Stamatakis, et al. 2005) and simultaneous NNIs (Guindon and Gascuel 2003)) and a highly-optimized implementation of likelihood functions (Flouri, et al. 2015) for better computational efficiency.

These four programs offer different tradeoffs between accuracy and speed in fast phylogenetic inference, and they may exhibit different behaviors toward diverse phylogenomic data sets whose properties (e.g. taxon number and gene number) and evolutionary characteristics (e.g. age of lineage, taxonomic range, and evolutionary rate) vary. Therefore, a good understanding of their relative performance across diverse empirical phylogenomic data matrices is critical to the success of phylogenetic inference when computational resources are limited. This is particularly relevant for large-scale studies using data matrices of ever-increasing data volumes and complexities. So far, these four programs have only been evaluated using simulated data (Guindon, et al. 2010; Price, et al. 2010; Liu, et al. 2011), which might not well approximate real data, and relatively small empirical data sets containing ~10 to ~200 gene alignments (Guindon, et al. 2010; Price, et al. 2010; Liu, et al. 2011; Money and Whelan 2012; Nguyen, et al. 2015; Chernomor, et al. 2016), which might lack generality. In these studies, RAxML and PhyML showed largely similar performance in identifying trees of higher likelihood scores (Guindon, et al. 2010; Money and Whelan 2012), while IQ-TREE exhibited improved efficiency compared to both RAxML and PhyML (Nguyen, et al. 2015; Chernomor, et al. 2016). On the other hand, FastTree was found to be much faster than RAxML and PhyML but reported lower likelihood scores for data sets with both small and large numbers of sequences (Guindon, et al. 2010; Price, et al. 2010; Liu, et al. 2011). However, it remains unclear if these patterns would hold for large empirical data sets and for species tree estimation based on genome-scale data.

To comprehensively evaluate the four fast ML-based phylogenetic programs (table 1), we used a large collection of 19 empirical phylogenomic data sets representing a wide range of properties, including data type, numbers of taxa and genes, and taxonomic range for diverse animal, plant, and fungal lineages (table 2; for details on the source of each data set, see supplementary table S1). For each of these data sets, we compared the performance of all programs for single-gene tree inference and, for coalescent-based and concatenation-based species tree inference, the two major current approaches to inferring species phylogenies from phylogenomic data (Liu, et al. 2015). In the coalescent approach, the species tree is estimated by considering all individually inferred single-gene trees using coalescent methods that take into account that the histories of genes may differ from those of species due to incomplete lineage sorting (fig. 1A), whereas in the concatenation approach, the species tree is estimated from the supermatrix derived by concatenating all single-gene alignments (fig. 1B).

In single-gene tree estimation, we found that, although the more comprehensive analysis strategy (ten RAxML searches per alignment) performed considerably better than fast strategies (one tree search per alignment using RAxML, PhyML, or IQ-TREE), all produced results of comparable quality when the inferred gene trees were used for coalescent-based species tree inference. For the concatenation-based species tree inference, we found that, in some cases, IQ-TREE recovered trees with higher likelihood scores than RAxML/ExaML, although both showed the best performance for most data sets. Importantly, IQ-TREE exhibited comparable or better speed in both coalescent-based and concatenation-based species tree inference compared with RAxML/ExaML. In contrast, FastTree produced significantly worse single-gene and species trees than the other three programs even when allowed to run multiple times, whereas PhyML did not scale well to supermatrices because the concatenation-based species tree inferences failed to complete for multiple data sets. Overall, our benchmarking of the four fast ML-based phylogenetic programs against 19 state-of-the-art data matrices is highly informative for the design of efficient data analysis strategies in phylogenomic studies.

Results and Discussion

A comprehensive collection of empirical data

For a comprehensive evaluation of the four fast ML-based phylogenetic programs, we retrieved 19 data sets from 14 recently published phylogenomic studies (table 2; see supplementary table S1 for detailed sources of each data set), representing a wide range of characteristics: 1) they include both amino acid and nucleotide data sets (nine and ten, respectively); 2) they contain either many taxa (e.g. D6, 200 taxa and 259 genes (Prum, et al. 2015)), many genes (e.g. D5a, 48 taxa and 14,448 genes (Jarvis, et al. 2014)), or both (e.g. A2, 144 taxa and 1,478 genes (Misof, et al. 2014)); 3) they cover three major taxonomic groups (i.e. animals, plants, and fungi) and various depths within each group (e.g. data sets D1 (Song, et al. 2012), A4 (Chen, et al. 2015), and A6 (Whelan, et al. 2015) cover mammals, vertebrates, and metazoans, respectively); and 4) they consist of sequence data derived from different technologies (e.g. some data sets were built entirely on whole genome sequences (Song, et al. 2012; Jarvis, et al. 2014; Shen, et al. 2016b; Tarver, et al. 2016), while some others contained mostly transcriptome sequencing data (Misof, et al. 2014; Wickett, et al. 2014; Yang, et al. 2015)). In addition, these data sets were assembled and curated in state-of-the-art phylogenomic studies and thus are of high quality. Therefore, these data sets are well suited for benchmarking the performance of fast phylogenetic programs in the context of phylogenomics.

Performance Test I: Single-gene tree inference

In the first test, we examined the performance of four fast ML-based phylogenetic programs (i.e. RAxML, PhyML, IQ-TREE, and FastTree) in inferring single-gene trees (fig. 1A). We designed five strategies: four in which each program was used to infer each gene tree from a single starting tree (these were named *RAxML*, *PhyML*, *IQ-TREE*, and *FastTree*), as well as one (named *RAxML-10*) in which RAxML was used to infer each gene tree from ten replicates (five, including the starting tree used in *RAxML*, were obtained via parsimony and the other five were random starting trees).

The five strategies were compared for the likelihood scores and topologies of their single-gene tree inferences, as well as for their computational speeds. Since the true evolutionary histories are unknown for the empirical data used here, we identified the tree with the highest likelihood score for each alignment (hereafter referred to as the "best-observed" tree) among trees inferred by the five strategies and the trees reported in previous studies, if available. These "best-observed" trees were used as the reference in the comparisons of likelihood score and topology.

Likelihood score maximization – We first examined the performance of the five strategies in likelihood score maximization on single-gene alignments (supplementary table S2) by calculating the frequencies with which each of the five strategies had the highest score (fig. 2). *RAxML-10* had the highest frequency of finding the highest likelihood scores (90.37%) and reported the highest likelihood scores in ≥80% of the alignments in all data sets except for D5b, highlighting the benefit of using multiple starting trees. *IQ-TREE* was the second best strategy with an overall frequency of 50.24%. Importantly, the performances of *RAxML-10* and *IQ-TREE* varied substantially among data sets; whereas their performances were very similar on several data sets (e.g. A1 and D1), in others *RAxML-10* outperformed *IQ-TREE* by large margins (e.g. A2, D2a, and D2b). *RAxML* and *PhyML* were third and fourth, respectively, and had considerably lower overall frequencies (38.71% and 25.36%). *RAxML* performed better than *IQ-TREE* on only four (A2, A5, D2a, and D2b) data sets, whereas *PhyML* performed better than *IQ-TREE* on only one (A5) data set. However, none of these three strategies (i.e. *RAxML*, *PhyML*, and *IQ-TREE*) performed well on these data sets. Between *RAxML* and *PhyML*, the former found higher likelihood scores more often than the latter.

In comparison, the likelihood scores obtained by *FastTree* were much lower than those of the other four strategies; the program produced the highest likelihood scores in only 1.67% of all alignments. However, *FastTree* also had substantial advantages in computational speed compared to the others (see below). Since FastTree can initiate tree searches using distinct starting trees, we performed additional FastTree analyses for selected data sets, consisting of 100 tree searches for each alignment starting from 50 parsimony trees and 50 random trees. The results show that *FastTree* was still outperformed by other strategies even after compensating for the differences in runtime (supplementary table S3).

To further investigate the relative performance of *RAxML*, *PhyML*, and *IQ-TREE*, we carried out pairwise comparisons between each of them and the more comprehensive strategy *RAxML-10*. *IQ-TREE* found equally good or better likelihood scores than *RAxML-10* for more than half of the alignments in 13 / 19 data sets, followed by *RAxML* (7 / 19) and *PhyML* (3 / 19) (supplementary fig. S1). Overall, *IQ-TREE*, *RAxML*, and *PhyML* found trees with likelihood scores equal or better to those of *RAxML-10* in 54.04%, 40.81%, and 25.80% of all alignments, respectively. A similar pattern was also observed in pairwise comparisons between *RAxML*, *PhyML*, and *IQ-TREE* (supplementary fig. S2); *IQ-TREE* found trees with higher likelihood scores more often than both *RAxML* (for 14 out of 19 data sets) and *PhyML* (for 15 out of 19 data sets), while *RAxML* outperformed *PhyML* for all data sets. Cumulatively, *IQ-TREE* found higher likelihood scores for an additional 13.18% of alignments than *RAxML*, while *IQ-TREE* and *RAxML* respectively found higher likelihood scores for an additional 35.08% and 28.30%, respectively, of alignments than *PhyML*.

Topological accuracy – We also assessed the topological accuracy of the five strategies by comparing their tree inferences on each alignment against the corresponding "best-observed" tree (i.e. the tree with the highest likelihood score, which was used to approximate the true ML tree). Overall, there was a strong positive correlation between the differences in likelihood scores and the topological distances (measured by the normalized Robinson-Foulds, or nRF, distance (Robinson and Foulds 1981)) when comparing inferred trees to the best-observed trees (Spearman's correlations of 0.85 for all alignments and above 0.90 for most data sets, p-values < 2.2×10^{-16} in all cases). In other words, strategies that yielded likelihood scores closest or equal to the best-observed likelihood scores tended to be those whose topologies were also closest or identical to the best-observed topologies (supplementary table S4; see fig. 3 for data set A8 as an example).

Among the five strategies, *RAxML-10* showed the best performance in topological accuracy with median nRF distances of 0 for almost all data sets (supplemental table S4); this was unsurprising since *RAxML-10* contributed most of the best-observed trees. *IQ-TREE*, *RAxML*, and *PhyML* also performed relatively well, with median nRF distances less than 0.01, 0.06, and 0.13, respectively, for most data sets. Here again, *FastTree* was behind the other strategies as it led to median nRF distances greater than 0.3 for most data sets.

Computational speed – To compare the computational speed of the five strategies, we plotted the runtimes of *RAxML-10*, *PhyML*, *IQ-TREE*, and *FastTree* against that of *RAxML* (fig. 4; supplementary table S5). We found strong positive correlations between the speeds of strategies over a wide range of runtimes (Spearman' correlation ≥ 0.91 for all combinations of data types and strategies, *p*-values < 2.2×10^{-16} in all cases). As expected, *RAxML-10*, which conducts ten RAxML-based searches, took about ten times longer than *RAxML* (supplementary table S6). Interestingly, *PhyML* was ~1.5 times faster than *RAxML* on protein alignments, but ~3.1 times slower on DNA alignments. On the contrary, *IQ-TREE* was faster than *RAxML* for both protein and DNA data (~1.6 and ~1.3 times faster, respectively). Lastly, *FastTree* was substantially more time-efficient than *RAxML* on both DNA alignments (~47.9 times faster) and protein alignments (~95.4 times faster). In addition, the time advantage of *FastTree* was greater for alignments requiring longer runtimes; for instance, our linear regression analysis suggests that *FastTree* might run ~162.0 times faster than *RAxML* on the largest single protein alignments but only ~9.6 times faster on the smallest ones.

Overall, our results at the level of single-gene tree inference are consistent with previous, smaller-scale studies on the better efficiency of IQ-TREE relative to RAxML and PhyML (all using one search per alignment) (Nguyen, et al. 2015), and the inferior performance of FastTree in likelihood score maximization as compared to other programs (Guindon, et al. 2010). However, in contrast to previous observations (Guindon, et al. 2010), we found that RAxML consistently outperformed PhyML in all data sets. This difference might be due to the small number of alignments examined in the previous study (Guindon, et al. 2010) and the numerous updates of both programs since then.

Performance Test II: coalescent-based species tree inference

In the second test, we assessed the fast ML-based phylogenetic programs in the context of the "two-step" coalescent-based species tree inference, in which single-gene trees were first estimated from individual alignments by each examined strategy and then used collectively to infer the species tree under a coalescent model (fig. 1A) (Liu, et al. 2015). Here, we used the single-gene trees produced in the Performance Test I as input for the ASTRAL program (Mirarab and Warnow 2015), which was used to infer coalescent-based species trees. The five strategies were then compared for the accuracy of their species tree inferences by using the species tree estimated from the best-observed gene trees as the reference.

We first determined for each data set the topological distances between the species tree inferred from the best-observed single-gene trees and those inferred from the gene trees inferred by each of the five strategies. *RAxML-10*, *RAxML*, *PhyML*, and *IQ-TREE* displayed comparably high levels of topological accuracy in their species tree estimations (median nRF distances ranged between 0.02 and 0.04 across data sets), whereas *FastTree* had lower topological accuracy (median nRF distances of 0.121). Nonetheless, for most strategies and data sets, the species tree estimates were much more accurate than the corresponding single-gene tree inferences (table 3; supplementary table S7).

We further assessed the confidence levels (measured by quartet-based posterior probability, or PP, support (Sayyari and Mirarab 2016)) of the incongruent bipartitions or splits identified in the abovementioned species tree comparison in the set of single gene trees inferred by *FastTree*. Worryingly, the incongruent splits between the species tree inferred using *FastTree*-generated gene trees as input and the best-observed species tree received significantly higher PP supports (fig. 5; see supplementary table S8 for the results of Wilcoxon rank-sum tests), the median PP values of which were 0.81 for protein data sets and close to 1 for DNA data sets. Both of these values were much higher than those of other four strategies, which were all below 0.60 and 0.71 for protein and DNA data sets, respectively.

Performance Test III: Concatenation-based species tree inference

In the third test, we examined the relative performance of the four programs in concatenation analysis of 17 taxon-rich and gene-rich supermatrices (we conducted concatenation analyses on 17, rather than 19, data matrices because: a) D5a and D5b correspond to different partitioning strategies from the same supermatrix (Jarvis, et al. 2014), and b) D2a does not have a corresponding supermatrix available from the original study (Misof, et al. 2014)) (fig. 1B; table 2). Here, we again focused on the programs' performance on likelihood score maximization, topological accuracy, and computational speed. However, as PhyML required exceedingly high runtime, memory, or crashed on multiple data sets, its results are not included in the evaluation. In addition to our analyses, all the supermatrices have also been previously extensively analyzed using either RAxML or ExaML (e.g. (Jarvis, et al. 2014; Misof, et al. 2014; Wickett, et al. 2014)). Therefore, we included the reported likelihood scores and topologies – we refer to them as "RAxML/ExaML-published" trees – in our examination of relative performance.

Likelihood score maximization – Consistent with the pattern observed in single-gene tree analyses, RAxML and IQ-TREE achieved substantially higher likelihood scores than FastTree on supermatrix analyses (fig. 6; supplementary table S9). Interestingly, IQ-TREE found the highest likelihood scores in all 17 data sets and outperformed both our RAxML and previous RAxML/ExaML-published results on 7 and 8 data sets, respectively. Remarkably, IQ-TREE consistently yielded the highest likelihood scores in all independent replicates (except for the analyses of data set D2a), while RAxML replicates were often trapped at suboptimal solutions (supplementary table S10). Moreover, the highest likelihood scores were usually found quite early in the IQ-TREE analyses (supplementary table S10), further suggesting its high efficiency in concatenation analysis.

In comparison, RAxML/ExaML did not yield the highest likelihood scores for several data sets (fig. 6; supplementary table S9). One possible explanation is that, due to its "lazy SPR" heuristic, RAxML might report trees that are not optimal in terms of strict NNI or SPR rearrangement (Stamatakis 2015). Indeed, the best ML trees can be recovered by simply re-optimizing the RAxML-generated (or RAxML/ExaML-published) results using a function built in RAxML itself for four (or six) data sets (fig. 6; supplementary table S9). In addition, many of the differences in likelihood scores between trees inferred by RAxML/ExaML and IQ-TREE (the best ML trees) were small; three and five of the RAxML and previously published trees, respectively, were found to be equally good as the corresponding IQ-TREE trees as determined by approximately unbiased (AU) tests (fig. 6; supplementary table S9) (Shimodaira 2002). After taking these two factors into account, the likelihood scores of only one of our RAxML-generated trees and of two RAxML/ExaML-published trees that were significantly worse than their corresponding IQ-TREE results. In contrast, FastTree yielded significantly, and sometimes substantially, worse likelihood scores for most data sets. Furthermore, FastTree obtained lower likelihood scores than ExaML and IQ-TREE, even when it was allowed to run multiple times from distinct starting trees (supplementary table S11).

Topological accuracy – For all data sets, we calculated the nRF distances between trees inferred by the three programs and the best ML trees. As shown in figure 6, the topological accuracy of the examined programs is in agreement with their performance in likelihood score maximization (see also supplementary table S9). RAxML-generated or RAxML/ExaML-published trees were identical or highly similar to the best ML trees, with the largest nRF distance being 0.064. Importantly, some of the differences between the results of RAxML/ExaML and IQ-TREE correspond to contentious relationships in phylogenomic studies (e.g. in data set A4: the relative positions of pigeon, falcon, and other Neoaves; and in data set D3a: the relationships between Chloranthales, Eudicots, and Magnoliids) (Shen, et al. 2017). Furthermore, some of these differences disappeared (and nRF distances became smaller) after the NNI-based re-optimization of RAxML/ExaML results. FastTree trees, on the other hand, showed much greater nRF distances from the best trees. We also evaluated the confidence levels (measures by Shimodaira–Hasegawa approximate likelihood ratio test, or SH-aLRT support (Guindon, et al. 2010)) of trees that were significantly worse than the best ML trees. Figure 7 shows that large proportions of the incongruent splits in FastTree trees were highly supported.

Computational speed – We compared the runtimes of ExaML, IQ-TREE, and FastTree on ten selected supermatrix data sets; each program was used to analyze each data set three independent times. The results are summarized in figure 8 (see also supplementary table S12). Overall, FastTree was significantly and substantially faster than ExaML and IQ-TREE (Wilcoxon signed-rank test, *p*-values < 0.01 for all pairwise comparisons), whereas the last two programs were on par with each other with respect to speed (Wilcoxon signed-rank test, *p*-value=0.56). Interestingly, IQ-TREE was faster on five of the six protein data sets, while ExaML was faster on all four DNA supermatrices.

These results suggest that IQ-TREE is a very appealing alternative to RAxML/ExaML, which is currently the default choice in most concatenation-based phylogenomic studies. This finding might not be entirely surprising because IQ-TREE represents the latest development in fast phylogenetic programs and has implemented a novel data structure to facilitate concatenation analysis (Chernomor, et al. 2016). For RAxML and ExaML, our findings indicate that their results, even after multiple independent searches, should not be directly taken as the best answers and instead should be checked for potential improvements. On the other hand, together with the results of the coalescent-based test, our benchmarking suggests that FastTree is not suitable for production-level phylogenomic analyses. The exceptional runtime of FastTree might make it an attractive option for exploratory investigations, yet the results should still be interpreted with care.

Impact of data properties on the relative performance of fast phylogenetic programs

In this benchmarking, we noticed several data properties that appear to have an influence on the relative performance of the examined programs. The first one is the number of sequences in the data set; in single-gene analyses, while IQ-TREE outperformed RAxML and PhyML in most instances, it did not do so on some of the data sets that had the largest numbers of taxa (A2 and D2a/b, 144 taxa; A5, 100 taxa; D6, 200 taxa; supplementary fig. S2). A potential explanation could be that IQ-TREE uses NNI as its topological rearrangement mechanism (Nguyen, et al. 2015), whereas RAxML and PhyML are both based on SPR (Stamatakis 2006; Guindon, et al. 2010). It is well recognized that SPR explores a greater proportion of tree space than NNI (Whelan and Morrison 2017) and that it does so in a manner proportional to the sequence number (SPR examines $O(n^2)$ neighbors for each tree instead of $O(n)$ neighbors as by NNI). Therefore, while IQ-TREE exhibited better performance on data sets with fewer taxa through a combination of NNI rearrangement and stochastic algorithm, NNI might become a limiting factor on its performance on larger data sets.

Interestingly, in concatenation analyses, IQ-TREE found equally good or better trees than RAxML/ExaML for all data sets (fig. 6; supplementary table S9), including for the ones on which RAxML performed better in single-gene tree inference. The only difference between concatenation and single-gene tree analyses was the number of sites analyzed, a property that is strongly correlated with phylogenetic signal (Rokas, et al. 2003; Shen, et al. 2016a). Similarly, in single-gene tree inference, IQ-TREE showed much better performance over RAxML on data set D5b than on D5a (supplementary fig. S2); D5b was derived from concatenating single-gene alignments in D5a into a smaller number of longer partitions (Mirarab, et al. 2014), resulting in enhanced phylogenetic signal (measured by average bootstrap support, or ABS, of gene tree) (supplementary fig. 3A). Compared with the single-gene data sets, these concatenated data matrices probably correspond to much simpler tree spaces in which the NNI algorithm might be sufficient. Following this line of reasoning, however, suggests that the performance of the SPR-based RAxML/ExaML programs will become more favorable as the numbers of taxa included in phylogenomic data sets continue to increase beyond the numbers in the data sets than we analyzed in this study.

Consistent with this explanation, we found that the relative performance of SPR-based (RAxML and PhyML) and NNI-based (IQ-TREE) programs was indeed associated with the phylogenetic signal of alignment data. For instance, we compared the ABS values of the best-observed single-gene trees recovered by *RAxML* only, by *IQ-TREE* only, or by both programs, and found that they exhibited lower, intermediate, and higher ABS values, respectively (p-values < 2.2×10^{-16} for all Wilcoxon rank-sum tests; fig. 9). This trend held across all data sets (supplementary fig. 3A). We also observed the same pattern in the comparison between *PhyML* and *IQ-TREE* (supplementary fig. 3B), but not between *RAxML* and *PhyML* (supplementary fig. 3C). Investigating the relationship between the performance of fast phylogenetic programs and the strength of phylogenetic signal, which is in turn correlated with many other factors (Shen, et al. 2016a), is an interesting area of future research.

Lastly, in agreement with previous studies (Guindon, et al. 2010; Nguyen, et al. 2015), we found that some programs displayed different time efficiency on protein and DNA data sets. For example, in single-gene analyses, PhyML was ~1.5 times faster on protein alignments but ~3.1 times slower on DNA alignments in comparison with RAxML (fig. 4; supplementary table S6). Similarly, in concatenation analyses, IQ-TREE required shorter runtimes than RAxML/ExaML on most protein data sets, while the opposite was true for DNA data sets. Such differential behavior may be attributed to the distinct algorithmic designs and/or software implementations of the programs on protein and DNA data (Guindon, et al. 2010).

Conclusion

In this study, we systematically examined and compared the performance of four popular, ML-based fast phylogenetic programs. As our evaluation covered standard phylogenetic and phylogenomic approaches (gene tree inference, as well as coalescent-based and concatenation-based species tree inference), assessed key parameters of inference (likelihood score, topology, and computational speed), and examined a comprehensive collection of empirical state-of-the-art phylogenomic data sets, our findings are directly relevant for the experimental design and execution of real-world phylogenetic and, particularly, phylogenomic studies.

Materials and Methods

Empirical phylogenomic data sets

The 19 data sets were retrieved from their respective sources as listed in supplementary table S1. They were used in this study without any filtering on their contents, with two operations performed when necessary: 1) file split – some data sets (e.g. D2) have only the concatenated alignments available, hence they needed to be split up to obtain single gene alignments; and 2) format conversion – alignments in the data sets are provided in either the "FASTA" or the "Phylip" formats, and had to be converted into the other format to be compatible with all examined phylogenetic programs (e.g. FastTree requires the "FASTA" format and PhyML requires the "Phylip" format). Similarly, all partition model files were transformed into the desired format for each phylogenetic program. Both the original and the actual files used for this study, as well as all the inferred trees are available from the figshare repository (https://figshare.com/account/home#/projects/22040, last accessed May 24, 2017).

Single-gene tree inference

For single-gene tree inference, model selection analysis was first performed for each amino acid alignment to determine the best-fit model using the "TESTONLY" option of IQ-TREE v1.4.2 (Nguyen, et al. 2015). The set of candidate models included all amino acid substitution models supported by RAxML, with and without empirical amino acid frequencies, and with the GAMMA correction for among site heterogeneity of evolutionary rates (Yang 1994) always enforced. For nucleotide alignments, the GTR model with empirical base frequencies and GAMMA distribution was used since it is the choice of almost all phylogenomic studies. Further details on the commands used for the model selection and all the analyses described below are available in the Supplementary Text.

Then each alignment was analyzed by single-threaded versions of the four fast phylogenetic programs. For the purpose of benchmarking, one tree search was conducted using each program under the same model settings (see below for FastTree as the only exception). We also performed additional RAxML searches with multiple parsimony and random starting trees, which represents a common strategy used in phylogenomic studies. In total, five strategies of phylogenetic analysis were assessed:

1) *RAxML-10*: Two analyses were carried out for each alignment using RAxML v8.2.0 (Stamatakis 2014); one included five independent searches starting from parsimony trees and the other five starting from random trees. A random number seed was generated independently and fed into each analysis. The BFGS optimization method was turned off in the analyses of nucleotide alignments since it has been reported previously to produce unstable results (Church, et al. 2015). The likelihood scores of the trees inferred by the two analyses were compared to determine the final result of *RAxML-10* and the tree with the highest likelihood was selected; ; in cases where two trees had equally high likelihood scores but different topologies, a random selection was made from the two trees (see the "Assessment of tree inferences" section for detailed procedure on likelihood score and topological distance calculations);

2) *RAxML*: One search was carried out for each alignment using RAxML v8.2.0 (Stamatakis 2014) with a parsimony starting tree. The analysis was initiated using the same random seed number as the analysis based on parsimony starting tree in *RAxML-10*, and thus can be considered as a subset of the tree inferences conducted in *RAxML-10*. Therefore, *RAxML-10* will always produce equal or better results than *RAxML*. All other settings were the same as *RAxML-10*;

3) *PhyML*: One search was carried out for each alignment using PhyML v20160530 (Guindon, et al. 2010) with a parsimony starting tree. The "SPR" algorithm was selected for tree topology search. Certain amino acid substitution models (e.g. JTTDCMut and mtZOA) were specified as custom models since they were not supported by PhyML natively. Unlike in RAxML analyses, random number seeds were generated automatically by PhyML;

4) *IQ-TREE*: One search was carried out for each alignment using IQ-TREE v1.4.2 with default settings except for the model. Similar to PhyML, IQ-TREE generates random seed numbers automatically;

5) *FastTree*: One search was carried out for each alignment using FastTree v2.1.9 (Price, et al. 2010) with the default heuristic NJ starting tree. The "-spr 4", "-mlacc 2", and "-slownni" options were specified to enable more thorough heuristic tree search. Unlike the other programs, FastTree only supports three amino acid substitution models (i.e. JTT, WAG, and LG). Therefore, the best-fit model among the three was selected for each FastTree analysis of amino acid

alignment. Moreover, the algorithm of FastTree is deterministic, thus independent analyses of the same alignment will always lead to the same result.

Once all single-gene tree estimations were completed, each alignment was associated with at least five gene trees, which included the trees inferred by the five above-mentioned strategies and, for most data sets, previously reported single-gene trees from respective publications. The gene trees of each alignment were then compared to identify the one with the best likelihood score, which is referred to as the "*best-observed*" tree; the tree with the highest likelihood score was selected to be the best-observed tree, or, if multiple trees had the same likelihood score, a random selection was made among them (see the "Assessment of tree inferences" section for detailed procedure on likelihood score and topological distance calculations).

Coalescent-based species tree inference

Each of the 19 data sets was analyzed following the "two-step" procedure of coalescent-based species tree inference (Liu, et al. 2015); single-gene trees were first estimated using fast ML-based phylogenetic programs (see above) and were then used to infer the species tree with the coalescent-based approach implemented in the ASTRAL program, v4.10.12 (Mirarab and Warnow 2015). In total, six coalescent-based species trees were estimated for each data set, five of which were based on single-gene trees produced by the five strategies, and the sixth one was based on the "best-observed" trees.

Concatenation-based species tree inference

Supermatrices consisting of all single-gene alignments and corresponding model files indicating partition scheme as well as model assignments are available for all data sets except for D2a and D5b. Concatenation-based species tree inferences were performed on these supermatrices using parallelized versions of all phylogenetic tools whenever possible due to the heavy computation being required. Edge-linked partitioned analyses (i.e. branch-lengths shared across partitions) were performed on each supermatrix using both RAxML and IQ-TREE. The RAxML analyses were conducted using RAxML-MPI v8.2.3 (available through the CIPRES Scientific Gateway), each consisting of six to eight tree searches with parsimony starting trees, while five independent IQ-TREE searches were carried out for each supermatrix using IQ-TREE-OMP v1.4.2. FastTreeMP v2.1.9 was run once per supermatrix with the thorough search parameters (see above); partition schemes were not used since FastTree does not support partitioned analysis. PhyML v20160530 was also used to analyze the supermatrices but failed on multiple data sets (the analyses either collapsed or did not finish after more than one week of computation).

Assessment of tree inferences

In order to evaluate the performance of different fast phylogenetic programs, their inferred trees were compared from the following three aspects:

1) Likelihood: With respect to likelihood score maximization, a program was considered to perform better than another if it yielded a log-likelihood score that was more than 0.01 higher than the other. To ensure the fairness of the comparison, the likelihood scores of all trees were re-calculated using RAxML v8.2.0 with models set to the best-fit models and “GTR+G” for amino acid and nucleotide single-gene alignments, respectively, or the respective partition schemes for supermatrices. Trees of the same topology are presumed to have the same likelihood score. The BFGS optimization method was turned off in the analyses of nucleotide alignments. Independent likelihood score re-calculations were conducted for all trees using IQ-TREE v.1.4.2 to control for potential biases since RAxML itself is one of the programs to be assessed. The results were essentially the same (data not shown);

2) Topology: Our benchmarking is based on empirical data sets whose true underlying histories were unknown, thus preventing a direct measurement of the topological accuracy of programs. Thus, we compared the trees inferred by various strategies/programs against the tree with the best likelihood score observed for each alignment by calculating the pairwise Robinson-Foulds (RF) distances (Robinson and Foulds 1981) between them. To allow for comparison across alignments, the RF distances were normalized by the total numbers of internodes in respective pairs of trees. The reliabilities of coalescent-based and concatenation-based species tree estimations were evaluated using the local posterior probability measure (Sayyari and Mirarab 2016) implemented in ASTRAL v4.10.12 and the SH-aLRT test (Guindon, et al. 2010) implemented in IQ-TREE v1.4.2, respectively;

3) Speed: Computational efficiency is another critical factor affecting the choice of phylogenetic programs, especially when the availability of computational resource is a concern. The aforementioned phylogenetic analyses were conducted on multiple different computational

platforms, each equipped with different types of CPUs, thus preventing a direct comparison of the runtimes. To address this issue, we selected 10% of single-gene alignments randomly from each data set and redid all relevant phylogenetic analyses on Vanderbilt University's ACCRE cluster (http://www.accre.vanderbilt.edu/) using the same type of computing nodes. Similarly, a subset of supermatrices were selected and re-analyzed by ExaML v3.0.17 and IQ-TREE-OMP v1.4.2 (each with three replicates) on the same type of ACCRE nodes.

Computational resources

In this study, we conducted more than 670,000 tree inferences on about 45,000 single-gene alignments and supermatrices, which costed more than 300,000 CPU hours of computational time in total. This huge amount of phylogenetic analyses was made possible by using three supercomputing resources, including the Advanced Computing Center for Research and Education (ACCRE) at the Vanderbilt University, the University of Wisconsin-Madison Center for High Throughput Computing (CHTC), and the CIPRES Scientific Gateway at the San Diego Supercomputer Center (Miller, et al. 2010). Single-gene analyses were distributed between ACCRE and CHTC. For supermatrices, RAxML analyses were performed using the "RAxML-HPC v.8 on XSEDE" interface on CIPRES, while the other analyses were carried out on ACCRE

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Table 1. Overview of the four fast ML-based phylogenetic programs evaluated in this study.

Programs	Optimality criterion	Starting tree	Topological moves	Supported models		Partitioned analysis
				AA	DNA	
RAxML v8.2.0 (ExaML v3.0.17)	ML	Parsimony / random / custom	SPR	Common and custom models	JC69, K80, HKY85, GTR	Y
PhyML v20160530	ML	Parsimony / random / custom	Interleaved NNI and SPR	Common and custom models	Common and custom models	Y
IQ-TREE v1.4.2	ML	BIONJ and multiple parsimony / random / custom	NNI and stochastic perturbation	Common and custom models	Common and custom models	Y
FastTree v2.1.9	ML	Heuristic NJ	NNI and SPR (ME) followed by NNI (ML)	JTT, WAG, LG	JC69, GTR	N

Note: ML – maximum likelihood; ME – minimum evolution; NJ – neighbor joining; NNI – nearest neighbor interchange; SPR – subtree pruning and re-grafting.

Table 2. Overview of the 19 phylogenomic data sets included in this study.

Study	Data set		Genes	Taxa	Taxonomic group	Data type
	AA	DNA				
(Nagy, et al. 2014)	A1		594	60	Fungi	Genome
(Misof, et al. 2014)	A2		1,478	144	Insects	Transcriptome
		D2a[1,2]				
		D2b[2]				
(Wickett, et al. 2014)	A3		844	103	Land plants	Transcriptome
		D3a[2]				
		D3b[3]				
(Chen, et al. 2015)	A4		4,682	58	Vertebrates	Transcriptome
(Struck, et al. 2015)	A5		679	100	Worms	Transcriptome
(Borowiec, et al. 2015)	A6		1,080	36	Metazoans	Genome
(Whelan, et al. 2015)	A7		210	70	Metazoans	Transcriptome
(Yang, et al. 2015)	A8		1,122	95	Caryophyllales	Transcriptome
(Shen, et al. 2016b)	A9		1,233	96	Yeasts	Genome
(Song, et al. 2012)		D1	424	37	Mammals	Genome
(Xi, et al. 2014)		D4	310	46	Flowering plants	Transcriptome
(Jarvis, et al. 2014)		D5a[4]	14,446	48	Birds	Genome
		D5b[4]	2,022	48		
(Prum, et al. 2015)		D6	259	200	Birds	Target Enrichment
(Tarver, et al. 2016)		D7	11,178	36	Mammals	Genome

[1] Date set D2a does not have a corresponding supermatrix from the original study;

[2] DNA data sets D2a and D3a include the codon-based alignments corresponding to the amino acid alignments in data sets D2 and D3, respectively;

[3] DNA data sets D2b and D3b include the full codon-based alignments corresponding to the amino acid alignments in data sets D2 and D3, respectively, with the third codon positions removed;

[4] Data set D5b were derived from data set D5a through statistical binning (Mirarab, et al. 2014), and the

two data sets correspond to the same supermatrix.

Table 3. Normalized Robinson-Foulds distances between the coalescent-based species trees estimated from gene trees inferred by various strategies and the "best-observed" gene trees.

Data set		Analysis strategies				
		RAxML_10	*RAxML*	*IQ-TREE*	*PhyML*	*FastTree*
Amino acid	A1	0.035	0.053	0.053	0.070	0.123
	A2	0.021	0.057	0.028	0.028	0.106
	A3	0.030	0.030	0.020	0.050	0.090
	A4	0.000	0.000	0.000	0.000	0.000
	A5	0.031	0.144	0.144	0.175	0.258
	A6	0.000	0.000	0.030	0.030	0.121
	A7	0.027	0.055	0.041	0.055	0.123
	A8	0.000	0.011	0.000	0.000	0.054
	A9	0.011	0.043	0.032	0.011	0.043
Nucleotide	D1	0.000	0.000	0.000	0.000	0.000
	D2a	0.035	0.035	0.071	0.085	0.206
	D2b	0.035	0.014	0.035	0.028	0.128
	D3a	0.030	0.040	0.030	0.030	0.140
	D3b	0.010	0.000	0.000	0.040	0.090
	D4	0.023	0.023	0.023	0.047	0.186
	D5a	0.000	0.000	0.022	0.044	0.400
	D5b	0.044	0.067	0.044	0.044	0.267
	D6	0.025	0.066	0.061	0.071	0.142
	D7	0.000	0.000	0.000	0.000	0.000

Figure legends

Figure 1. Schematics of the (A) single-gene tree inference test as well as the coalescent-based and (B) concatenation-based species tree inference tests used to evaluate the performance of fast phylogenetic programs in phylogenomic analysis.

Figure 2. Performance of fast phylogenetic programs in single-gene tree inferences. The bar-plots show the frequencies with which each of the six analysis strategies produced the best likelihoods for single-gene alignments in each of the (A) protein and (B) DNA data sets.

Figure 3. The performances of fast phylogenetic programs with respect to likelihood maximization and topological accuracy are positively correlated. Dots in the scatter plot correspond to trees inferred by various analysis strategies from single-gene alignments in data set A8. Log-likelihood score differences between inferred trees and the "best-observed" trees are plotted against the corresponding topological distances. The log-likelihood score differences are shown in logarithmic scale (with the addition of a small value of 0.01). The violin plots on the top and right show the distributions of log-likelihood differences (top) and topological distances (right), respectively, for trees inferred by each strategy.

Figure 4. Runtime comparisons of fast phylogenetic programs in single-gene tree inferences. The runtimes required by each strategy to analyze a randomly selected subset of all protein (top row) and DNA (bottom row) alignments are plotted against the corresponding runtimes of *RAxML*. All runtimes (in seconds) are shown in logarithmic scale.

Figure 5. Incongruent splits in coalescent-based species trees estimated by *RAxML-10*, *RAxML*, *PhyML*, and *IQ-TREE* are weakly supported. The violin plots show the distribution of local posterior probabilities for incongruent splits in coalescent-based species trees estimated by various analysis strategies. Here, incongruent splits are defined as the splits that are not present in species trees estimated from best-observed single-gene trees. The areas of violin plots are proportional to the total numbers of incongruent splits. The grey dots and bars in each violin plot indicate the median and the first/third quartiles of the local posterior probabilities, respectively.

**Figure 6. Likelihood score differences (A) and normalized Robinson-Foulds distances (B) between

concatenation-based species trees inferred by various fast phylogenetic programs and the best-observed trees. In panel (A), the log-likelihood score differences are shown in logarithmic scale (with the addition of a small value of 0.01), and the likelihood scores that are not significantly different from the best-observed scores are shown in grey. In panel (B), the nRF distances of ExaML/RAxML-published and RAxML-generated trees that can be further improved by NNI rearrangements are shown in grey.

Figure 7. Many incongruent splits in concatenation-based species trees estimated by FastTree receive strong support. The jitter plots show the distribution of SH-aLRT supports for incongruent splits in concatenation-based species trees estimated by various fast phylogenetic programs. Here, incongruent splits are defined as the splits that are not present in the species trees with the best likelihoods. The species trees inferred by IQ-TREE contain no incongruent splits and therefore the data for IQ-TREE is not shown.

Figure 8. Runtime comparisons of fast phylogenetic programs in concatenation-based species tree inferences. The bar-plots show the runtimes (averaged over three replicates) required by RAxML, IQ-TREE, and FastTree to analyze 10 selected supermatrices.

Figure 9. The strength of phylogenetic signal in the data has an impact on the relative performance of *RAxML* and *IQ-TREE*. The violin plots show the distributions of average bootstrap values of alignments for which the best likelihood scores were found by either *RAxML* or *IQ-TREE*, or both strategies at the same time. The average bootstrap values are taken from previously reported phylogenies for the alignments are used here as a measure of the strength of phylogenetic signal.

Figure 1

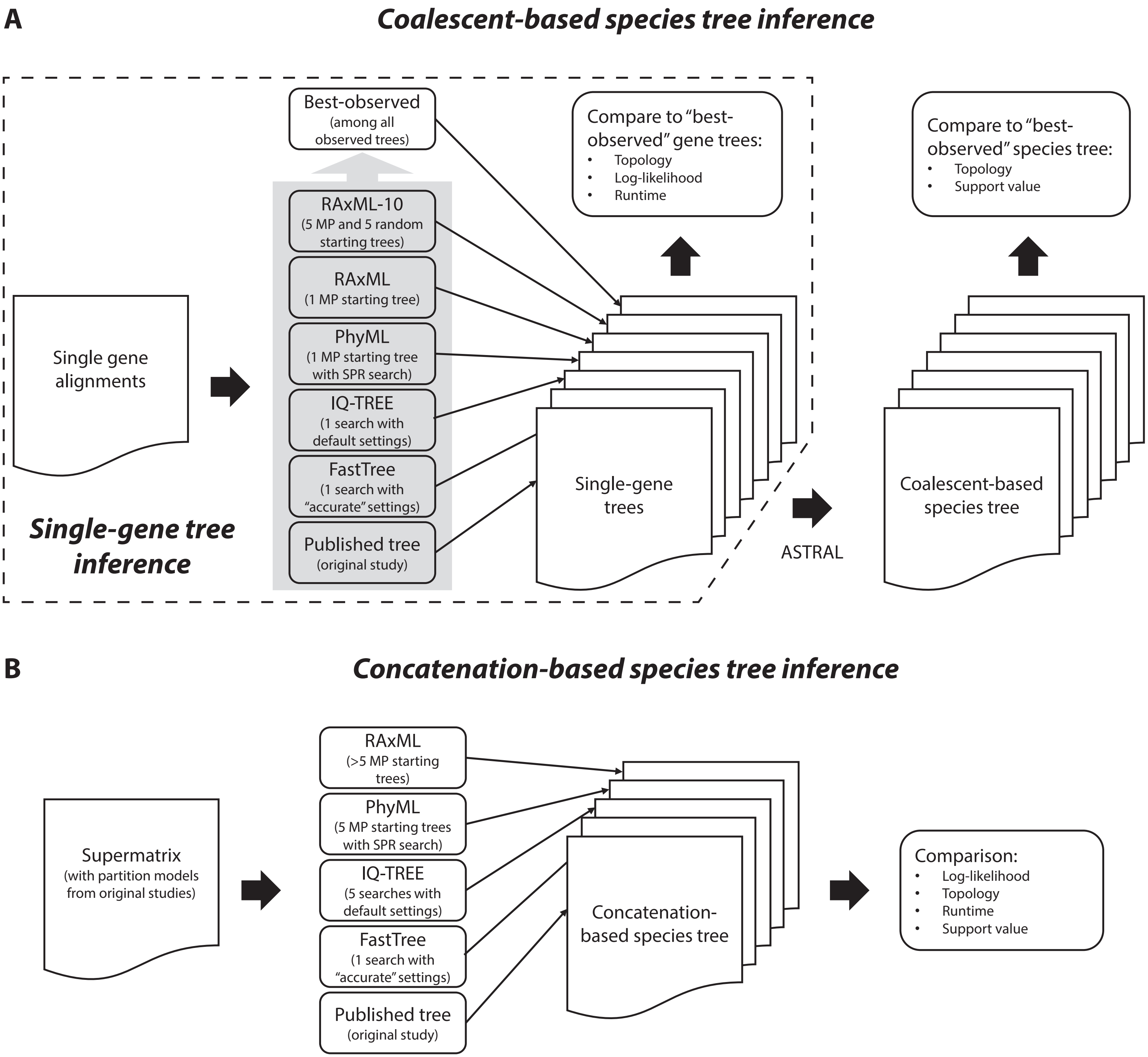

A
Coalescent-based species tree inference
Single gene alignments
Best-observed
(among all observed trees)
RAxML-10
(5 MP and 5 random starting trees)
RAxML
(1 MP starting tree)
PhyML
(1 MP starting tree with SPR search)
IQ-TREE
(1 search with default settings)
FastTree
(1 search with "accurate" settings)
Published tree
(original study)
Single-gene trees
Compare to "best-observed" gene trees:
• Topology
• Log-likelihood
• Runtime
Single-gene tree inference
ASTRAL
Coalescent-based species tree
Compare to "best-observed" species tree:
• Topology
• Support value
B
Concatenation-based species tree inference
Supermatrix
(with partition models from original studies)
RAxML
(>5 MP starting trees)
PhyML
(5 MP starting trees with SPR search)
IQ-TREE
(5 searches with default settings)
FastTree
(1 search with "accurate" settings)
Published tree
(original study)
Concatenation-based species tree
Comparison:
• Log-likelihood
• Topology
• Runtime
• Support value

Figure 2

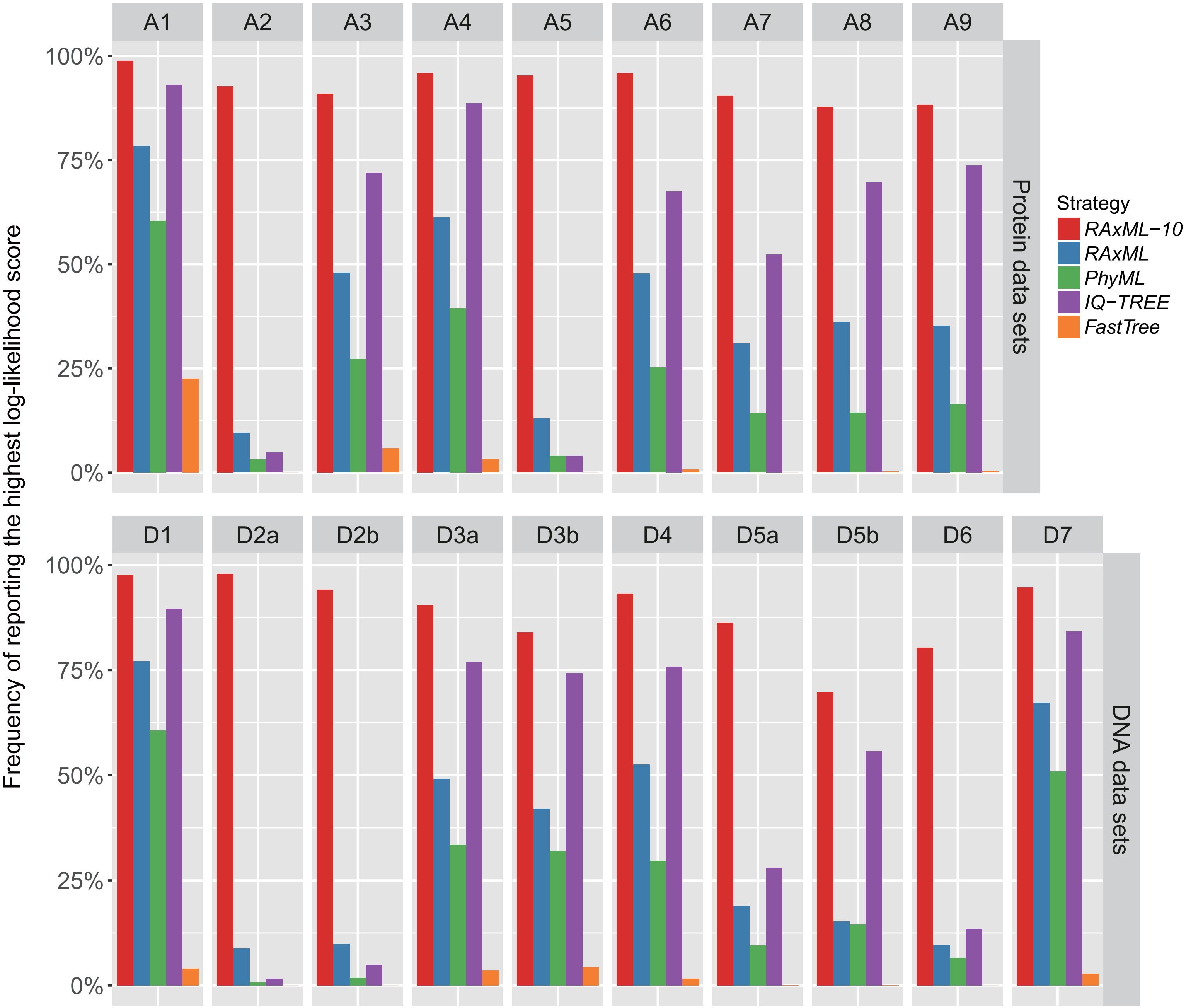
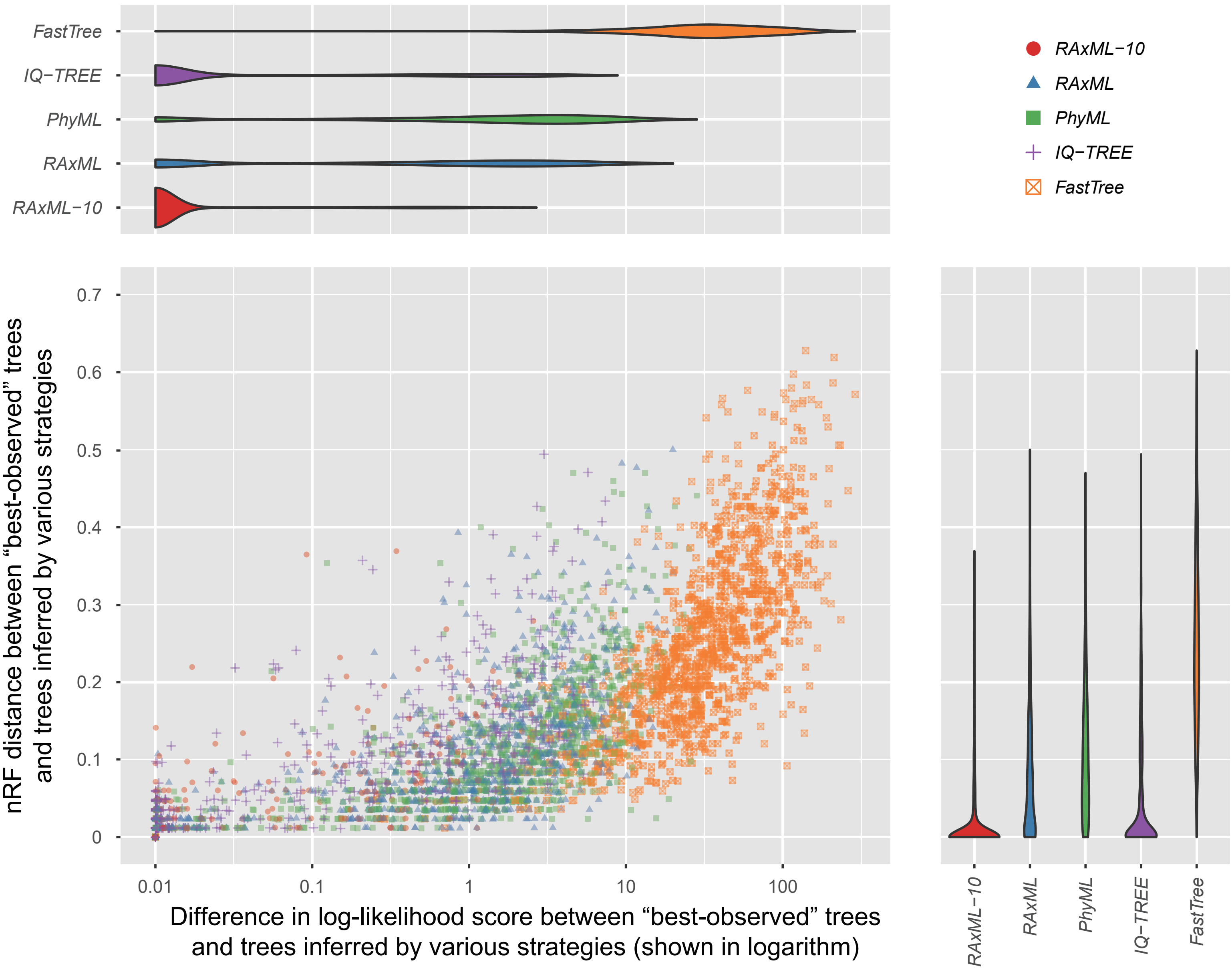

A1
A2
A3
A4
A5
A6
A7
A8
A9
D1
D2a
D2b
D3a
D3b
D4
D5a
D5b
D6
D7
100%
75%
50%
25%
0%
Frequency of reporting the highest log-likelihood score
Protein data sets
DNA data sets
Strategy
RAxML−10
RAxML
PhyML
IQ−TREE
FastTree

Figure 3



FastTree
IQ−TREE
PhyML
RAxML
RAxML−10
● RAxML−10
▲ RAxML
■ PhyML
+ IQ−TREE
⊠ FastTree
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
nRF distance between “best-observed” trees and trees inferred by various strategies
0.01
0.1
1
10
100
Difference in log-likelihood score between “best-observed” trees and trees inferred by various strategies (shown in logarithm)
RAxML−10
RAxML
PhyML
IQ−TREE
FastTree

Figure 4

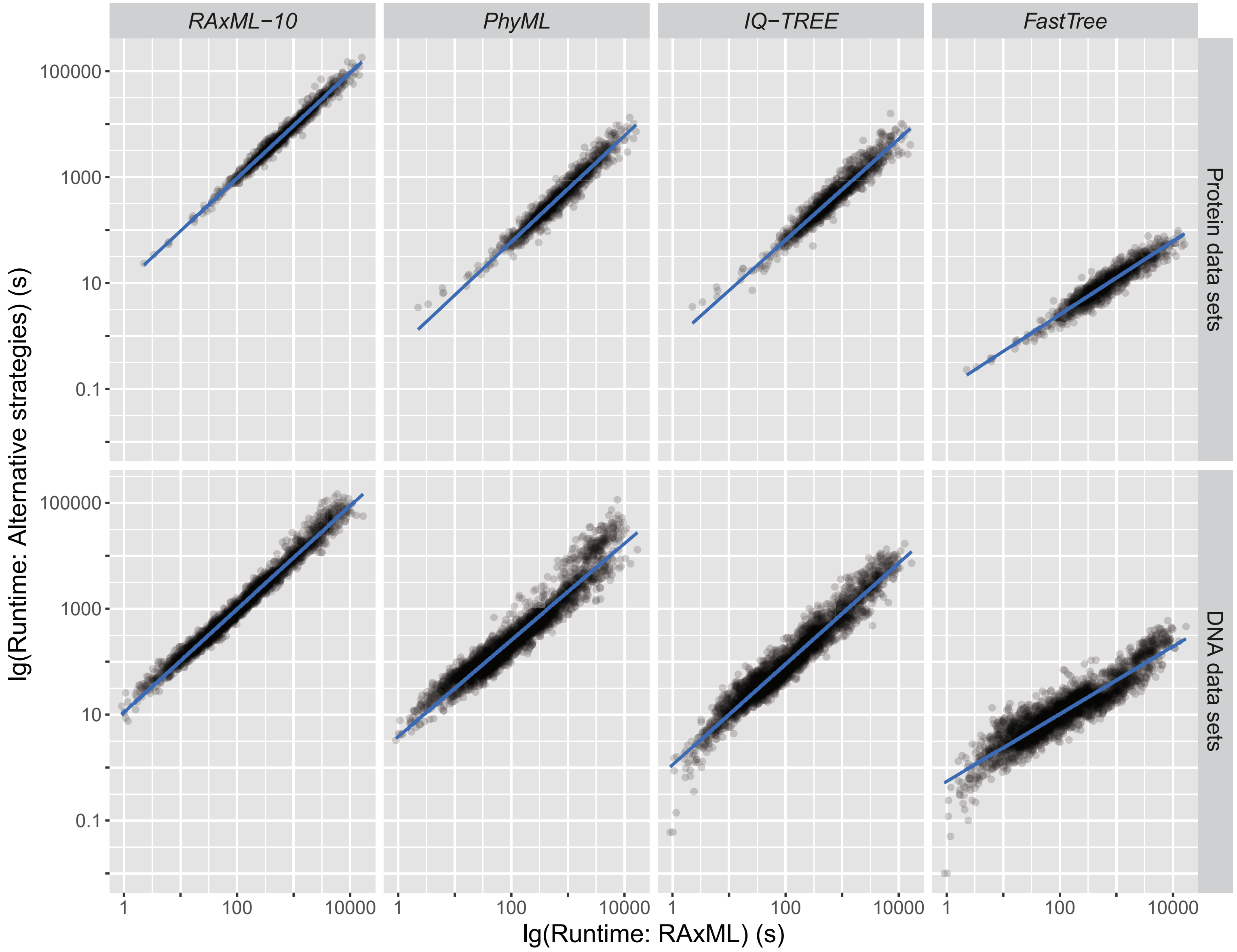

RAxML−10
PhyML
IQ−TREE
FastTree
Protein data sets
DNA data sets
lg(Runtime: Alternative strategies) (s)
100000
1000
10
0.1
100000
1000
10
0.1
1
100
10000
1
100
10000
1
100
10000
1
100
10000
lg(Runtime: RAxML) (s)

Figure 5

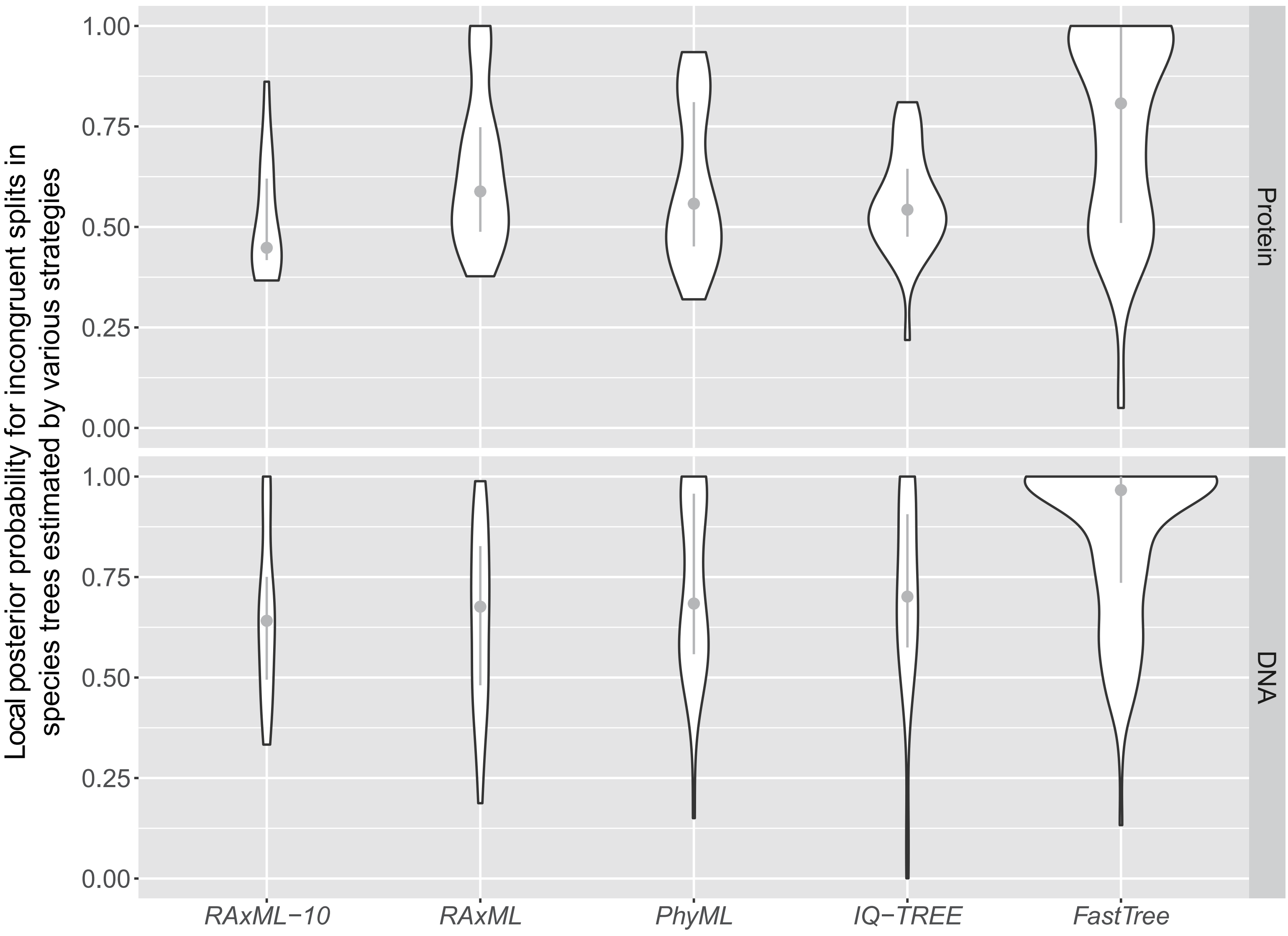

Local posterior probability for incongruent splits in species trees estimated by various strategies
1.00
0.75
0.50
0.25
0.00
Protein
DNA
RAxML−10
RAxML
PhyML
IQ−TREE
FastTree

Figure 6

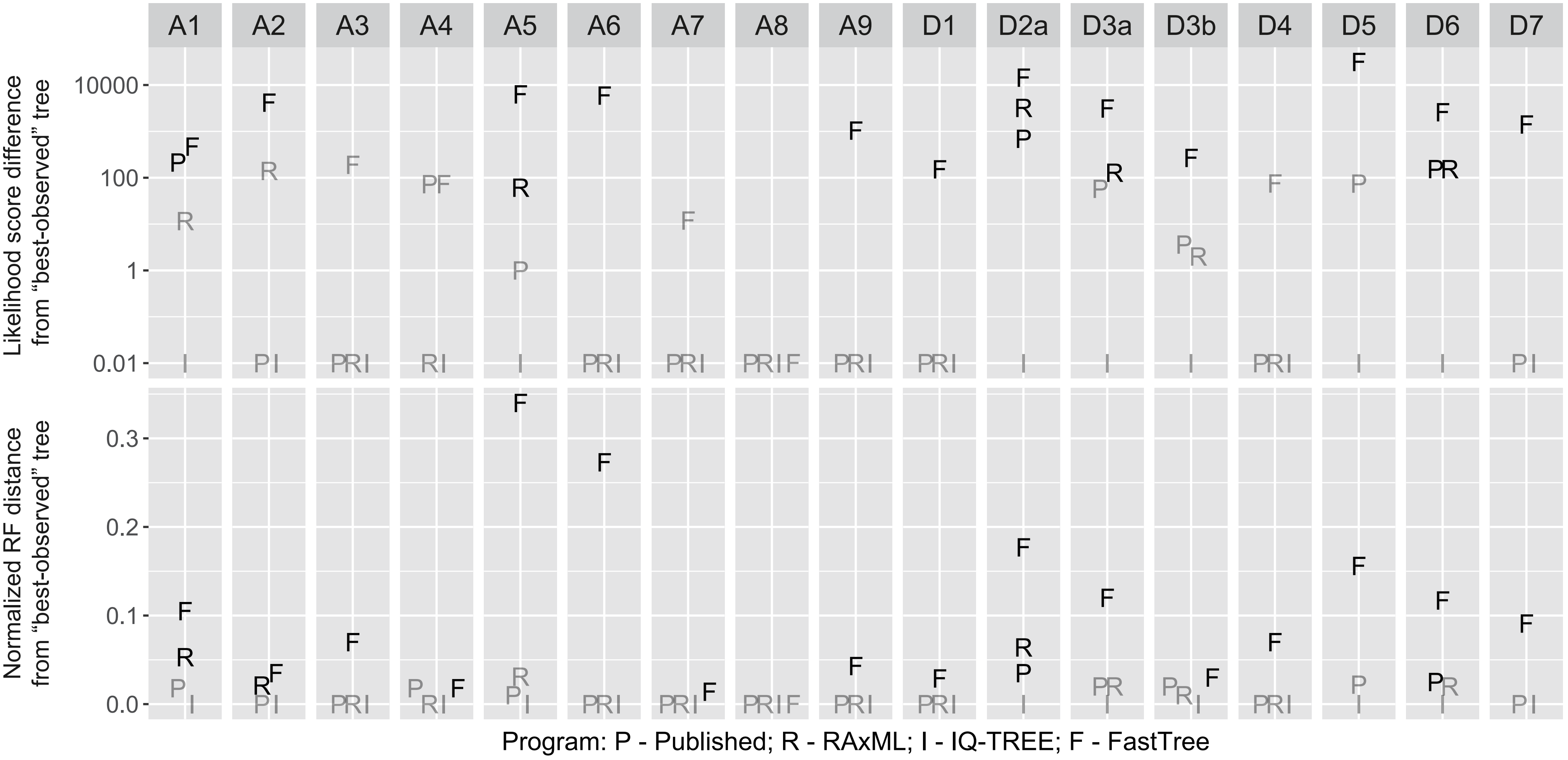

A1
A2
A3
A4
A5
A6
A7
A8
A9
D1
D2a
D3a
D3b
D4
D5
D6
D7
Likelihood score difference from "best-observed" tree
10000
100
1
0.01
Normalized RF distance from "best-observed" tree
0.3
0.2
0.1
0.0
P
R
I
F
Program: P - Published; R - RAxML; I - IQ-TREE; F - FastTree

Figure 7

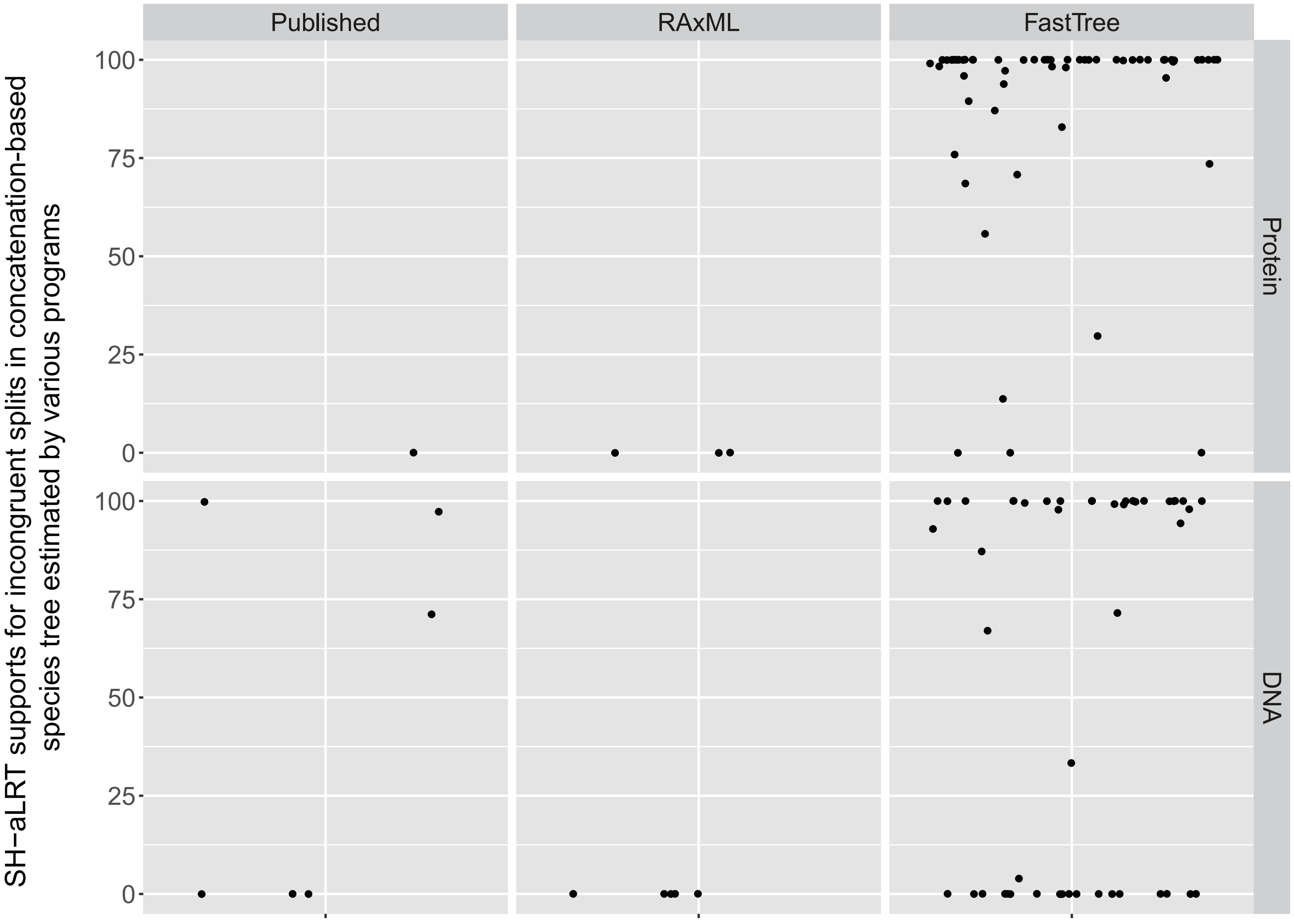

Published
RAxML
FastTree
Protein
DNA
100
75
50
25
0
SH−aLRT supports for incongruent splits in concatenation-based species tree estimated by various programs

Figure 8

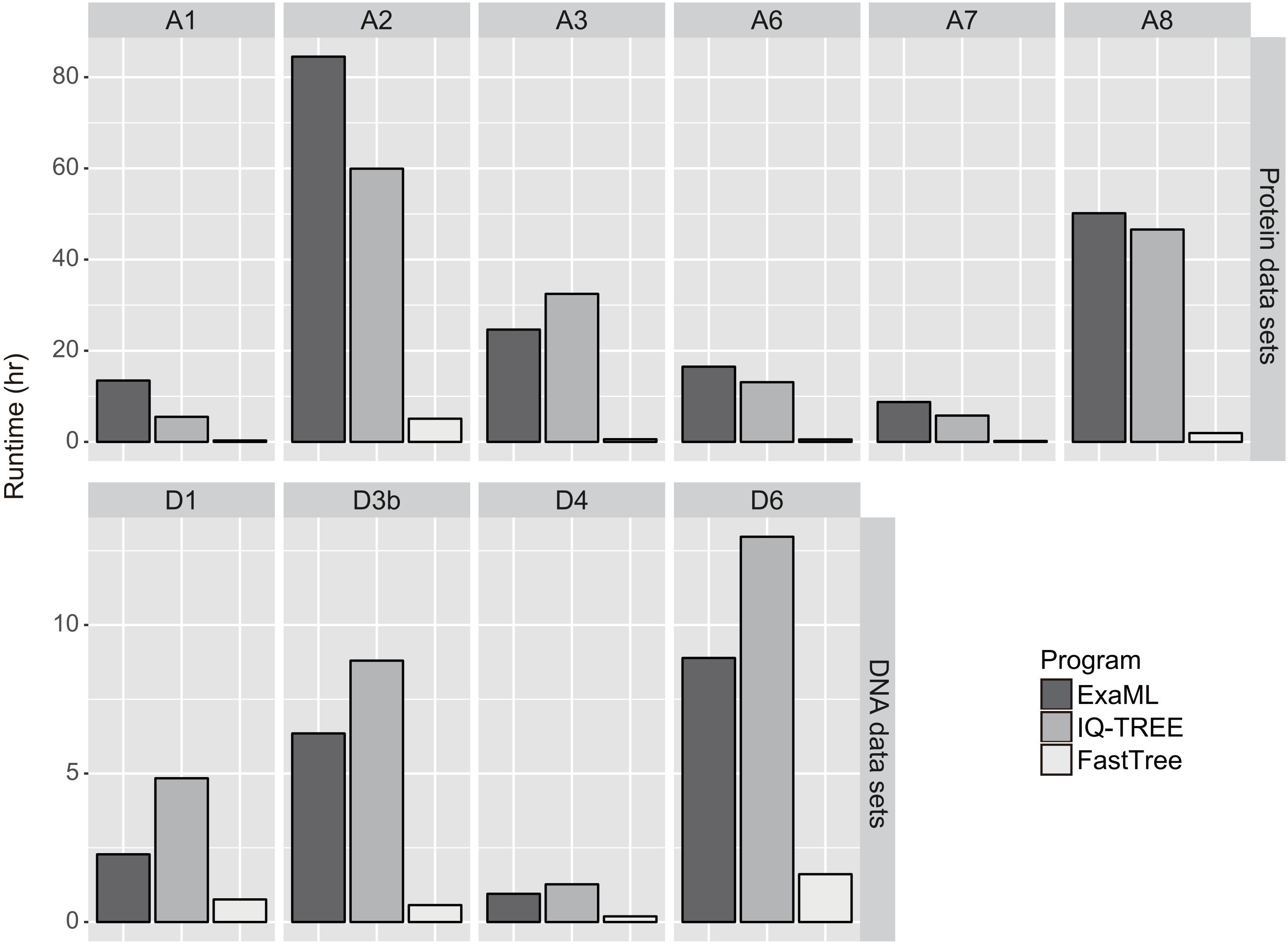
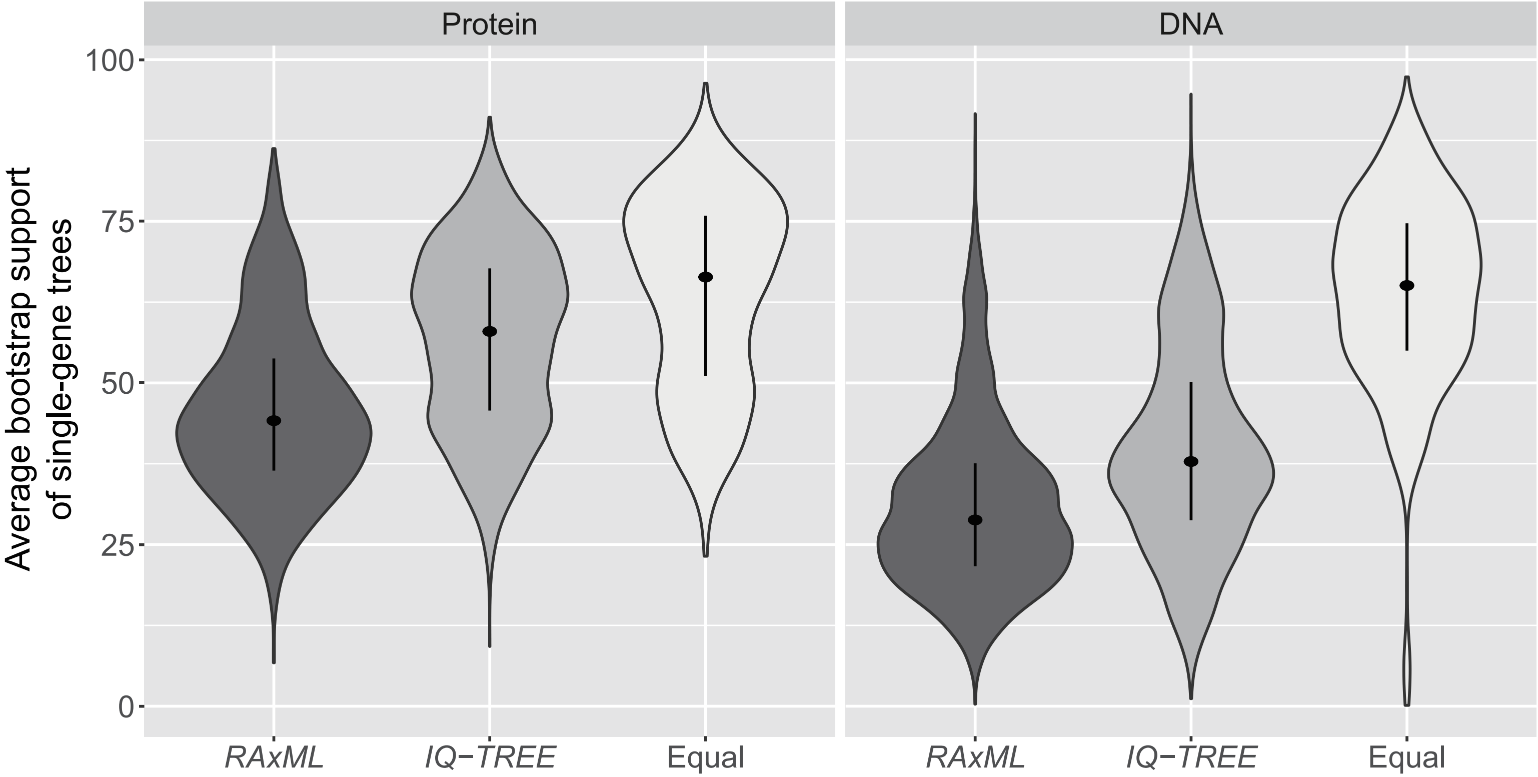

A1
A2
A3
A6
A7
A8
Protein data sets
D1
D3b
D4
D6
DNA data sets
Runtime (hr)
0
20
40
60
80
0
5
10
Program
ExaML
IQ-TREE
FastTree

Figure 9


Protein
DNA
Average bootstrap support of single-gene trees
100
75
50
25
0
RAxML
IQ−TREE
Equal
RAxML
IQ−TREE
Equal