Using paralogs for phylogenetic inference mitigates the effects of long-branch attraction

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Abstract

Traditionally, the inference of species trees has relied on orthologs, or genes related through speciation events, to the exclusion of paralogs, or genes related through duplication events. This has led to a focus on using only gene families with a single gene-copy per species, as these families are likely to be composed of orthologs. However, recent work has demonstrated that phylogenetic inference using paralogs is accurate and allows researchers to take advantage of more data. Here, we investigate a case in which using larger gene families actually increases accuracy compared to using single-copy genes alone. Long-branch attraction is a phenomenon in which taxa with long branches may be incorrectly inferred as sister taxa due to homoplasy. The most common solution to long-branch attraction is to increase taxon sampling to break up long branches. Sampling additional taxa is not always feasible, perhaps due to extinction or access to high-quality DNA. We propose the use of larger gene families with additional gene copies to break up long branches. Using simulations, we demonstrate that using larger gene families mitigates the impacts of long-branch attraction across large regions of parameter space. We also analyze data from Chelicerates, with a focus on assessing support for a sister relationship between scorpions and pseudoscorpions. Previous work has suggested that the failure to

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recover this relationship is due to long-branch attraction between pseudoscorpions and other lineages. Using data from larger gene families increases support for a clade uniting scorpions and pseudoscorpions, further highlighting the potential utility of these gene families in phylogenetic inference.

Key words: gene duplication, homoplasy, maximum parsimony, maximum likelihood, Chelicerates.

A major aim of phylogenetics is to reconstruct evolutionary relationships among species. Advances in sequencing technologies in recent decades have provided access to thousands of loci, which promises to help resolve recalcitrant nodes in the tree of life (Scornavacca et al., 2020). While phylogenetic methods are powerful, they are sometimes prone to systematic biases, in which incorrect trees are inferred due to unmodelled processes. One of the most well-studied such biases is long-branch attraction (LBA). LBA is a systematic bias in which phylogenetic methods preferentially infer a sister relationship between distantly related taxa with long branches. This occurs because a large number of homoplasious substitutions along long branches leads to high apparent similarity between such taxa (Felsenstein, 1978) (Figure 1A).

LBA was first described in the context of parsimony (Felsenstein, 1978; Hendy and Penny, 1989), which is highly susceptible to LBA. Probabilistic approaches—such as maximum likelihood—can somewhat mitigate LBA by accounting for the possibility of homoplasy due to multiple substitutions (Felsenstein, 1978). However, using inadequate substitution models can still leave these methods susceptible to LBA (Gaut and Lewis, 1995; Philippe et al., 2005b; Szánthó et al., 2023). Several solutions have been suggested to mitigate LBA (reviewed in Bergsten 2005), including using more appropriate models of nucleotide substitution (Gaut and Lewis, 1995; Szánthó et al., 2023; Sullivan and Swofford, 1997; Lartillot et al., 2007), excluding quickly evolving sites or genes (Brinkmann et al., 2005; Duchêne et al., 2022; Philippe et al., 2005a; Sanderson et al., 2000), and/or adding

taxa to break up long branches (Hendy and Penny, 1989; Graybeal, 1998) (Figure 1B). The latter approach has proven particularly powerful, but sampling additional taxa may be challeging (e.g., due to available resources) or impossible (e.g., due to extinction) in many cases. Here, we explore an alternative approach to breaking up long branches—considering data from larger gene families.

Traditionally, phylogenomics has relied on the use of orthologs (genes related through speciation events) to the exclusion of paralogs (genes related through duplication events). In a typical work flow, researchers begin with a set of coding genes from each sampled taxon. Ortholog clustering approaches (e.g., OrthoMCL; Li et al. 2003) are then used to identify gene families, or groups of closely related genes (sometimes called "orthogroups"). These families include both orthologs and paralogs. The traditional approach is to use only those gene families in which there is a single gene-copy present in each species, as these single copy (SC) genes are hypothesized to be orthologs. Since single-copy orthologs are related only through speciation events, their history should match the species history in the absence of other processes (e.g., incomplete lineage sorting). While this approach has proven powerful in phylogenomics, it results in a massive amount of data loss (Emms and Kelly, 2018). Recent work has explored the potential of using data from larger gene families, either by using tree-based approaches to extract orthologs from these larger gene families (e.g., Yang and Smith 2014; Willson et al. 2022) or by using large gene families directly for inference (e.g., Legried et al. 2021; Zhang et al. 2020, reviewed in Smith and Hahn 2021). Theoretical (Legried et al., 2021; Zhang et al., 2020), simulation (Legried et al., 2021; Zhang et al., 2020; Yan et al., 2022), and empirical (Smith et al., 2022) studies have demonstrated that phylogenetic inference using data from larger gene families is highly accurate and allows researchers to use substantially more data.

While previous work has focused on the ability to use more data as the primary reason to analyze larger gene families, here we explore another potential benefit. Just as taxon sampling can be used to break up long branches, gene sampling could also break up

long branches (Figure 1C). Specifically, analyzing gene families in which duplication events occur on long branches in the tree should mitigate long-branch attraction. Here, we use simulations to evaluate the impact of including data from larger gene families on inferences using maximum parsimony (MP) and maximum likelihood (ML) in regions of parameter space in which LBA occurs. We explore these effects using both all gene families together and focusing only on gene families with duplications on the long branches of interest. Finally, we analyze data from Chelicerates to evaluate the impacts of including larger gene families on support for a sister relationship between scorpions and pseudoscorpiones, a relationship that has previously been suggested to have been obscured by LBA (Ontano et al., 2021).

Materials and Methods

Simulations

We simulated gene families using SimPhy v1.0.2 (Mallo et al., 2015). As a species tree, we used the tree shown in Figure 1A. To vary the strength of long-branch attraction, we varied the length of the internal branch, r, and the magnitude of the substitution rate multiplier, q. Specifically, we explored a grid of values for r and q. We considered 20 values of each parameter evenly spaced throughout the grid, and we simulated 100 gene trees per parameter combination. In the presence of loss (see below), we may not always have 100 trees per condition, because loss can lead to scenarios in which the four focal species are not all present. We considered r values ranging from 100,000 to 1,000,000 generations. For MP, we considered q values ranging from 10 to 30, while for ML, we considered q values ranging from 40 to 100 (because of the greater sensitivity of MP to LBA; Swofford et al. 2001). We set the length of the external branches, p, to 250,000. We considered a duplication rate of 1e-6, a nucleotide substitution rate of 1e-7, and a population size of 10,000. We performed two sets of experiments—one with the loss rate set to 0 and a

second with the loss rate set equal to the duplication rate. We required a minimum of four leaves in each gene tree.

After simulating gene family trees in SimPhy, we simulated sequence data on each gene family tree using SeqGen v1.3.4 (Rambaut and Grass, 1997). We used the HKY model of sequence evolution to simulate sequences 2,000 base-pairs in length. We inferred trees from these data using either MP or ML. For MP inference, we used MPBoot v1.1.0 (Hoang et al., 2018) with default settings. For ML inference, we used IQTree v2.3.2 (Minh et al., 2020), and set the nucleotide substitution model to the HKY model used to simulate data.

To quantify the accuracy of our inferences, we used ASTRAL-IV v1.19.4.5 (Zhang et al., 2018; Zhang and Mirarab, 2022) as implemented in ASTER (available from https://github.com/chaoszhang/ASTER) to calculate quartet concordance factors. These numbers report the fraction of quartets from the inferred gene trees that match the species tree shown in Figure 1A. Since our simulations include only four taxa, the quartet concordance factor is equivalent to the proportion of quartets supporting the species tree topology (averaged across all trees simulated for each condition). Long-branch attraction will lead to decreased quartet concordance, so we expect concordance factors to be lowest when long-branch attraction has the largest impacts on inference. Notably, gene trees could also differ from the species tree due to incomplete lineage sorting (ILS); however, we expect this to contribute minimally to discordance given the population sizes used here, and the amount of ILS should be constant across simulation conditions. We also recorded whether each replicate was a single-copy gene, whether the inferred tree included only lineage-specific duplicates (after midpoint rooting), and whether the inferred tree included lineage-specific duplicates on the long branches (after midpoint rooting). Finally, we calculated whether true trees included lineage-specific duplicates on long branches, and, if so, the distance of these duplicates from the furthest leaf.

In addition to these experiments, we performed an experiment in which there was increased discordance due to duplication and loss. In our original simulations, duplications

could only occur in the ancestor of a single lineage, in the ancestor of A and B, or in the ancestor of C and D. Because of this, duplications could not generate quartet discordance. On the other hand, if duplications occur in the ancestor of the four focal species, additional discordance may result (Supporting Figure S1), because sampled quartets may include paralogs, or genes related through duplication events. When paralogs are sampled, gene tree quartets may disagree with the species tree even in the absence of gene tree inference error and ILS. Thus, we expect quartet concordance to decrease primarily due to two factors in these simulations: gene tree estimation error and gene duplication and loss. To allow for duplications in the ancestor of the focal species in our simulations, we included an outgroup when simulating data in SimPhy. The outgroup was removed prior to gene tree inference and subsequent analyses. While this can lead to discordance when the full dataset is analyzed using quartet-based approaches, discordance can be avoided by using tree-based decomposition approaches or by sampling only gene families with lineage-specific duplications. For this set of experiments, we varied the ratio between total tree height and the ingroup height, O (Fig. S1). We considered O values ranging from 1.01 to 5. Because these simulations can lead to much larger trees, we considered duplication and loss rates equal to 5e-7. We set the length of the internal branch r equal to 300,000, and allowed q to vary as above. All other parameters were set as above. Simulation of sequence data, gene tree inference, and calculations of concordance factors followed the methods described above.

Chelicerate data

To evaluate whether using paralogs also mitigates LBA in an empirical dataset, we used data from Chelicerates. Previous work has suggested that the lack of support for a sister relationship between pseudoscorpions and scorpions is due to LBA (Ontano et al., 2021), specifically LBA between pseudoscorpions and other long-branch chelicerates. We downloaded 19 whole genomes from NCBI (Sayers et al., 2022), i5k (Thomas et al., 2020),

and other sources (Nong et al., 2021; Shingate et al., 2020) (Table S1). For one species, *Tachypleus gigas*, no CDSs were available, so we generated a CDS annotation from the GFF and genome using gffread (Pertea and Pertea, 2020).

Generally, we followed the methodology used to infer gene families from coding sequences used by Thomas et al. (2024). Briefly, we extracted the coding sequence of the longest isoform of each gene using a python script available from https://github.com/gwct/isofilter-gff. We then used FastOrtho with an inflation parameter of 3 (https://github.com/olsonanl/FastOrtho) to cluster genes into gene families. Next, we aligned the sequences of each gene family using mafft v7.526 (Katoh et al., 2009) with a maximum of 5,000 iterations. We then used Gblocks v.0.91b with default parameters to remove poorly aligned positions (Castresana, 2000). Additionally, we used TrimAl to further trim alignments (Capella-Gutiérrez et al., 2009). Finally, we removed all alignments less than 300 base-pairs long.

We inferred gene trees from all Chelicerate alignments using MP and ML. For MP inference, we used MPBoot v1.1.0 (Hoang et al., 2018) with default parameters. For ML inference, we used IQTree v2.3.2 (Minh et al., 2020), selecting the best-fit model automatically using ModelFinder (Kalyaanamoorthy et al., 2017). Finally, to assess support for a sister relationship between the scorpion, *Centruroides sculpturatus*, and the pseudoscorpion, *Cordylochernes scorpioides*, we again used ASTRAL to calculate quartet concordance factors on the tree from Thomas et al. (2024), except that we added *Cordylochernes scorpioides* to the tree as sister to *Centruroides sculpturatus*. We calculated concordance factors for six datasets. First, we calculated concordance factors for our trees inferred using MP either including only single-copy genes, all gene families, or only gene families with at least one lineage-specific duplication in pseudoscorpions. Second, we calculated concordance factors for our ML trees for the same three sets of gene families. We calculated confidence intervals by bootstrapping the data. Specifically, we resampled trees from the original dataset with replacement 100 times and calculated concordance

factors from each resampled dataset.

Results

Using paralogs mitigates long-branch attraction in simulations

We first conducted simulations to demonstrate the effects of long-branch attraction when using single-copy genes. As expected, the accuracy of inferred trees decreases as the length of the internal branch (r) decreases, and as the branch length multiplier (q) increases (Fig. 2). This pattern is observed for both MP and ML, but is much more pronounced under MP (Fig. 2A) than under ML (Fig. 2C). (Note the difference in the q-values used on the y-axes between MP and ML.)

Next, we inferred trees using data from all gene families, which includes many duplicates that can break up the long branches. Using all gene families resulted in increased accuracy for both MP (Fig. 2B) and ML (Fig. 2D). In general, the accuracy stayed high in regions of parameter space in which accuracy was already high when using single-copy genes, but using all gene families extended the parameter space across which accurate trees could be inferred. The above results are all for simulations with no gene loss, but the same trends held when the loss rate, μ, was set equal to the duplication rate, λ (Supporting Fig. S2).

Duplicates that occur very near the present may be less effective at breaking up long branches compared to duplicates that occur at intermediate ages. To further explore the role of lineage-specific duplicates in mitigating LBA, we calculated the maximum distance between a lineage-specific duplication node in species B or D to the furthest leaf of the node on simulated gene trees. We found that the age of the oldest LSD was larger for gene families that were concordant with the species tree (Fig. 3).

Finally, we explored the impact of adding duplications in the ancestor of the focal taxa, which can increase discordance due to gene duplication and loss (Smith and Hahn,

2021). As expected, these duplications have no impact when analyzing single-copy genes, as paralogs are unlikely to be included. Specifically, the amount of concordance observed in Fig. 4A is equivalent to that seen in 2A with a fixed r of 750,000. However, when analyzing all gene families, additional opportunities for discordance due to duplication and loss generally lead to lower quartet concordance. The larger the value of O—i.e. the longer the branch subtending the ingroup—the higher the probability of duplications on that branch. At small to medium values of q, increasing O leads to more discordance (Fig. 4). The situation is more complicated when q is large. Under MP, when q is large, the systematic biases due to long-branch attraction lead to concordance factors near zero. In this scenario, adding noise actually increases the proportion of quartets that agree with the species tree. Thus, the added noise due to duplication and loss increases concordance relative to that seen in the case of extreme systematic bias (Fig. 4B).

To avoid issues with duplication and loss complicating relationships, researchers need not rely only on single-copy genes. Instead, researchers can filter their data to include only lineage-specific duplicates. Furthermore, if the goal is to mitigate LBA, then using only those gene families with gene duplications on the long branches should be particularly effective. Notably, no knowledge of true gene family trees is needed to do this: researchers can use simple filtering approaches to include only gene families with two gene copies in the taxa of interest. When we calculated accuracy using only those gene families with relevant lineage-specific duplicates, concordance was again increased relative to the single-copy genes (Fig. 4C,F).

Including data from larger gene families increases support for a sister relationship between pseudoscorpions and scorpions

LBA has been proposed to decrease support for a sister relationship between scorpions and pseudoscorpions (Ontano et al., 2021), so we expect that support for this relationship will be higher under ML than under MP (since MP is more susceptible to

LBA). Our results support this prediction, with more trees supporting their sister relationship when inferred using ML, though the confidence intervals are overlapping ("SC" row in Table 1). Furthermore, if using larger gene families helps to mitigate LBA, then we expect that support for this relationships will be higher when all gene families are analyzed compared to when only single-copy genes are analyzed. Again, our results support this prediction, with higher support for sister relationships between scorpions and pseudoscorpions when all gene families are used ("All" row in Table 1); however, confidence intervals are again overlapping with estimates from single-copy datasets. Finally, we expect support for this sister relationship to be highest when we only analyze lineage-specific duplicates, as this should allow us to break up long branches without adding noise due to gene duplication and loss. While our results support this prediction when using ML ("LSD" row in Table 1), the same was not true under MP, and confidence intervals always overlapped with those found when analyzing all gene families.

Discussion

Traditionally, phylogenetic inference has relied on orthologs, primarily by restricting analyses to single-copy genes. Recent work has demonstrated that using data from larger gene families allows researchers to use more data without sacrificing accuracy (Smith et al., 2022; Yan et al., 2022; Legried et al., 2021; Zhang et al., 2020). While this work has generally focused on the advantages of using larger gene families when single-copy genes are limited in number, here we focus on a case in which individual gene trees inferred from larger gene families have increased accuracy.

LBA appears to be common across the tree of life (e.g., Ontano et al. (2021); Cai et al. (2024); Szánthó et al. (2023)). While several solutions have been proposed, no single solution is without its challenges. Adding more taxa is an effective solution (Hendy and Penny, 1989; Graybeal, 1998), but is not always possible due to extinction or a lack of available samples. Using more complex substitution models has also proven powerful in

many contexts, particularly when taking into account heterogeneity in the substitution process (Lartillot et al., 2007; Szánthó et al., 2023). However, estimating parameters of complex models that account for rate heterogeneity (e.g., CAT models; Lartillot and Philippe 2004) is challenging and computationally expensive, and may not always result in more accurate inferences (Whelan and Halanych, 2017). Thus, novel approaches to mitigating LBA are still needed.

Many recent approaches to accurate phylogenetic inference combine data from multiple gene trees to infer a species tree (e.g., ASTRAL, Zhang and Mirarab 2022; ASTRID, Vachaspati and Warnow 2015). These methods are explicitly avoiding biases associated with concatenating gene trees (Kubatko and Degnan, 2007), but may themselves suffer from issues inherent in inferring gene trees from short alignments (Bryant and Hahn, 2020). If gene trees are not inferred accurately, then the species tree may also not be inferred accurately (Molloy and Warnow, 2018), especially for branches deep in time (Rosenzweig et al., 2022). Using data from larger gene families offers potential for mitigating LBA in individual gene trees with minimal costs. In particular, using gene families with only lineage-specific duplicates makes it possible to break up long branches without adding any additional noise due to gene duplication and loss. Our simulation studies support the idea that using such gene families should increase accuracy without increasing noise (Fig. 4), hopefully also making the inference of species trees from such gene trees more accurate.

Our empirical analysis of chelicerates was less decisive. While support for pseudoscorpions and scorpions as sister lineages increased when using all gene families or lineage-specific duplicates, the increase was not large. This may be due in part to the relatively small size of our datasets, particularly the dataset including only lineage-specific duplicates: only 183-187 genes (depending on the method used) showed lineage-specific duplicates in pseudoscorpions (Table 1). In addition, though LBA was proposed to be the cause of phylogenetic uncertainty in the chelicerates (Ontano et al., 2021), this is by no

Data	MP (95% CI)	ML (95% CI)	# gene families
SC	0.146 (0.119, 0.174)	0.187 (0.157, 0.216)	2787
All	0.157 (0.139, 0.177)	0.198 (0.178, 0.215)	4348
LSD	0.154 (0.120, 0.197)	0.205 (0.164, 0.251)	187 (MP), 183 (ML)

Table 1. Quartet concordance for a sister relationship between pseudoscorpions and scorpions across maximum parsimony (MP) and maximum likelihood (ML) datasets with only single copy genes (SC), all gene families (All), and only gene families with a lineage-specific duplication on the branch leading to pseudoscorpions (LSD). Confidence intervals were calculated using a nonparametric bootstrap.

means certain. Applications to more systems in which LBA is thought to act (e.g. Bergsten 2005) would offer additional tests of our method.

Our results suggest a potential path forward to mitigating long-branch attraction using genomic data. Future research should explore the impacts of other methods for inferring trees from larger gene famlies, including methods that do not simply use all orthologs and paralogs together. Methods that either account for gene duplication and loss explicitly (e.g., ASTRAL-Pro; Zhang et al. 2020) or decompose larger trees into more manageable sub-trees ((Yang and Smith, 2014; Smith et al., 2022)) could further remove noise even while taking advantage of duplicated genes. We anticipate that applying such approaches would provide more ways to mitigating LBA using data from larger gene families.

Acknowledgements

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Data Availability

All scripts and input files used in this study are available at `https://github.com/SmithLabBio/paralogs_lba`.

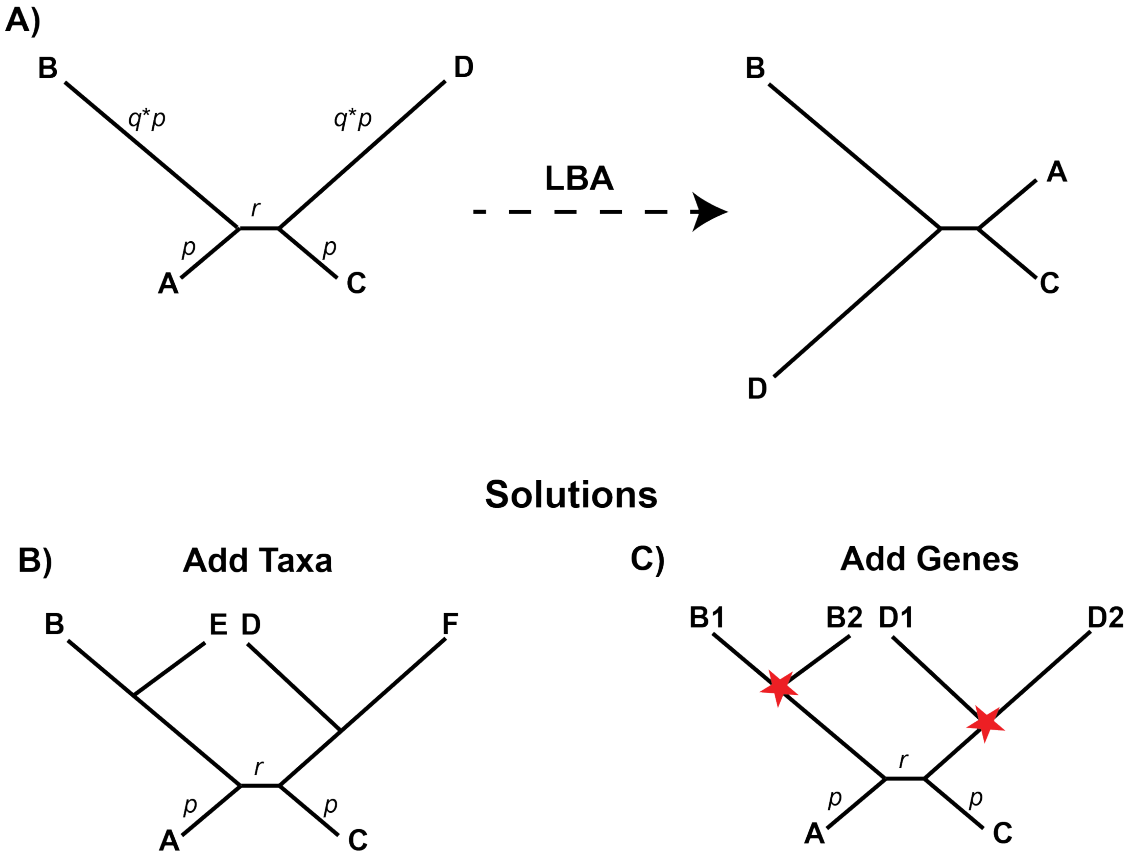


Fig. 1. Long-branch attraction (LBA) is a systematic bias that leads phylogenetic methods to incorrectly infer taxa with long branches as sister taxa (A). LBA has often been mitigated by including more taxa to break up long branches (B), but this may not always be possible. Here, we propose using gene duplicates to break up long branches (C). Red stars indicate duplication events. For simulations, we use the tree in A as the species tree.

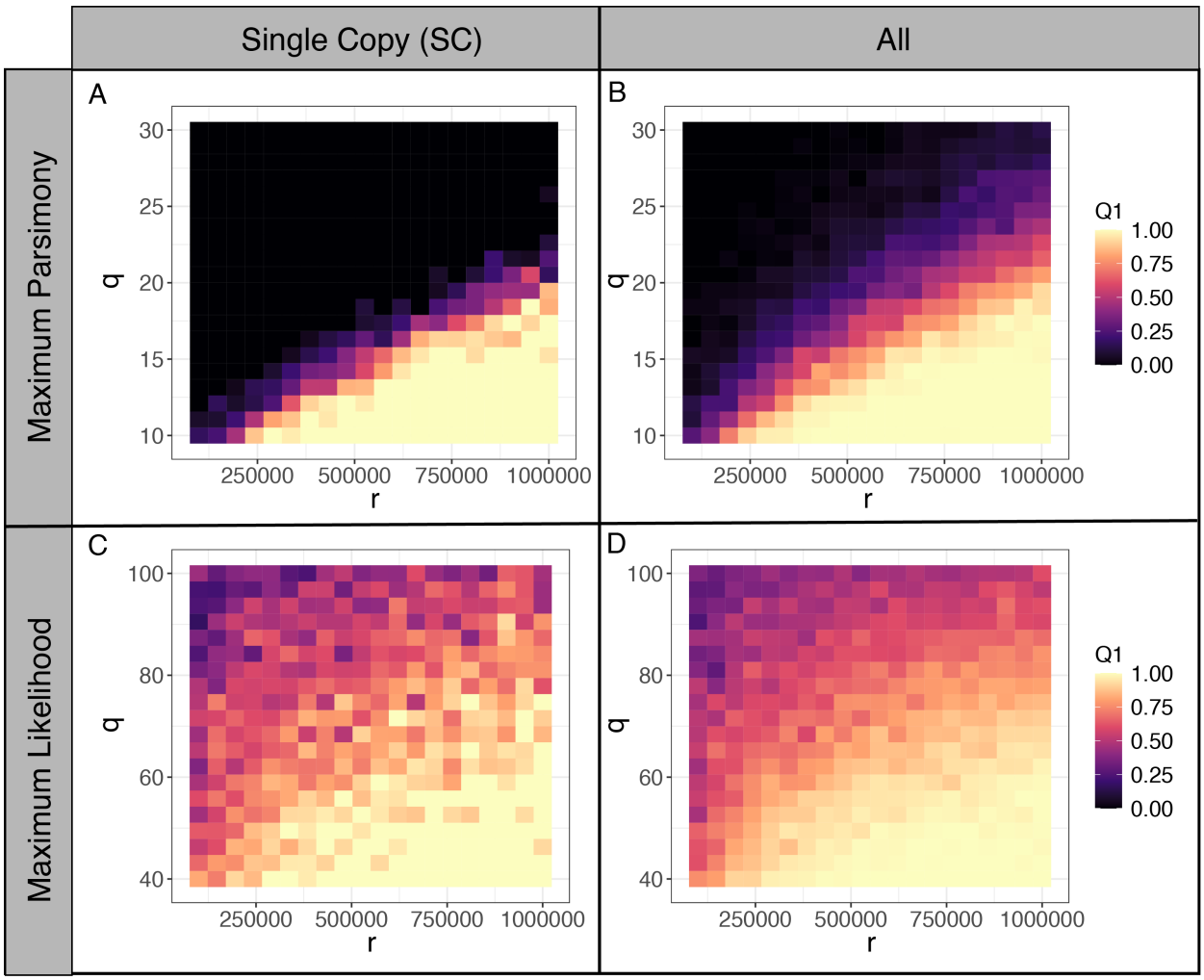


Fig. 2. Quartet concordance across values of the long branch multiplier, q, and the internal branch length, r. A) Maximum parsimony (MP) with single copy (SC) genes. B) MP with all gene families. C) ML with SC genes. D) ML with all gene families. Parameter values used in simulations: λ=1e-6; μ=0; p=250,000; substitution rate = 1e-7; $N_e = 10000$; $L = 2000$

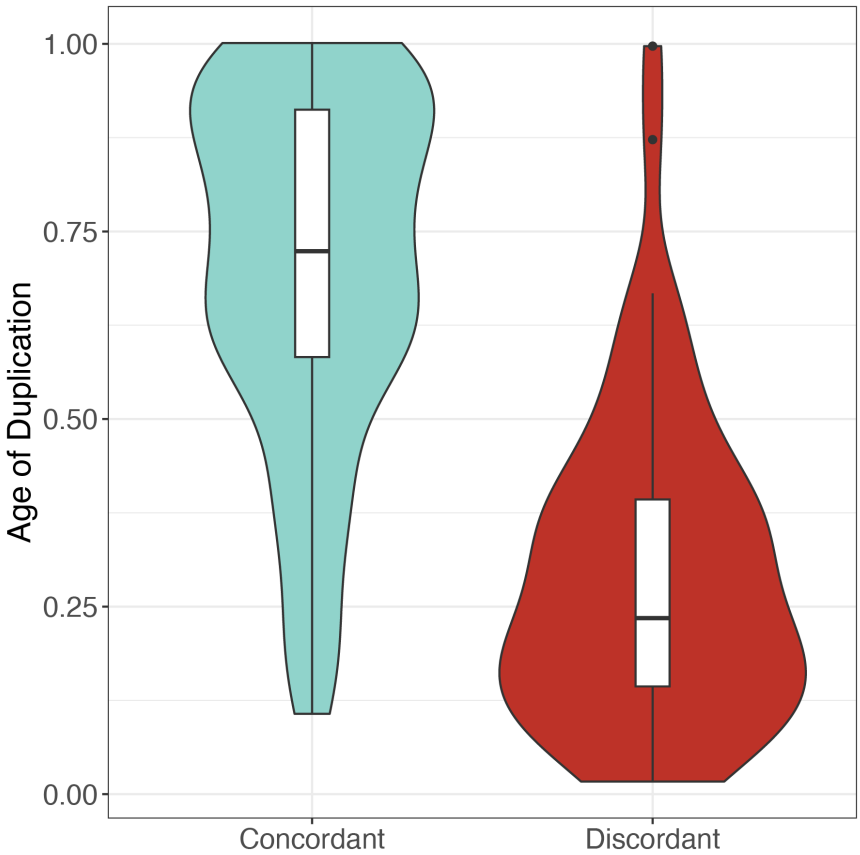


Fig. 3. The age (calculated as the distance to the furthest leaf divided by the total branch length) of the oldest duplicate on branches leading to the long branch taxa B and D. Only gene families with lineage-specific duplicates on the long branches were included. Parameter values used in simulations: λ=1e-6; μ=0; p=250,000; substitution rate = 1e-7; $N_e = 10000$; $L = 2000$; $q = 20$; $r = 750,000$.

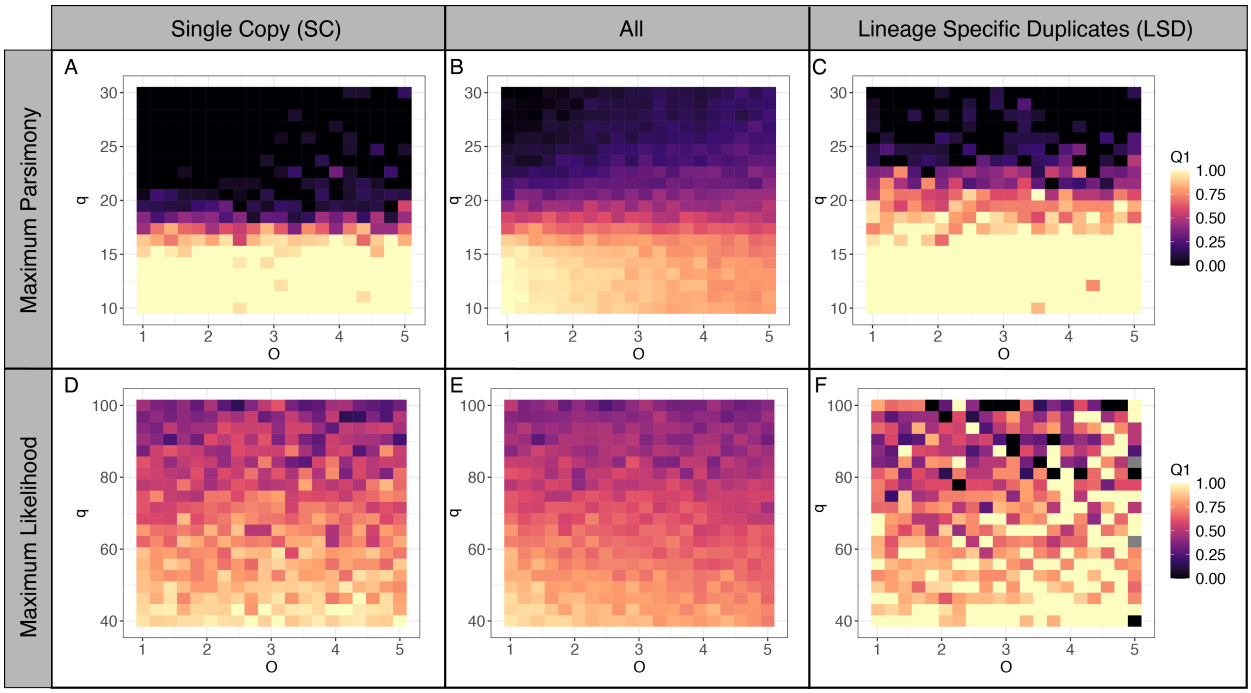
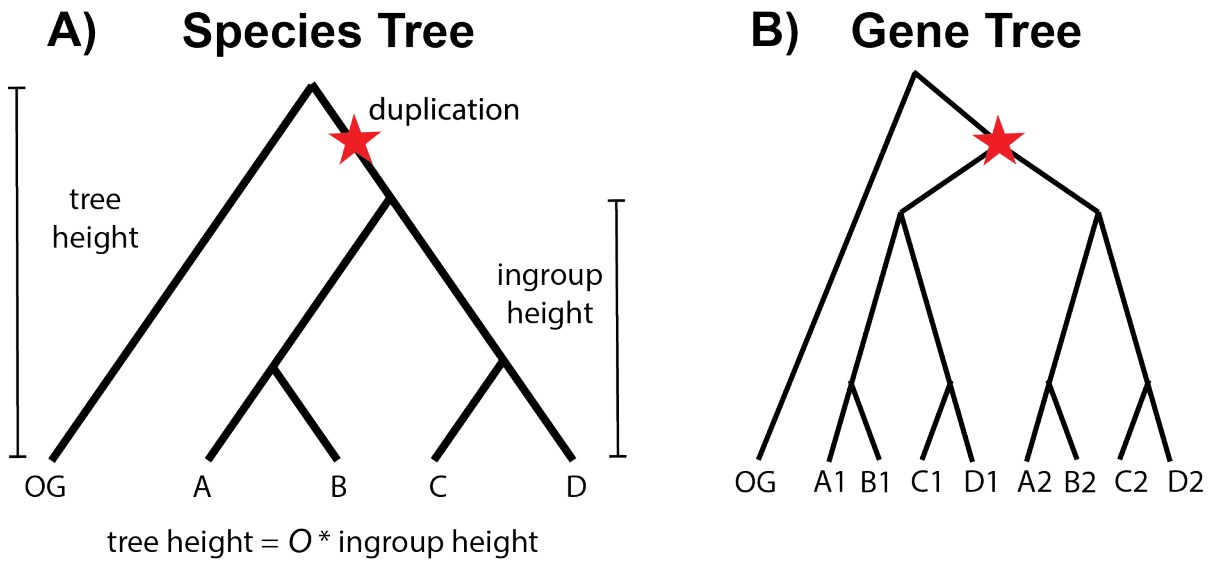


Fig. 4. Quartet concordance across values of the long branch multiplier, q, and the ratio between total tree height and the ingroup height, O. A) Maximum parsimony (MP) with single copy (SC) genes. B) MP with all gene families. C) MP with gene families that include a lineage-specific duplicate in the long-branch taxa and no duplications that are not lineage-specific. D) ML with SC genes. E) ML with all gene families. F) ML with gene families that include a lineage-specific duplicate in the long-branch taxa and no duplications that are not lineage-specific. Parameter values used in simulations: λ=5e-7; μ=5e-7; p=250,000; r = 750,000 for MP and r = 300,000 for ML; substitution rate = 1e-7; $N_e = 10000$; $L = 2000$



C) Sampled Quartets

Orthologs:
((A1,B1),(C1,D1));
((A2,B2),(C2,D2));

Paralogs (Match Species Tree):
((A1,B1),(C1,D2));
((A2,B2),(C2,D1));
((A1,B1),(C2,D1));
((A2,B2),(C1,D2));
((A1,B2),(C1,D1));
((A2,B1),(C2,D2));
((A2,B1),(C1,D1));
((A1,B2),(C2,D2));
((A1,B1),(C2,D2));
((A2,B2),(C1,D1));

Paralogs (Alternate 1):
(A1,C1),(B2,D2));
(A2,C2),(B1,D1));

Paralogs (Alternate 2):
(B1,C1),(A2,D2));
(B2,C2),(A1,D1));

Fig. S1. When data are simulated on a species tree including an outgroup (A), duplications can occur in the ancestor of the focal taxa (B). This leads to discordance, as evidenced by looking at the quartets that can be sampled in this case (C). When sampling quartets for a set of species (A,B,C,D), we sample a single copy from each species. In some cases, we sample four orthologs. However, we often sample paralogs. These paralogs can either have topologies that match the species tree, or topologies that disagree with the species tree (Alternate 1 or Alternate 2). Red stars indicate gene duplication events.

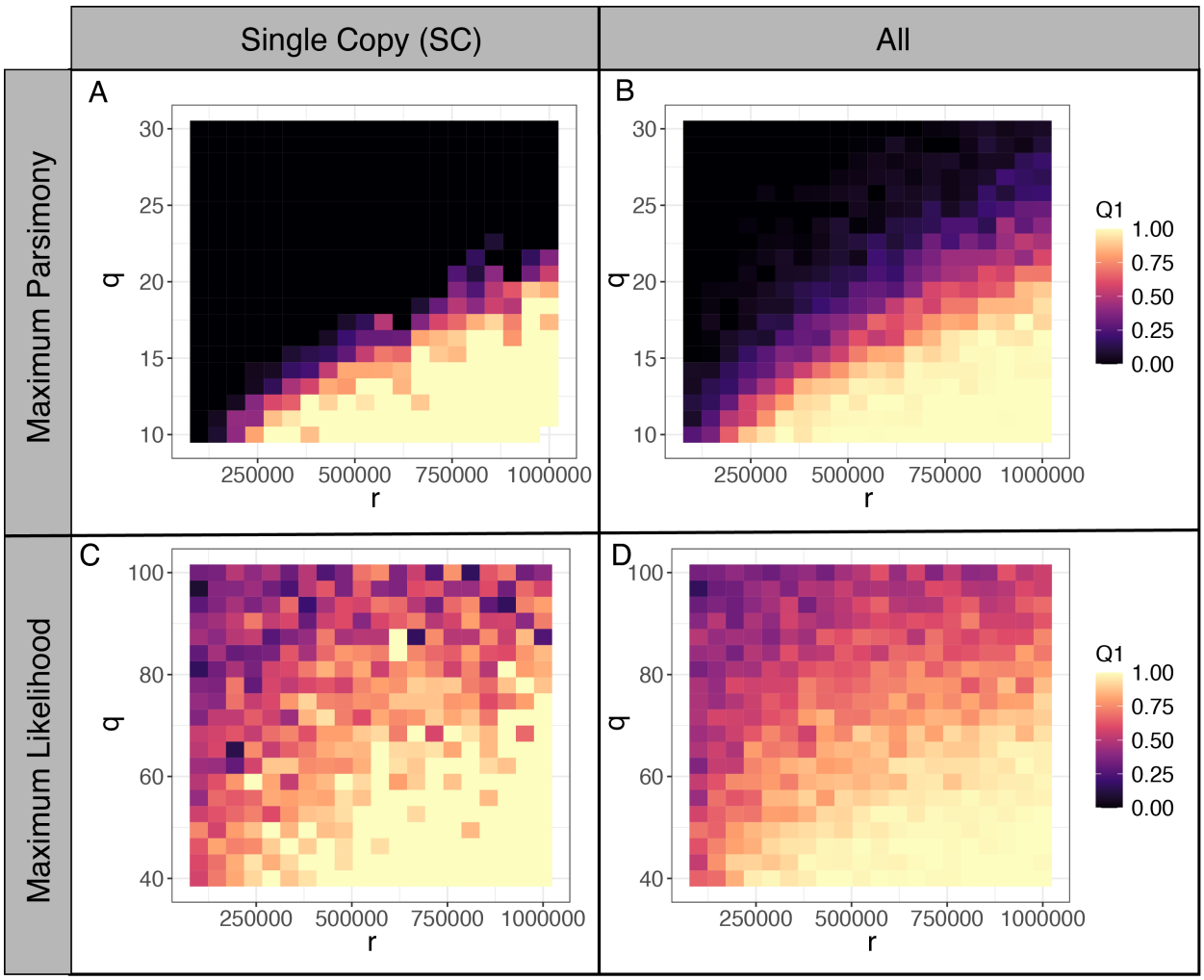


Fig. S2. Quartet concordance across values of the long branch multiplier, q, and the internal branch length, r. A) Maximum parsimony (MP) with single copy (SC) genes. B) MP with all gene families. C) ML with SC genes. D) ML with all gene families. Parameter values used in simulations: λ=1e-6; μ=1e-6; p=250,000; substitution rate = 1e-7; $N_e = 10000$; $L = 2000$

Species name	NCBI Accession	Source	Link (non-NCBI)	Notes
Argiope bruennichi	GCA_015342795.1	NCBI		
Bombyx mori	GCA_000151625.1	NCBI		
Carcinoscorpius rotundicauda		FigShare	https://doi.org/10.6084/m9.figshare.13172414.v2	
Cordylochernes scorpioides	GCA_030710605.1	NCBI		
Centruroides sculpturatus	GCA_000671375.2	NCBI		
Drosophila melanogaster	GCA_000001215.4	NCBI		
Haemaphysalis longicornis	GCA_013339765.2	NCBI		
Ixodes scapularis	GCA_000208615.1	NCBI		
Latrodectus hesperus		i5k	https://i5k.nal.usda.gov/content/data-downloads	Current Genome Assembly Official or Primary Gene Set BCM_version_0.5.3 consensus_gene_set
Limulus polyphemus	GCA_000517525.1	NCBI		
Loxosceles reclusa		i5k	https://i5k.nal.usda.gov/content/data-downloads	Current Genome Assembly Official or Primary Gene Set BCM_version_0.5.3 consensus_gene_set
Metaseiulus occidentalis	GCA_000255335.2	NCBI		
Nephila clavipes	GCA_002102615.1	NCBI		
Parasteatoda tepidariorum	GCA_000365465.3	NCBI		
Stegodyphus mimosarum	GCA_000611955.2	NCBI		
Sarcoptes scabiei	GCA_000828355.1	NCBI		
Tachypleus gigas		Dryad	https://doi.org/10.5061/dryad.2jm63xsmc	
Tetranychus urticae	GCA_000239435.1	NCBI		
Varroa destructor	GCA_002443255.1	NCBI		

Table S1. Whole genomes used for the Chelicerate analyses.

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