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Title: Analyzing contentious relationships and outlier genes in phylogenomics

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ABSTRACT

Despite the wealth of evolutionary information available from genomic and transcriptomic data, recalcitrant relationships in phylogenomic studies remain throughout the tree of life. Recent studies have demonstrated that conflict is common among gene trees, and less than one percent of genes may ultimately drive species tree inference in supermatrix analyses. In this study, we examined plant and vertebrate datasets where supermatrix and coalescent-based species trees conflict. Using a two-topology site-specific log-likelihood test, we identified two highly influential genes in each dataset. While the outlier genes in the vertebrate dataset have been shown to be the result of errors in orthology detection, we demonstrate that the outlier genes from the plant dataset may be the result of biological processes rather than model or methodological errors. When the outlier genes were removed from each supermatrix, the inferred trees matched the topologies obtained from coalescent analyses. While most tests of this nature limit the comparison to a small number of fixed topologies, often two topologies, gene tree topologies generated under processes such as incomplete lineage sorting are unlikely to precisely match these topologies. We therefore examined edges across a set of trees and recover more support for the resolution favored by coalescent analyses. These results suggest that by expanding beyond fixed-topology comparisons, we can dramatically improve our understanding of the underlying signal in phylogenomic datasets by asking more targeted edge-based questions.

INTRODUCTION

Recent studies have highlighted that small changes to a dataset can yield conflicting hypotheses at particular recalcitrant relationships with high support (i.e., 100% support from nonparametric bootstrap (BS) or posterior probability (PP) values). Prominent examples of this include many charismatic lineages such as the root of placental mammals (Morgan et al. 2013; Romiguier et al. 2013), early branching within Neoaves (Jarvis et al. 2014; Prum et al. 2015), and the earliest diverging lineage of extant angiosperms (Zanis et al. 2002; Wickett et al. 2014; Xi et al. 2014). The resolution of these relationships is critical to understanding the evolutionary history of their respective clades (e.g., patterns of biochemical, morphological, and life history evolution).

Finding the underlying causes of uncertainty in phylogenomic datasets is an essential step toward resolving problematic relationships. Recently, authors have developed means of exploring conflict between gene trees and species trees specifically for phylogenomic datasets (Salichos et al. 2014; Smith et al. 2015; Kobert et al. 2016), aiding in the identification of regions of species trees with considerable uncertainty despite strong statistical support from traditional support measures. Two studies have shown that the disproportionate influence of just one or two genes “outlier genes” on a supermatrix analysis is capable of altering tree topology inference (Brown and Thomson 2017; Shen et al. 2017)(Brown and Thomson 2017; Shen et al. 2017)(Brown and Thomson 2017; Shen et al. 2017). Using a Bayes factor approach Brown and Thomson (2017) reanalyzed a series of published datasets and found that the transcriptome data from Chiari et al. (2012) contained outlier genes. When the outlier genes were included in phylogenetic reconstruction, a clade of turtles+crocodilians was inferred to be sister to birds with 100% PP. The same topology was previously inferred using ML with nucleotide data in the original study by Chiari et al. (2012), but was dismissed in favor of a coalescent reconstruction that placed turtles sister to birds+crocodilians. When Brown and Thomson (2017) removed the outlier genes, the reduced supermatrix infers the same topology as the coalescent reconstruction with 100% PP. Another recently published study compared gene-wise likelihoods across multiple topologies to examine contentious relationships across the tree of life and found disproportionate influence of genes at all contentious relationships examined (Shen et al. 2017).

Given the prevalence of outlier genes in phylogenomic datasets, and the continued focus on contentious relationships in the tree of life, it is imperative that we develop methods for analyzing conflict and selecting among alternative resolutions for recalcitrant relationships. We build upon the discussions of Brown and Thomson (2017) and Shen et al. (2017) by addressing whether these outlier genes violate models of evolution. Furthermore, we present a method that expands on topology comparisons to instead pursue edge-based questions. Typically, site-wise and gene-wise log-likelihood analyses of phylogenomic datasets are performed in a pairwise manner on two or more fixed alternate topologies (e.g., Castoe et al. 2009; Smith et al. 2011; Shen et al. 2017). However, given widespread gene tree discordance (e.g., due to incomplete lineage sorting), it may be more realistic to assume that many alternative topologies are supported within larger genomic datasets (e.g., Smith et al. 2015; Pease et al. 2016; Walker et al. 2017). Additionally, when the research question involves a single relationship and not the entirety of the tree, it may be more appropriate to examine targeted edges instead of resolved topologies (Lee and Hugall 2003). This allows for any processes that may be causing conflict in the non-focal parts of the tree to be accommodated without influencing the relationships of interest. Here, we compare results from two-topology gene-wise log-likelihood analyses and a novel approach of gene-wise edge (MGWE) analysis (see Methods below). We examine vertebrate (Chiari et al. 2012; Brown and Thomson 2017) and carnivorous Caryophyllales datasets (Walker et al. 2017) (the latter hereafter referred to as the carnivory dataset). Both datasets contain contentious relationships, outlier genes, and, in their respective original studies, the authors dismissed the supermatrix topology for the topology inferred using a coalescent method. In both cases we find that the use of an edge based approach results in stronger support for the topology hypothesized to be correct by researchers in the original study.

METHODS

Data collection

We obtained the 248 genes that were codon-aligned and analyzed by Brown and Thomson (2017) from the Dryad deposit (http://dx.doi.org/10.5061/dryad.8gm85) of the original study (Chiari et al. 2012) that focused on resolving the placement of turtles among amniotes. The coding DNA sequences of the 1237 one-to-one orthologs from Walker et al. (2017) to infer the relationships among carnivorous Caryophyllales (Eudicots: Superasterids) are available from Dryad (http://datadryad.org/resource/doi:10.5061/dryad.vn730). All programs used in this analysis may be found at https://bitbucket.org/jfwalker/maximizelikelihoods and the code to conduct the MGWE analysis may be found at https://github.com/jfwalker/SiteSpecificLogLikelihood.

Species trees

Brown and Thomson (2017) used Bayesian analyses to obtain the topologies from the Chiari et al. (2012) data set. As our study focused on the use of maximum likelihood (ML) for detecting overly influential genes, we ensured that ML phylogenetic reconstruction would recapitulate the previous species tree results. To construct a supermatrix tree for the vertebrate dataset, the 248 individual vertebrate genes used in Brown and Thomson (2017) were concatenated using the Phyx program pxcat (Brown et al. 2017). The species tree was inferred in RAxML v8.2.1 (Stamatakis 2014) using the GTR+ Γ model of evolution, and edge support was assessed from 200 rapid bootstrap replicates. Supermatrix trees for the vertebrate dataset were inferred both with all genes present, and again with the previously identified two outlier genes (8916 and 11434) removed (see below). The ML tree inferred from all the data from the carnivory dataset was downloaded from (http://dx.doi.org/10.5061/dryad.33m48) while a novel ML tree was inferred from a reduced supermatrix that excluded two highly informative genes (cluster575 and cluster3300; see below).

Gene tree construction and analysis of conflict

Individual gene trees for both datasets were inferred using ML with the GTR+ Γ model of evolution as implemented in RAxML. A SH-like test (Anisimova et al. 2011), as implemented in RAxML, was performed to assess gene tree edge support. As this test examines alternative topologies by nearest-neighbor interchange (NNI), it is possible that during the test a topology with a higher likelihood is found (i.e., an 'NNI-optimal' topology). When a better topology was found during the test performed for this study, that topology was used in downstream analyses. We used the pxrr program in the Phyx package (Brown et al. 2017) to root all gene trees on the outgroup (*Protopterus* for the vertebrate dataset, and *Beta vulgaris* and *Spinacia oleraceae* for the carnivory dataset) and we excluded gene trees where an outgroup was not present. We mapped conflict onto the supermatrix tree using phyparts (Smith et al. 2015) with SH-like support of < 80 treated as uninformative. We chose 80 as a support cutoff due to the traditional cutoff of (95) being shown as overly conservative with this test (Guindon et al. 2010). Gene tree conflict was visualized using the script phypartspiecharts.py (available from https://github.com/mossmatters/MJPythonNotebooks). We conducted more detailed conflict analyses used for edge comparisons discussed below using pxbp as part of the Phyx package (Brown et al. 2017).

Calculating two-topology gene-wise log-likelihoods

The alternate topologies (supermatrix and coalescent) and data matrices for the vertebrate and carnivory datasets were obtained from the original studies, Chiari et al. (2012) and Walker et al. (2017), respectively. We calculated site-wise log-likelihood scores for the two topologies in RAxML using the GTR+ Γ model of evolution, with the data partitioned by gene. The differences in site-wise log-likelihoods between the candidate topologies were then calculated using scripts available from https://bitbucket.org/jfwalker/maximizelikelihoods and https://github.com/jfwalker/SiteSpecificLogLikelihood.

Maximum gene-wise edge calculations

In addition to pairwise topological comparisons, we also examined the maximum gene-wise edges (MGWE) (Fig 1.). For a single gene and a single focal edge, the MGWE is the likelihood of a gene tree with the highest likelihood that also displays the edge of interest. When calculating the MGWE for a focal edge across multiple genes, this approach does not require each gene to have the same topology, just that the likelihood comes from a tree that displays the edge of interest. This contrasts with a standard fixed topology comparison where the topology for each gene would be required to be the same (e.g., supermatrix vs. coalescent topology). Unlike the fixed topology approach the MGWE allows for genes to have conflicting relationships outside of the edge of interest. Here, we are interested in comparing the MGWE for sets of alternative and conflicting edges in order to determine if, by relaxing the requirement for each gene to share the topology, we gain insight into the signal for conflicting relationships. One could calculate the MGWE on any number of edges, and we consider the dominant alternative edges as identified in the literature.

While there are several ways that MGWEs could be calculated, we restricted the tree space under under consideration by circumscribing a set of empirically-supported topologies (TREESET) consisting of the supermatrix-inferred topology, coalescent inferred topology, and individual gene trees that contained all taxa. We then identified the conflicting trees and pooled trees based upon shared conflicting relationships for the edges of interest (EDGE). We then calculated the maximum likelihood for each gene and for each topology.

For the edges of interest, we calculated the MGWEs by retaining the likelihood for the tree with the highest likelihood that displayed the focal EDGE. This became the representative likelihood for that EDGE. We then summed the representative likelihoods together. That value, however, is not comparable between edges because a different number of trees may be compared (Theobald 2010). Therefore, we calculated AIC scores (-2ln(L) + 2k) for each EDGE. This, effectively, allowed for comparisons between more parameter rich models and parameter poor models. The parameters, k, were calculated based on the number of taxa, n, and the number of genes in the analysis, g. The branch length parameters equal $2 \times n - 3$ and the GTR + Γ model of evolution = 6 (where base frequencies were empirical and not estimated). The supermatrix ML analyses that assumed a single set of branch lengths on one topology and model parameters to be unlinked across genes consisted of $2 \times n - 3 + 6 \times g$ parameters. For each EDGE, because branch lengths were calculated for each gene tree, the parameters consisted of the sum of the number of parameters used for each gene: g x (2 x n – 3 + 6). In addition to calculating AICs for the coalescent and supermatrix topologies with a single set of branch lengths across the gene set, we also calculated AICs allowing the branch lengths to vary across genes. This calculation results in the same number of parameters as the EDGE calculations. Here, we are focused on addressing conflicting signal between edges of interest and so the increase in the number of parameters is acceptable considering our examination of gene trees. However, future work could attempt to limit the expansion of the number of parameters for each EDGE by sharing branch length estimates or model parameters across genes.

Testing for paralogy in carnivory dataset

The homolog trees created from amino acid data in the study by Walker et al. (2017) were downloaded from Dryad (http://datadryad.org/resource/doi:10.5061/dryad.vn730). We matched the sequences from the outlier genes to their corresponding sequence in the amino acid homolog trees. This allowed us to examine whether a nucleotide cluster contained homology errors that may be exposed by the slower evolving amino acid dataset.

RESULTS

Gene tree conflict and log-likelihood analysis reveals genes of disproportionate influence

Our ML analysis of the vertebrate dataset recovered the same supermatrix topology (Fig. 2) as found with ML by Chiari et al. (2012) and Bayesian inference by Brown and Thomson (2017). The difference in log-likelihood between the supermatrix and coalescent topologies for the vertebrate dataset was 4.01. Ninety-three of 248 gene trees could be rooted on the outgroup *Protopterus* and only five of these had all taxa represented (Supplementary Table 1). We found low support for relationships within gene trees (SH <80) and significant gene tree conflict (Fig. 2). Of the gene trees with high support (SH >80), seven resolved turtles+crocodilians as sister to birds (hereafter referred to as the vertebrate supermatrix topology) and nine resolved crocodilians+birds sister to turtles (hereafter referred to as the vertebrate coalescent topology).

The two-topology gene-wise log-likelihood comparison showed that 105 genes had a higher likelihood score for the vertebrate supermatrix topology while 143 supported the vertebrate coalescent topology (Figs. 3A, 4A). Two genes (ENSGALG00000008916 and ENSGALG00000011434, referred to here as 8916 and 11434, respectively), appeared as outliers, exhibiting a disproportionate influence on the overall likelihood of the supermatrix (Fig. 3A). The outlier genes identified with maximum likelihood analyses matched those previously identified as outliers using Bayes factors (Brown and Thomson 2017). These two genes both supported the vertebrate supermatrix topology with log-likelihood scores of 79.55 and 46.01 greater than the alternative coalescent tree topology, respectively. The difference in log-likelihood between the two topologies of the non-outlier genes ranged from |0.006| to |19.891| with an average of 3.31 for all genes in the analysis. The removal of the vertebrate genes 8916 and 11434, as shown by Brown and Thomson (2017), recovered the coalescent topology, albeit with low bootstrap support (BS = 12; Supplementary Fig. 1).

Previous work on the carnivory dataset demonstrated that the placement of the *Ancistrocladus+Drosophyllum* clade (Fig. 2) contained significant conflict and is strongly influenced by species sampling (Walker et al. 2017). The log-likelihood difference between the supermatrix and coalescent topologies was 74.94 in favor of the former. The two-topology log-likelihood comparison between the dominant topologies on the carnivory dataset (Fig. 3B) showed that 623 genes supported *Ancistrocladus+Drosophyllum* sister to all other carnivorous plants (hereafter referred to as carnivory supermatrix topology) while 614 genes supported *Ancistrocladus+Drosophyllum* sister to *Nepenthes alata+Nepenthes ampullaria* (hereafter referred to as carnivory coalescent topology; Figs. 3A & 4D). Two genes (cluster575 and cluster3300) contributed disproportionately to the overall likelihood. Individually these two genes have a difference in log-likelihood scores between the two topologies of 33.06 and 16.63, respectively, and support the carnivory supermatrix topology. When we reanalyzed the supermatrix with cluster575 and cluster3300 removed, the carnivory coalescent topology was recovered, with 100% BS support (Supplementary Fig. 1). The difference between the two topologies in log-likelihood of the non-outlier genes ranged from |0.001| to |12.82| with an average of 2.82 for all genes in the analysis.

Edge based analysis changes supported topology

We compared MGWE and two topology gene-wise likelihoods involving the contentious bird, crocodilian, and turtle relationships in the vertebrate dataset (Fig. 4B). We found seven unique topologies with the necessary species coverage to conduct the analyses: five gene tree topologies from Chiari et al. (2012) and the two dominant species tree topologies. The set of seven trees included three major conflicting edges for the relationship in question: the two resolutions found in the supermatrix and coalescent trees, and birds sister to crocodilian+mammals+turtles. 91 genes supported the vertebrate supermatrix edge, 144 genes supported the vertebrate coalescent edge, and 13 genes supported the third conflicting edge (Fig. 4B). When comparing the supermatrix analysis with a single set of branch lengths, to that where it was treated as a sum of gene tree likelihoods, we found a superior AIC score for the sum of gene tree likelihoods (Table 1). The MGWE AIC scores for the summed likelihoods of the supermatrix (three source trees), the coalescent (three source trees), and the third conflicting edge (one source tree) were highest for the coalescent edge and out of all tested models the coalescent edge was inferred to be the best (Table 1).

For the carnivory dataset, we found 168 unique tree topologies to include in the tree set. The 168 tree topologies contained 41 conflicting edges for the relationship in question with 3 dominant edges. The MGWE analyses found 499 genes supported the supermatrix edge, 466 genes supported the coalescent edge, and 272 genes supported 15 additional edges (Figs. 2D, 3E). When we further compared the MGWE AIC scores for the supermatrix (44 source trees), the coalescent (56 source trees), and for the third edge (24 source trees) we found the coalescent edge to have the best AIC score out of all tested models (Table 1).

Outlier gene examination

For the carnivory dataset, we explored the possibility that the strongly conflicting genes cluster575 and cluster3300 reflected methodological error in the assembly pipeline, as is the case for the genes identified by Brown and Thomson (2017) for the vertebrate dataset. However, both the alignment and inferred phylogram for each gene revealed no obvious problems or potential sources of systematic error (sparse alignment, abnormally long branch lengths, etc.). We also explored whether compositional heterogeneity could explain the strongly conflicting results (i.e., that the relationships were not truly conflicting, but instead incorrectly modeled). However, both RY-coding in RAxML and explicit modeling of multiple equilibrium frequencies (2, 3, or 4 composition regimes) across the tree in p4 v1.0 (Foster 2004) failed to overturn the inferred relationships. We further explored the possibility of misidentified orthology. By examining the homolog tree produced from amino acid data, we identified the ortholog from the nucleotide data to be complete (i.e., an ortholog within the homolog amino acid tree). We found that with the slower amino acid data the sequences in the nucleotide cluster575 were inferred as a single monophyletic ortholog within a duplicated homolog (Supplementary Fig. 2). The discrepancies that appeared between the amino acid dataset and the CDS dataset were found to be either different in-paralogs/splice sites maintained during the dataset cleaning procedure or short sequences that were not identified as homologs in the coding DNA sequence (CDS) dataset (Supplementary Table 2 and Supplementary Fig. 2).

DISCUSSION

Biological processes including substitution saturation, hybridization, horizontal gene transfer, and incomplete lineage sorting can contribute to conflicting signal and may explain both conflict and lack of support widely found in phylogenomic datasets (Salichos et al. 2014; Smith et al. 2015; Kobert et al. 2016). In addition to these biological processes, other data set assembly issues such as limited taxonomic coverage for each gene may also contribute to conflict and low support in these data sets. For example, while the carnivory dataset had extensive data overlap, the vertebrate dataset only had five gene regions that contained sequence data for every species (Supplementary Table 1). To further complicate the challenges facing phylogenomic analyses, high support values, especially from concatenated runs, can mask significant underlying conflict (Lee and Hugall, 2003; Ryan et al. 2013; Salichos et al. 2014; Smith et al. 2015; Kobert et al. 2016; Pease et al. 2017). Both datasets examined here recovered high support for two different topologies depending on the inclusion or exclusion of two genes with disproportionate influence on the likelihood (Brown and Thomson 2017; Walker et al. 2017). In the case of the carnivory dataset, the inferred topology changes with the inclusion or exclusion of just 0.0016% of the genes.

To address these challenges, several approaches have been outlined in the literature. Recently, the discovery of outlier genes has resulted in the necessity to closely examine gene tree topologies and likelihoods (Brown and Thomson 2017; Shen et al. 2017). Outlier genes may be the result of biological processes or methodological errors, and due to their high influence of species tree inference should be thoroughly examined. Previously, the outlier genes in a vertebrate dataset were found to be the result of errors in orthology detection and not biological processes (Brown and Thomson 2017). We explored, in this study, the potential sources of error for the outlier genes in a dataset of carnivorous plants. While the genomic resources are not available to fully examine the carnivorous outlier genes (e.g., we do not yet have synteny or information on gene loss), our analyses did not detect any obvious problems with alignment, compositional heterogeneity, or homology. We found one gene, cluster575, to be an ortholog of a gene that experienced a duplication event prior to the divergence of both ingroup and outgroup taxa (Supplementary Fig. 3). While we cannot rule out every possible source of error, we also cannot identify a source of methodological error, suggesting the possibility that the conflicting topology is the result of real (albeit unknown) biological processes.

Fixed topological and pairwise examinations explored by most authors (Castoe et al. 2009; Smith et al. 2011; Shen et al. 2017), have been very informative for the identification of not only outlier genes, but also for phylogenetic signal for and against conflicting phylogenetic relationships. However, for many reasons, these fixed topological examinations, where a single topology is assumed to underlie all genes, may not be optimal. Conflict among gene trees is common and expected from processes such as incomplete lineage sorting, hybridization, and other processes. For instance, Jarvis et al. (2014) reported that no gene trees from a genomic data set of 48 species of birds matched the inferred species tree. Furthermore, such a result becomes increasingly likely as sampling breadth (both taxa within a clade as well as the age of the clade itself) increases. The results of a fixed-topology analysis may be driven by the resolution of a part of the phylogeny other than the area of interest, as fixed-topology analyses condition on fully bifurcating trees that necessarily resolve conflict in the entire tree.

To overcome these limitations, instead of fixed singular topologies, we examined edges across a set of empirically supported candidate topologies, as defined by the set of inferred gene trees and the two tree hypotheses in question. By examining edges, we accommodate for uncertainty across the rest of the tree, regardless of the process generating that uncertainty. We examined this with both a vertebrate dataset and carnivorous plants dataset discussed above. The vertebrate dataset contained three alternative edges for the relationship of interest while the carnivory dataset contained 41 different edges representing 168 topologies. The MGWE analysis and AIC scores of both the vertebrate dataset and the carnivory dataset both suggested a better fit of the coalescent edge than the supermatrix edge (Table 1). Also, in both cases, we found that the AIC score supported the higher parameterized model, as opposed to a single fixed topology and branch lengths. While we do not suggest that this is the best fit model and only the best of the ones analyzed here, this indicates that future studies may benefit from allowing more heterogeneity than is typically involved in a concatenation analysis. This will require careful examination of some of the complexity involved in these large phylogenomic analyses. For example, there is the issue of how missing data is handled in these calculations (e.g.,Stamatakis and Alachiotis 2010). Furthermore, the models explored could potentially have significantly reduced parameters by sharing topologies and branch lengths across some gene regions, including potentially scaling branch lengths proportionally (e.g., as is possible with the -spp option in the program iqtree). Nevertheless, the exploratory analyses presented here provide additional evidence that a simple concatenation approach with these large datasets masks important heterogeneity that can be analyzed further to help inform phylogenetic resolution.

The results presented here contribute to a growing body of literature that address the question of how phylogenomic analyses should proceed in the presence of highly influential outlier genes, conflicting topologies, and ever expanding datasets (Wickett et al. 2014; Pease et al. 2016; Brown and Thomson 2017; Shen et al. 2017; Yang et al. 2017). For example, some authors have noted, and it is the case here, that supermatrix analyses may be more susceptible to the problem of strong outliers (Shen et al. 2017; Walker et al. 2017). In these studies, the resolutions inferred using a coalescent method were generally favored. When the dominant process generating gene tree conflict is incomplete lineage sorting, coalescent methods should perform better (i.e., when gene tree diversity is modeled correctly). Some coalescent methods that weigh all gene tree equally (e.g., Mirarab and Warnow 2015), may overcome the problem of outlier genes even if incomplete lineage sorting is not the dominant source of conflict simply by eliminating the disproportionate influence of one or two outlying genes. Here, we demonstrate with two empirical examples that the coalescent resolution had higher support when examining edges without using an explicit coalescent method.

While we continue to uncover the patterns and processes that generate conflicting signal within phylogenomic datasets, it is imperative that we explore new methods that accommodate conflict. Phylogenomic studies often focus sampling efforts around particularly recalcitrant nodes, and it is important we develop methods designed with the same purpose. Here we focus on conflicting edges and explore the MGWE method as a means of analyzing these conflicting edges while allowing for topological heterogeneity outside of the relationships of interest. This approach helps accommodate the biological realities of heterogeneity among lineages, conflicting signal both for in and outside the relationship of interest, and evolutionary processes that violate assumptions by supermatrix and coalescent models. This approach, however, is just a start and future research should examine how to better incorporate the underlying heterogeneity that has emerged from our large data sets over the last few years. We believe further investigation into edge based testing is warranted to better understand how we may incorporate the process based conflict of phylogenomics into our analyses.

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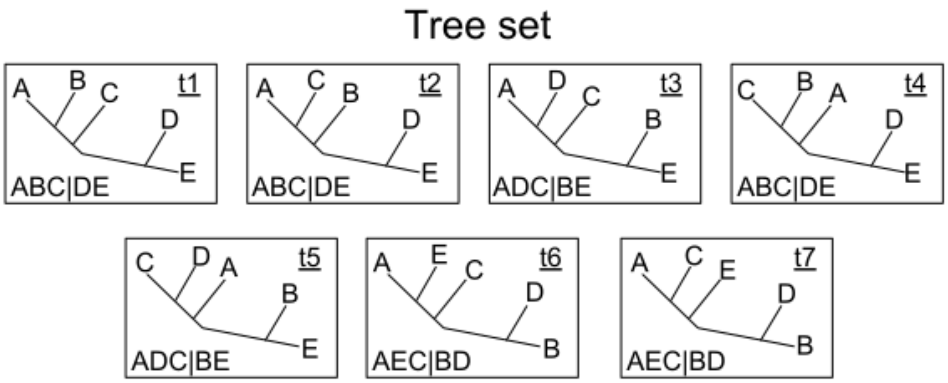


MGWE

Edge	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5
ABC | DE	-20.00 (t1)	-10.00 (t4)	-11.00 (t2)	-14.00 (t1)	-9.00 (t1)
ADC | BE	-90.00 (t3)	-5.00 (t5)	-4.00 (t5)	-50.00 (t3)	-20.00 (t5)
AEC | BD	-15.00 (t6)	-11.00 (t7)	-7.00 (t6)	-8.00 (t7)	-10.00 (t6)

Figure 1. Outline for the MGWE procedure. The inferred tree set is depicted at the top, with the tree number in the top right hand corner of each box, and the edge representing the relationship of interest in the bottom left hand corner. The MGWE shows the best likelihood for each edge at each gene, with the tree from which that likelihood was obtained in the box in parentheses next to the likelihood score.

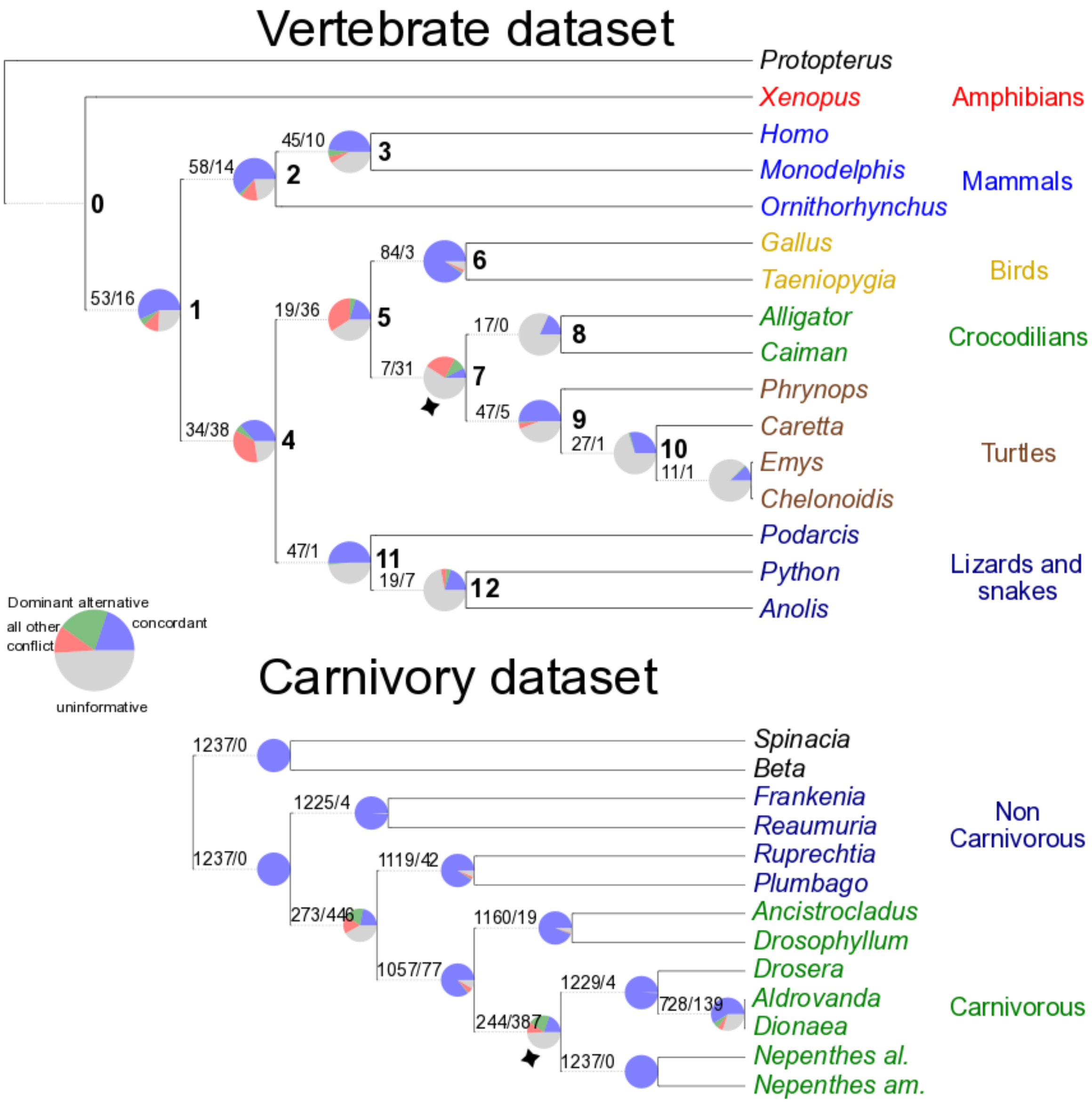


Figure 2. Maximum likelihood trees inferred by RAxML for the Chiari et al. 2012 (vertebrate) and Walker et al. 2017 (carnivorous Caryophyllales) datasets. Conflict analysis for the vertebrate (A) and carnivory (B) datasets. The vertebrate analysis includes the 93 genes that contained the outgroup (*Protopterus*), and the carnivory analysis includes 1237 genes all of which had the outgroups (*Spinacia oleraceae* and *Beta vulgaris*). Blue represents gene trees that are concordant with the relationship, grey represents uninformative genes (SH-like < 80 or no taxon representation for the edge),

green represents the dominant alternate topology, and red represents all other conflict. Numbers on edges represent concordance/conflict. Bold numbers at the nodes of the vertebrate dataset correspond to edge numbers in Supplementary Table 1.

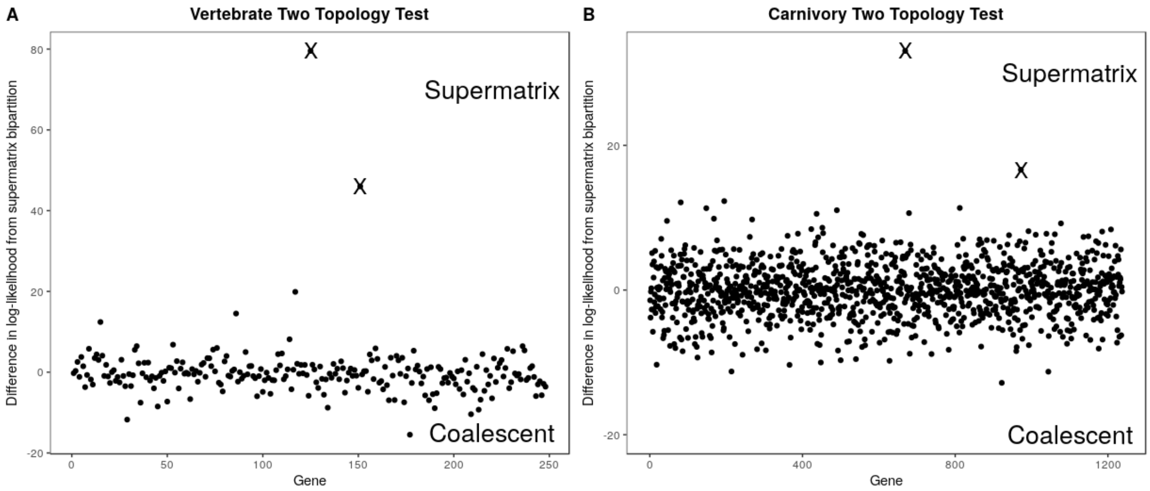


Figure 3. Identification of outlier genes using gene-wise likelihood test. A&B) Show the results of the two-topology test on the vertebrate and carnivory dataset, respectively, using the coalescent (negative values) and supermatrix (positive values) topologies as the comparison. The genes identified as outliers from the analysis are marked with an X.

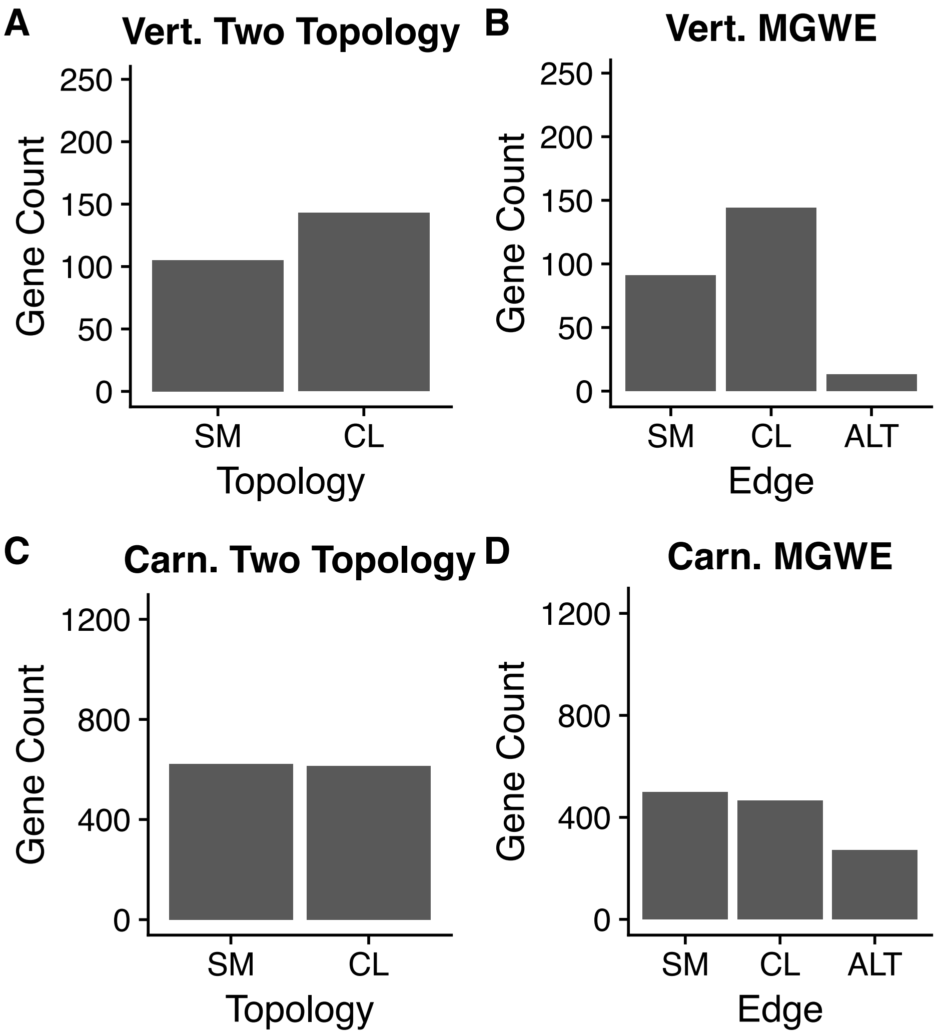


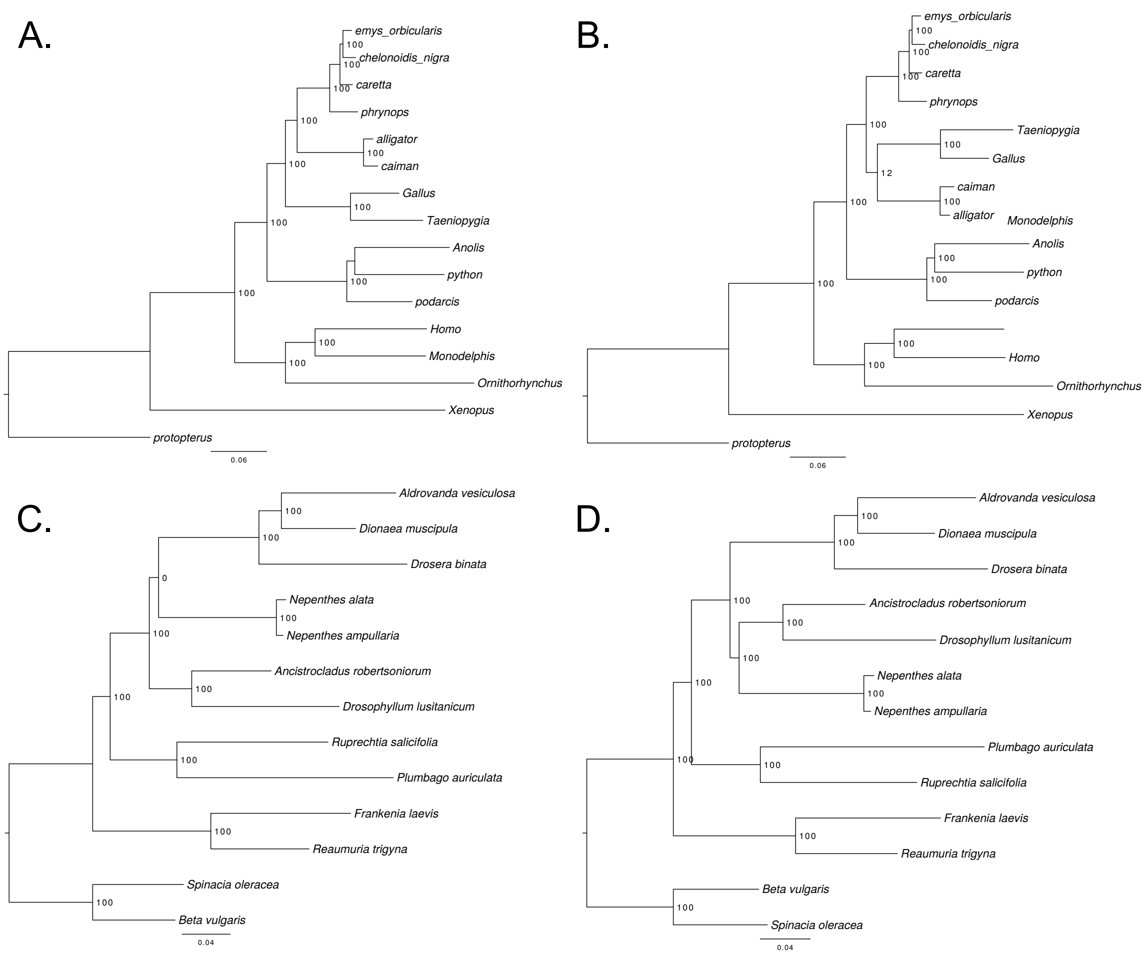
Figure 4. Bar plot representing gene counts for the two-topology and MGWE methods. A&C) represent counts of genes that support the supermatrix inferred maximum likelihood (ML) topology and the maximum quartet support species tree (MQSST), for the vertebrate and carnivory datasets respectively. B&D) Show the results of the MGWB analysis for support of the edge found in the ML analysis, the conflicting edge from the MQSST analysis, and the sum of all genes supporting an alternative conflict from an edge in the TREE SET.

Table 1. Results of model testing the various topologies and edges.

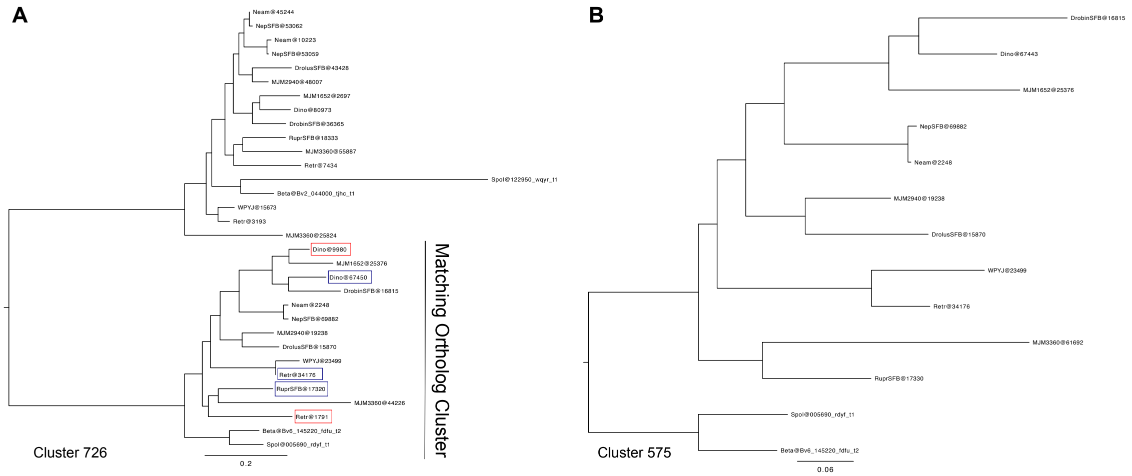
	Relationship	Type	Likelihood	k	AIC	ΔAIC
Vertebrate	Supermatrix	Topology	-1,047,406.05	1517	2,097,846.11	22374.01
		As Gene Trees	-1,031,489.81	6442	2,075,863.63	391.53
		Edge	-1,031,423.65	6442	2,075,731.31	259.20
	Coalescent	Topology	-1,047,410.07	1517	2,097,854.15	22382.04
		As Gene Trees	-1,031,450.71	6442	2,075,785.43	313.32
		Edge	**-1,031,294.05**	**6442**	**2,075,472.10**	**0**
	Dominant Alternative	Edge	-1,033,773.81	6442	2,080,431.62	4959.52
Carnivory	Supermatrix	Topology	-13,305,055.20	7445	26,625,000.40	36618.41
		As Gene Trees	-13,205,130.14	35873	26,595,640.58	7258.59
		Edge	-13,258,387.61	35873	26,588,521.23	139.24
	Coalescent	Topology	-13,305,130.14	7445	26,625,150.28	36768.28
		As Gene Trees	-13,262,019.55	35873	26,595,785.10	7403.10
		Edge	**-13,258,317.99**	**35873**	**26,588,381.99**	**0**
	Dominant Alternative	Edge	-13,260,106.83	35873	26,591,959.66	3577.67

*In the type column, "Topology" represents the supermatrix or coalescent topology with a single set of branch lengths, "As Gene Trees" is the supermatrix or coalescent topology with branch lengths varying among genes, and "Edge" is the MGWE analysis. The top AIC score is bolded.

APPENDICES



Supplementary Figure 1. Species trees inferred using maximum likelihood from the different supermatrices. Support at each node was obtained from 200 rapid bootstrap replicates. A) Species tree for vertebrate dataset inferred with all 248 genes included in the supermatrix. B) Species tree for the vertebrate dataset inferred with 8916 and 11434 removed from the supermatrix. C) carnivorous Caryophyllales species tree inferred from all 1237 genes. D) carnivorous Caryophyllales species tree inferred with cluster575 and cluster3300 removed from the supermatrix.



Supplementary Figure 2. Homolog tree for Amino Acid clustered (726) and CDS clustered (575) highly influential gene in the carnivorous Caryophyllales dataset. Different genes identified in the ortholog clusters are circled on cluster 726. Genes circled in red represent ones that are shorter and were not identified as orthologous in the CDS dataset and genes circled in blue represent alternate paralogs or introsplice sites used between the two clustering analyses.

Supplementary Table 1. Number of gene trees in which all the species for a given edges are present. edges correspond to node labels on Fig. 1.

Edge number	Genes containing all species for the edge
0	5
1	5
2	246
3	248
4	5
5 (All turtle, crocodilians, and birds)	6
6	248
7	6
8	23
9	36
10	45
11	69
12	51
13	94
edge of turtles sister to birds+crocodilians	36

Supplementary Table 2. Sources of discrepancy between the orthologs detected in highly influential nucleotide cluster575 and in matching amino acid homolog cluster726.

Ortholog in 575	Ortholog in 726	Seq length of 575 (Nuc)	Seq length of 726 (Nuc)	Reason for misidentification
Dino@67443 (*Dionaea*)	Dino@67450	2793	2991	Different copy of the in-paralog or intron splice site was retained
Dino@67443 (*Dionaea*)	Dino@9980	2793	510	Not identified as homologs in blast
RuprSFB@17320 (*Ruprechtia*)	RuprSFB@17330	2787	2787	Different copy of the in-paralog or intron splice site was retained
MJM3360@61692 (*Plumbago*)	MJM3360@44226	2211	2403	Different copy of the in-paralog or intron splice site was retained
Retr@34176 (*Reaumuria*)	Retr@1791	1044	546	Not identified as homologs in blast