Long-branch attraction in species tree estimation: inconsistency of partitioned likelihood and topology-based summary methods

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Abstract

With advances in sequencing technologies, there are now massive amounts of genomic data from across all life, leading to the possibility that a robust Tree of Life can be constructed. However, “gene tree heterogeneity”, which is when different genomic regions can evolve differently, is a common phenomenon in multi-locus datasets, and reduces the accuracy of standard methods for species tree estimation that do not take this heterogeneity into account. New methods have been developed for species tree estimation that specifically address gene tree heterogeneity, and that have been proven to converge to the true species tree when the number of loci and number of sites per locus both increase (i.e., the methods are said to be “statistically consistent”). Yet, little is known about the biologically realistic condition where the number of sites per locus is bounded. We show that when the sequence length of each locus is bounded (by any arbitrarily chosen value), the most common approaches to species tree estimation that take heterogeneity

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into account (i.e., traditional fully partitioned concatenated maximum likelihood and newer approaches, called summary methods, that estimate the species tree by combining gene trees) are not statistically consistent, even when the heterogeneity is extremely constrained. The main challenge is the presence of conditions such as long branch attraction that create biased tree estimation when the number of sites is restricted. Hence, our study uncovers a fundamental challenge to species tree estimation using both traditional and new methods.

Introduction

Species trees are a key aspect of much biological research, including the detection of co-evolution, the inference of the ancestral traits, and the dating of speciation events [34]. The availability of sequence data collected from diverse species representing a broad spectrum of life has led to the expectation that the construction of a robust Tree of Life should be possible using statistical estimation methods, such as maximum likelihood. These estimations are increasingly based on large numbers of loci (sometimes thousands) selected from across the genomes of different species [25, 16, 29, 41, 5, 23].

By and large, however, the methods used for species tree estimation have been designed for gene tree estimation, which is a simpler statistical estimation problem. For gene tree estimation, the assumption is that the input sequences have all evolved down a single model tree (called the “gene tree”) under a sequence evolution model, such as Cavender-Farris-Neyman [6, 13, 32], Jukes-Cantor [17], or the Generalised Time Reversible (GTR) model [38]. The estimation of the gene tree under these models from the aligned sequence data is a well-studied problem, and many statistically consistent methods have been developed under these models [37]. Species tree estimation is much more complex, since gene trees can differ from the species tree due to multiple causes, including incomplete lineage sorting (ILS), as modelled by the multi-species coalescent (MSC) model [24]. Indeed, many recent phylogenetic analyses of genome-scale biological datasets for birds [16], land plants [41], worms [5], and other organisms, have revealed substantial heterogeneity across the genes that is consistent with ILS.

The construction of the species tree when there is gene tree heterogeneity due to ILS can be seen as a statistical estimation problem under a two-phase model of sequence evolution where gene trees evolve within a species tree under the MSC model, and then gene sequences evolve down each gene tree under a sequence evolution model.
evolution model. For example, under the MSC+JC model where true gene trees evolve within the species tree under the MSC model and gene sequences evolve down the gene trees under the Jukes-Cantor (JC) model, the estimation of species trees from gene sequence data needs to use the properties of the evolutionary models in order to be statistically consistent. One such approach for species tree estimation is to estimate gene trees for each locus, and then combine these gene trees into a species tree using a coalescent-based summary method (that takes gene tree incongruence due to ILS into account); such approaches can be proven to converge in probability to the true species tree as the number of genes and number of sites per gene both increase. Thus, for example, statistically consistent species tree estimation is possible under the MSC+JC model when gene trees are estimated using Jukes-Cantor maximum likelihood and then combined into a species tree using an appropriate coalescent-based summary method. Examples of these summary methods that enable statistically consistent species tree estimation include MP-EST [21], NJst [20], ASTRID [39], ASTRAL [26, 27], STEM [18], STEAC [22], STAR [22], and GLASS [31].

In contrast, many species trees are estimated using “unpartitioned maximum likelihood”, where the gene sequence alignments are concatenated into a single supermatrix, and a tree is then estimated on that supermatrix under the assumption that all the sites evolve under the same model tree. As shown by [35], this approach is not statistically consistent and can even be positively misleading in the presence of gene tree heterogeneity due to ILS.

Although unpartitioned concatenated analysis with maximum likelihood (CA-ML) is known to be statistically inconsistent and coalescent-based species tree methods can be statistically consistent, performance in practice (and in particular on simulated datasets) has been mixed, with CA-ML sometimes more accurate than leading summary methods [19, 33, 28, 2, 10, 30]. One of the challenges to using summary methods is gene tree estimation error, resulting in part from limited sequence lengths per gene [3]. The “statistical binning” approach [28] was designed to improve the accuracy of species trees estimated using summary methods by binning sequences from different genes together using statistical techniques for detecting strongly supported incongruence (e.g., using bootstrap support on estimated gene trees) and then estimate new gene trees on the combined datasets. As shown in [2], weighted statistical binning (an improved version of the original statistical binning approach) followed by appropriate summary methods is statistically consistent under the MSC+JC model.

Note however that the guarantees of statistical consistency provided so far have nearly always made the following assumptions: every locus is recombination-
free, the number of sites per locus increases without bound, and the number of loci increases without bound. These assumptions are unrealistic, since recombination-free loci are generally short. Therefore, of greater relevance to practice is the question of statistical consistency where the number of recombination-free loci increases, but the number of sites per locus is bounded by some \( L \in \mathbb{Z}_+ \). We investigate this question for the following methods:

- fully partitioned maximum likelihood,
- topology-based summary methods (i.e., methods that combine gene tree topologies), and
- weighted statistical binning pipelines followed by topology-based summary methods.

We address this question under the MSC+CFN model, where the CFN is the symmetric two-state sequence evolution model (i.e., the two-state version of the Jukes-Cantor model); the results we find extend to nucleotide sequence evolution models, but the proofs are simplest under the CFN model. Perhaps surprisingly, our results are negative: for all \( L \), none of the approaches is statistically consistent under the MSC+CFN model and can even be positively misleading. Furthermore, this problematic behavior occurs even when all the genes evolve down a single model CFN tree. Therefore, expectations of accurate species trees using any of these methods given large amounts of data may be unfounded.

The key challenge to species tree estimation is long branch attraction, a phenomenon that can confound maximum likelihood tree estimation when sequence lengths for each genomic region are finite. In fact, we show that many species tree estimation methods that are statistically consistent when the number of genomic regions and their lengths both increase become inconsistent when only the number of regions increases, and the sequence length for each genomic region is bounded (however arbitrarily). These results suggest that all common approaches to species tree estimation are far from being mathematically rigorous, even under highly simplified model conditions where there is no heterogeneity between the loci. This is a very substantial limitation for multilocus phylogeny estimation methods in general, and shows that new approaches for species tree estimation method are needed.
Multi-locus evolution under the MSC

Our analysis is based on the MSC+CFN model. A CFN model tree is a binary tree \((T, \Lambda)\) with topology \(T\) and branch lengths \(\Lambda\). Under the assumption that the tree has \(n\) leaves, each site (character) \(\chi\) refers to the length-\(n\) vector of character states corresponding the same homologous site for each taxon. The possible character states are \(\{0, 1\}\) and evolutionary changes are modeled by a continuous-time Markov process with instantaneous rate matrix \(Q = \begin{pmatrix} -1/2 & 1/2 \\ 1/2 & -1/2 \end{pmatrix}\). In particular, the probability of a change along a branch of length \(\lambda\) is parametrized as \(p = \frac{1}{2} \left( 1 - e^{-2\lambda} \right) \). Under the MSC+CFN model, each locus \(j\) evolves independently on a random gene tree \((T_j, \Lambda_j)\), which is derived from the multispecies coalescent on a species tree \((S, \Gamma, \theta)\), where the \(\Gamma_e\)s are the branch lengths in units of \(\theta_e = 2N_e\mu_e\) with \(N_e\) and \(\mu_e\) the effective population size and mutation rate of branch \(e\). That is, on each branch \(e\) of \(S\), looking backwards in time, lineages entering the branch coalesce at rate \(2/\theta_e\) according to the Kingman coalescent. The remaining lineages at the top of the branch enter the ancestral population, and so on.

We assume that all \(m\) loci evolve on the same species tree and that each locus has a constant, finite sequence length \(L\). Let \(\chi_{ij}\) represent site \(i\) on locus \(j\), where \(1 \leq i \leq L\) and \(1 \leq j \leq m\), and let \(\chi_{-j}\) represent the set of all characters for locus \(j\). We refer to the \(\chi_{-j}\) as \(j\)-th locus sequences. Denote the entire set of characters on all loci as \(X\).

Inconsistency of partitioned maximum likelihood

Let \(\mathcal{L}(T^0, \Lambda, \chi)\) denote the likelihood function for a single site \(\chi\) under the CFN model on \((T^0, \Lambda)\), and let \(\ell = \log \mathcal{L}\) be the log-likelihood. Under fully partitioned maximum likelihood, we seek a single binary tree topology \(T^0\) but allow each locus to have its own branch length parameter \(\Lambda_j\); hence, the general likelihood function over all sites and all loci is

\[
\ell^*(T^0, \Lambda_1, \ldots, \Lambda_m, X) = \sum_{j=1}^{m} \sum_{i=1}^{L} \ell(T^0, \Lambda_j, \chi_{ij}),
\]

and a maximum likelihood topology is any element of the set

\[
\arg \max_{T^0} \max_{\Lambda_1, \ldots, \Lambda_m} \ell^*(T^0, \Lambda_1, \ldots, \Lambda_m, X).
\]

(1)
Theorem 1 (Inconsistency of partitioned ML). Under the MSC+CFN model, fully partitioned maximum likelihood on loci with a bounded number of sites is not statistically consistent and is even positively misleading. That is, for any length $L \in \mathbb{N}$, there is a species tree with topology, branch lengths and mutation rates such that, given data generated under the MSC+CFN model, as the number of loci $m \to \infty$, the maximum likelihood topology is unique and is different from the true species tree topology with probability going to 1.

The proof of this theorem is provided below.

Inconsistency of topology-based summary methods  Summary methods have been developed that are designed to address heterogeneity between gene tree topologies due to ILS, and are statistically consistent under the MSC model. We consider topology-based summary methods that take as input unrooted gene trees, and only use their topologies and not any additional information (e.g., sequence data, branch lengths, bootstrap support).

- We assume that the tree provided for a given gene sequence alignment is its maximum likelihood gene tree, and if there is a tie for the best maximum likelihood tree topology, then a random best-scoring tree is selected.

When the number of species is four, then the summary method is selecting the best unrooted tree topology from the three possible unrooted tree topologies, also referred to as quartet trees. By [1], under the MSC the most probable quartet tree is the true species tree for any four species (i.e., there is no anomaly zone on unrooted four-leaf species trees). Hence, in the four species case, we will make the assumption that the summary method will return the tree topology that appears the most frequently among its input gene trees, as this is a statistically consistent technique for estimating the unrooted species tree on four leaves. We refer to this most frequent quartet tree as the “dominant” quartet tree. That is, we restrict ourselves to the following “reasonable” property of a summary method $A$:

- When $n = 4$, as the number of loci $m$ increases then with probability converging to 1, $A(T_1, \ldots, T_m) = t$ where $t$ is the quartet tree that appears with the highest frequency in the input $T_1, \ldots, T_m$; if there are ties, then $A$ picks uniformly at random between the most frequent quartet trees.

We will say that the summary method $A$ is reasonable if it satisfies this property. Many of the popular summary methods (e.g., ASTRAL and BUCKy) are reasonable in that sense.
Theorem 2 (Inconsistency of reasonable summary methods). Under the MSC+CFN model, any reasonable summary method $A$ with maximum likelihood input trees on loci with a bounded number of sites is not statistically consistent. That is, for any length $L \in \mathbb{N}$, there is a species tree with topology, branch lengths, and mutation rates, such that given data generated under the MSC+CFN model, as the number of loci $m \to \infty$, the topology produced by $A$ is unique and is different from the true species tree topology with probability going to 1.

Inconsistency of weighted statistical binning followed by a summary method

The “statistical binning” method, and its improved version “weighted statistical binning”, were developed to address challenges in species tree estimation that result from gene tree estimation error. In [2] it was shown that statistical binning was inconsistent under the MSC+CFN model but that weighted statistical binning (WSB) was statistically consistent. Those proofs depend crucially on the number of sites per locus increasing to infinity, and so this previous work did not address the case we consider here, where each site has length bounded by $L$.

In a WSB pipeline, estimated gene trees with bootstrap support are provided for every locus, and then an incompatibility graph is computed for that set of gene trees with branch support. The graph is used to partition the genes into sets (called “bins”) and then “supergene trees” are computed using a fully partitioned maximum likelihood analysis on each bin. These supergene trees are then given to the selected summary method as input, and a species tree is returned. In a weighted statistical binning pipeline, each supergene tree is replicated by the number of genes in its associated bin. The incompatibility graph depends on a parameter $B$, as follows: two gene trees are considered to be incompatible if there is a pair of edges, one from each tree, each with bootstrap support strictly greater than $B$, that conflict. Hence, if $B = 1$, then no two trees can be considered incompatible.

Theorem 3 (Inconsistency of WSB pipeline followed by reasonable summary method). Under the multi-locus MSC+CFN model, with a single site evolving down each gene tree, the WSB pipeline followed by a reasonable summary method is not statistically consistent.

The proof of this theorem is given in the Appendix, and establishes that when each locus has a single site then there is a $B < 1$ and a tree with topology, branch lengths, and mutation rates such that, given data generated under the MSC+CFN model, as the number of loci $m \to \infty$, the distribution produced by the WSB pipeline with support threshold $B$ is flat. Hence, the application of $A$ to this distribution will not converge to the true species tree topology with probability going
to 1. In other words, the WSB pipeline is not statistically consistent under the MSC+CFN model because uninformative genes can swamp the bins and produce a flat distribution.

The following modification to the WSB pipeline (which we refer to as the WSB* pipeline) to remove all genes that have no branches with bootstrap support above $B$ addresses this problem in that the distribution is no longer flat:

- Remove all gene trees that do not support any internal edge above the bootstrap threshold $B$ from the analysis before doing any binning.

However, we still show:

**Theorem 4** (Inconsistency of WSB pipeline followed by reasonable summary method). *The WSB* pipeline followed by $\mathcal{A}$ is not only not statistically consistent but is positively misleading. That is, for any length $L \in \mathbb{N}$, there is a $B < 1$ and a species tree with topology, branch lengths and mutation rates such that, given data generated under the MSC+CFN model, as the number of loci $m \to \infty$, the topology produced by $\mathcal{A}$ after going through the WSB* pipeline with support threshold $B$ is unique and is different from the true topology with probability going to 1.

**Theoretical framework**

Our analysis in fact establishes a stronger—perhaps more counter-intuitive—result. We show that partitioned maximum likelihood, topology-based summary methods, and weighted statistical binning pipelines are statistically inconsistent for multi-locus evolution where there is no gene tree heterogeneity at all, when all the loci have only $L$ sites for any arbitrarily selected $L$. By a continuity argument, we also establish that these negative results imply that these methods, which were designed to address heterogeneity across the genome resulting from ILS, are also statistically inconsistent under the MSC+CFN model.

**Setting for analysis** Fix $\mathcal{T}^0$ to be the four-taxon topology $ab|cd$ on $\{a, b, c, d\}$ and let $\Lambda^0$ denote a vector of branch lengths on $\mathcal{T}^0$ under the CFN model. Specifically, denote the endpoint of the middle edge on the $ab$ side as $e$, and on the $cd$ side as $f$ (see Figure 1). For this tree, denote the length of branch $ae$ as $\lambda^0_a$, $be$ as $\lambda^0_b$, $cf$ as $\lambda^0_c$, $df$ as $\lambda^0_d$ and $ef$ as $\lambda^0_m$. For a branch length $\lambda$, we will also use the
parametrization $\phi = -\frac{1}{2} \log \lambda$ in terms of which the probability of a change along this branch is

$$p = \frac{1}{2} \left(1 - e^{-2\lambda}\right) = \frac{1}{2} (1 - \phi),$$

and the probability of no change is $q = \frac{1}{2} (1 + \phi)$. See [37, Section 8.6] for more details on this standard parameterization. Denote the $p$-, $q$-, and $\phi$-parameters as defined above for each branch using the same subscripts. We choose $\Lambda^0$ to construct a Felsenstein zone tree (i.e., a four-leaf model tree where some tree estimation methods are positively misleading, as shown in [14]) where, for a parameter $\rho > 0$, $p^0_a = p^0_c = \rho$ and $p^0_b = p^0_d = p^0_m = \rho^3$. Note that for any $\rho > 0$, we can set $\lambda^0_a = \lambda^0_c = -\frac{1}{2} \log(1 - 2\rho)$ and $\lambda^0_b = \lambda^0_d = -\frac{1}{2} \log(1 - 2\rho^3)$ to satisfy this relationship. We assume that the characters $\chi_{\cdot j}$, $j = 1, 2, \ldots$, are generated under the CFN model on $(T^0, \Lambda^0)$. We also denote the alternate topologies by $T^* = ac|bd$ and $T^1 = ad|bc$.

**Basic claims**  Our main theorems are implied respectively by the following basic claims.

**Claim 1** (Partitioned ML: Felsenstein zone). Assume that the length-$L$ locus sequences $\chi_{\cdot j}$, $j = 1, 2, \ldots$, are generated under the CFN model on $(T^0, \Lambda^0)$ and let $\hat{T}_j$ be the fully partitioned maximum likelihood topology obtained from the sequences of the first $j$ loci. For any length $L \geq 1$, there is $\rho > 0$ small enough such that, with probability one, $\hat{T}_j \rightarrow T^*$ as $j \rightarrow +\infty$.

**Claim 2** (Reasonable summary methods: Felsenstein zone). Assume that the length-$L$ locus sequences $\chi_{\cdot j}$, $j = 1, 2, \ldots$, are generated under the CFN model on
and let $\hat{T}_j$ be the topology obtained from a reasonable summary method $A$ on the sequences of the first $j$ loci using maximum likelihood. For any length $L \geq 1$, there is $\rho > 0$ small enough such that, with probability one, $\hat{T}_j \to T^*$ as $j \to +\infty$.

Claim 3 (WSB* pipeline: Felsenstein zone). Let $1 - \frac{2}{3} \left(\frac{1}{L}\right)^L \leq B < 1$. Assume that the length-$L$ locus sequences $\chi_j$, $j = 1, 2, \ldots$, are generated under the CFN model on $(T^0, \Lambda^0)$ and let $\hat{T}_j$ be the topology obtained from the WSB* pipeline with threshold $B$ followed by a reasonable summary method $A$ on the sequences of the first $j$ loci. There is $\rho > 0$ small enough such that, with probability one, $\hat{T}_j \to T^*$ as $j \to +\infty$.

While the claims above are established under the multi-locus CFN model with a single tree, we show in the Appendix that these results also apply to the MSC+CFN model by choosing a species tree which is highly likely to produce gene trees matching the species tree.

Analysis of partitioned ML We describe the main ideas used to prove Claim. We proceed as follows:

(a) By choosing $\rho$ small enough, we show that we can restrict the analysis to the five most common dataset types, which we refer to as locus patterns.

(b) We then show that, for these locus patterns, the likelihood on $T^*$ dominates the likelihood on $T^0, T^1$, and that this domination is strict in one case.

Under our choice of branch lengths, as $\rho \to 0$, the five most common locus patterns, which we refer to as dominant (see Lemma below for justification), are:

1. All constant sites: Every character has the same state on all four taxa, but that state can change from one character to another (e.g. $x^a = x^b = x^c = x^d = 0001010$). We let $\mathcal{X}_0$ be the set of such datasets and we let $Q_0$ be the probability of observing any $x \in \mathcal{X}_0$ under $(T^0, \Lambda^0)$.

2. One singleton site on $a$ or $c$: All sites are constant except for one, on which either $a$ or $c$ is different from all others (e.g. $x^a = 0111110, x^b = x^c = x^d = 1111110$). We let $\mathcal{X}_{11}$ be the set of such datasets and we let $Q_{11}$ be the probability of observing any $x \in \mathcal{X}_{11}$ under $(T^0, \Lambda^0)$. 

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3. **Two identical singleton sites on a or c:** All sites are constant except for two, each of which has the same taxon a or c different from the others (e.g. \(x^a = 0011110, x^b = x^c = x^d = 1111110\)). We let \(\mathcal{X}_2\) be the set of such datasets and we let \(Q_2\) be the probability of observing any \(x \in \mathcal{X}_2\) under \((T^0, \Lambda^0)\).

4. **Two different singleton sites on a and c:** All sites are constant except for two, one of which has a different character state on a and the other a different character state on c (e.g. \(x^a = 1001110, x^c = 0101110, x^b = x^d = 0001110\)). We let \(\mathcal{X}_2\) be the set of such datasets and we let \(Q_2\) be the probability of observing any \(x \in \mathcal{X}_2\) under \((T^0, \Lambda^0)\).

5. **One site with a 2/2-split ac|bd:** \(L - 1\) sites are constant with a single site having a and c different from b and d (e.g. \(x^a = x^c = 1001110, x^b = x^d = 0001110\)). We let \(\mathcal{X}_{12}\) be the set of such datasets and we let \(Q_{12}\) be the probability of observing any \(x \in \mathcal{X}_{12}\) under \((T^0, \Lambda^0)\).

Note that above only the last pattern is informative and it supports the split in \(T^*\) rather than \(T^0\). Let \(\tilde{\mathcal{X}}\) be the set of all remaining locus patterns.

**Lemma 1** (Dominant patterns and their likelihood contributions).

(a) The probabilities of observing the dominant locus patterns are bounded as follows:

\[
Q_0 = \left(\frac{1}{2}\right)^L - O(\rho), \quad Q_{11} = O(\rho), \quad Q_2 = O(\rho^2),
\]

\[
Q_{2\neq} = O(\rho^2) \text{ and } Q_{12} = \left(\frac{1}{2}\right)^L \rho^2 + O(\rho^3).
\]

Moreover, for all \(x \in \tilde{\mathcal{X}}\), the probability of observing \(x\) under the CFN model on \((T^0, \Lambda^0)\) is \(O(\rho^3)\).

(b) For all \(x \in \mathcal{X}_0 \cup \mathcal{X}_{11} \cup \mathcal{X}_{2=} \cup \mathcal{X}_{2\neq}\), it holds that

\[
\sup_{\Lambda} \ell(T^*, \Lambda, x) - \sup_{\Lambda} \ell(T^0, \Lambda, x) \geq 0,
\]

while, for all \(x \in \mathcal{X}_{12}\),

\[
\sup_{\Lambda} \ell(T^*, \Lambda, x) - \sup_{\Lambda} \ell(T^0, \Lambda, x) \geq K_{12} > 0,
\]

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for some positive constant $K_{12}$ dependent only on $L$. The same holds if one replaces $T^0$ with $T^1$ above.

Note that the big-O notation implicitly includes the contribution from $L$, which we treat as a constant. The detailed proofs of Lemma 1 and Claim 1 are provided in the Appendix. Claim 2 follows from a similar argument, which is also detailed in the Appendix.

**Analysis of WSB* pipeline** Our analysis of the WSB* pipeline follows along similar lines. Our key additional observation is that, by choosing an appropriate bootstrap threshold, we ensure that the only loci passed on to the summary method are “saturated,” that is all their sites correspond to an equivalent character. The rest of the analysis is similar to Claim 2 and relies on the fact that the loci passed on to the summary method are dominated by the “wrong split.” Formally, we say that two characters are equivalent if they are identical up to switching 0s and 1s. We say that a locus pattern $x$ is saturated if all characters in $x$ are equivalent. On four taxa, there are only three types of saturated patterns:

1. **All-constant**: Every character has the same value on all four taxa (e.g. $x^a = x^b = x^c = x^d = 0001010$). We let $\mathcal{P}_0^s$ be the set of such datasets and we let $Q_0^s$ be the probability of observing any $x \in \mathcal{P}_0^s$ under $(T^0, \Lambda^0)$.

2. **All-singleton on a fixed taxon**: All sites have the same taxon different from all others (e.g. $x^a = 0101111, x^b = x^c = x^d = 1010000$). We let $\mathcal{P}_1^s$ be the set of such datasets and we let $Q_1^s$ be the probability of observing any $x \in \mathcal{P}_1^s$ under $(T^0, \Lambda^0)$.

3. **All-2/2-split with a fixed split**: All sites have two fixed taxa—say, $a$ and $c$—identical while being different from the other two taxa—$b$ and $d$—(e.g. $x^a = x^c = 1010111, x^b = x^d = 0101000$). We let $\mathcal{P}_{ac|bd}$ be the set of such datasets for the split $ac|bd$ and we let $Q_{ac|bd}^s$ be the probability of observing any $x \in \mathcal{P}_{ac|bd}^s$ under $(T^0, \Lambda^0)$ (and similarly for the other possible splits). For short, we refer to this type of datasets as split-saturated genes.

**Lemma 2** (Saturated genes).

(a) Under the WSB* pipeline with threshold $B \geq 1 - \frac{2}{3} \left( \frac{1}{T} \right)^L$, the only length-$L$ locus sequences passed on to the summary method are the ones in $\mathcal{P}_{ac|bd}^s$. 

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Moreover,
\[ Q^{s}_{ac|bd} = \left( \frac{1}{2} \right)^{L} \rho^{2L} + \mathcal{O}(\rho^{2L+1}), \]
while
\[ Q^{s}_{ab|cd} = \mathcal{O}(\rho^{3L}), \quad Q^{s}_{ad|bc} = \mathcal{O}(\rho^{3L}). \]

(b) For any \( x \in \mathcal{X}_{ab|cd} \), the topology \( ab|cd \) is the unique ML optimizer. And similarly for the other splits.

The detailed proofs of Lemma 2 and Claim 3 are provided in the Appendix.

**Discussion**

Our results show that fully partitioned maximum likelihood is inconsistent (even positively misleading) even when there is no gene tree heterogeneity at all (i.e., when all loci evolve down a common CFN model tree), and hence by continuity under the multi-locus MSC+CFN model. The inconsistency result occurs because each locus has at most \( L \) sites (for an arbitrarily selected bound \( L \)), and the loci all evolve down gene trees that have long branch attraction (LBA). It is well known that maximum likelihood is statistically consistent even in the presence of LBA, but our results show that LBA is sufficient to bias fully partitioned ML towards the same wrong tree on each locus, and hence towards the same wrong tree for the partitioned concatenation analysis.

The same argument is used to establish that reasonable summary methods and weighted statistical binning pipelines that use these reasonable summary methods can be positively misleading when each locus has only \( L \) sites, even when there is no gene tree heterogeneity. Hence, summary methods and weighted statistical binning pipelines do not solve this challenge, either. All the methods we addressed in this study can be seen as partitioned analyses – partitioned maximum likelihood estimates numeric parameters for each locus but keeps the tree topology the same across the loci, and summary methods estimate the gene trees independently across the loci.

The fundamental challenge to multi-locus species tree estimation using these partitioned analyses (whether partitioned maximum likelihood or summary methods) is that maximum likelihood tree estimation is impacted by conditions such as LBA when the number of sites is not allowed to increase.
It is interesting to consider unpartitioned maximum likelihood under the same set of conditions. When all the loci evolve down the same CFN model tree, even though each locus has only $L$ sites, as the number of loci increases, the unpartitioned maximum likelihood analysis will converge to the true tree; thus, unpartitioned maximum likelihood analysis is consistent under this setting. On the other hand, when there is gene tree heterogeneity resulting from ILS (as modelled by the MSC), then unpartitioned ML is inconsistent and can be positively misleading [35]. Hence, unpartitioned maximum likelihood can be statistically consistent under one setting and inconsistent (and even positively misleading) under another. In other words, unpartitioned maximum likelihood is not the solution to the challenge raised by this study.

Our analysis does not apply to multilocus methods that estimate the species tree directly from sequence data—without a gene tree reconstruction step. These include for instance METAL [11], SNAPP [4], SVDquartets [8, 9], and *BEAST [15]. In particular, METAL has been shown to be consistent on finite-length genes under some assumptions on the multispecies coalescent [11]. It is also worthwhile pointing out that our results, while being based on the MSC, are likely to hold more generally for other sources of gene tree discordance, including horizontal gene transfer (HGT). Indeed, as long as rates of HGT are low enough, in the Felsenstein zone similar conclusions about inconsistency will follow for partitioned ML and summary-based methods.

**Conclusion**

Prior to this study, many coalescent-based species tree estimation methods were assumed to be statistically consistent under this regime, but no proofs had been provided. This study now establishes that all the standard methods used in phylogenomic species tree estimation are statistically inconsistent.

Moreover, only a very small number of methods have been proven to be statistically consistent for bounded $L$. Some of the summary methods described in [36] are statistically consistent for $L = 1$, but the proofs depend on the strict molecular clock. Similarly, SVDquartets [7] (a site-based method for estimating quartet trees from a single site per locus) is based on an identifiability result that depends on the strict molecular clock; however, the species tree estimation method itself has not yet established to be statistically consistent under the MSC even when the strict molecular clock holds.

Furthermore, when the strict molecular clock assumption does not hold, very
few methods are statistically consistent for bounded $L$. METAL [11] is one of the few coalescent-based methods that does not require a molecular clock, and that has been proven to be statistically consistent under the MSC+CFN model. It should be noted however that the model of evolution in [11] allows mutation rates to vary across branches of the species tree, but those rates must be the same across loci, a major constraint. Much remains to be understood about the important theoretical question of fixed locus length consistency of multilocus method in general.

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Inconsistency of WSB on a single site

Here we show that the weighted statistical binning pipeline (as defined in [2]) is inconsistent for any positive $L$, for some four-species model tree. We begin with a lemma.

**Lemma 3.** Let $S$ be a model species tree with four species $a, b, c, d$, and suppose every locus has only one site. In a weighted statistical binning pipeline with bootstrap support threshold $B \geq \frac{1}{3}$, there will at most three bins (one for each of the three possible binary topologies on four leaves), and the bin associated with topology $ab|cd$ will have all the ML-informative genes that support $ab|cd$.

**Proof.** Because there is only one site for each gene, the ML-informative genes have bootstrap support of 100%. Hence, no two ML-informative genes can be placed in the same bin if they support different tree topologies. Therefore, for any bin, the ML-informative genes placed in the bin will support the same topology. Also, the ML-uninformative genes produce trees with bootstrap support equal to $\frac{1}{3}$, since every tree topology has equal maximum likelihood score. These genes are therefore considered compatible with every other gene, since the bootstrap support threshold $B \geq \frac{1}{3}$.

Since there are only three tree topologies, the incompatibility graph is the union of a complete 3-partite graph (defined by the ML-informative genes) and a collection of isolated vertices (defined by the ML-uninformative genes). Hence, the incompatibility graph can be 3-colored. Since statistical binning seeks the minimum vertex coloring for the incompatibility graph, it will partition the genes into three bins, with one bin for each binary tree topology. Hence, the ML-informative genes are partitioned into three sets based on the tree topology they support. \qed
We continue with an analysis of WSB pipelines followed by reasonable summary methods, beginning with the case of a single site per gene. The following result implies Theorem 3.

**Theorem 5.** Suppose every gene has only one site, and let \((S, \Gamma, \theta)\) be a MSC+CFN model species tree with leaves \(a, b, c, d\). Let \(B \geq \frac{1}{3}\). If for all binary trees \(t\) on \(a, b, c, d\) the probability that a random gene is ML-informative and supports \(t\) is at most \(\frac{1}{3}\), then weighted statistical binning followed by a reasonable summary method will be statistically inconsistent.

**Proof.** The argument will establish that under the conditions of the theorem, as the number of genes increases, the WSB binning process will converge to a flat distribution on the three possible tree topologies on \(a, b, c, d\), so that any reasonable summary method will be inconsistent.

By Lemma 3 in a weighted statistical binning pipeline, there will be three bins (one for each binary tree topology), and the bin for binary tree \(t\) will have all genes that are ML-informative and support the split for \(t\), and may also have ML-uninformative genes. Furthermore, the ML-uninformative genes can be distributed to the bins arbitrarily, since their bootstrap support is exactly \(\frac{1}{3}\) and \(B \geq \frac{1}{3}\).

Since every gene has only one site, the supergene alignment associated to the bin for \(ab|cd\) will consist of sites that all split \(ab|cd\). Hence, when a fully partitioned ML analysis is applied to the bin for \(t\), the resultant supergene tree will be the tree \(t\). In a WSB pipeline, the supergene trees for each bin will be replicated as many times as the number of genes in the bin for \(t\). These trees are the newly computed gene trees that will be passed to the reasonable summary method.

The division of genes into bins attempts to achieve balanced bins, so that the number of genes in each bin should be as close to the same as possible. Therefore, if the probability that a gene is ML-uninformative is sufficiently high, then it will be possible to achieve balanced bins, and the distribution of newly computed gene trees will converge to the flat distribution. Since reasonable summary methods cannot infer the species tree from flat distributions, this means that when the probability of being ML-uninformative is sufficiently high, then WSB pipelines based on reasonable summary methods will not be statistically consistent. \(\square\)
B Proofs of the main results

We provide detailed proofs of the main claims.

Key lemmas

Proof of Lemma 1 (a) Under our choice of branch lengths, as \( \mu \to 0 \), the five most common locus site patterns are:

1. All constant sites: Every character has the same value on all four taxa (e.g. \( x^a = x^b = x^c = x^d = 000101 \)). For any such \( x \in \mathcal{X}_0 \), \( x \) occurs with probability

\[
Q_0 = \left[ \frac{1}{2} (1 - \rho^3)^3 (1 - \rho)^2 + \mathcal{O}(\rho) \right]^L = \left( \frac{1}{2} \right)^L - \mathcal{O}(\rho),
\]

where the first term in the brackets corresponds to the case of no substitution, while the second term accounts for all possibilities with at least one substitution. For convenience we denote the expression in brackets—the probability of a single site being identical on all four taxa—as \( q_0 \).

2. One singleton site on \( a \) or \( c \): All sites are constant except for one, on which either \( a \) or \( c \) is different from all others (e.g. \( x^a = 01 \ldots, x^b = x^c = x^d = 11 \ldots \)). Any dataset with this locus site pattern occurs with probability

\[
Q_{11} = q_0^{L-1} \left[ \frac{1}{2} (1 - \rho^3)^3 (1 - \rho)\rho + \mathcal{O}(\rho^2) \right] = \mathcal{O}(\rho),
\]

where the first term in the brackets corresponds to the case of a single substitution along the edge leading to the differing taxon, while the second term accounts for all possibilities involving at least two substitutions.

3. Two identical singleton sites on \( a \) or \( c \): All sites are constant except for two, each of which has the same taxon \( a \) or \( c \) different from the others (e.g. \( x^a = 001 \ldots, x^b = x^c = x^d = 111 \ldots \)). Any dataset with this locus site pattern occurs with probability

\[
Q_{2=} = q_0^{L-2} \left[ \frac{1}{2} (1 - \rho^3)^3 (1 - \rho)\rho + \mathcal{O}(\rho^2) \right]^2 = \mathcal{O}(\rho^2),
\]

which follows from the same computation as in the one singleton case.
4. **Two different singleton sites on a and c:** All sites are constant except for two, one of which has a different character on a and the other a different character on c (e.g. $x^a = 100\ldots, x^c = 010\ldots, x^b = x^d = 000\ldots$). Any dataset with this locus site pattern occurs with probability

$$Q_{2\neq} = q_0^{L-2} \left[ \frac{1}{2} (1 - \rho^3)^3 (1 - \rho) \rho + \mathcal{O}(\rho^2) \right]^2 = \mathcal{O}(\rho^2),$$

which follows from the same computation as in the one singleton case.

5. **One site with a 2/2-split ac|bd:** $L - 1$ sites are constant with a single site having a and c different from b and d (e.g. $x^a = x^c = 100\ldots, x^b = x^d = 000\ldots$). Any dataset with this locus site pattern occurs with probability

$$Q_{12} = q_0^{L-1} \left[ \frac{1}{2} (1 - \rho^3)^2 \rho^2 + \mathcal{O}(\rho^3) \right] = \left( \frac{1}{2} \right)^L \rho^2 + \mathcal{O}(\rho^3), \quad (3)$$

where the first term in the brackets corresponds to the case of substitutions along the edges leading to the differing taxa, while the second term accounts for all possibilities with at least one substitution along the other edges.

Any remaining locus site pattern must include either a change along one of the short branches, which involves multiplication by $\rho^3$, or three changes along one of the long branches, which also means multiplication by $\rho^3$. Thus all $x$ in $\tilde{X}$ have probability $\mathcal{O}(\rho^3)$. That concludes the proof of the claim in (a).

(b) It remains to prove (b). For each locus site pattern we will put an upper bound on the maximum of the likelihood function for topology $T^0 = ab|cd$, and show that in every case the alternate topology $T^* = ac|bd$ has maximum likelihood greater than or equal to this upper bound, and in at least one case is strictly greater.

Some remarks about notation first. Note that the labels we have used for the branch lengths of $T^0$ can be used similarly regardless of the topology of the tree: $\lambda_{mn}$ represents the middle branch in any topology, and the others represent the branch leading to their respective taxon. Also we use $\Lambda$ and $\Phi$ interchangeably, where $\Phi$ is the corresponding collection of $\phi$-parameters as defined in (2). Finally we will use the following property of the $\phi$-parametrization \[37\]: the $\phi$’s multiply along paths; indeed, we have for instance,

$$\mathbf{P}_{x \sim (T^0, \Phi^0)} [x^a_1 \neq x^b_1]$$
\[(1 - p_a^0)p_b^0 + p_a^0(1 - p_b^0)\]
\[= \frac{1}{2}(1 + \phi_a^0)^\frac{1}{2}(1 - \phi_b^0) + \frac{1}{2}(1 - \phi_a^0)^\frac{1}{2}(1 + \phi_b^0)\]
\[= \frac{1}{2}(1 - \phi_a^0 \phi_b^0). \quad (4)\]

Finally, because by inclusion the probability of observing \(\chi_{a1}\) is at most the probability of observing \(\chi_{a1}\), which is simply \((\frac{1}{2})^L\) by independence of the sites, we have
\[
\sup_{\Lambda} \ell(T, \Lambda, \chi_{a1}) \leq \log \left( \frac{1}{2} \right)^L = -L \log 2. \quad (5)
\]

We divide up the proof of by locus site pattern.

1. **All constant sites**: Recall from (5) that, for any \(T\) (and, in particular, for \(T^0\)),
\[
\sup_{\Lambda} \ell(T, \Lambda, x) \leq -L \log 2.
\]
For \(x \in X_0\), that can always (in particular, for \(T^*\)) be achieved by setting all branch lengths to 0.

2. **One singleton site on \(a\) or \(c\)**: Without loss of generality, assume the non-constant site is site 1 and that it has \((x_1^a, x_1^b, x_1^c, x_1^d) = (1, 0, 0, 0)\). Assume also that \((x_i^a, x_i^b, x_i^c, x_i^d) = (0, 0, 0, 0)\) for all \(i = 2, \ldots, L\). We can put the following upper bound on the likelihood function for \(T^0\). Letting \(\phi_{ab} = \phi_a \phi_b\) and using (4), we have
\[
\mathbf{P}_{x \sim (T^0, \Phi)} (x_1^a = 1, x_1^b = x_1^c = x_1^d = 0) \times \mathbf{P}_{x \sim (T^0, \Phi)} (x_i^a = x_i^b = x_i^c = x_i^d = 0)^{L-1} \]
\[\leq \mathbf{P}_{x \sim (T^0, \Phi)} (x_1^a = 1 \neq x_1^b) \mathbf{P}_{x \sim (T^0, \Phi)} (x_i^a = 0 = x_i^b)^{L-1} \]
\[\leq \frac{1}{2} \left( \frac{1 - \phi_{ab}}{2} \right) \left[ \frac{1}{2} \left( \frac{1 + \phi_{ab}}{2} \right) \right]^{L-1}, \quad (6)\]

where the first inequality follows by inclusion. To derive our upper bound, we maximize the expression on the last line as a function of \(\phi_{ab}\). Taking the log, differentiating and equating to 0, we get
\[
\frac{-1}{1 - \phi_{ab}} + (L - 1) \frac{1}{1 + \phi_{ab}} = 0
\]
that is, \( \phi_{ab} = \frac{L-2}{L} \). Plugging this back above, we get the upper bound

\[
\sup_{\Phi} \ell(T^0, \Phi, x) \leq L \log \left( \frac{1}{2} \right) + \log \left( \frac{1}{L} \right) + (L-1) \log \left( \frac{1}{1-L} \right).
\]

On the other hand, for \( T^* \) (or, in fact, any topology), setting \( \lambda_b = \lambda_c = \lambda_m = 0 \) and \( \lambda_a \) so that \( p_a = \frac{1}{L} \), we get the matching bound

\[
P_{x \sim (T^*, \Phi)} (x^a = 1, x^b = x^c = x^d = 0) \\
\times P_{x \sim (T^*, \Phi)} (x^a_i = x^b_i = x^c_i = x^d_i = 0)^{L-1} \\
= \frac{1}{2} \left( \frac{1}{L} \right) \left[ \frac{1}{2} \left( 1 - \frac{1}{L} \right) \right]^{L-1},
\]

which establishes the required lower bound on \( \sup_{\Phi} \ell(T^*, \Phi, x) \).

3. Two identical singleton sites on \( a \) or \( c \): For this locus site pattern, the argument is identical to the previous locus site pattern, with the difference that the exponents in (6) are \( 2 \) and \( L-2 \), and accordingly throughout, giving an optimal \( \phi_{ab} \) of \( \frac{L-4}{L} \) and the upper bound \( L \log(1/2) + 2 \log \left( \frac{2}{L} \right) + (L-2) \log \left( 1 - \frac{2}{L} \right) \). This can likewise be achieved with topology \( T^* \) (or, in fact, any topology) if \( \lambda_b = \lambda_c = \lambda_d = \lambda_m = 0 \) and \( \lambda_a \) is set so that \( p_a = \frac{2}{L} \).

4. Two different singleton sites on \( a \) and \( c \): Assume that \( (x^a_1, x^b_1, x^c_1, x^d_1) = (1, 0, 0, 0) \), \( (x^a_1, x^b_1, x^c_1, x^d_1) = (0, 0, 1, 0) \) and \( (x^a_1, x^b_1, x^c_1, x^d_1) = (0, 0, 0, 0) \) for all \( i = 3, \ldots, L \), without loss of generality. (Recall that the case of two different singletons not involving \( a \) and \( c \) has negligible probability of being observed by part (a) and is therefore not considered here.) We will use the following property of the CFN model: on \( T^0 \), because the path joining \( a, b \) and the path joining \( c, d \) are disjoint, the event \( \{x^c_1 = x^d_1\} \) is independent of the states \( x^a_1 \) and \( x^b_1 \). This is immediate by the symmetry of the CFN model and the Markov property [37]. (Indeed, conditioning on the state at \( f \) has no effect on the agreement between \( c \) and \( d \).) Using this fact as well as inclusion and (4), we get

\[
P_{x \sim (T^0, \Phi)} (x^a_1 = 1, x^b_1 = x^c_1 = x^d_1 = 0) \\
\times P_{x \sim (T^0, \Phi)} (x^a_1 = 1, x^b_1 = x^c_1 = x^d_1 = 0) \\
\times P_{x \sim (T^0, \Phi)} (x^a_i = x^b_i = x^c_i = x^d_i = 0)^{L-2}
\]

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\[ \leq P_{x \sim (T_0, \Phi)} (x_1^a \neq x_1^b, x_1^c = x_1^d) \]
\[ \times P_{x \sim (T_0, \Phi)} (x_1^a = 0 = x_1^b, x_1^c \neq x_1^d) \]
\[ \times P_{x \sim (T_0, \Phi)} (x_1^a = 0 = x_1^b, x_1^c = x_1^d)^{L-2} \]
\[ = [P_{x \sim (T_0, \Phi)} (x_1^a \neq x_1^b) P_{x \sim (T_0, \Phi)} (x_1^c = x_1^d)] \]
\[ \times [P_{x \sim (T_0, \Phi)} (x_1^a = 0 = x_1^b) P_{x \sim (T_0, \Phi)} (x_1^c \neq x_1^d)] \]
\[ \times [P_{x \sim (T_0, \Phi)} (x_1^a = 0 = x_1^b) P_{x \sim (T_0, \Phi)} (x_1^c = x_1^d)]^{L-2} \]
\[ = \frac{1}{2} \left( \frac{1 - \phi_{ab}}{2} \right) \left( \frac{1 + \phi_{cd}}{2} \right) \]
\[ \times \frac{1}{2} \left( \frac{1 + \phi_{ab}}{2} \right) \left( \frac{1 - \phi_{cd}}{2} \right) \]
\[ \times \left[ \frac{1}{2} \left( \frac{1 + \phi_{ab}}{2} \right) \left( \frac{1 + \phi_{cd}}{2} \right) \right]^{L-2} \]
\[ = \left( \frac{1}{2} \right)^L \left( \frac{1 - \phi_{ab}}{2} \right) \left( \frac{1 + \phi_{ab}}{2} \right)^{L-1} \]
\[ \times \left( \frac{1 - \phi_{cd}}{2} \right) \left( \frac{1 + \phi_{cd}}{2} \right)^{L-1}, \]

where \( \phi_{ab} = \phi_a \phi_b \) and \( \phi_{cd} = \phi_c \phi_d \). Maximizing this last expression over \( \phi_{ab} \) and \( \phi_{cd} \) proceeds as in (6). We then get the upper bound

\[ \sup_{\Phi} \ell (T_0, \Phi, x) \leq L \log \left( \frac{1}{\sqrt{L}} \right) + 2 \log \left( \frac{1}{L} \right) + 2(L - 1) \log \left( 1 - \frac{1}{L} \right). \]

On the other hand, for \( T^* \) (or, in fact, any topology), setting \( \lambda_b = \lambda_d = \lambda_m = 0 \) and \( \lambda_a = \lambda_c \) so that \( p_a = p_c = \frac{1}{L} \), we get

\[ P_{x \sim (T^*, \Phi)} (x_1^a = 1, x_1^b = x_1^c = x_1^d = 0) \]
\[ \times P_{x \sim (T^*, \Phi)} (x_1^c = 1, x_1^a = x_1^b = x_1^d = 0) \]
\[ \times P_{x \sim (T^*, \Phi)} (x_1^a = x_1^b = x_1^c = x_1^d = 0)^{L-2} \]
\[ = \frac{1}{2} \left( \frac{1}{L} \right) \left( 1 - \frac{1}{L} \right) \times \frac{1}{2} \left( \frac{1}{L} \right) \left( 1 - \frac{1}{L} \right) \]
\[ \times \left[ \frac{1}{2} \left( 1 - \frac{1}{L} \right)^2 \right]^{L-2}, \]

On the other hand, for \( T^* \) (or, in fact, any topology), setting \( \lambda_b = \lambda_d = \lambda_m = 0 \) and \( \lambda_a = \lambda_c \) so that \( p_a = p_c = \frac{1}{L} \), we get

\[ P_{x \sim (T^*, \Phi)} (x_1^a = 1, x_1^b = x_1^c = x_1^d = 0) \]
\[ \times P_{x \sim (T^*, \Phi)} (x_1^c = 1, x_1^a = x_1^b = x_1^d = 0) \]
\[ \times P_{x \sim (T^*, \Phi)} (x_1^a = x_1^b = x_1^c = x_1^d = 0)^{L-2} \]
\[ = \frac{1}{2} \left( \frac{1}{L} \right) \left( 1 - \frac{1}{L} \right) \times \frac{1}{2} \left( \frac{1}{L} \right) \left( 1 - \frac{1}{L} \right) \]
\[ \times \left[ \frac{1}{2} \left( 1 - \frac{1}{L} \right)^2 \right]^{L-2}, \]
which establishes the required lower bound on \( \sup_{\Phi} \ell(T^*, \Phi, x) \).

5. One site with a 2/2-split ac|bd: Without loss of generality, we assume that \((x_1^a, x_1^b, x_1^c, x_1^d) = (1, 0, 1, 0)\) and \((x_i^a, x_i^b, x_i^c, x_i^d) = (0, 0, 0, 0)\) for all \(i = 2, \ldots, L\). Arguing as in the previous case,

\[
\begin{align*}
&\mathbb{P}_{x \sim (T^0, \Phi)} (x_1^a = x_1^c = 1, x_1^b = x_1^d = 0) \\
&\quad \times \mathbb{P}_{x \sim (T^0, \Phi)} (x_i^a = x_i^b = x_i^c = x_i^d = 0)^{L-1} \\
&\leq \mathbb{P}_{x \sim (T^0, \Phi)} (x_1^a = 1 \neq x_1^b, x_1^c \neq x_1^d) \\
&\quad \times \mathbb{P}_{x \sim (T^0, \Phi)} (x_i^a = 0 = x_i^b, x_i^c = x_i^d)^{L-1} \\
&= \left[ \mathbb{P}_{x \sim (T^0, \Phi)} (x_1^a = 1 \neq x_1^b) \right] \left[ \mathbb{P}_{x \sim (T^0, \Phi)} (x_i^a = 0 = x_i^b) \right]^{L-1} \\
&= \frac{1}{2} \left( \frac{1 - \phi_{ab}}{2} \right) \left( \frac{1 - \phi_{cd}}{2} \right) \\
&\quad \times \left[ \frac{1}{2} \left( \frac{1 + \phi_{ab}}{2} \right) \left( \frac{1 + \phi_{cd}}{2} \right) \right]^{L-1} \\
&= \left( \frac{1}{2} \right)^L \left( \frac{1 - \phi_{ab}}{2} \right) \left( \frac{1 + \phi_{ab}}{2} \right)^{L-1} \\
&\quad \times \left( \frac{1 - \phi_{cd}}{2} \right) \left( \frac{1 + \phi_{cd}}{2} \right)^{L-1} \\
&= \left( \frac{1}{2} \right)^L \left( \frac{1 - \phi_{ab}}{2} \right) \left( \frac{1 + \phi_{ab}}{2} \right)^{L-1} \\
&\quad \times \left( \frac{1 - \phi_{cd}}{2} \right) \left( \frac{1 + \phi_{cd}}{2} \right)^{L-1},
\end{align*}
\]

where, again, \(\phi_{ab} = \phi_a \phi_b\) and \(\phi_{cd} = \phi_c \phi_d\). This bound matches the bound we obtained in the previous case. Hence, we once again get the upper bound

\[
\sup_{\Phi} \ell(T^0, \Phi, x) \\
\leq L \log \left( \frac{1}{2} \right) + 2 \log \left( \frac{1}{L} \right) + 2(L - 1) \log \left( 1 - \frac{1}{L} \right).
\]

However, in this case, we claim that the maximum likelihood under \(T^*\) is strictly greater. Indeed, letting \(\lambda_a = \lambda_b = \lambda_c = \lambda_d = 0\) and setting \(\lambda_m\) such that \(p_m = \frac{1}{L}\), we get

\[
\begin{align*}
&\mathbb{P}_{x \sim (T^*, \Phi)} (x_1^a = x_1^c = 1, x_1^b = x_1^d = 0) \\
&\quad \times \mathbb{P}_{x \sim (T^*, \Phi)} (x_i^a = x_i^b = x_i^c = x_i^d = 0)^{L-1}
\end{align*}
\]
\[
\begin{align*}
    &= \frac{1}{2} \left( \frac{1}{L} \right) \times \left[ \frac{1}{2} \left( 1 - \frac{1}{L} \right) \right]^{L-1},
\end{align*}
\]

so
\[
\sup_{\Phi} \ell(T^*, \Phi, x)
\geq L \log \left( \frac{1}{2} \right) + \log \left( \frac{1}{L} \right) + (L - 1) \log \left( 1 - \frac{1}{L} \right).
\]

Therefore
\[
\sup_{\Phi} \ell(T^*, \Phi, x) - \sup_{\Phi} \ell(T^0, \Phi, x)
\geq - \log \left( \frac{1}{L} \right) - (L - 1) \log \left( 1 - \frac{1}{L} \right)
=: K_{12} > 0,
\]

where the last equality is a definition.

In all the above cases, a similar argument still applies if one replaces \(T^0\) with \(T^1\) (by exchanging the roles of \(b\) and \(d\) throughout). That concludes the proof of the claim in (b).

\(\square\)

**Proof of Lemma 2** (a) The expressions for \(Q^s_{ac|bd}\), \(Q^s_{ab|cd}\) and \(Q^s_{ad|bc}\) come from taking \(L = 1\) in Lemma 1 (a) and raising to the power \(L\). Specifically, it was shown in (3) that observing a single site splitting \(a, c\) from \(b, d\) has probability of the form \((1/2)\rho^2 + O(\rho^3)\). Since a saturated locus contains \(L\) sites with the same probability, we raise this expression to the power \(L\) to obtain
\[
Q^s_{ac|bd} = \left( \frac{1}{2} \right)^L \rho^{2L} + O(\rho^{2L+1}).
\]

Similarly, it was observed in the proof of Lemma 1 (a) that observing a single site splitting \(a, b\) from \(c, d\) (or \(a, d\) from \(b, c\)) has probability \(O(\rho^3)\). Raising to the power \(L\) gives \(Q^s_{ab|cd}\), \(Q^s_{ad|bc} = O(\rho^{3L})\).

For the first part of the claim, we consider several cases.

- Suppose that sequence dataset \(x\) contains at least one uninformative character (i.e., a constant site or a singleton). Then, in computing bootstrap supports, there is probability at least \((1/L)^L\) of resampling a dataset containing only that particular uninformative site. We have shown in the proof
of Lemma $\text{I}(b)$ (see cases 1 and 2 with $L = 1$) that all topologies have an equal ML score on such a site and therefore on such a resampled dataset (since the probability of observing a dataset of this type is the probability of observing a single site to the power $L$). Hence each topology is supported with probability $1/3$. Hence the bootstrap support for the ML-optimizer for $x$ is at most $1 - (2/3)(1/L)^L \leq B$ and $x$ is rejected by WSB*.

- Suppose that sequence dataset $x$ contains two different informative characters (i.e., two different splits). One of those splits is incompatible with the ML-optimizer (possibly random) for $x$. Then, in computing bootstrap supports, there is probability at least $(1/L)^L$ of resampling a dataset containing only that incompatible split. From the argument in Lemma $\text{I}(b)$ again (case 5 with $L = 1$), the incompatible split is then the ML-optimizer of such a resampled dataset. Hence the bootstrap support for the ML-optimizer for $x$ is at most $1 - (1/L)^L < 1 - (2/3)(1/L)^L \leq B$ and $x$ is rejected by WSB*.

- Suppose finally that sequence dataset $x$ contains only characters equivalent to a given split. Then all resampled datasets are saturated for that split as well. From the argument in Lemma $\text{I}(b)$ again (case 5 with $L = 1$), that split is the unique ML-optimizer for $x$. Hence the bootstrap support for the ML-optimizer for $x$ is $1 > B$ and $x$ is passed along by WSB* to the summary method.

(b) This was proved in (a).

\[ \Box \]

**Partitioned ML on CFN model**

*Proof of Claim $\text{I}$.* Using Lemma $\text{I}$ we are now ready to prove Claim $\text{I}$.

We first show that, for a fixed topology, as the number of loci grows to infinity the maximum likelihood value converges almost surely to the expected value of the maximum likelihood value on a single locus.

**Lemma 4** (Convergence of the partitioned log-likelihood). Let $T'$ be a fixed topology on the four taxa with branch lengths $\Lambda'$. Let also $T''$ be a fixed topology on the four taxa (possibly, but not necessarily, equal to $T'$). If the length-$L$ locus sequence datasets $\chi_{j}$, $j = 1, 2, \ldots$, are generated under the CFN model on $(T', \Lambda')$,
then it holds that

\[
\frac{1}{m} \sum_{j=1}^{m} \sup_{\Lambda_j} \ell(\mathcal{T}'', \Lambda_j, \chi_j) \to \mathbb{E}_{\chi_1 \sim (\mathcal{T}', \Lambda')} \left[ \sup_{\Lambda} \ell(\mathcal{T}'', \Lambda, \chi_1) \right] \in [-4L \log 2, -L \log 2],
\]

almost surely as \( m \to +\infty \). Above, the subscript \( \chi_1 \sim (\mathcal{T}', \Lambda') \) indicates that the expectation is taken over a single locus under the CFN model on \((\mathcal{T}', \Lambda')\).

**Proof.** For a given topology and data set there is a unique maximum likelihood value, though the branch lengths at which it is attained may not themselves be unique. For any given locus \( j \), there are a finite number of four-sequence data sets \( \chi_j \) of length \( L \) that can occur under the CFN model. As the number of loci approaches infinity, the frequency of each data set approaches its expected value by the Strong Law of Large Numbers (SLLN) (see, e.g., [12]). To check that the conditions of the SLLN are satisfied, note that the log-likelihood is non-positive. In fact, by taking branch lengths to \(+\infty\) under the CFN model, we have for any topology \( \mathcal{T} \) on \{a, b, c, d\} and any locus data set \( \chi_1 \)

\[
\sup_{\Lambda} \ell(\mathcal{T}, \Lambda, \chi_1) \geq \log \left( \frac{1}{2} \right)^{4L} = -4L \log 2.
\]

On the other hand, because by inclusion the probability of observing \( \chi_1 \) is at most the probability of observing \( \chi_{a1} \), which is simply \( \left( \frac{1}{2} \right)^L \) by independence of the sites, we also have

\[
\sup_{\Lambda} \ell(\mathcal{T}, \Lambda, \chi_1) \leq \log \left( \frac{1}{2} \right)^L = -L \log 2.
\]

So the expectation on the RHS of (7) lies in the interval \([-4L \log 2, -L \log 2]\).

Hence, in view of Lemma [4] our goal is to show that there is \( \rho > 0 \) small enough such that the expected log-likelihood under \((\mathcal{T}^0, \Lambda^0)\) is higher for \( \mathcal{T}^* \) than it is for \( \mathcal{T}^0 \) or \( \mathcal{T}^1 \). That is, it suffices to establish the following claim.

**Lemma 5** (Expected locus-wise maximum likelihood on a fixed topology: key inequality). **There exists \( \rho > 0 \) such that**

\[
\mathbb{E}_{\chi_1 \sim (\mathcal{T}^0, \Lambda^0)} \left[ \sup_{\Lambda} \ell(\mathcal{T}^0, \Lambda, \chi_1) \right]
\]
\begin{align}
< E_{\chi \sim (T^0, \Lambda^0)} \left[ \sup_{\Lambda} \ell(T^*, \Lambda, \chi) \right], \tag{10} 
\end{align}

and

\begin{align}
\mathbb{E}_{\chi \sim (T^0, \Lambda^0)} \left[ \sup_{\Lambda} \ell(T^1, \Lambda, \chi) \right] \\
< \mathbb{E}_{\chi \sim (T^0, \Lambda^0)} \left[ \sup_{\Lambda} \ell(T^0, \Lambda, \chi) \right]. \tag{11}
\end{align}

**Proof.** Let \( \mathcal{X} \) be the set of all possible single-locus datasets. To prove Lemma 5, we expand the expectations in (10) over \( \mathcal{X} \). In other words, we seek to show that

\begin{align}
\sum_{x \in \mathcal{X}} P_{\chi \sim (T^0, \Lambda^0)}(\chi_1 = x) \\
\times \left\{ \sup_{\Lambda} \ell(T^*, \Lambda, x) - \sup_{\Lambda} \ell(T^0, \Lambda, x) \right\} > 0. \tag{12}
\end{align}

We then use Lemma 1 as follows. By (a),

\begin{align}
\sum_{x \in \tilde{\mathcal{X}}} P_{\chi \sim (T^0, \Lambda^0)}(\chi_1 = x) \\
\times \left| \sup_{\Lambda} \ell(T^*, \Lambda, x) - \sup_{\Lambda} \ell(T^0, \Lambda, x) \right| = \mathcal{O}(\rho^3). \tag{13}
\end{align}

Indeed, any locus site pattern in \( \tilde{\mathcal{X}} \) has probability \( \mathcal{O}(\rho^3) \). Moreover, recall from (8) and (9) that the expression in absolute value is bounded by \( 3L \log 2 \). In addition, by (a) and (b), we then arrive at

\begin{align}
\sum_{x \in \mathcal{X}} P_{\chi \sim (T^0, \Lambda^0)}(\chi_1 = x) \\
\times \left\{ \sup_{\Lambda} \ell(T^*, \Lambda, x) - \sup_{\Lambda} \ell(T^0, \Lambda, x) \right\} \\
\geq K_{12} \left\{ \left( \frac{1}{2} \right)^L \rho^2 + \mathcal{O}(\rho^3) \right\} + \mathcal{O}(\rho^3) > 0,
\end{align}

for \( \rho > 0 \) small enough.

The same argument applies for (11).

\( \square \)

Combining Lemmas 4 and 5 gives Claim 1.
Reasonable summary methods on CFN model

Proof of Claim 2. Using Lemma 1, we are now ready to prove Claim 2.

By definition of a reasonable summary method, on a four-taxon dataset, \( A \) outputs the most common quartet topology (breaking ties uniformly at random). We also assume that for genes with multiple optimal ML topologies, a highest scoring topology is picked uniformly at random. We denote by \( \hat{R}(\chi_{-j}) \) be the ML gene tree on the \( j \)-th locus sequence dataset. The law of large numbers immediately gives the following.

Lemma 6 (Convergence of frequencies). Let \( T' \) be a fixed topology on the four taxa with branch lengths \( \Lambda' \). Let also \( T'' \) be a fixed topology on the four taxa (possibly, but not necessarily, equal to \( T' \)). If the length-\( L \) locus sequence datasets \( \chi_{-j}, j = 1, 2, \ldots \), are generated under the CFN model on \( (T', \Lambda') \), then it holds that

\[
\frac{1}{m} \sum_{j=1}^{m} 1 \left[ \hat{R}(\chi_{-j}) = T'' \right] \to P_{\chi_{-1} \sim (T', \Lambda')} \left[ \hat{R}(\chi_{-1}) = T'' \right],
\]

almost surely as \( m \to +\infty \). Above, \( 1[\mathcal{E}] \) is 1 if event \( \mathcal{E} \) occurs, and 0 otherwise.

Hence, in view of Lemma 6, our goal is to show that there is \( \rho > 0 \) small enough such that, under \( (T^0, \Lambda^0) \), \( T^* \) is more likely to be the ML gene tree topology than \( T^0 \) or \( T^1 \). That is, it suffices to establish the following claim.

Lemma 7 (Locus-wise maximum likelihood on a fixed topology: key inequality). There exists \( \rho > 0 \) such that

\[
P_{\chi_{-1} \sim (T^0, \Lambda^0)} \left[ \hat{R}(\chi_{-1}) = T^0 \right] < P_{\chi_{-1} \sim (T^0, \Lambda^0)} \left[ \hat{R}(\chi_{-1}) = T^* \right],
\]

and

\[
P_{\chi_{-1} \sim (T^0, \Lambda^0)} \left[ \hat{R}(\chi_{-1}) = T^1 \right] < P_{\chi_{-1} \sim (T^0, \Lambda^0)} \left[ \hat{R}(\chi_{-1}) = T^* \right].
\]

Proof. By Lemma 1(b), for all \( x \in \mathcal{X}_0 \cup \mathcal{X}_{11} \cup \mathcal{X}_{2=} \cup \mathcal{X}_{2\neq} \), all three topologies are ML-optimal, while for all \( x \in \mathcal{X}_{12} \), \( T^* \) alone is ML-optimal. Moreover, by Lemma 1(a), all other patterns are negligible. Hence, we get

\[
P_{\chi_{-1} \sim (T^0, \Lambda^0)} \left[ \hat{R}(\chi_{-1}) = T^* \right]
\]
\[ \geq \frac{1}{3} 2^L \left[ Q_0 + 4Q_{11} + 4Q_{2=} + 3Q_{2\neq} \right] + 2^L Q_{12} + \mathcal{O}(\rho^3), \]

while

\[ P_{\chi_1 \sim (T^0, \Lambda^0)} \left[ \hat{R}(\chi_1) = T^0 \right] \leq \frac{1}{3} 2^L \left[ Q_0 + 4Q_{11} + 4Q_{2=} + 3Q_{2\neq} \right] + \mathcal{O}(\rho^3), \]

and similarly for \( T^1 \). The result then follows from the fact that

\[ Q_{12} = \left( \frac{1}{2} \right)^L \rho^2 + \mathcal{O}(\rho^3). \]

Combining Lemmas 6 and 7 gives Claim 2.

\[ \square \]

**WSB* pipeline on CFN model**

*Proof of Claim 3.* Using Lemma 2, we are now ready to prove Claim 3.

We begin with two basic results.

**Lemma 8.** In a WSB* pipeline with bootstrap support threshold \( B \geq 1 - \frac{2}{3} \left( \frac{1}{L} \right)^L \), there will be at most three bins (one for each of the three possible binary topologies on four leaves), and the bin associated with topology \( ab|cd \) will have all the saturated genes that support \( ab|cd \) (and similarly for \( ac|bd \) and \( ad|bc \)).

*Proof.* By Lemma 2(b), the genes saturated for a given split have bootstrap support of 100%. Hence, no two such genes can be placed in the same bin if they support different tree topologies. Therefore, for any bin, the genes placed in the bin will support the same topology. By Lemma 2(a), all other genes are discarded.

Since there are only three tree topologies, the incompatibility graph is the union of a complete 3-partite graph (defined by the split-saturated genes). Hence, the incompatibility graph can be 3-colored. Since statistical binning seeks the minimum vertex coloring for the incompatibility graph, it will partition the genes into three bins, with one bin for each binary tree topology. Hence, the split-saturated genes are partitioned into three sets based on the tree topology they support.

\[ \square \]
Lemma 9. (Lemma 2 from [2]:) Let \( S \) be a set of taxa, and let \( S_i \) be a set of DNA sequences for \( S \), with \( i = 1, 2, \ldots, p \). Suppose that tree topology \( t \) is an optimal solution for GTR maximum likelihood for each \( S_i \) (allowing various GTR parameters for different \( i = 1, 2, \ldots, p \)). Then \( t \) will be an optimal solution to a fully partitioned GTR maximum likelihood analysis on a concatenation of \( S_1, S_2, \ldots, S_p \).

Corollary 1. The set of newly computed gene trees computed during a WSB* pipeline has the same distribution as the original set of ML gene trees obtained from the split-saturated genes.

Proof. By Lemma 8, the split-saturated genes are partitioned into three bins for the different tree topologies. By Lemma 9, fully partitioned maximum likelihood on each supergene alignment produces the tree topology associated with the bin. In a WSB* pipeline, the supergene tree for each bin is copied by as many genes as in the bin. Hence, the distribution defined by the newly computed gene trees is identical to the distribution defined by original ML gene trees.

The rest of the argument follows as in the proof of Claim 2. By Lemma 2 (a), under our four-taxon model species tree with topology \( ab|cd \), the most probable estimated quartet tree on split-saturated genes is \( ac|bd \). After removing all the loci that are not split-saturated, we are left only with genes that split 2/2. As the number of loci increases, with probability going to 1 the most frequent estimated quartet tree will be \( ac|bd \). Therefore by Corollary 1 in a WSB* pipeline with bootstrap support threshold \( B \geq 1 - \frac{2}{3} \left( \frac{1}{L} \right)^L \), the most frequent supergene tree computed by weighted statistical binning is identical to the most frequent estimated quartet tree in the input, and will converge to \( ac|bd \) as the number of loci increases by the law of large numbers. Hence, WSB* pipelines followed by reasonable summary methods will be positively misleading under this model.

Extension to MSC+CFN model

In this section, we extend the main claims to the MSC+CFN model. The key idea is to choose a species tree that is highly likely to produce, on any given locus, sequence data whose distribution is close to that of a fixed gene tree in the Felsenstein zone.

When a character of length \( L \), \( \chi_j \), is generated under the CFN model on \((T, \Lambda)\), we write \( \chi_j \sim D_g^j[T, \Lambda] \). Formally, \( D_g^j[T, \Lambda] \) is a probability distribution over sequence datasets in \( \{0,1\}^{n \times L} \), that is, containing \( n \) sequences of length \( L \) taking values in \( \{0,1\} \), where \( n \) is the number of leaves in \( T \). The subscript \( g \)
is meant to refer to the fact that this is a distribution obtained from a single gene tree.

We also consider sequence datasets generated by the MSC+CFN model. Consider a species tree \((S, \Gamma, \theta)\) with \(n\) leaves. Each gene \(j = 1, \ldots, m\) has a genealogical history represented by its gene tree \(T_j\) distributed according to the following process: looking backwards in time, on each branch \(e\) of the species tree, the coalescence of any two lineages is exponentially distributed with rate \(2/\theta_e\), independently from all other pairs; whenever two branches merge in the species tree, we also merge the lineages of the corresponding populations, that is, the coalescence proceeds on the union of the lineages. More specifically, the probability density of a realization of this model for \(m\) independent genes is

\[
\prod_{j=1}^{m} \prod_{e \in E} \exp \left( -\frac{O^e_j}{2} \left[ \gamma^{e,O^e_j+1}_j - \gamma^{e,O^e_j}_j \right] \frac{2}{\theta_e} \right) \prod_{\ell=1}^{I^e_j-O^e_j} \exp \left( -\frac{\ell}{2} \left[ \gamma^{e,\ell}_j - \gamma^{e,\ell-1}_j \right] \frac{2}{\theta_e} \right),
\]

where, for gene \(j\) and branch \(e\), \(I^e_j\) is the number of lineages entering \(e\), \(O^e_j\) is the number of lineages exiting \(e\), and \(\gamma^{e,\ell}_j\) is the \(\ell\)th coalescence time in \(e\); for convenience, we let \(\gamma^{e,0}_j\) and \(\gamma^{e,I^e_j-O^e_j+1}_j\) be respectively the divergence times of \(e\) and of its parent population (which depend on \(\Gamma\)).

When a character of length \(L\), \(\chi_j\), is generated under the MSC+CFN model on \((S, \Gamma, \theta)\), we write \(\chi_j \sim D^L_s[S, \Gamma, \theta]\). Formally, \(D^L_s[S, \Gamma, \theta]\) is a probability distribution over sequence datasets in \(\{0, 1\}^{n \times L}\), where \(n\) is the number of leaves in \(S\). The subscript \(s\) is meant to refer to the fact that this is a distribution obtained from the MSC on a species tree.

As in the main text, fix \(T^0\) to be the four-taxon topology \(ab|cd\) on \(\{a, b, c, d\}\) and let \(\Lambda^0\) denote a vector of branch lengths on \(T^0\). Denote the endpoint of the middle edge on the \(ab\) side as \(e\), and on the \(cd\) side as \(f\). For this tree, denote the length of branch \(ae\) as \(\lambda^0_a\), \(be\) as \(\lambda^0_b\), \(cf\) as \(\lambda^0_c\), \(df\) as \(\lambda^0_d\) and \(ef\) as \(\lambda^0_m\). For a branch length \(\lambda\), recall that we also use the parametrization \(\phi = -\frac{1}{2} \log \lambda\) in terms of which the probability of a change along this branch is

\[
p = \frac{1}{2} \left( 1 - e^{-2\lambda} \right) = \frac{1}{2} \left( 1 - \phi \right),
\]

and the probability of no change is \(q = \frac{1}{2} (1 + \phi)\). We choose \(\Lambda^0\) to construct a Felsenstein zone tree where, for a parameter \(\rho > 0\), \(p^0_a = p^0_c = \rho\) and \(p^0_b = p^0_d = \frac{1}{2} (1 + \phi)\).
\( p^0_m = \rho^3 \). Note that for any \( \rho > 0 \), we can set \( \lambda^0_a = \lambda^0_b = -\frac{1}{2} \log(1 - 2\rho) \) and \( \lambda^0_c = \lambda^0_m = -\frac{1}{2} \log(1 - 2\rho^3) \) to satisfy this relationship. We also denote the alternate topologies by \( T^* = ac|bd \) and \( T^1 = ad|bc \).

**Claim 4** (Species tree in the Felsenstein zone). For all \( \epsilon > 0 \), there is a species tree \((S^0, \Gamma^0, \theta^0)\) with leaves \(\{a, b, c, d\}\) and a probability distribution \(\mathcal{R}\) over \(\{0, 1\}^4 \times L\) such that

\[
D^L_s[S^0, \Gamma^0, \theta^0] = (1 - \epsilon) D^L_g[T^0, \Lambda^0] + \epsilon \mathcal{R}.
\]

**Proof.** We let \(S^0\) be the balanced species tree with split \(ab|cd\) and root \(r\). Denote the endpoint of the edge incident to the root on the \(ab\) side as \(e\), and on the \(cd\) side as \(f\). For this tree, denote the length of branch \(ae\) as \(\gamma^0_a\), \(be\) as \(\gamma^0_b\), \(cf\) as \(\gamma^0_c\), \(df\) as \(\gamma^0_d\), \(er\) as \(\gamma^0_e\) and \(fr\) as \(\gamma^0_f\). And similarly for \(\theta^0\). The branch \(r\infty\) above the root \(r\) has infinite length and parameter \(\theta^0_r\). We take \(\theta^0_a = \theta^0_b = \theta^0_c = \theta^0_d = 1\), \(\gamma^0_a = \lambda^0_a\), \(\gamma^0_b = \lambda^0_b\), \(\gamma^0_c = \lambda^0_c\), \(\gamma^0_d = \lambda^0_d\). Finally we let \(\gamma^0_e = \gamma^0_f = \alpha + \lambda^0_m/2\) and \(\theta^0_e = \theta^0_f = \theta^0_r = \beta\). Take \(\alpha\) and \(\beta\) small enough that:

- coalescences in \(er, fr\) and \(r\infty\) occur within \(\alpha\) of \(e, f\), and \(r\) respectively;
- no mutation occurs within \(\alpha\) above \(e, f\) and \(r\) respectively;

with probability at least \(1 - \epsilon\). Conditioned on the event above, the distribution of sequence dataset is precisely \(D^L_g[T^0, \Lambda^0]\). The result follows.

We are now ready to prove the main theorems.

**Proof of Theorem** \([7]\) We take \((S^0, \Gamma^0, \theta^0)\) as in Claim \([4]\) for \(\epsilon > 0\) to be determined below. We think of the first \(m\) loci as divided into two subsets: \(M^0_m\) coming from distribution \(D^L_g[T^0, \Lambda^0]\) and \(M^R_m\) coming from \(\mathcal{R}\). By the law of large numbers, we have

\[
\frac{|M^0_m|}{m} \to 1 - \epsilon \quad \text{and} \quad \frac{|M^R_m|}{m} \to \epsilon.
\]

We then apply the argument in the proof of Claim \([1]\) to the samples in \(M^0_m\) and take \(\epsilon\) small enough that the contribution of \(M^R_m\) to the partitioned log-likelihood is in the limit \(m \to +\infty\) smaller than the expected gap between \(T^*\) and \(T^0\).

The proofs of Theorems \([2]\) and \([4]\) follow from similar arguments.