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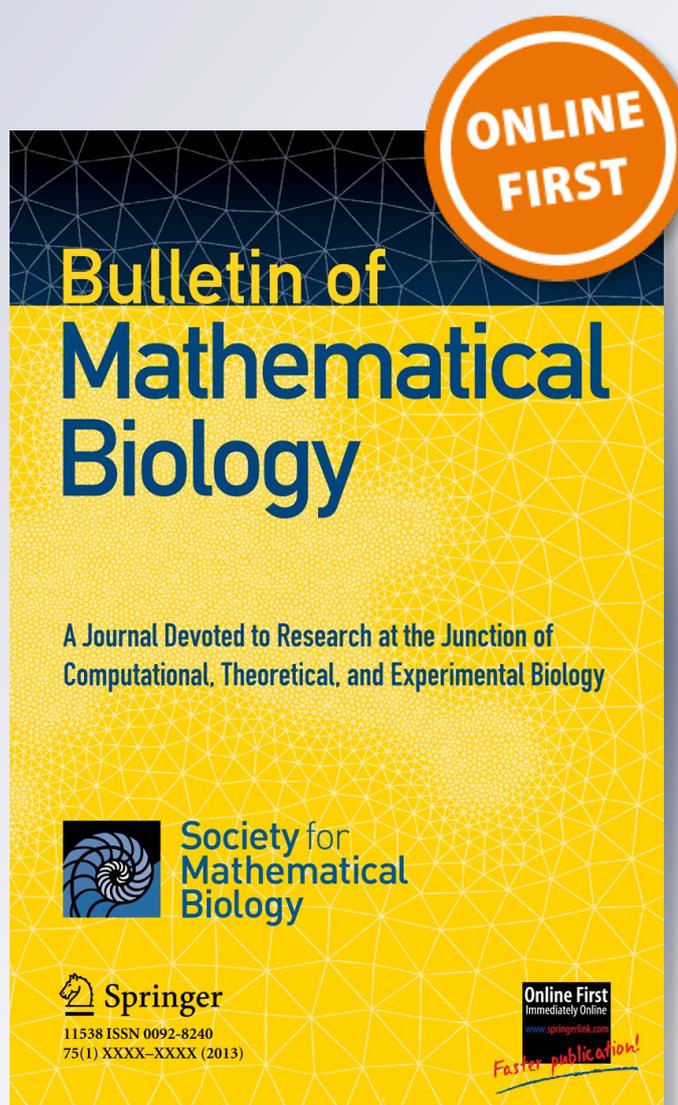
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Split Probabilities and Species Tree Inference Under the Multispecies Coalescent Model

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Abstract Using topological summaries of gene trees as a basis for species tree inference is a promising approach to obtain acceptable speed on genomic-scale datasets, and to avoid some undesirable modeling assumptions. Here we study the probabilities of splits on gene trees under the multispecies coalescent model, and how their features might inform species tree inference. After investigating the behavior of split consensus methods, we investigate split invariants—that is, polynomial relationships between split probabilities. These invariants are then used to show that, even though a split is an unrooted notion, split probabilities retain enough information to identify the rooted species tree topology for trees of 5 or more taxa, with one possible 6-taxon exception.

Keywords Multispecies coalescent model · Split probability · Species tree identifiability

Mathematics Subject Classification 92D15

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1 Introduction

As advances in technology have allowed for the collection of genomic-scale data across a collection of organisms, it has been frequently observed that phylogenetic trees inferred from single genes for a fixed taxon set often differ from one another. Improving inference of species relationships requires addressing such gene tree discordance in a principled way. While there are several biological processes that might cause this discord, including hybridization or other forms of horizontal gene transfer, *incomplete lineage sorting* is an especially common source of gene tree incongruence when times between speciation events are short and/or population sizes are large. Incomplete lineage sorting is modeled by the *multispecies coalescent model*, an extension of the standard coalescent model describing gene tree formation within a single population.

Many methods of species tree inference based on the multispecies coalescent have been proposed. The Bayesian approaches of the software *BEAST (Heled and Drummond 2010) and Mr. Bayes/BEST (Liu and Pearl 2007; Ronquist et al. 2012) perform simultaneous gene tree and species tree inference under a combined coalescent and sequence evolution model, from data composed of the site pattern frequencies for individual genes. The SVD quartets method (Chifman and Kubatko 2015; Long and Kubatko 2017) also utilizes a combined model, but bases inference on site pattern frequencies across the full genome, with no assignment of sites to genes. Nonetheless, it still gives a statistically consistent estimate of the species tree under the plausible assumption of independence of gene length and gene tree.

Alternatively, gene trees inferred by traditional phylogenetic methods can be used as input for a subsequent inference of a species tree, in methods such as Rooted triple Consensus (Ewing et al. 2008), STEM (Kubatko et al. 2009), STAR (Liu et al. 2009), NJ_{st}/U-STAR/ASTRID (Liu and Yu 2011; Allman et al. 2016; Vachaspati and Warnow 2015), MP-EST (Liu et al. 2010), BUCKy (Larget et al. 2010) and ASTRAL-II (Mirarab and Warnow 2015). While theoretical justification for these two-stage approaches generally ignores gene tree inference error, they can be applied to much larger data sets (more taxa and more genes) than the computationally intensive Bayesian algorithms, and have exhibited strong performance in simulations. Such scalability makes them highly attractive and motivates further exploration of their underpinnings.

A fundamental issue for any inference of a species tree is how to relate the timescale used in the multispecies coalescent model on a species tree to those in sequence evolution models used on gene trees. The coalescent timescale can be measured in number of generations divided by population size, while the sequence evolution model is generally in number of substitutions per site. Assumptions such as a constant mutation rate over the gene tree (implying all gene trees are ultrametric) and a constant population size over the species tree are sometimes made, despite their implausibility. While these assumptions can be relaxed somewhat through more elaborate modeling, it is difficult to test the robustness of inference when they are violated.

An alternative way of addressing this difficult time scale issue is to simply discard all metric information inferred about gene trees, and only use their topological features to infer a species tree. Although discarding such information is undesirable if one can

validly relate time scales, one can view it as a conservative approach to avoid reliance on unjustified assumptions.

Some methods go further, and only consider summaries of the inferred topological gene trees, such as displayed quartets (unrooted 4-taxon trees), rooted triples (rooted 3-taxon trees), clades (all taxa descended from an internal node of the rooted tree) or splits (bipartitions of taxa induced by an edge in the tree). Among current methods, Rooted triple consensus, MP-EST, BUCKy, STAR, ASTRAL-II, and NJ_{st} /U-STAR/ASTRID are all of this type [while for Rooted triple Consensus, MP-EST, BUCKy and ASTRAL-II this is obvious from their formulations, for STAR and NJ_{st} the connection to clades and splits was established in [Allman et al. \(2013\)](#) and [Allman et al. \(2016\)](#)]. These methods all use topological summaries of a sample of gene trees, rather than the full trees, in ways allowing for statistically consistent species tree inference when the gene trees are sampled from the multispecies coalescent model without error.

In this work, we undertake a theoretical study of the probabilities of splits on gene trees arising from the multispecies coalescent, with the aim of better understanding how species tree inference may be performed from gene tree split information. This parallels several previous works, in which we have shown that rooted species tree topologies are identifiable from unrooted gene tree topologies or from clades displayed on gene trees, and unrooted species tree topologies are identifiable from gene tree quartets.

A pleasant outcome of our study is that gene tree split probabilities generally retain enough information on the species tree that they determine both its topology and its root, despite the fact that splits themselves are an unrooted notion. Specifically, for all species trees on 5 or more taxa, with one 6-taxon exception, for generic edge lengths, the rooted species tree topology is identifiable. Translating to an empirical setting, this means that one should be able to develop a statistically consistent method of inference of the rooted species tree from the frequencies of splits on a collection of unrooted gene trees. This would be an extension of the NJ_{st} /U-STAR/ASTRID method, which only infers an unrooted species tree topology from the same information. Such a method would avoid any issues with erroneous rooting of gene trees by inclusion of an outgroup, which has long been known as a source of additional error (see [Philippe et al. 2011](#) for a recent discussion), and allow rooting even when no appropriate outgroup is available. A recent work ([Alanzi and Degnan 2017](#)) pursued this through approximate Bayesian computation, with split distributions used as one measure to estimate a posterior distribution of rooted species trees. Since the method by which we show the species tree root is identifiable depends only on linear relationships between split probabilities, other approaches taking advantage of this simple form may also be possible.

After setting notation in Sect. 2, we begin our study of split probabilities under the multispecies coalescent in Sect. 3 with some basic observations. These include an analysis of the behavior of greedy split consensus from gene tree splits, concluding that it is not a statistically consistent method of species tree inference even on trees with as few as 5 taxa. Mathematical arguments for this appear in Appendix A section.

Since split probabilities are complicated expressions that are difficult to compute for trees with more than 6 taxa, in Sect. 4 we turn our attention to relationships between such split probabilities—that is, rather than focus on *explicit* formulas for

them, we look for *implicit* formulas they must satisfy. Our methods thus are mathematically the same as those used for studying pattern probabilities under sequence evolution models through *phylogenetic invariants*, so we adopt the same terminology of referring to equalities as invariants. Our previous work (Allman et al. 2016) on relationships between the split probabilities and the NJ_{st}/U-STAR/ASTRID inference method quickly leads to a number of linear invariants and inequalities the split probabilities must satisfy, tied to the quartets displayed on the species tree. These depend only on the unrooted species tree, and thus give no information on its root. However, building on results in Allman et al. (2011a), we then find additional split invariants that depend on the clades displayed on the species tree, which thus give some information about the root location. While our theoretical work gives only linear invariants, independent invariants of higher degree also exist. Unfortunately a computational determination of them was successful only for 5-taxon trees, and their structure remains unclear.

In Sect. 5 we build on the results on linear invariants from Sect. 4, to prove a main result: the collection of split probabilities under the multispecies coalescent model determines the species tree topology, including the root location (with one exception). This *identifiability* result, Theorem 3, holds generically, i.e., for all edge lengths on the species tree not in a set of measure zero. For most trees testing whether the invariants found in the previous section vanish is sufficient for locating the root; however, for certain trees these tests leave several possibilities for the branching pattern near the root of the tree. Motivated by known invariants, we formulate some linear inequalities that resolve these ambiguities in all cases, except for a particular unrooted 6-taxon tree shape. Establishing that these inequalities hold is accomplished by a laborious technical argument, which is relegated to Appendix B section.

2 Notation

Let \mathcal{X} be a finite set of taxa, whose elements are denoted by lower case letters a, b, c, \dots , etc. For any specific gene, we denote a single sample from each taxon by the corresponding upper case letter A, B, C, \dots , etc. If $\mathcal{A} \subseteq \mathcal{X}$ is a subset of taxa, the corresponding subset of genes is $\mathcal{A}_g \subseteq \mathcal{X}_g$.

By a species tree $\sigma = (\psi, \lambda)$ on \mathcal{X} we mean a rooted topological phylogenetic tree ψ , with leaves bijectively labeled by \mathcal{X} , together with an assignment of edge weights λ to its internal edges. These edge weights are specified in coalescent units, so that the multispecies coalescent model on σ leads to a probability distribution on rooted gene trees with leaves labeled by \mathcal{X}_g (for more on the multispecies coalescent model as we use it, see Allman et al. 2011b). Since we limit ourselves to the situation where one individual is sampled per taxon, no coalescent events can occur in pendant edges of a species tree, so the lengths of those edges are inconsequential and omitted from our notation (if more than one individual is sampled per taxon, one can create an “extended species tree” as in Allman et al. (2011a) by grafting several pendant edges of unspecified length to the leaf labeled by that taxon, and assigning a length to the formerly pendant edge, to again be in the framework set here).

The gene trees sampled from the coalescent are rooted metric binary phylogenetic trees on \mathcal{X}_g , though marginalization over edge lengths and root locations induces a distribution on unrooted topological binary gene trees. Unrooted topological gene trees will be denoted by T , and the probability of an unrooted topological gene tree under the multispecies coalescent on σ is denoted by $\mathbb{P}_\sigma(T)$, or simply $\mathbb{P}(T)$ when σ is clear from context.

A *split* of a set of taxa \mathcal{X} is a bipartition $\mathcal{A} \sqcup \mathcal{B}$ into nonempty subsets, denoted $\mathcal{A}|\mathcal{B} = \mathcal{B}|\mathcal{A} = Sp(\mathcal{A}) = Sp(\mathcal{B})$. If σ is a species tree on \mathcal{X} then by a split *on* σ we mean a split of \mathcal{X} formed by deleting a single edge of ψ and grouping taxa according to the connected components of the resulting graph. We similarly refer to splits of \mathcal{X}_g , and splits of \mathcal{X}_g on specific gene trees T . For small sets of taxa, it will often be convenient to use juxtaposition of elements to represent sets, rather than standard set notation. Thus $ac = \{a, c\}$ and $Sp(ac) = Sp(\{a, c\})$.

In a *trivial* split, one of the partition blocks is a singleton set. Trivial splits for taxa \mathcal{X} appear on every phylogenetic tree on \mathcal{X} . For $\mathcal{A} \subset \mathcal{X}$ we will denote the complementary set of \mathcal{A} by $\overline{\mathcal{A}} = \mathcal{X} \setminus \mathcal{A}$, so that $\mathcal{A}|\overline{\mathcal{A}}$ is a split when $\emptyset \neq \mathcal{A} \subsetneq \mathcal{X}$.

For a species tree σ on \mathcal{X} , by the probability of a split $\mathcal{A}|\mathcal{B}$ of \mathcal{X} under the multispecies coalescent we mean

$$\mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) = \sum_T \mathbb{P}_\sigma(T) \delta_{\mathcal{A}|\mathcal{B}}(T) \tag{1}$$

where $\delta_{\mathcal{A}|\mathcal{B}}(T)$ is 1 if $\mathcal{A}_g|\mathcal{B}_g$ is a split on T , and 0 otherwise, and the sum runs over all binary unrooted topological phylogenetic trees on \mathcal{X}_g . Thus the probability of a split is the probability that an observation of a gene tree displays the corresponding split. Note that trivial splits have probability 1 for every species tree, since they are on every binary gene tree.

We will also need to refer to clades and quartets of taxa. A *clade* is simply a subset $\mathcal{A} \subseteq \mathcal{X}$. A clade \mathcal{A} is *on* the species tree σ if it equals the set of all leaf descendants of some node in the tree. A *quartet* is a 4-element subset of \mathcal{X} partitioned into 2-element sets, denoted as $ab|cd$, with $a, b, c, d \in \mathcal{X}$. A quartet $ab|cd$ is *on* σ if the unrooted tree with leaves labeled a, b, c, d induced from σ has an edge separating a, b from c, d .

Finally, given a rooted topological tree on \mathcal{X} , and a subset $\mathcal{Y} \subseteq \mathcal{X}$, we use $\text{MRCA}(\mathcal{Y})$ to denote the *most recent common ancestor* of \mathcal{Y} , i.e., the least ancestral node in the tree which is ancestral to all elements of \mathcal{Y} . For a node v in the tree, we use $\text{desc}_\mathcal{X}(v)$ to denote the set of taxa descended from v . Thus a clade $\mathcal{Y} \subseteq \mathcal{X}$ is on the tree if and only if $\mathcal{Y} = \text{desc}_\mathcal{X}(\text{MRCA}(\mathcal{Y}))$.

3 Basic Observations

While in principle it is straightforward to compute the probabilities of gene tree splits for a fixed species tree, in practice the work required can be formidable. For an n -taxon species tree, using the definition in Eq. (1), one first must compute probabilities of each of the $(2n - 3)!! = 1 \cdot 3 \dots (2n - 3)$ unrooted topological gene trees. This

can be accomplished by work of [Degnan and Salter \(2005\)](#) or [Wu \(2012\)](#) in finding probabilities of all rooted topological gene trees, and then marginalizing over the root locations. For a given split one must still sum over all unrooted gene trees displaying that split. If the split has blocks of size k and $n-k$, then there are $(2k-3)!!(2n-2k-3)!!$ such unrooted trees.

Using this approach, we computed split probabilities for all species trees on 6 or fewer taxa for use in computations discussed in later sections, but went no further. Indeed, this approach does not seem to be tractable except for small species trees. On the other hand, since the U-STAR inference methods implemented in ASTRID are based on split frequencies ([Vachaspati and Warnow 2015](#); [Allman et al. 2016](#)) and perform well on large data sets, theoretical study of these probabilities is still strongly warranted.

As a first step, analogous to Proposition 1 of [Allman et al. \(2011a\)](#) for clade probabilities, we have the following.

Lemma 1 *If $|\mathcal{X}| = n$, then the sum of the non-trivial split probabilities is $n - 3$.*

Proof First considering all splits of \mathcal{X} , including trivial ones,

$$\begin{aligned} \sum_{\mathcal{A}|\mathcal{B}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) &= \sum_{\mathcal{A}|\mathcal{B}} \sum_T \mathbb{P}_\sigma(T) \delta_{\mathcal{A}|\mathcal{B}}(T) = \sum_T \mathbb{P}_\sigma(T) \sum_{\mathcal{A}|\mathcal{B}} \delta_{\mathcal{A}|\mathcal{B}}(T) \\ &= \sum_T \mathbb{P}_\sigma(T) (2n - 3) = 2n - 3. \end{aligned}$$

Since the n trivial splits of \mathcal{X} all have probability 1, removing them from the sum gives the claim. □

Another analog of a result for clade probabilities, Theorem 3 of [Allman et al. \(2011a\)](#), is the content of the next Proposition. T. Warnow first asked if this might hold, and C. Ané independently provided a proof ([Ané 2016](#)).

Proposition 1 *Let $\epsilon \geq 0$ be fixed, and let σ be a binary species tree on \mathcal{X} with internal edge lengths $\lambda_i > \epsilon$. Consider any split $\mathcal{A}|\mathcal{B}$ of \mathcal{X} .*

Then under the multispecies coalescent model if

$$\mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) \geq (1/3) \exp(-\epsilon)$$

then $\mathcal{A}|\mathcal{B}$ is displayed on σ .

Furthermore, if $(1/3) \exp(-\epsilon)$ is replaced with any smaller number, this statement is no longer true: For any $\alpha < (1/3) \exp(-\epsilon)$, there exists a species tree σ with branch lengths $\lambda_i > \epsilon$ and a split $\mathcal{A}|\mathcal{B}$ of \mathcal{X} not displayed on σ with $\mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) > \alpha$.

Proof The first statement holds for trivial splits, since they have probability 1 and are displayed on every binary σ .

Now consider a non-trivial split $\mathcal{A}|\mathcal{B}$ not displayed on σ . Then there exist $a_1, a_2 \in \mathcal{A}$, $b_1, b_2 \in \mathcal{B}$ so the quartet $a_1 a_2 | b_1 b_2$ is not displayed on σ . Thus, by ([Allman](#)

et al. 2011b, Sect. 4.1) the probability that an unrooted gene tree displays the quartet $A_1A_2|B_1B_2$ is

$$\mathbb{P}_\sigma(A_1A_2|B_1B_2) = (1/3) \exp(-\ell) < (1/3) \exp(-\epsilon)$$

where $\ell > \epsilon$ is the sum of the lengths of all branches in σ that form the central edge in the induced quartet tree on a_1, a_2, b_1, b_2 . But since displaying the split $\mathcal{A}_g|\mathcal{B}_g$ is a subevent of displaying $A_1A_2|B_1B_2$, this implies that if $\mathcal{A}|\mathcal{B}$ is not displayed on σ then

$$\mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) < (1/3) \exp(-\epsilon),$$

establishing the first claim.

For the second claim, we construct an example. For any non-trivial split $\mathcal{A}|\mathcal{B}$, pick $a \in \mathcal{A}$, $b \in \mathcal{B}$, and let $\mathcal{A}' = \mathcal{A} \setminus \{a\}$, $\mathcal{B}' = \mathcal{B} \setminus \{b\}$. Pick any binary rooted tree σ_1 on \mathcal{A}' , and any binary rooted tree σ_2 on \mathcal{B}' , with internal branch lengths greater than ϵ , and consider the tree

$$\sigma = (((a, b) : \lambda_1, \sigma_1 : \lambda_2) : \lambda_3, \sigma_2 : \lambda_4).$$

Note $\mathcal{A}|\mathcal{B}$ is not a split on this tree. However, if λ_2, λ_4 are sufficiently large, then the lineages for each of the groups \mathcal{A}'_g and \mathcal{B}'_g are almost certain to have coalesced within the branches of those lengths. But then using results on the probabilities of unrooted topological gene trees for 4-taxon species trees from Allman et al. (2011b), we deduce that the probability of a gene tree displaying the split $\mathcal{A}_g|\mathcal{B}_g$ can be made arbitrarily close to $(1/3) \exp(-\lambda_1)$. If $\alpha < (1/3) \exp(-\epsilon)$, there is a choice of $\lambda_1 > \epsilon$ so that $\alpha < (1/3) \exp(-\lambda_1)$. Thus we can ensure $\mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) > \alpha$. \square

Setting $\epsilon = 0$ in the previous proposition yields the following.

Corollary 1 *Suppose σ is a binary species tree on \mathcal{X} , with positive edge lengths, and $\mathcal{A}|\mathcal{B}$ a split of \mathcal{X} . Then under the multispecies coalescent model if*

$$\mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) \geq 1/3$$

then $\mathcal{A}|\mathcal{B}$ is a split on σ .

This proposition has implications for a greedy split consensus approach to inferring splits in a species tree. Recall that in this method, one first orders splits observed in a gene tree sample by decreasing frequency, arbitrarily (or randomly) breaking ties if necessary. Proceeding in order down the list, splits are accepted if they are compatible with all previously accepted ones. For a large sample of gene trees from the multispecies coalescent, a fully resolved unrooted tree is likely to be returned, since all splits have positive probability. The above corollary implies that if one only allows the acceptance of splits of frequency greater than 1/3, then this method will not be misleading; as the size of the gene tree sample grows, the probability of accepting

only splits on the species tree goes to 1. While a tree displaying the accepted splits may not be fully resolved, one can have confidence in the splits that are displayed.

To show that accepting splits below a frequency 1/3 cutoff in greedy split consensus would not lead to consistent species tree inference, we investigate 5-taxon trees in more detail. Up to permutation of taxon labels, there are three species trees to consider:

$$\begin{aligned} \text{balanced tree} & \quad \sigma_{\text{bal}} = (((a, b):x, c):y, (d, e):z) \\ \text{pseudocaterpillar tree} & \quad \sigma_{\text{ps}} = (((a, b):x, (d, e):y):z, c) \\ \text{caterpillar tree} & \quad \sigma_{\text{cat}} = (((a, b):x, c):y, d):z, e) \end{aligned}$$

Although we use the same variables x, y, z to denote the three internal edge lengths in each tree, note that these have no relationship across the species trees. All split probabilities can be expressed as polynomials in the transformed edge lengths

$$X = \exp(-x), Y = \exp(-y), Z = \exp(-z).$$

Note that with this transformation, values of X close to 1 correspond to small branch lengths x , and values of X close to 0 correspond to large branch lengths x .

The following two propositions are proved in Appendix A section.

Proposition 2 *For the 5-taxon balanced and pseudocaterpillar species trees, $\sigma = \sigma_{\text{bal}}, \sigma_{\text{ps}}$, with positive branch lengths,*

$$\mathbb{P}_{\sigma}(Sp(ab)), \mathbb{P}_{\sigma}(Sp(de)) > \mathbb{P}_{\sigma}(S)$$

for each of the eight other non-trivial splits S , so the splits displayed on the species tree have the highest probability of appearing on gene trees.

When restricted to these species trees, as the sample size goes to infinity greedy split consensus infers the correct unrooted species tree topology with probability approaching 1.

Proposition 3 *For the 5-taxon caterpillar species tree $\sigma = \sigma_{\text{cat}}$ with positive branch lengths, $\mathbb{P}_{\sigma}(Sp(ab)) > \mathbb{P}_{\sigma}(S)$ for all non-trivial splits $S \neq Sp(de)$, and $\mathbb{P}_{\sigma}(Sp(de)) > \mathbb{P}_{\sigma}(Sp(ce))$.*

If $\mathbb{P}_{\sigma}(Sp(de)) > \mathbb{P}_{\sigma}(Sp(cd))$ for such a species tree, as the sample size goes to infinity greedy split consensus infers the correct unrooted species tree topology with probability approaching 1.

However, if $\mathbb{P}_{\sigma}(Sp(de)) < \mathbb{P}_{\sigma}(Sp(cd))$, it infers the incorrect unrooted species tree topology $((a, b), e, (c, d))$ with probability approaching 1. The parameter region in which this occurs is

$$18 + XY^3Z^6 + 2XY^3 - 3XY - 18Y < 0. \tag{2}$$

Figure 1 shows the surface dividing the regions of parameter space on which greedy split consensus is misleading from that on which it is not. We refer to the region behind the surface, in which greedy consensus on splits is expected to return the incorrect species tree, as the *too-greedy zone*.

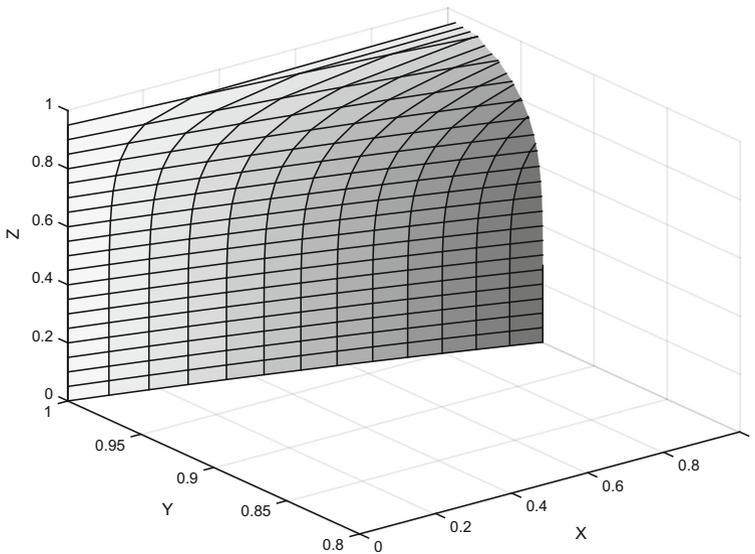


Fig. 1 The boundary of the too-greedy zone for the 5-taxon caterpillar tree σ_{cat} . Greedy consensus for split probabilities is inconsistent for all choices of parameters behind the surface, and consistent in front of the surface. For example, if $(X, Y, Z) \approx (.9, .96, .5)$, so species tree branch lengths are $(x, y, z) \approx (0.1054, 0.0408, 0.6931)$, then greedy consensus with a large number of gene trees is expected to return the incorrect tree $((a, b), e, (c, d))$

The analogous expression for the boundary of the five-taxon unrooted anomaly zone (the branch lengths for which the most likely unrooted gene tree does not match the unrooted caterpillar species tree) is Degnan (2013, Eq. (4))

$$18 + XY^3Z^6 + 2XY^3 + 9XY - 12X - 18Y < 0. \tag{3}$$

We note that if inequality (2) holds, then inequality (3) holds as well. This means that for the five-taxon caterpillar, the too-greedy zone is a subset of the unrooted anomaly zone. This relationship is also true for the rooted 4-taxon caterpillar case: the rooted too-greedy zone is a subset of the rooted anomaly zone. In both the rooted and unrooted cases for four and five taxa, respectively, when greedy consensus is misleading, it returns the anomalous gene tree. We leave it as an open question whether the too-greedy zones for larger trees are also subsets of the corresponding anomaly zones.

One can show that the minimum value of Y on the boundary surface in Fig. 1 occurs when $X = 1$, $Y \approx 0.93498735$, and $Z = 0$, so $x = 0$, $y \approx 0.06722228$, $z = \infty$. For values of y larger than this, regardless of the values of x and z , branch length parameters are outside the too-greedy zone, and greedy split consensus is expected to return the correct species tree.

Moreover, if $Z = 0$ so that z is an infinite branch length in σ_{cat} , the too-greedy zone for splits coincides exactly with the too-greedy zone for clade consensus on the 4-taxon tree $((a, b), c), d$ (Degnan et al. 2009). This is as expected, since placing

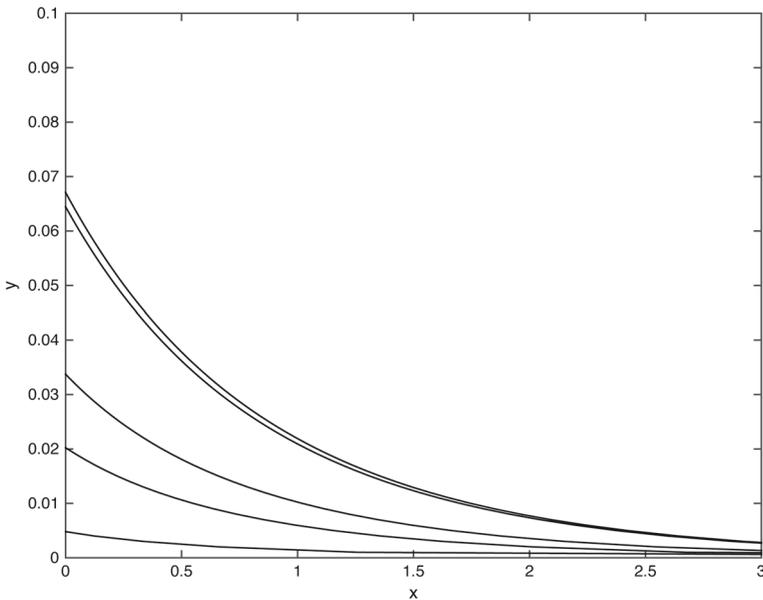


Fig. 2 For $z = 0.01, .05, .1, .5, 1$, curves giving, from bottom to top, the boundary of the too-greedy zone in the xy -plane for the 5-taxon caterpillar tree. Note x, y, z are edge lengths in coalescent units. The too-greedy zone for a value of z is the region below the curve, illustrating that the region grows with z . For $z > 1$ the curve is visually indistinguishable from that for $z = 1$

the root of the 5-taxon caterpillar species tree “at infinity” makes non-trivial splits for it correspond exactly to clades in the 4-taxon caterpillar, by viewing e as an outgroup and noting that the lineage from e must coalesce last.

As was pointed out to us (Ané 2016), the shape of the surface in Fig. 1 has an interesting consequence. As Z increases from 0 to 1, the too-greedy zone in the XY -plane becomes smaller. Equivalently, in terms of branch lengths, as z increases from 0 to ∞ , the too-greedy zone in the xy plane becomes larger, as shown in Fig. 2. Thus smaller values of z result in greedy split consensus performing well for more choices of branch lengths x and y .

If one views the taxon e as an outgroup on σ_{cat} , this means that an outgroup that is closely related to all other taxa results in better performance of greedy split consensus than one that is more distantly related. While an extremely distantly related outgroup ($z = \infty$) enables determination of the root of each gene tree, so that knowing the splits on 5-taxon gene trees is equivalent to knowing the clades on 4-taxon trees omitting the outgroup, this actually reduces the ability of greedy split consensus to determine the correct unrooted topology of the species tree. Indeed, the too-greedy zone for clade consensus on a 4-taxon caterpillar as shown in Figure 3 of Degnan et al. (2009) matches precisely that for $z = \infty$ in Fig. 2.

These comments have analogs for the unrooted anomaly zones for five taxa. In particular, the left side of inequality (3) is decreasing in z , so that increasing that branch length results in more values of x and y with (x, y, z) in the anomaly zone.

4 Linear Invariants and Inequalities for Split Probabilities

A useful concept for understanding probabilistic models in phylogenetics has been that of a *phylogenetic invariant*. An invariant of this sort is a multivariate polynomial that when evaluated at probabilities arising from the model gives 0, regardless of the particular parameter values associated with the model instance. Equivalently, it is a polynomial relationship between the probabilities that holds for all parameter values, and thus gives information about the probabilities implicitly.

A *split invariant* for a species tree topology is a polynomial in the probabilities of splits under the multispecies coalescent model that vanishes for all edge length assignments to the species tree. More completely, a split invariant associated with an n -taxon species tree topology ψ is a multivariate polynomial in $2^{n-1} - 1$ indeterminates (one for every split) which evaluates to zero at any vector of split probabilities $\mathbb{P}_\sigma(\mathcal{A}|\mathcal{B})$ arising from $\sigma = (\psi, \lambda)$, regardless of the values of λ . Since probabilities of trivial splits are always 1 under the coalescent, we can and will consider only invariants in variables for the $2^{n-1} - n - 1$ non-trivial splits.

The *trivial split invariant*, which is valid for all ψ , is

$$n - 3 - \sum_{\substack{\text{non-trivial} \\ \text{splits } \mathcal{A}|\mathcal{B}}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}). \tag{4}$$

That this evaluates to 0 when the split probabilities arise from the multispecies coalescent on some species tree is established by Lemma 1.

4.1 Linear Invariants and Inequalities for Unrooted Species Trees

In this section we explore linear relationships, both invariants and inequalities, between split probabilities that are tied to the U-STAR algorithm (Allman et al. 2016). Since U-STAR allows a distance method to be used to infer a species tree, these have a rather direct correspondence to equalities and inequalities defining tree metrics.

From equation (7) of Allman et al. (2016), we know the expected U-STAR distance on a species tree σ under the multispecies coalescent can be expressed as

$$D(a, b) = \sum_{\substack{\text{splits } \mathcal{A}|\mathcal{B} \\ \text{separating } a, b}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}),$$

where a split is said to *separate* two taxa if they lie in different sets of the bipartition. Moreover, the expected U-STAR distance is a tree metric on σ . Using this in the 4-point condition for tree metrics implies a collection of linear equalities and inequalities in split probabilities that must hold for *unrooted* species trees. That is, these equalities and inequalities hold regardless of the location of the root on the species tree.

To state the result, we will say that a split $\mathcal{A}|\mathcal{B}$ of \mathcal{X} separates two nonempty disjoint subsets $\mathcal{Y}_0, \mathcal{Y}_1 \subset \mathcal{X}$ provided $\mathcal{Y}_i \subseteq \mathcal{A}$ and $\mathcal{Y}_{1-i} \subseteq \mathcal{B}$ for one of $i = 0, 1$.

Theorem 1 Suppose σ is an n -taxon species tree on \mathcal{X} , and $a, b, c, d \in \mathcal{X}$ are any four taxa for which σ induces the quartet tree $ab|cd$. Then

$$\begin{aligned} \sum_{\substack{\text{splits } \mathcal{A}|\mathcal{B} \\ \text{separating } ac, bd}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) &= \sum_{\substack{\text{splits } \mathcal{A}|\mathcal{B} \\ \text{separating } ad, bc}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) \\ &\leq \sum_{\substack{\text{splits } \mathcal{A}|\mathcal{B} \\ \text{separating } ab, cd}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}). \end{aligned}$$

Proof If a, b, c, d are 4 taxa on a metric tree displaying the quartet $ab|cd$, then for the associated tree metric d the 4-point condition (Semple and Steel 2003) states that

$$d(a, c) + d(b, d) = d(a, d) + d(b, c) \geq d(a, b) + d(c, d).$$

The equality of this condition applied to the U-STAR expected distance gives

$$\begin{aligned} &\sum_{\substack{\text{splits } \mathcal{A}|\mathcal{B} \\ \text{separating } a, c}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) + \sum_{\substack{\text{splits } \mathcal{A}|\mathcal{B} \\ \text{separating } b, d}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) \\ &= \sum_{\substack{\text{splits } \mathcal{A}|\mathcal{B} \\ \text{separating } a, d}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}) + \sum_{\substack{\text{splits } \mathcal{A}|\mathcal{B} \\ \text{separating } b, c}} \mathbb{P}_\sigma(\mathcal{A}|\mathcal{B}). \end{aligned}$$

Any split for which all four of a, b, c, d appear in the same split set does not appear in this equation. If one of a, b, c, d is separated from the other three in a split, then that split probability occurs exactly once on each side of the equation and can be canceled. If two of a, b, c, d are separated from the others, several cases must be considered. First, if ab is separated from cd by a split, that split probability occurs in all four sums, and so can be canceled. Second, if ac is separated from bd by a split, that split probability occurs in both sums on the right, and not on the left. Third, if ad is separated from bc by a split, that split probability occurs in both sums on the left, and not on the right. Thus after all canceling and division by 2, we obtain the claimed equality.

Similar reasoning shows the 4-point inequality yields the inequality of the theorem. □

Since the invariants of Theorem 1 depend only on displayed quartets, they hold for all rooted versions of a fixed unrooted species topology.

Example 1 We apply the theorem to a species tree with unrooted topology $((a, b), c, (d, e))$. Using abbreviated notation such as $s_{ab} = \mathbb{P}(Sp(ab)) = \mathbb{P}(ab|cde)$ for the 10 non-trivial split probabilities, by Theorem 1 for each quartet on the species tree, we obtain an equality and inequality:

$$\begin{aligned} ab|cd &: s_{ad} + s_{bc} = s_{ac} + s_{bd} \leq s_{ab} + s_{cd}, \\ ab|ce &: s_{ae} + s_{bc} = s_{ac} + s_{be} \leq s_{ab} + s_{ce}, \\ ab|de &: s_{ad} + s_{be} = s_{ae} + s_{bd} \leq s_{ab} + s_{de}, \\ ac|de &: s_{ad} + s_{ce} = s_{ae} + s_{cd} \leq s_{ac} + s_{de}, \\ bc|de &: s_{bd} + s_{ce} = s_{be} + s_{cd} \leq s_{bc} + s_{de}. \end{aligned}$$

The first three equalities here span a space of dimension 2, as do the last three. The middle three are a basis for the span of them all.

One can compute all split probability invariants for the unrooted 5-taxon tree in *Singular* (Decker et al. 2016), by computing and intersecting the ideals of invariants for the 7 rooted versions of the tree. Doing so shows that the linear invariants given here together with the trivial invariant span the full space of linear invariants for the unrooted tree. There is also a quadratic invariant in a Gröbner basis for the ideal intersection,

$$(s_{ac} - s_{bc})(s_{ad} - s_{ae}) = 0,$$

which reflects the fact that for each of the rooted trees either $s_{ac} = s_{bc}$ or $s_{ad} = s_{ae}$. As will be explained in the next section, these equalities arise as cherry-swapping invariants, since each of the 7 trees either has ab or de as a 2-clade. In addition, there are 14 higher-degree invariants (not shown) in the basis for the ideal, of total degree ranging from 3 to 8.

Singular code for these and all other computations in this work is provided in the Online Resource.

Example 2 For each of the 2 unrooted shapes of binary 6-taxon trees one can similarly compute all linear split invariants. For the unrooted tree shape with 2 cherries, exemplified by $((a, b), c), d, (e, f)$, there is one additional linear split invariant, outside the span of those of Theorem 1:

$$s_{ace|bdf} - s_{acf|bde} + s_{ade|bcf} - s_{adf|bce} = 0.$$

This also can be explained by the cherry-swapping invariants of the next section, since any rooted version of this tree will have at least one of ab or ef as a 2-clade.

For the unrooted shape with 3 cherries, exemplified by $((a, b), (c, d), (e, f))$, in addition to the linear invariants of the above theorem one finds

$$s_{ace|bdf} = s_{acf|bde} = s_{ade|bcf} = s_{adf|bce}.$$

All equalities can be explained by the fact that any rooted version of the tree has at least 2 of the 2-clades ab , cd , and ef , and determining the cherry-swapping invariants these clades imply.

4.2 Linear Invariants for Rooted Species Trees

Next we investigate linear split invariants that depend on the rooted species tree. More specifically, we construct a family of such invariants associated with each non-trivial clade on the species tree. The existence of these *clade-induced split invariants*, as given in Theorem 2 below, will form the basis of arguments in Sect. 5 that the root of the species tree can be identified from split probabilities.

Theorem 2 *Let $\mathcal{A} \subset \mathcal{X}$ be a subset of taxa with $|\mathcal{A}|, |\bar{\mathcal{A}}| \geq 2$. Choose $\emptyset \neq \mathcal{C} \subsetneq \bar{\mathcal{A}}$, and distinct $a, b \in \mathcal{A}$. Let $\mathcal{A}' = \mathcal{A} \setminus \{a, b\}$.*

Then if \mathcal{A} is a clade on σ ,

$$\sum_{S \subseteq \mathcal{A}'} \mathbb{P}_\sigma(Sp(S \cup \{a\} \cup \mathcal{C})) - \sum_{S \subseteq \mathcal{A}'} \mathbb{P}_\sigma(Sp(S \cup \{b\} \cup \mathcal{C})) = 0. \tag{5}$$

We note that this theorem applies to any species tree, even if non-binary. Moreover, since a non-binary species tree σ can be thought of as any of its binary resolutions with length 0 assigned to any introduced edges, the clade probabilities arising from such a σ will satisfy the polynomials of the theorem for every binary resolution. Thus in the statement of the theorem the phrase “if \mathcal{A} is a clade on σ ” can be replaced with “if \mathcal{A} is a clade on a binary resolution of σ .”

Proof of Theorem 2 We derive this result in part using ideas developed for the construction of invariants for clade probabilities in Allman et al. (2011a).

Let $Cl(\mathcal{A})$ represent the event that \mathcal{A}_g is a clade on an observed gene tree. Then note that

$$\mathbb{P}_\sigma(Sp(\mathcal{A})) = \mathbb{P}_\sigma(Cl(\mathcal{A})) + \mathbb{P}_\sigma(Cl(\bar{\mathcal{A}})) - \mathbb{P}_\sigma(Cl(\mathcal{A}), Cl(\bar{\mathcal{A}})),$$

where $\mathbb{P}_\sigma(Cl(\mathcal{A}), Cl(\bar{\mathcal{A}}))$ denotes the probability of the two clades simultaneously being displayed on a gene tree. Thus Eq. (5) will follow from establishing the three equalities:

$$\sum_{S \subseteq \mathcal{A}'} \mathbb{P}_\sigma(Cl(S \cup \{a\} \cup \mathcal{C})) - \sum_{S \subseteq \mathcal{A}'} \mathbb{P}_\sigma(Cl(S \cup \{b\} \cup \mathcal{C})) = 0, \tag{6}$$

$$\sum_{S \subseteq \mathcal{A}'} \mathbb{P}_\sigma(Cl(\overline{S \cup \{a\} \cup \mathcal{C}})) - \sum_{S \subseteq \mathcal{A}'} \mathbb{P}_\sigma(Cl(\overline{S \cup \{b\} \cup \mathcal{C}})) = 0, \tag{7}$$

$$\begin{aligned} & \sum_{S \subseteq \mathcal{A}'} \mathbb{P}_\sigma(Cl(S \cup \{a\} \cup \mathcal{C}), Cl(\overline{S \cup \{a\} \cup \mathcal{C}})) \\ & - \sum_{S \subseteq \mathcal{A}'} \mathbb{P}_\sigma(Cl(S \cup \{b\} \cup \mathcal{C}), Cl(\overline{S \cup \{b\} \cup \mathcal{C}})) = 0, \end{aligned} \tag{8}$$

That Eq. (6) holds is Theorem 6 of Allman et al. (2011a). To establish Eq. (7), for any $\mathcal{S} \subseteq \mathcal{A}'$, let $\tilde{\mathcal{S}} = \mathcal{A}' \setminus \mathcal{S}$, and $\tilde{\mathcal{C}} = (\overline{\mathcal{A}}) \setminus \mathcal{C}$. With this notation

$$\overline{\mathcal{S} \cup \{a\} \cup \mathcal{C}} = \tilde{\mathcal{S}} \cup \{b\} \cup \tilde{\mathcal{C}},$$

where $\tilde{\mathcal{S}} \subseteq \mathcal{A}'$ and $\emptyset \neq \tilde{\mathcal{C}} \subsetneq \mathcal{X} \setminus \mathcal{A}$. We thus see Eq. (7) is another instance of the Eq. (6) (it is essential here that \mathcal{C} be a proper subset of $\overline{\mathcal{A}}$, so that $\tilde{\mathcal{C}}$ is nonempty; this is why \mathcal{A} must exclude at least 2 taxa).

Establishing Eq. (8) requires more argument. Using the above notation, it can be restated as

$$\sum_{\mathcal{S} \subseteq \mathcal{A}'} \mathbb{P}_\sigma \left(Cl(\mathcal{S} \cup \{a\} \cup \mathcal{C}), Cl(\tilde{\mathcal{S}} \cup \{b\} \cup \tilde{\mathcal{C}}) \right) - \sum_{\mathcal{S} \subseteq \mathcal{A}'} \mathbb{P}_\sigma \left(Cl(\mathcal{S} \cup \{b\} \cup \mathcal{C}), Cl(\tilde{\mathcal{S}} \cup \{a\} \cup \tilde{\mathcal{C}}) \right) = 0. \tag{9}$$

We establish Eq. (9) by showing a version of it conditioned on the partition of \mathcal{A} corresponding to lineages present at the MRCA(\mathcal{A}) in the species tree. Now consider any realization of the remainder of the coalescent process (i.e., on σ with edges below MRCA(\mathcal{A}) removed) which displays the clades $\mathcal{S}_g \cup \{A\} \cup \mathcal{C}_g$ and $\tilde{\mathcal{S}}_g \cup \{B\} \cup \tilde{\mathcal{C}}_g$. First note that A and B are in different partition sets at MRCA(\mathcal{A}), or these clades could not be formed. But then this realization has the same probability as one where the lineages for B and A are exchanged. This exchange leads to a gene tree displaying clades $\hat{\mathcal{S}}_g \cup \{B\} \cup \mathcal{C}_g$ and $\tilde{\hat{\mathcal{S}}}_g \cup \{A\} \cup \tilde{\mathcal{C}}_g$. Here $\hat{\mathcal{S}}$ is still a subset of \mathcal{A}' , but generally differs from \mathcal{S} because some elements of \mathcal{S} are in the partition sets with A and B at MRCA(\mathcal{A}). Conditioned on the partition, this establishes a bijective correspondence between equiprobable realizations of the coalescent contributing to the two sums in the equality, and thus the conditioned equality holds. Summing over all possible partitions of \mathcal{A} , weighted by their probabilities, gives the unconditioned Eq. (9). \square

Example 3 Here we explicitly give the clade-induced split invariants of Theorem 2 for 5-taxon species trees, and compare them to the full set of invariants for such trees.

Caterpillar tree ((($(a, b), c), d), e)$: Consider the 2-clade $\mathcal{A} = \{a, b\}$, so $\mathcal{A}' = \emptyset$. With $\mathcal{C} = \{c\}, \{d\}$, and $\{e\}$, using the notation introduced in Example 1, Eq. (5) yields

$$s_{ac} - s_{bc} = 0, \quad s_{ad} - s_{bd} = 0, \quad s_{ae} - s_{be} = 0.$$

We refer to these as *cherry-swapping split invariants*, since they hold because a, b form a 2-clade, and their lineages are thus exchangeable under the coalescent model. Two-element choices of \mathcal{C} give the same equalities, up to sign, as the ones already listed.

For the clade $\{a, b, c\}$ using a and b as the two singleton taxa, we find

$$s_{ad} + s_{acd} - s_{bd} - s_{bcd} = 0, \quad s_{ae} + s_{ace} - s_{be} - s_{bce} = 0,$$

both of which were already implied by the cherry-swapping invariants. But using a and c as the singleton taxa, we get

$$s_{ad} + s_{abd} - s_{cd} - s_{bcd} = 0, \quad s_{ae} + s_{abe} - s_{ce} - s_{bce} = 0. \tag{10}$$

However, equations (10) differ only by sign. An additional invariant, with a and b interchanged from equations (10), is obtained by choosing b, c as the singletons. Alternately, it follows from equations (10) using the ‘‘cherry-swapping’’ exchangeability of lineages for a and b .

A computation with `Singular` shows these together with the trivial invariant span the space of all linear split invariants for this rooted tree. Note that the tree $((((a, b), c), e), d)$ would produce exactly the same set of invariants, so by evaluating linear invariants one would not be able to identify the root of such a caterpillar tree. This is an instance of Corollary 4 (a) below.

Balanced tree $((a, b), c), (d, e)$: This tree has all the clades excluding at least 2 taxa that the caterpillar does, but in addition displays $\{d, e\}$. Thus all the invariants listed for the caterpillar hold, as well as additional ones from this cherry. For instance,

$$s_{ad} - s_{ae} = 0.$$

These span the space of linear split invariants for this tree, as computed by `Singular`.

Pseudocaterpillar $((a, b), (d, e)), c$: This tree has only two clades that exclude at least two taxa, namely $\{a, b\}$ and $\{d, e\}$, yielding the invariants

$$\begin{aligned} s_{ac} - s_{bc} = 0, \quad s_{ad} - s_{bd} = 0, \quad s_{ae} - s_{be} = 0, \\ s_{ad} - s_{ae} = 0, \quad s_{bd} - s_{be} = 0, \quad s_{cd} - s_{ce} = 0. \end{aligned}$$

Note that of these six invariants, the middle four are linearly dependent, with a 3-dimensional span. The span of all six invariants is 5-dimensional. All can be explained by cherry-swapping.

For the 5-taxon pseudocaterpillar, a computation with `Singular` produces a 7-dimensional space of linear invariants (including the trivial linear invariant), with

$$s_{ab} + 2s_{bc} - 2s_{ce} - s_{de} = 0. \tag{11}$$

as the additional generator. Note that neither of Theorems 1 and 2 give equalities involving $\mathbb{P}_\sigma(Sp(ab))$ or $\mathbb{P}_\sigma(Sp(de))$ for this tree, so they cannot provide an explanation for this invariant. One will be given in Proposition 5 below.

For the 5-taxon trees, our `Singular` computations found a Gröbner basis for all invariants in split probabilities. For the pseudocaterpillar, there were only linear polynomials, indicating that those given above imply all higher-degree invariants. For the caterpillar and balanced trees this is not the case, though we have no theoretical understanding of the form of the higher-degree invariants. In particular, the balanced tree has an additional quadratic invariant under our choice of Gröbner basis, and the pseudocaterpillar has one quadratic and three cubic invariants.

Table 1 Dimensions of the vector spaces of non-trivial linear invariants for 6-taxon species trees

These are defined on a space of $\binom{2^5 - 6 - 1}{1} = 25$ non-trivial split probabilities

Species tree	Dim
$(((((a, b), c), d), e), f)$	11
$(((((a, b), (c, d)), e), f)$	13
$(((((a, b), c), (d, e)), f)$	14
$(((((a, b), c), d), (e, f))$	14
$((((a, b), (c, d)), (e, f))$	15
$((((a, b), c), (d, (e, f)))$	15

Example 4 For each of the six shapes for 6-taxon species trees, we computed invariants for the split probabilities using Singular. In order to make the computations terminate, we limited the degree to 8. For all shapes we found that the clade-induced split invariants given by Theorem 2 spanned the space of linear invariants; that is, there were no “extra” linear invariants such as that found for the 5-taxon pseudocaterpillar species trees. Table 1 shows the dimension of the space of non-trivial linear invariants, which depends upon the rooted topology.

For the 6-taxon trees, we found no nonlinear invariants of degree less than our bound. However, a dimension argument indicates higher-degree invariants must exist: There are 25 non-trivial split probabilities. After accounting for the trivial split invariant of Eq. (4), the space the linear invariants cut out is of dimension 24 minus that shown in Table 1. Since the variety of split probabilities has dimension at most 4 (the number of internal edges on the species tree), higher-degree invariants must exist to further restrict the space cut out by the linear ones.

5 Identifiability of the Rooted Species Tree from Split Probabilities

We first show that the clade-induced split invariants of the last section, which vanish if a species tree has a particular clade, do not vanish for generic parameter choices if the species tree lacks that clade (with some exceptions). This is the main ingredient in obtaining Theorem 4, that the rooted topological species tree is recoverable from split probabilities in most circumstances.

The following lemma is key to our argument.

Lemma 2 *Let ψ be a binary rooted topological species tree on \mathcal{X} , and $\mathcal{X} = \mathcal{A} \sqcup \mathcal{D}$ a disjoint union of subsets with $|\mathcal{A}|, |\mathcal{D}| \geq 2$. Suppose*

1. \mathcal{A} is not a clade on ψ ,
2. \mathcal{D} is not a 2-clade on ψ .

Then there exists some $\emptyset \neq \mathcal{C} \subsetneq \mathcal{D}$, $a, b \in \mathcal{A}$, and some choice of edge lengths λ on ψ such that the clade-induced split invariant of Theorem 2, Eq. (5) does not vanish on the split probabilities arising under the multispecies coalescent model on $\sigma = (\psi, \lambda)$.

Proof We consider two cases, according to whether or not \mathcal{D} is a clade displayed on ψ .

First suppose \mathcal{D} is not a clade on ψ . Pick a minimal clade displayed on ψ that contains at least one element of \mathcal{A} and at least one element of \mathcal{D} , and let v be its MRCA. Let w_1 and w_2 be the children of v . Note that the minimality of the clade implies one of the w_i , say w_1 , has as its leaf descendants only elements of \mathcal{A} , and the other, say w_2 , has as its leaf descendants only elements of \mathcal{D} . Also observe that $\mathcal{A} \setminus \text{desc}_{\mathcal{X}}(v)$ is nonempty, since otherwise the leaf descendants of w_1 would have to be all of \mathcal{A} , contradicting that \mathcal{A} is not a clade on the tree. Similarly, $\mathcal{D} \setminus \text{desc}_{\mathcal{X}}(v)$ is nonempty. These statements furthermore imply v is not the root of the tree, so it has a parent u .

Choose $a \in \text{desc}_{\mathcal{X}}(w_1) \subsetneq \mathcal{A}$, $b \in \mathcal{A} \setminus \text{desc}_{\mathcal{X}}(v)$, and $\emptyset \neq \mathcal{C} = \text{desc}_{\mathcal{X}}(w_2) \subsetneq \mathcal{D}$. Let all edge lengths on ψ below v have positive length $x \approx 0$, edge (u, v) have length $y \gg 0$, and the remaining edges have any finite positive length. Then a gene tree arising from the coalescent model will have probability ≈ 0 of displaying $Sp(\mathcal{S} \cup \{b\} \cup \mathcal{C})$ for $\mathcal{S} \subseteq \mathcal{A}$, since any displayed split of non-negligible probability with $\{B\} \cup \mathcal{C}_g$ in a partition set will with probability ≈ 1 contain all of $\text{desc}_{\mathcal{X}}(v)$, and hence A . Thus all negative terms in Eq. (5) are negligible. On the other hand, there is a positive term in that equation for $\mathbb{P}(Sp(\text{desc}_{\mathcal{X}}(v)))$, which has value ≈ 1 . Thus Eq. (5) does not hold.

Next we consider the case when \mathcal{D} is a clade displayed on ψ , but, by condition (2), \mathcal{D} has at least 3 elements.

We first consider a particular form for ψ , and will then reduce the general tree to this form. To this end, suppose $\mathcal{D} = \{d_1, d_2, d_3\}$ and ψ is the rooted caterpillar tree

$$(((\dots(((d_1, d_2), d_3), a), c_1) \dots), c_n), b)$$

with at least 5 taxa, $\mathcal{A} = \{a, b, c_1, \dots, c_n\}$ with $n \geq 0$, a, b chosen as shown. Let $w = \text{MRCA}(\mathcal{D})$, and v its parent, so v is also the parent of $a \in \mathcal{A}$. Let $\mathcal{C} = \{d_3\}$. Choose all internal edge lengths of ψ to be $x \gg 0$, except for those below v which we choose to be $y \approx 0$. Then all rooted gene trees realizable with non-negligible probability will be formed by $\mathcal{D}_g \cup \{A\}$ coalescing into some rooted gene tree in the branch above v , with this subtree then joining to the remaining taxa in \mathcal{A} in a tree that otherwise exactly matches the caterpillar structure of ψ . Thus the event $Sp(\mathcal{S} \cup \{a\} \cup \mathcal{C})$ is non-negligibly realizable only for $\mathcal{S} = \emptyset$, by gene trees with the rooted subtree on $\mathcal{D}_g \cup \{A\}$ having $\{D_3, A\}$ as a clade. But the probability of this 2-clade forming is $\approx 4/18 = 2/9$, since of the 18 ranked rooted trees on 4 taxa, four have any given 2-clade. On the other hand, the event $Sp(\mathcal{S} \cup \{b\} \cup \mathcal{C})$ is non-negligibly realizable only for $\mathcal{S} = \{c_1, \dots, c_n\}$, by gene trees where the 4-taxon rooted subtree on $\mathcal{D}_g \cup \{A\}$ has D_3 as an outgroup. Such gene trees occur with probability $3/18 = 1/6$. Thus the invariant of Eq. (5) evaluates to $\approx 2/9 - 1/6$, and is thus not zero.

Now for the general case, in which \mathcal{D} is a clade with 3 or more elements, by picking some internal edges of ψ within the subtree on \mathcal{D} to have lengths $\gg 0$ and ≈ 0 we can ensure that with probability ≈ 1 that \mathcal{D} coalesces into exactly 3 lineages by $\text{MRCA}(\mathcal{D})$. Similarly by picking edge lengths $\gg 0$ for those edges leading off of the path between $\text{MRCA}(\mathcal{D})$ and the root of ψ to groups of elements in \mathcal{A} , we can ensure with probability ≈ 1 that these groups have coalesced before reaching that path. Then the argument above for the caterpillar tree applies with lineages for groups of taxa replacing the individual ones. \square

That condition (2) of the above lemma is necessary is shown by the following.

Proposition 4 *Let ψ be a species tree topology on \mathcal{X} , and $\mathcal{X} = \mathcal{A} \sqcup \mathcal{D}$ a disjoint union of subsets with $|\mathcal{D}| = 2$. Then if \mathcal{D} is a clade on ψ , the polynomials defined for \mathcal{A} by Eq. (5) in Theorem 2 all vanish, regardless of whether \mathcal{A} is a clade on ψ .*

Proof Since $\mathcal{D} = \{d_1, d_2\}$, the clade-induced split invariants for \mathcal{A} in Eq. (5) require that \mathcal{C} be a singleton set, which we may assume is $\mathcal{C} = \{d_1\}$. Since \mathcal{D} is a 2-clade, by exchangeability of lineages under the coalescent implies

$$\begin{aligned} \mathbb{P}_\sigma(Sp(\mathcal{S} \cup \{a\} \cup \{d_1\})) &= \mathbb{P}_\sigma(Sp(\mathcal{S} \cup \{a\} \cup \{d_2\})) \\ &= \mathbb{P}_\sigma(Sp(\tilde{\mathcal{S}} \cup \{b\} \cup \{d_1\})), \end{aligned}$$

where the last equality is obtained by taking the complementary split set. Thus Eq. (5) holds, since terms cancel in pairs. \square

From Theorem 2, we obtain the following.

Corollary 2 *Let ψ be a rooted binary species tree topology on at least 5 taxa \mathcal{X} , where $\mathcal{X} = \mathcal{A} \sqcup \mathcal{D}$ is a disjoint union of subsets with $|\mathcal{A}|, |\mathcal{D}| \geq 2$. If \mathcal{A} is not a clade on ψ and \mathcal{D} is not a 2-clade, then for generic choices of internal edge lengths λ (i.e., all except those in some set of measure zero) there exists some $\emptyset \neq \mathcal{C} \subsetneq \mathcal{D}$, $a, b \in \mathcal{A}$, such that the corresponding clade-induced split invariant of Eq. (5) does not vanish on the split probabilities arising under the multispecies coalescent on $\sigma = (\psi, \lambda)$.*

Proof The non-trivial split probabilities arising from the coalescent on σ can be expressed as polynomials in the $\exp(-\lambda_i)$. We can thus view the set of all vectors of split probabilities as the image of $(0, 1)^{n-2}$ under a polynomial map, which is therefore a semi-algebraic set. By Lemma 2, there is an invariant which does not vanish at some point in this set, so the composition of the invariant with the polynomial map is not identically zero on $(0, 1)^{n-2}$. Since this composition is a polynomial, its non-vanishing at a single point implies the set where it vanishes has measure zero in $(0, 1)^{n-2}$. Mapping this set to interior edge lengths by $x = -\log(X)$ shows the set of edge lengths for which the invariant vanishes has measure zero. \square

Corollary 3 *Let ψ be a rooted binary species tree topology on a set \mathcal{X} of at least 5 taxa. For generic edge lengths λ , all clades on ψ excluding at least three taxa can be identified by evaluating clade-induced split invariants on the probabilities of splits under the multispecies coalescent on $\sigma = (\psi, \lambda)$.*

Clades on ψ excluding exactly two taxa can similarly be identified if their complement is not a 2-clade.

Proof For any subset of $\mathcal{A} \subsetneq \mathcal{X}$ excluding at least three taxa, if we find any invariant given by Theorem 2 that fails to vanish on the split probabilities for $\sigma = (\psi, \lambda)$, then \mathcal{A} is not a clade on ψ . If all such invariants vanish, then by Corollary 2, either \mathcal{A} is a clade on ψ , or λ lies in a set of measure zero (which is dependent on $\mathcal{A}, \mathcal{C}, a$, and b used in defining the invariant).

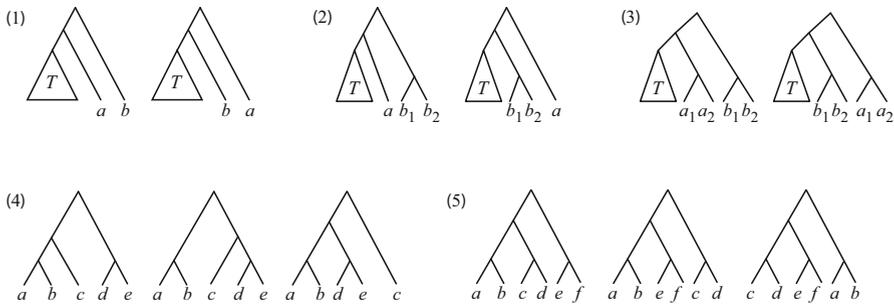


Fig. 3 For generic species tree edge lengths, split invariants can be used to determine rooted species tree topologies, up to the 5 ambiguous cases shown here, as proved in Corollary 4

Thus, considering all such \mathcal{A} , we can determine all clades excluding at least three taxa, unless the edge lengths λ lie in a set of measure zero (the finite union of sets of measure zero for each clade, each of which is a finite intersection of sets of measure zero for each invariant for that clade).

Finally, suppose \mathcal{A} excludes only two taxa, with complement \mathcal{D} . Then for generic edge lengths the non-vanishing of an appropriate invariant can detect whether \mathcal{D} is a 2-clade. If it is not, then using this knowledge, the vanishing of all clade split invariants associated with \mathcal{A} will identify it as a clade. \square

We now are able to use split invariants to fully identify rooted species trees in some cases, and find only 2 or 3 possible rootings in others. Although this result will be strengthened in Theorem 3 below by also using some inequalities, equalities alone lead to the following result.

Corollary 4 *A binary rooted species tree topology can be identified from split probabilities via the clade-induced split invariants of Eq. (5) for generic edge lengths on all species trees on 5 or more taxa, except in the following cases of indeterminacy. Here T denotes a rooted subtree on 3 or more taxa, which is identifiable, and lower case letters denote other taxa.*

1. $((T, a), b), ((T, b), a)$
2. $((T, a), (b_1, b_2)), ((T, (b_1, b_2)), a)$
3. $((T, (a_1, a_2)), (b_1, b_2)), ((T, (b_1, b_2)), (a_1, a_2)),$
4. $((a, b), c), (d, e), ((a, b), (c, (d, e))), ((a, b), (d, e)), c$
5. $((a, b), (c, d)), (e, f), (((a, b), (e, f)), (c, d)), (((c, d), (e, f)), (a, b))$

The various cases enumerated in the corollary are depicted in Fig. 3.

Proof Given all split probabilities computed from a species trees with generic edge lengths, we may test every subset of \mathcal{X} omitting 3 or more taxa to see if it is a clade on ψ , using Corollary 3. We then form a list of all such clades (including trivial ones) on ψ .

If two clades on this list form a bipartition of \mathcal{X} , then we have determined all clades on ψ , hence its rooted topology.

If no pair of clades on this list partition \mathcal{X} , but we find three clades on the list that do, denote them by \mathcal{A} , \mathcal{B} , and \mathcal{C} with $|\mathcal{A}| \geq |\mathcal{B}| \geq |\mathcal{C}|$. Since there are at least 5 taxa, we cannot have $|\mathcal{A}| = 1$. If $|\mathcal{A}| = 2$ then $|\mathcal{B}| = 2$, $|\mathcal{C}| = 1$ or 2, yielding cases 4 and 5. If $|\mathcal{A}| \geq 3$, then we know $\mathcal{B} \cup \mathcal{C}$ is not a clade, since otherwise it would have appeared on the list, leading to a bipartition of \mathcal{X} . Thus either $\mathcal{A} \cup \mathcal{B}$ or $\mathcal{A} \cup \mathcal{C}$ is a clade. Note that $|\mathcal{B}| \neq 1$, else \mathcal{A} would not omit at least 3 taxa. If $|\mathcal{B}| = 2$, then we obtain cases 2, and c. If $|\mathcal{B}| \geq 3$, then $\mathcal{A} \cup \mathcal{B}$ is a clade, since $\mathcal{A} \cup \mathcal{C}$ and $\mathcal{B} \cup \mathcal{C}$ were not found on the list. Since all subclades of \mathcal{A} , \mathcal{B} , and \mathcal{C} appear in the list, all clades on ψ are determined.

If there is no partition \mathcal{X} into two or three clades on the list, then there must be one with four, since if five or more were needed then the union of each pair would omit at least 3 taxa and at least one such union is a detectable clade. Denote the four clades by \mathcal{A} , \mathcal{B} , \mathcal{C} , and \mathcal{D} , with $|\mathcal{A}| \geq |\mathcal{B}| \geq |\mathcal{C}| \geq |\mathcal{D}|$. We also must have $|\mathcal{C}| = |\mathcal{D}| = 1$, since otherwise the union of each pair of sets would omit at least 3 taxa, and hence would already have been tested for being a clade. It is now enough to determine the clade structure formed by the union of these sets, since all subclades of them are already known.

If $|\mathcal{B}| = 1$, then $|\mathcal{A}| \geq 2$, and none of $\mathcal{B} \cup \mathcal{C}$, $\mathcal{B} \cup \mathcal{D}$, and $\mathcal{C} \cup \mathcal{D}$ are clades since they omit at least 3 taxa and did not appear on the list of known clades. Thus the four clades must form a rooted unbalanced 4-leaf tree with \mathcal{A} in the cherry. We can then use invariants to check which of $\mathcal{A} \cup \mathcal{B}$, $\mathcal{A} \cup \mathcal{C}$, $\mathcal{A} \cup \mathcal{D}$ is a clade, since we know their complement is not a 2-clade. This results in case 1.

If $|\mathcal{B}| \geq 2$, then every pairwise union of the four clades except $\mathcal{A} \cup \mathcal{B}$ would have been tested, so $\mathcal{A} \cup \mathcal{B}$ must be a clade. As $\mathcal{C} \cup \mathcal{D}$ is not a clade, this also falls into case 1. □

The identifiability results of Corollary 4 are based solely on the use of clade-induced linear invariants, that is on certain linear equalities. Using other linear invariants and inequalities, we can strengthen these results. For example, those trees in case 4 of Corollary 4 can be distinguished by considering the sign (+/-) or vanishing (= 0) of the linear invariant given below. Indeed, this linear expression in split invariants is equivalent to that of the computationally determined expression (11), and the following proposition gives a theoretical justification for its existence. However, this linear invariant appears to be a special one for a single 5-taxon tree, with no analogs for other trees.

Proposition 5 *The expression*

$$\begin{aligned} & \mathbb{P}_\sigma(\text{Sp}(ab)) + \mathbb{P}_\sigma(\text{Sp}(ac)) + \mathbb{P}_\sigma(\text{Sp}(bc)) \\ & - \mathbb{P}_\sigma(\text{Sp}(de)) - \mathbb{P}_\sigma(\text{Sp}(cd)) - \mathbb{P}_\sigma(\text{Sp}(ce)) \end{aligned} \tag{12}$$

evaluates to 0 for the species tree $((((a, b), (d, e)), c)$. Assuming all species tree edge lengths are finite and positive, expression (12) is positive for the species tree $((((a, b), c), (d, e)))$ and negative for the species tree $((a, b), (c, (d, e)))$.

Proof For the species tree $((((a, b), (d, e)), c)$, let e_1 be the edge immediately above MRCA(a, b), and e_2 the edge above MRCA(d, e) in the species tree. To show expression (12) evaluates to 0, it is enough to show this conditioned on disjoint and exhaustive

events. To this end, we compute (12) conditioned on whether coalescent events occur on edges e_1 and e_2 .

Given that no coalescence occurs on either e_1 or e_2 , the probabilities of $Sp(ab)$ and $Sp(de)$ are equal by exchangeability. Similarly, the other 4 split probabilities appearing in formula (12) are all equal. Thus all terms cancel.

Given that coalescences occurs on both e_1 and e_2 , then the probabilities of $Sp(ab)$ and $Sp(de)$ are both 1. The other 4 probabilities are all 0, so again all terms cancel.

Assuming that a coalescent event occurred on exactly one of e_1 and e_2 , without loss of generality we may assume it is on e_1 . Then $Sp(ab)$ has probability 1, while $Sp(ac)$ and $Sp(bc)$ have probability 0. The next coalescent event produces the only other non-trivial split of the gene tree, which must be one of $Sp(CD)$, $Sp(CE)$, $Sp(DE)$. Thus $\mathbb{P}(Sp(cd)) + \mathbb{P}(Sp(ce)) + \mathbb{P}(Sp(de)) = 1$, and again we find the expression gives 0.

For the species tree $((a, b), c), (d, e))$, let e_1 be the edge above $MRCA(a, b)$, e_2 that above $MRCA(a, b, c)$, and e_3 that above $MRCA(d, e)$. We will again consider disjoint exhaustive events, and show that conditioned on the number of coalescent events on these edges expression (12) is always nonnegative, and sometimes positive. Thus, the unconditioned expression is positive.

If there are exactly 2 coalescences on the edges e_1, e_2 , then in the formation of a gene tree a compound lineage ABC enters the population above the root, and exactly one of $Sp(AB)$, $Sp(AC)$, $Sp(BC)$ form. Moreover, $Sp(DE)$ will be present on any unrooted version of such a gene tree, and $Sp(CD)$, $Sp(CE)$ absent. Thus, in the conditional probability, the first three terms of expression (12) sum to 1, and the last three terms to -1 , for a total of 0.

If there is exactly 1 coalescence on the edges e_1, e_2 , then again exactly one of $Sp(AB)$, $Sp(AC)$, $Sp(BC)$ must form on a gene tree, and the sum of the first three probabilities in (12) is 1. If $Sp(AB)$ formed, then exactly one of $Sp(DE)$, $Sp(CD)$, $Sp(CE)$ forms, and the expression in (12) is zero. If $Sp(AB)$ does not form, say instead $Sp(AC)$ does, then neither $Sp(CD)$ nor $Sp(CE)$ can appear on any such gene tree, while $Sp(DE)$ forms with probability less than 1 since e_3 has finite length. In this case, expression (12) is positive. Similarly, if $Sp(BC)$ forms, then the expression is positive.

If there are no coalescences on e_1, e_2 , or e_3 , then all 5 lineages of the taxa arrive at the root of the species tree distinct. Then by exchangeability one sees the probability of every split $Sp(xy)$ is the same, so the expression evaluates to 0.

If there are no coalescences on e_1, e_2 , but there is one on e_3 , then $Sp(DE)$ forms, but not $Sp(CD)$ nor $Sp(CE)$. Thus the last three terms yield -1 . As the lineages at the root of the species tree will be A, B, C , and a combined DE , exactly one of $Sp(AB)$, $Sp(AC)$, $Sp(BC)$ forms, so the first three terms add to 1, and all terms cancel.

The claim for the species tree $((a, b), (c, (d, e)))$ follows by interchanging taxon names from $((a, b), c), (d, e))$. \square

The following proposition identifies species tree roots in case 1 of Corollary 4.

Proposition 6 *Let \mathcal{X} be a set of at least 5 taxa, $a, b \in \mathcal{X}$, and T any rooted species tree topology on $\mathcal{X}' = \mathcal{X} \setminus \{a, b\}$. Let $c \in \mathcal{X}'$. Suppose ψ is one of the species trees $((T, a), b)$, $((T, b), a)$, or $(T, (a, b))$, and $\sigma = (\psi, \lambda)$ has positive length edges*

incident to the root. Then

$$\mathbb{P}(Sp(ac)) - \mathbb{P}(Sp(bc)) \begin{cases} > 0 & \text{if and only if } \psi = ((T, a), b), \\ = 0 & \text{if and only if } \psi = (T, (a, b)), \\ < 0 & \text{if and only if } \psi = ((T, b), a). \end{cases}$$

The intuition behind this proposition is rather simple. The polynomial $\mathbb{P}(Sp(ac)) - \mathbb{P}(Sp(bc))$ is a clade-induced split invariant for the tree $(T, (a, b))$, identifying by its vanishing that ab is a clade. One might reasonably hope that the hyperplane defined by this invariant's vanishing separates collections of split probabilities for the two alternative trees $((T, a), b)$ and $((T, b), a)$. That this is true is established by a rather technical proof which appears in Appendix B section.

For case 2, we follow a similar tack, focusing on a split invariant for the clade ab_1b_2 on the tree $(T, (a, (b_1, b_2)))$. The proof of the following is also in Appendix B section.

Proposition 7 *Let \mathcal{X} be a set of at least 6 taxa, $a, b_1, b_2 \in \mathcal{X}$, and T any rooted species tree topology on $\mathcal{X}' = \mathcal{X} \setminus \{a, b_1, b_2\}$. Let $c \in \mathcal{X}'$. Suppose ψ is one of the species trees $((T, a), (b_1, b_2))$, $((T, (b_1, b_2)), a)$, or $(T, (a, (b_1, b_2)))$, and $\sigma = (\psi, \lambda)$ has positive length edges incident to the root. Then*

$$\mathbb{P}(Sp(ac)) + \mathbb{P}(Sp(ab_2c)) - \mathbb{P}(Sp(b_1c)) - \mathbb{P}(Sp(b_1b_2c)) \begin{cases} > 0 & \text{if and only if } \psi = ((T, a), (b_1, b_2)) \\ = 0 & \text{if and only if } \psi = (T, (a, (b_1, b_2))) \\ < 0 & \text{if and only if } \psi = ((T, (b_1, b_2)), a) \end{cases}$$

For case 3, we similarly have the following, also proved in the appendix section.

Proposition 8 *Let \mathcal{X} be a set of at least 7 taxa, $a_1, a_2, b_1, b_2 \in \mathcal{X}$, and T any rooted species tree topology on $\mathcal{X}' = \mathcal{X} \setminus \{a_1, a_2, b_1, b_2\}$. Let $c \in \mathcal{X}'$. Suppose ψ is one of the species trees $((T, (a_1, a_2)), (b_1, b_2))$, $((T, (b_1, b_2)), (a_1, a_2))$, or $(T, ((a_1, a_2), (b_1, b_2)))$, and $\sigma = (\psi, \lambda)$ has positive length edges incident to the root. Then,*

$$\mathbb{P}(Sp(a_1c)) + \mathbb{P}(Sp(a_1a_2c)) + \mathbb{P}(Sp(a_1b_2c)) + \mathbb{P}(Sp(a_1a_2b_2c)) - \mathbb{P}(Sp(b_1c)) - \mathbb{P}(Sp(b_1a_2c)) - \mathbb{P}(Sp(b_1b_2c)) - \mathbb{P}(Sp(b_1a_2b_2c)) \begin{cases} > 0 & \text{if and only if } \psi = ((T, (a_1, a_2)), (b_1, b_2)) \\ = 0 & \text{if and only if } \psi = (T, ((a_1, a_2), (b_1, b_2))) \\ < 0 & \text{if and only if } \psi = ((T, (b_1, b_2)), (a_1, a_2)) \end{cases}$$

We summarize these results with the following.

Theorem 3 *For any species tree on 5 or more taxa with generic edge lengths, the rooted species tree topology is identifiable from split probabilities by testing linear equalities and inequalities, with the possible exception of case 5 of Corollary 4, the 6-taxon rooted trees with three 2-clades.*

Note that we do not claim that there do not exist linear inequalities that could be used to identify the root in case 5, only that we have not found any among the candidates we considered for this purpose. Moreover, nonlinear split invariants for those trees might be useful for root identification, but they are of higher degree than we were able to compute and remain unknown. While the practical import of this special case is small, understanding it better is desirable nonetheless.

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A Greedy Split Consensus on 5-Taxon Trees: Proofs

Here we prove Propositions 2 and 3 from Sect. 3.

With $\mathcal{X} = \{a, b, c, d, e\}$, there are 10 non-trivial splits, each with blocks of size 2 and 3. We use notation for split probabilities given in Example 1. Computations, assisted by the software COAL (Degnan and Salter 2005), produce the formulas in Table 2 for these split probabilities on the 3 species tree shapes, σ_{bal} , σ_{pc} , and σ_{cat} .

Proof of Proposition 2 Given the equalities of split probabilities in Table 2, we need only show that $s_{ab}, s_{de} \geq s_{ac}, s_{ad}, s_{cd}$ for each of the trees σ_{bal} and σ_{ps} , for a total of 12 inequalities. Note that positive branch lengths imply $0 < X, Y, Z < 1$.

Table 2 Split probabilities for gene trees arising on the 5-taxon species trees under the multispecies coalescent model

	σ_{bal}	σ_{pc}
s_{ab}	$1 - \frac{2}{15}XY^3Z - \frac{2}{3}X$	$1 - \frac{1}{45}XYZ^6 - \frac{1}{9}XY - \frac{2}{3}X$
s_{ac}, s_{bc}	$-\frac{2}{15}XY^3Z + \frac{1}{3}X$	$\frac{1}{30}XYZ^6 - \frac{1}{6}XY + \frac{1}{3}X$
$s_{ad}, s_{ae}, s_{bd}, s_{be}$	$\frac{1}{30}XY^3Z + \frac{1}{6}XYZ$	$-\frac{1}{45}XYZ^6 + \frac{2}{9}XY$
s_{cd}, s_{ce}	$\frac{1}{30}XY^3Z - \frac{1}{6}XYZ + \frac{1}{3}YZ$	$\frac{1}{30}XYZ^6 - \frac{1}{6}XY + \frac{1}{3}Y$
s_{de}	$1 + \frac{1}{3}XY^3Z - \frac{1}{3}XYZ - \frac{2}{3}YZ$	$1 - \frac{1}{45}XYZ^6 - \frac{1}{9}XY - \frac{2}{3}Y$
		σ_{cat}
s_{ab}		$1 - \frac{1}{45}XY^3Z^6 - \frac{1}{9}XY^3 - \frac{2}{3}X$
s_{ac}, s_{bc}		$-\frac{1}{45}XY^3Z^6 - \frac{1}{9}XY^3 + \frac{1}{3}X$
s_{ad}, s_{bd}		$-\frac{1}{45}XY^3Z^6 + \frac{1}{18}XY^3 + \frac{1}{6}XY$
s_{ae}, s_{be}		$\frac{1}{30}XY^3Z^6 + \frac{1}{6}XY$
s_{cd}		$-\frac{1}{45}XY^3Z^6 + \frac{1}{18}XY^3 - \frac{1}{6}XY + \frac{1}{3}Y$
s_{ce}		$\frac{1}{30}XY^3Z^6 - \frac{1}{6}XY + \frac{1}{3}Y$
s_{de}		$1 + \frac{1}{30}XY^3Z^6 + \frac{1}{6}XY^3 - \frac{1}{3}XY - \frac{2}{3}Y$

While each of the inequalities can be checked without machine assistance, as an example we use the software Maple to verify one of them: $s_{de} > s_{cd}$ for σ_{bal} . Note

$$s_{de} - s_{cd} = 1 + \frac{1}{6}XY^3Z - \frac{1}{6}XYZ - YZ.$$

The Maple command

```
solve({1+(1/6)*X*Y^3*Z-(1/6)*X*Y*Z-Y*Z>0,
      0<X, 0<Y, 0<Z, X<1, Y<1, Z<1})
```

produces as output

```
{0<X, 0<Y, 0<Z, X<1, Y<1, Z<1}
```

verifying the claim. □

Proof of Proposition 3 That $s_{ab} > s_{xy}$ for $xy \neq de$ can be verified as in the preceding proof.

Suppose now that $s_{de} \geq s_{ab}$. Then since s_{ab} is larger than all the remaining split probabilities by the above calculations, the true non-trivial splits on the species tree have the highest probability, and greedy consensus for gene tree splits is consistent.

Now assume instead that $s_{ab} > s_{de}$, so s_{ab} is the strict maximum of the split probabilities. Under the greedy consensus algorithm, splits incompatible with $Sp(ab)$ are discarded and only the splits s_{cd} , s_{ce} , and s_{de} remain as candidate splits for acceptance by the algorithm.

It can be verified that $s_{de} > s_{ce}$ for all $0 < X, Y, Z < 1$. However,

$$s_{de} - s_{cd} = 1 + \frac{1}{18}XY^3Z^6 + \frac{1}{9}XY^3 - \frac{1}{6}XY - Y = F(X, Y, Z)$$

can have either sign. If $F(X, Y, Z) > 0$, then greedy consensus will return the correct species tree. If $F(X, Y, Z) < 0$, it will return the tree $((a, b), e, (c, d))$. □

B Identifiability of Trees: Additional Proofs

The proofs we give of Propositions 6, 7, and 8 depend on a careful analysis of probabilities under the coalescent. That of Proposition 6 is the simplest, and serves as a model for the others.

B.1 Proof of Proposition 6

The proof of Proposition 6 depends on several lemmas.

We begin with a definition. Consider a non-binary rooted species tree $((x_1, x_2, \dots, x_k):L, y)$ formed by attaching a single outgroup taxon y to a claw tree with k taxa x_i , with edge length $L > 0$. Under the multispecies coalescent model we will be interested in the case where the gene lineages, one for each x_i , have coalesced ℓ times, from k to $k - \ell$ lineages, by the time they reach the root of the tree, and then further

coalescences occur with the y lineage in the root population, until a single tree is formed. For $\mathcal{A} \subset \mathcal{X} = \{x_1, x_2, \dots, x_k, y\}$. We denote the probability that a resulting gene tree displays a split $Sp(\mathcal{A}_g)$ as

$$p(\mathcal{A} \mid k, \ell).$$

Note that this probability does not depend on branch lengths in the species tree, since $L > 0$ and we have conditioned on ℓ . Furthermore, since the x_i lineages are exchangeable under the coalescent model on this tree, $p(\mathcal{A} \mid k, \ell)$ actually depends on \mathcal{A} only through the number of $x_i \in \mathcal{A}$ and whether $y \in \mathcal{A}$, but not on the particular $x_i \in \mathcal{A}$.

By an m -split, we mean a split of taxa where one block of the partition has size m . We now give recursions and base cases for the probability of various 2-splits for the above species tree.

- Lemma 3**
1. $p(x_1x_2 \mid k, 0) = p(x_1y \mid k, 0)$ for $k \geq 2$,
 2. $p(x_1x_2 \mid 3, \ell) = p(x_1y \mid 3, \ell) = \frac{1}{3}$ for $\ell = 0, 1, 2$,
 3. $p(x_1y \mid k, 0) = \frac{1}{\binom{k+1}{2}} + \frac{\binom{k-1}{2}}{\binom{k+1}{2}} p(x_1y \mid k-1, 0)$ for $k \geq 3$,
 4. $p(x_1x_2 \mid k, \ell) = \frac{1}{\binom{k}{2}} + \frac{\binom{k-2}{2}}{\binom{k}{2}} p(x_1x_2 \mid k-1, \ell-1)$, for $k \geq 4, k > \ell \geq 1$,
 5. $p(x_1y \mid k, \ell) = \frac{\binom{k-1}{2}}{\binom{k}{2}} p(x_1y \mid k-1, \ell-1)$ for $k \geq 3, k > \ell \geq 1$.

Proof These all follow directly from properties of the coalescent model. We give reasoning for several, leaving the rest to the reader.

For claim (1), observe no coalescent events occur below the root of the tree, so exchangeability of lineages at the root implies the statement.

For claim (4), note that for the split $Sp(X_1X_2)$ to form, the first coalescent event must either be between the x_1 and x_2 lineages, which occurs with probability $1/\binom{k}{2}$, or be between x_i lineages with $i \neq 1, 2$, which occurs with probability $\binom{k-2}{2}/\binom{k}{2}$, with the split forming subsequently. \square

We next establish some probability bounds.

Lemma 4 For $k \geq 4, k > \ell \geq 0, p(x_1y \mid k, \ell) < \frac{1}{k}$.

Proof Lemma 3 (3) and (2) imply $p(x_1y \mid 4, 0) = \frac{1}{5} < \frac{1}{4}$. For $k > 4, \ell = 0$, Lemma 3 (3) and an inductive hypothesis then show

$$p(x_1y \mid k, 0) < \frac{1}{\binom{k+1}{2}} + \frac{\binom{k-1}{2}}{\binom{k+1}{2}} \frac{1}{k-1} = \frac{1}{k+1} < \frac{1}{k}.$$

For $\ell \geq 1$, first consider the case that $k - \ell = 1, 2$, or 3 . The using Lemma 3 (5) repeatedly and Lemma 3 (2) shows

$$p(x_1y \mid k, \ell) = \frac{\binom{k-1}{2}}{\binom{k}{2}} \frac{\binom{k-2}{2}}{\binom{k-1}{2}} \dots \frac{\binom{3}{2}}{\binom{4}{2}} p(x_1y \mid 3, \ell - k + 3)$$

$$= \frac{6}{k(k-1)} \cdot \frac{1}{3} = \frac{2}{k(k-1)} < \frac{1}{k}.$$

If instead $k - \ell \geq 4$, Lemma 3 (5) and what has already been established imply

$$\begin{aligned} p(x_{1y} | k, \ell) &= \frac{\binom{k-1}{2} \binom{k-2}{2}}{\binom{k}{2} \binom{k-1}{2}} \cdots \frac{\binom{k-\ell}{2}}{\binom{k-\ell+1}{2}} p(x_{1y} | k - \ell, 0) \\ &\leq \frac{(k-\ell)(k-\ell-1)}{k(k-1)} \frac{1}{k-\ell} < \frac{1}{k}. \end{aligned}$$

□

Next, we obtain a key inequality.

Lemma 5 For $k = 3, \ell = 0, 1, 2$ and for $k > 3, \ell = 0$,

$$p(x_1x_2 | k, \ell) - p(x_{1y} | k, \ell) = 0.$$

For $k \geq 4$ and $k > \ell \geq 1$,

$$p(x_1x_2 | k, \ell) - p(x_{1y} | k, \ell) > 0.$$

Proof For $k = 3, \ell = 0, 1, 2$ and for $k > 3, \ell = 0$, the claimed equalities follow from Lemma 3 (2) and (1), respectively.

For the inequality when $k \geq 4, k > \ell \geq 1$, by Lemma 3 (4) and (5),

$$\begin{aligned} &p(x_1x_2 | k, \ell) - p(x_{1y} | k, \ell) \\ &= \frac{1}{\binom{k}{2}} \left(1 + \binom{k-2}{2} p(x_1x_2 | k-1, \ell-1) - \binom{k-1}{2} p(x_{1y} | k-1, \ell-1) \right) \\ &= \frac{1}{\binom{k}{2}} \left(1 + \binom{k-2}{2} \left(p(x_1x_2 | k-1, \ell-1) - p(x_{1y} | k-1, \ell-1) \right) \right. \\ &\quad \left. - (k-2)p(x_{1y} | k-1, \ell-1) \right). \end{aligned} \tag{13}$$

Using Lemma 3 (2) in Eq. (13) shows

$$p(x_1x_2 | 4, \ell) - p(x_{1y} | 4, \ell) = \frac{1}{18} > 0$$

for $\ell = 1, 2, 3$, establishing the $k = 4$ case of the inequality.

For $k > 4$, by Lemma 3 (1) and Lemma 4 Eq. (13) yields

$$p(x_1x_2 | k, 1) - p(x_{1y} | k, 1) > \frac{1}{\binom{k}{2}} \left(1 + \binom{k-2}{2} 0 - (k-2) \frac{1}{k-1} \right) > 0.$$

This shows the inequality holds for $\ell = 1$, and provides base cases for an inductive proof for $\ell \geq 1$.

Finally, Eq. (13), an inductive hypothesis, and Lemma 4 show that for $\ell \geq 2$

$$p(x_1x_2 \mid k, \ell) - p(x_1y \mid k, \ell) > \frac{1}{\binom{k}{2}} \left(1 + \binom{k-2}{2} 0 - (k-2) \frac{1}{k-1} \right) > 0.$$

□

Proof of Proposition 6 That the equality holds for species tree $(T, (a, b))$ is an instance of Theorem 2. It is enough to establish the inequality for $((T, a), b)$, since that for $((T, b), a)$ will follow by interchanging taxon names.

On the species tree $((T, a), b)$, let v denote the MRCA of T and a . Observe that for the splits $Sp(AC)$ or $Sp(BC)$ to form, it is necessary that the c lineage not coalesce with any other below v . In any such realization of the coalescent process below v , lineages from taxa on T will have coalesced to $k - 1$ lineages by v , where $k \geq 3$. There the lineage from a enters, and ℓ coalescent events, $k > \ell \geq 0$ occur on the edge immediately ancestral to v .

To establish the inequality, we consider it conditioned on a number of disjoint and exhaustive events: For each possible k, ℓ , let $C = C(k, \ell)$ denote the event that $k - 1$ agglomerated lineages from T reach v , one of which is the lineage from c alone, and that ℓ coalescent events occur in the population immediately ancestral to v . Fixing $C = C(k, \ell)$, with $y = b, x_1 = c, x_2 = a$ we have

$$\begin{aligned} \mathbb{P}(Sp(ac) \mid C) &= p(x_1x_2 \mid k, \ell), \\ \mathbb{P}(Sp(bc) \mid C) &= p(x_1y \mid k, \ell). \end{aligned}$$

Lemma 5 thus shows $\mathbb{P}(Sp(ac \mid C) - \mathbb{P}(Sp(bc \mid C))$ is positive for $k \geq 4, k > \ell \geq 1$, and zero for other relevant cases. Multiplying by the probabilities of each $C = C(k, \ell)$ and summing, we obtain the desired unconditioned expression $\mathbb{P}(Sp(ac)) - \mathbb{P}(Sp(bc))$. Because T has at least three taxa, there are some positive summands from $k \geq 4, \ell \geq 1$, so the desired inequality holds. □

B.2 Proof of Proposition 7

While the proof of Proposition 7 follows the same line of reasoning as that of Proposition 6, there are further technical details. We first extend some of the results from the previous section to splits of size 3. These will be applied in arguments for the species tree $((T, (b_1, b_2)), a)$.

- Lemma 6**
1. $p(\mathcal{A} \mid k, 0) = p(\mathcal{B} \mid k, 0)$ for $|\mathcal{A}| = |\mathcal{B}|$,
 2. $p(x_1x_2x_3 \mid k, 0) = \frac{3}{\binom{k+1}{2}} p(x_1x_2 \mid k - 1, 0) + \frac{\binom{k-2}{2}}{\binom{k+1}{2}} p(x_1x_2x_3 \mid k - 1, 0)$ for $k \geq 4$,
 3. $p(x_1x_2x_3 \mid 3, \ell) = p(x_1x_2y \mid 3, \ell) = 1$, for $\ell = 0, 1, 2$,
 4. $p(x_1x_2x_3 \mid 4, \ell) = \frac{1}{2} p(x_1x_2 \mid 3, \ell - 1)$ for $\ell = 1, 2, 3$,

5. $p(x_1x_2x_3 | k, \ell) = \frac{3}{\binom{k}{2}}p(x_1x_2 | k - 1, \ell - 1) + \frac{\binom{k-3}{2}}{\binom{k}{2}}p(x_1x_2x_3 | k - 1, \ell - 1)$ for $k \geq 5, k > \ell \geq 1,$
6. $p(x_1x_2y | k, \ell) = \frac{1}{\binom{k}{2}}p(x_1y | k - 1, \ell - 1) + \frac{\binom{k-2}{2}}{\binom{k}{2}}p(x_1x_2y | k - 1, \ell - 1)$ for $k \geq 4, k > \ell \geq 1.$

Proof For claim (1), it suffices to note that $k + 1$ lineages enter the population above the root, with no coalescent events having occurred below, so the probabilities of any two m -splits are the same by exchangeability of lineages under the coalescent model.

For claim (2), again $k + 1$ lineages enter the root population, with no previous coalescence. For $Sp(X_1X_2X_3)$ to form, the first coalescent event above the root must be between a pair of lineages chosen from $x_1, x_2, x_3,$ or disjoint from them. It is between a pair chosen from them with probability $\frac{3}{\binom{k+1}{2}}$. Then, for $Sp(X_1X_2X_3)$ to form, this pair's lineage must join with the remaining x_i lineage. By claim (1), this has probability $p(x_1x_2 | k - 1, 0)$. Multiplying these probabilities, we obtain the first summand. The first coalescent event not involving any of the x_1, x_2, x_3 lineages, and then the desired split forming with 1 less lineage present gives the second summand.

The remaining verifications are left to the reader. □

Lemma 7 For $k \geq 4, k > \ell \geq 0,$

$$p(x_1y | k, \ell) + p(x_1x_2y | k, \ell) < \frac{1}{k - 2}.$$

Proof We first show the inequality for $\ell = 0,$ by induction on $k.$ From Lemmas 3 and 6, $p(x_1y | 4, 0) + p(x_1x_2y | 4, 0) = \frac{2}{5},$ establishing the base case of $k = 4.$

If $k \geq 5,$ an inductive hypothesis, Lemma 3 (3), Lemma 6 (1) and (2), and Lemma 4 yield

$$\begin{aligned} & p(x_1y|k, 0) + p(x_1x_2y|k, 0) \\ &= \frac{1}{\binom{k+1}{2}} \left(1 + (k + 1)p(x_1y|k - 1, 0) \right. \\ &\quad \left. + \binom{k - 2}{2} (p(x_1y|k - 1, 0) + p(x_1x_2y|k - 1, 0)) \right) \\ &< \frac{1}{\binom{k+1}{2}} \left(1 + \frac{k + 1}{k - 1} + \binom{k - 2}{2} \frac{1}{k - 3} \right) = \frac{k^2 + k + 2}{(k + 1)k(k - 1)} < \frac{1}{k - 2}. \end{aligned}$$

Next observe that for $\ell = 1, 2, 3,$ Lemmas 3 and 6 imply

$$p(x_1y | 4, \ell) + p(x_1x_2y | 4, \ell) = \frac{7}{18} < \frac{1}{4 - 2}.$$

With the $k = 4, \ell = 1, 2, 3$ cases and the $k \geq 4, \ell = 0$ cases already established, we now proceed by induction on $\ell.$ For $k \geq 5, k > \ell \geq 1$ by Lemma 3 (5), Lemma 6 (6),

Lemma 4, and an inductive hypothesis,

$$\begin{aligned}
 & p(x_1y \mid k, \ell) + p(x_1x_2y \mid k, \ell) \\
 &= \frac{\binom{k-1}{2} + 1}{\binom{k}{2}} p(x_1y \mid k-1, \ell-1) + \frac{\binom{k-2}{2}}{\binom{k}{2}} p(x_1x_2y \mid k-1, \ell-1) \\
 &= \frac{k-1}{\binom{k}{2}} p(x_1y \mid k-1, \ell-1) + \frac{\binom{k-2}{2}}{\binom{k}{2}} (p(x_1y \mid k-1, \ell-1) \\
 &\quad + p(x_1x_2y \mid k-1, \ell-1)) \\
 &< \frac{k-1}{\binom{k}{2}} \frac{1}{k-1} + \frac{\binom{k-2}{2}}{\binom{k}{2}} \frac{1}{k-3} = \frac{1}{k-1} < \frac{1}{k-2}.
 \end{aligned}$$

□

Lemma 8 *Let*

$$P(k, \ell) = p(x_1x_2 \mid k, \ell) + p(x_1x_2x_3 \mid k, \ell) - p(x_1y \mid k, \ell) - p(x_1x_2y \mid k, \ell).$$

Then for $k = 4, \ell = 0, 1, 2, 3$, and for $k \geq 5, \ell = 0, P(k, \ell) = 0$. For $k \geq 5, k > \ell \geq 1, P(k, \ell) > 0$.

Proof Note that for $k = 4$, the event $Sp(x_1x_2)$ is the same as $Sp(x_3x_4y)$, and $Sp(x_1x_2x_3)$ is the same as $Sp(x_4y)$, so using exchangeability of the x_i lineages we have

$$\begin{aligned}
 p(x_1x_2 \mid 4, \ell) &= p(x_1x_2y \mid 4, \ell), \\
 p(x_1x_2x_3 \mid k, \ell) &= p(x_1y \mid k, \ell).
 \end{aligned}$$

Thus $P(4, \ell) = 0$ for $\ell = 0, 1, 2, 3$. For $k \geq 5$, Lemma 6 (1) implies $P(k, 0) = 0$.

For $k \geq 5, \ell \geq 1$, by Lemmas 3 (4), (5) and 6 (5), (6), we find

$$\begin{aligned}
 P(k, \ell) &= \frac{1}{\binom{k}{2}} \left[1 + (k-2) (p(x_1x_2 \mid k-1, \ell-1) - p(x_1y \mid k-1, \ell-1)) \right. \\
 &\quad - (k-2) (p(x_1y \mid k-1, \ell-1) + p(x_1x_2y \mid k-1, \ell-1)) \\
 &\quad + 2p(x_1x_2 \mid k-1, \ell-1) + p(x_1x_2y \mid k-1, \ell-1) \\
 &\quad \left. + \binom{k-3}{2} P(k-1, \ell-1) \right].
 \end{aligned}$$

Using Lemmas 5 and 7, the nonnegativity of probabilities, and an inductive hypothesis that $P(k-1, \ell-1) \geq 0$, it follows that

$$P(k, \ell) > \frac{1}{\binom{k}{2}} \left(1 + (k-2) \cdot 0 - (k-2) \frac{1}{k-2} + 2 \cdot 0 + 0 + \binom{k-3}{2} 0 \right) = 0.$$

□

Lemma 9 Consider a species tree with topology $((T, (b_1, b_2)), a)$, where T is a subtree on at least three taxa, one of which is c . Suppose the edge above $(T, (b_1, b_2))$ has positive length. Then under the multispecies coalescent model,

$$\mathbb{P}(Sp(ac)) + \mathbb{P}(Sp(ab_2c)) - \mathbb{P}(Sp(b_1c)) - \mathbb{P}(Sp(b_1b_2c)) < 0.$$

Proof Let v denote the MRCA on the species tree of the taxa on T and the b_i .

To establish the claimed inequality, it is enough to show it holds when conditioned on whether b_1 and b_2 lineages have coalesced before reaching v or not. If they have coalesced before v to form a single lineage, then the events $Sp(ab_2c)$ and $Sp(b_1c)$ have probability zero. Thus using b for b_1b_2 , we wish to show

$$\mathbb{P}(Sp(ac)) - \mathbb{P}(Sp(bc)) < 0.$$

This follows immediately from Proposition 6.

We henceforth condition on the two b_i lineages being distinct at v . Noticing that all four probabilities in the expression of interest are 0 if the c lineage coalesces with any lineage below v , we further condition on the c lineage being distinct at v , where there are thus $k \geq 4$ lineages entering the population above v , and ℓ coalescent events occurring between v and the root.

Then, with $\mathcal{C} = \mathcal{C}(k, \ell)$ denoting the events we condition on,

$$\begin{aligned} \mathbb{P}(Sp(ac) | \mathcal{C}) &= p(x_1y | k, \ell), \\ \mathbb{P}(Sp(ab_2c) | \mathcal{C}) &= p(x_1x_2y | k, \ell), \\ \mathbb{P}(Sp(b_1c) | \mathcal{C}) &= p(x_1x_2 | k, \ell), \\ \mathbb{P}(Sp(b_1b_2c) | \mathcal{C}) &= p(x_1x_2x_3 | k, \ell). \end{aligned}$$

From Lemma 8 we find conditioned on \mathcal{C} that the expression is strictly negative for $k \geq 5, k > \ell \geq 1$, and zero for $k \geq 5, \ell = 0$ and $k = 4, \ell = 0, 1, 2, 3$. Thus weighting the conditioned expressions by the probabilities of the events \mathcal{C} and summing, we see the full expression is negative, as long as $k \geq 5$ and $\ell \geq 1$ is possible. Since T has at least 3 taxa, this only requires that the edge above v has positive length. \square

To handle the species tree $((T, a), (b_1, b_2))$ we proceed analogously, but consider a rooted species tree $((x_1, x_2, \dots, x_k):L, y_1, y_2)$ formed by attaching a trifurcating root to two outgroups y_1, y_2 and a claw tree with k taxa, with a positive edge length L . We will be interested in the case where the gene lineages, one for each x_i , have coalesced ℓ times, from k to $k - \ell$ lineages, by the time they reach the root of the tree, and then further coalescence occurs in the root population until a single tree is formed. With $\mathcal{X} = \{x_1, \dots, x_k, y_1, y_2\}$ and $\mathcal{A} \subset \mathcal{X}$, let

$$r(\mathcal{A} | k, \ell) = \mathbb{P}(Sp(\mathcal{A}) | k, \ell)$$

for this species tree. By exchangeability of lineages in the coalescent model, $r(\mathcal{A} | k, \ell)$ depends on \mathcal{A} only up to the number of x_i and the number of y_i it contains.

The reader who has followed previous arguments should be able to verify the following.

- Lemma 10**
1. $r(\mathcal{A} \mid k, 0) = r(\mathcal{B} \mid k, 0)$ for $|\mathcal{A}| = |\mathcal{B}|$,
 2. $r(x_1x_2 \mid 3, 0) = \frac{1}{5}$, $r(x_1x_2 \mid 3, \ell) = \frac{1}{3}$ for $\ell = 1, 2$,
 3. $r(x_1x_2 \mid k, 0) = \frac{1}{\binom{k+2}{2}} + \frac{\binom{k}{2}}{\binom{k+2}{2}}r(x_1x_2 \mid k - 1, 0)$ for $k \geq 3$,
 4. $r(x_1x_2 \mid k, \ell) = \frac{1}{\binom{k}{2}} + \frac{\binom{k-2}{2}}{\binom{k}{2}}r(x_1x_2 \mid k - 1, \ell - 1)$, for $k \geq 4, k > \ell \geq 1$,
 5. $r(x_1y_1 \mid 2, 0) = \frac{1}{3}$, $r(x_1y_1 \mid 2, 1) = 0$,
 6. $r(x_1y_1 \mid k, \ell) = \frac{\binom{k-1}{2}}{\binom{k}{2}}r(x_1y_1 \mid k - 1, \ell - 1)$ for $k \geq 3, k > \ell \geq 1$.
 7. $r(x_1x_2x_3 \mid 4, \ell) = \frac{1}{2}r(x_1x_2 \mid 3, \ell - 1)$ for $\ell = 1, 2, 3$,
 8. $r(x_1x_2x_3 \mid k, \ell) = \frac{3}{\binom{k}{2}}r(x_1x_2 \mid k - 1, \ell - 1) + \frac{\binom{k-3}{2}}{\binom{k}{2}}r(x_1x_2x_3 \mid k - 1, \ell - 1)$ for $k \geq 5, k > \ell \geq 1$,
 9. $r(x_1x_2y_1 \mid 2, 0) = r(x_1x_2y_1 \mid 2, 1) = 1$, $r(x_1x_2y_1 \mid 3, 1) = \frac{1}{9}$, $r(x_1x_2y_1 \mid 3, 2) = 0$,
 10. $r(x_1x_2y_1 \mid k, 0) = \frac{3}{\binom{k+2}{2}}r(x_1x_2 \mid k - 1, 0) + \frac{\binom{k-1}{2}}{\binom{k+2}{2}}r(x_1x_2y_1 \mid k - 1, 0)$ for $k \geq 3$,
 11. $r(x_1x_2y_1 \mid k, \ell) = \frac{1}{\binom{k}{2}}r(x_1y_1 \mid k - 1, \ell - 1) + \frac{\binom{k-2}{2}}{\binom{k}{2}}r(x_1x_2y_1 \mid k - 1, \ell - 1)$ for $k \geq 4, k > \ell \geq 1$,
 12. $r(x_1y_1y_2 \mid 2, 0) = r(x_1y_1y_2 \mid 2, 1) = 1$,
 13. $r(x_1y_1y_2 \mid k, \ell) = \frac{\binom{k-1}{2}}{\binom{k}{2}}r(x_1y_1y_2 \mid k - 1, \ell - 1)$ for $k \geq 3, k > \ell \geq 1$.

- Lemma 11**
1. $r(x_1y_1 \mid k, 0) \leq \frac{1}{k+2}$ for $k \geq 3$,
 2. $r(x_1y_1 \mid k, \ell) + r(x_1y_1y_2 \mid k, \ell) < \frac{1}{k-1}$ if $k \geq 3$ and $\ell = 0$, or if $k \geq 4$ and $k > \ell \geq 1$.

Proof For claim (1) first note that Lemma 10 (1) and (2) establish the $k = 3$ case. Then using Lemma 10 (3) one sees inductively that for $k > 3$,

$$\begin{aligned} r(x_1y_1 \mid k, 0) &\leq \frac{1}{\binom{k+2}{2}} + \frac{\binom{k}{2}}{\binom{k+2}{2}} \frac{1}{k+1} = \frac{k^2 + k + 2}{(k+2)(k+1)^2} \\ &\leq \frac{(k+1)^2}{(k+2)(k+1)^2} = \frac{1}{k+2}. \end{aligned}$$

For claim (2) when $\ell = 0$, note that by Lemma 10 (1), (2), (5), (9), and (10),

$$r(x_1y_1 \mid 3, 0) + r(x_1y_1y_2 \mid 3, 0) = \frac{1}{5} + \frac{3}{10} \cdot \frac{1}{3} + \frac{1}{10} \cdot 1 = \frac{2}{5} < \frac{1}{2},$$

so the base case of $k = 3$ holds. Then for $k > 3$, using Lemma 10 (1), (3), and (10) we have

$$r(x_1y_1 \mid k, 0) + r(x_1y_1y_2 \mid k, 0)$$

$$\begin{aligned} &= \frac{1}{\binom{k+2}{2}} + \frac{\binom{k}{2} + 3}{\binom{k+2}{2}} r(x_1 y_1 \mid k - 1, 0) + \frac{\binom{k-1}{2}}{\binom{k+2}{2}} r(x_1 y_1 y_2 \mid k - 1, 0) \\ &= \frac{1}{\binom{k+2}{2}} + \frac{k + 2}{\binom{k+2}{2}} r(x_1 y_1 \mid k - 1, 0) \\ &\quad + \frac{\binom{k-1}{2}}{\binom{k+2}{2}} \left(r(x_1 y_1 \mid k - 1, 0) + r(x_1 y_1 y_2 \mid k - 1, 0) \right). \end{aligned}$$

Using an inductive hypothesis and claim (1) of this proposition yields

$$\begin{aligned} &r(x_1 y_1 \mid k, 0) + r(x_1 y_1 y_2 \mid k, 0) \\ &< \frac{2}{(k + 2)(k + 1)} + \frac{2}{k + 1} \cdot \frac{1}{k + 1} + \frac{(k - 1)(k - 2)}{(k + 2)(k + 1)} \cdot \frac{1}{k - 2} \\ &= \frac{1}{k + 2} + \frac{2}{(k + 1)^2} < \frac{1}{k + 1} + \frac{2}{(k + 1)^2} < \frac{1}{k - 1}. \end{aligned}$$

Assume now $k \geq 4$ and $k > \ell \geq 1$, and consider first the case that $k - \ell = 1$ or 2. Applying Lemma 10 (6) and (13) repeatedly, we have

$$\begin{aligned} &r(x_1 y_1 \mid k, \ell) + r(x_1 y_1 y_2 \mid k, \ell) \\ &= \frac{3}{\binom{k}{2}} \left(r(x_1 y_1 \mid 3, \ell - k + 3) + r(x_1 y_1 y_2 \mid 3, \ell - k + 3) \right). \end{aligned}$$

From Lemma 10

$$\begin{aligned} r(x_1 y_1 \mid 3, 1) + r(x_1 y_1 y_2 \mid 3, 1) &= \frac{1}{9} + \frac{1}{3} = \frac{4}{9}, \\ r(x_1 y_1 \mid 3, 2) + r(x_1 y_1 y_2 \mid 3, 2) &= 0 + \frac{1}{3} = \frac{1}{3}, \end{aligned}$$

so for $k \geq 4$,

$$r(x_1 y_1 \mid k, \ell) + r(x_1 y_1 y_2 \mid k, \ell) < \frac{6}{k(k - 1)} \cdot \frac{4}{9} < \frac{1}{k - 1}.$$

If $k - \ell \geq 3$, then applying Lemma 10 (6) and (13) repeatedly gives

$$r(x_1 y_1 \mid k, \ell) + r(x_1 y_1 y_2 \mid k, \ell) = \frac{\binom{k-\ell}{2}}{\binom{k}{2}} \left(r(x_1 y_1 \mid k - \ell, 0) + r(x_1 y_1 y_2 \mid k - \ell, 0) \right).$$

Using what we proved above, this shows

$$r(x_1y_1 | k, \ell) + r(x_1y_1y_2 | k, \ell) < \frac{\binom{k-\ell}{2}}{\binom{k}{2}} \cdot \frac{1}{k-\ell-1} < \frac{1}{k-1}.$$

□

Lemma 12 *Let*

$$R(k, \ell) = r(x_1x_2 | k, \ell) + r(x_1x_2y_1 | k, \ell) - r(x_1y_1 | k, \ell) - r(x_1y_1y_2 | k, \ell).$$

Then for $k = 3, \ell = 0, 1, 2$, and for $k \geq 4, \ell = 0, R(k, \ell) = 0$. For $k \geq 4$ and $k > \ell \geq 1, R(k, \ell) > 0$.

Proof For $k = 3$, the events $Sp(x_1x_2)$ and $Sp(x_3y_1y_2)$ are the same, as are $Sp(x_1x_2y_1)$ and $Sp(x_3y_2)$, so using exchangeability of the x_i and of the y_i lineages

$$\begin{aligned} r(x_1x_2 | k, \ell) &= r(x_1y_1y_2 | k, \ell), \\ r(x_1x_2y_1 | k, \ell) &= r(x_1y_1 | k, \ell). \end{aligned}$$

Thus $R(3, \ell) = 0$ for $\ell = 0, 1, 2$. For $k \geq 3$, Lemma 10 (1) implies $R(k, 0) = 0$.

Now consider $k \geq 4, k > \ell \geq 1$. By Lemma 10 (4), (6), (11), and (13) we find

$$\begin{aligned} R(k, \ell) &= \frac{1}{\binom{k}{2}} \left(1 + r(x_1y_1 | k-1, \ell-1) + \binom{k-2}{2} R(k-1, \ell-1) \right. \\ &\quad \left. - (k-2)(r(x_1y_1 | k-1, \ell-1) + r(x_1y_1y_2 | k-1, \ell-1)) \right). \end{aligned}$$

An inductive hypothesis that $R(k-1, \ell-1) \geq 0$, Lemma 11, and the positivity of $r(x_1y_1 | k-1, \ell-1)$ then show

$$R(k, \ell) > \frac{1}{\binom{k}{2}} \left(1 + 0 + \binom{k-2}{2} 0 - (k-2) \frac{1}{k-2} \right) = 0.$$

□

Lemma 13 *Consider a species tree with topology $((T, a), (b_1, b_2))$, where T is a subtree on at least three taxa, one of which is c . Suppose the edge above (T, a) has positive length. Then under the multispecies coalescent model,*

$$\mathbb{P}(Sp(ac)) + \mathbb{P}(Sp(ab_2c)) - \mathbb{P}(Sp(b_1c)) - \mathbb{P}(Sp(b_1b_2c)) > 0.$$

Proof Let ρ denote the root of the species tree, and v the MRCA of the taxa on T and a .

To establish the claimed inequality, it is enough to show it holds when conditioned on whether the b_1 and b_2 lineages have coalesced before reaching ρ or not. If they have

coalesced below ρ to form a single lineage, then the events $Sp(ab_2c)$ and $Sp(b_1c)$ have probability zero. Thus using b for b_1b_2 , we wish to show

$$\mathbb{P}(Sp(ac)) - \mathbb{P}(Sp(bc)) > 0.$$

This follows immediately from Proposition 6.

We henceforth condition on the event that the lineages from b_1 and b_2 are distinct at ρ . Noticing that all four probabilities in the expression of interest are 0 if the c lineage coalesces with any lineage below v , we further condition on the event that the c lineage is distinct at v , so there are $k \geq 3$ distinct lineages at v , and that ℓ coalescent events occur on the edge above v . Calling this event $\mathcal{C} = \mathcal{C}(k, \ell)$,

$$\begin{aligned} \mathbb{P}(Sp(ac) \mid \mathcal{C}) &= r(x_1x_2 \mid k, \ell), \\ \mathbb{P}(Sp(ab_2c) \mid \mathcal{C}) &= r(x_1x_2y_1 \mid k, \ell), \\ \mathbb{P}(Sp(b_1c) \mid \mathcal{C}) &= r(x_1y_1 \mid k, \ell), \\ \mathbb{P}(Sp(b_1b_2c) \mid \mathcal{C}) &= r(x_1y_1y_2 \mid k, \ell). \end{aligned}$$

From Lemma 12 we find that conditioned on \mathcal{C} the expression of interest is strictly positive for $k \geq 4, k > \ell \geq 1$, and zero for $k = 3, \ell = 0, 1, 2$ and $k \geq 4, \ell = 0$. Weighting the conditioned expressions by the probabilities of the \mathcal{C} and summing we get the unconditioned expression. Since T has at least 3 taxa and the branch length above v has positive length, some of the summands corresponds to the event $\mathcal{C}(k, \ell)$ with $k \geq 4, k > \ell \geq 1$; thus the full expression is positive. \square

Finally, Proposition 7 follows from Theorem 2, Lemmas 9 and 13.

B.3 Proof of Proposition 8

To establish Proposition 8, we first extend the results of Lemma 10, and those that follow it, to splits of size 4.

A proof of the following is left to the reader.

Lemma 14 1. $r(x_1x_2x_3y_1 \mid 3, 0) = 1$,

2. $r(x_1x_2x_3y_1 \mid k, 0) = \frac{6}{\binom{k+2}{2}}r(x_1x_2y_1 \mid k-1, 0) + \frac{\binom{k-2}{2}}{\binom{k+2}{2}}r(x_1x_2x_3y_1 \mid k-1, 0)$ for $k \geq 4$,

3. $r(x_1x_2x_3y_1 \mid 4, \ell) = \frac{1}{2}r(x_1x_2y_1 \mid 3, \ell-1)$ for $\ell = 1, 2, 3$,

4. $r(x_1x_2x_3y_1 \mid k, \ell) = \frac{3}{\binom{k}{2}}r(x_1x_2y_1 \mid k-1, \ell-1) + \frac{\binom{k-3}{2}}{\binom{k}{2}}r(x_1x_2x_3y_1 \mid k-1, \ell-1)$ for $k \geq 5, k > \ell \geq 1$,

5. $r(x_1x_2y_1y_2 \mid 3, \ell) = 1$ for $\ell = 0, 1, 2$,

6. $r(x_1x_2y_1y_2 \mid k, \ell) = \frac{1}{\binom{k}{2}}r(x_1y_1y_2 \mid k-1, \ell-1) + \frac{\binom{k-2}{2}}{\binom{k}{2}}r(x_1x_2y_1y_2 \mid k-1, \ell-1)$ for $k \geq 4, k > \ell \geq 1$,

Lemma 15 Let $U(k, \ell) =$

$$r(x_1y_1 | k, \ell) + r(x_1x_2y_1 | k, \ell) + r(x_1y_1y_2 | k, \ell) + r(x_1x_2y_1y_2 | k, \ell).$$

Then $U(k, \ell) < \frac{1}{k-2}$ for $k \geq 4, k > \ell \geq 0$.

Proof We first take up the case that $\ell = 0$, and observe by Lemmas 10 and 14 that for $k \geq 4$,

$$U(k, 0) = \frac{1}{\binom{k+2}{2}} \left(1 + (2k+3) (r(x_1y_1 | k-1, 0) + r(x_1y_1y_2 | k-1, 0)) - r(x_1y_1y_2 | k-1, 0) + \binom{k-2}{2} U(k-1, 0) \right).$$

Since

$$U(3, 0) = \frac{1}{5} + \frac{1}{5} + \frac{1}{5} + 1 = \frac{8}{5},$$

we see

$$U(4, 0) = \frac{1}{15} \left(1 + 11 \left(\frac{1}{5} + \frac{1}{5} \right) - \frac{1}{5} + 1 \cdot \frac{8}{5} \right) = \frac{34}{75} < \frac{1}{4-2},$$

establishing the $k = 4, \ell = 0$ case. Proceeding inductively for $k \geq 5$, and using Lemma 11 (2), we have

$$U(k, 0) < \frac{1}{\binom{k+2}{2}} \left(1 + (2k+3) \frac{1}{k-2} - 0 + \binom{k-2}{2} \frac{1}{k-3} \right) = \frac{k^2 + 2k + 6}{k^2 + 3k + 2} \cdot \frac{1}{k-2} < \frac{1}{k-2}.$$

For $\ell > 0$, if $k \geq 4, k > \ell \geq 1$, Lemmas 10 and 14 show

$$U(k, \ell) = \frac{1}{\binom{k}{2}} \left(\binom{k-2}{2} U(k-1, \ell-1) + (k-1) (r(x_1y_1 | k-1, \ell-1) + r(x_1y_1y_2 | k-1, \ell-1)) \right). \tag{14}$$

In particular, since

$$U(3, 1) = \frac{1}{9} + \frac{1}{9} + \frac{1}{3} + 1 = \frac{14}{9},$$

$$U(3, 2) = 0 + 0 + \frac{1}{3} + 1 = \frac{4}{3},$$

then

$$\begin{aligned}
 U(4, 1) &= \frac{1}{6} \left(\frac{8}{5} + 3 \left(\frac{1}{5} + \frac{1}{5} \right) \right) = \frac{7}{15} < \frac{1}{4-2}, \\
 U(4, 2) &= \frac{1}{6} \left(\frac{14}{9} + 3 \left(\frac{1}{9} + \frac{1}{3} \right) \right) = \frac{13}{27} < \frac{1}{4-2}, \\
 U(4, 3) &= \frac{1}{6} \left(\frac{4}{3} + 3 \left(0 + \frac{1}{3} \right) \right) = \frac{7}{18} < \frac{1}{4-2},
 \end{aligned}$$

providing, along with the cases with $\ell = 0$, the base cases for induction. Now for $k \geq 5, k > \ell \geq 1$, we see from Eq. (14), Lemma 11 (2), and an inductive hypothesis that

$$\begin{aligned}
 U(k, \ell) &< \frac{1}{\binom{k}{2}} \left(\binom{k-2}{2} \frac{1}{k-3} + (k-1) \frac{1}{k-2} \right) \\
 &= \frac{k^2 - 2k + 2}{k(k-1)(k-2)} < \frac{1}{k-2}.
 \end{aligned}$$

□

Lemma 16 *Let*

$$\begin{aligned}
 S(k, \ell) &= r(x_1x_2 | k, \ell) + r(x_1x_2x_3 | k, \ell) + r(x_1x_2x_3y_1 | k, \ell) \\
 &\quad - r(x_1y_1 | k, \ell) - r(x_1y_1y_2 | k, \ell) - r(x_1x_2y_1y_2 | k, \ell).
 \end{aligned}$$

Then for $k = 4, \ell = 0, 1, 2, 3$ and for $k \geq 5, \ell = 0, S(k, \ell) = 0$. For $k \geq 5, k > \ell \geq 1, S(k, \ell) > 0$.

Proof Since for $k = 4$, the events $Sp(x_1x_2) = Sp(x_3x_4y_1y_2), Sp(x_1x_2x_3) = Sp(x_4y_1y_2)$, and $Sp(x_1x_2x_3y_1) = Sp(x_4y_2)$, so using exchangeability of the x_i and of the y_i lineages we have

$$\begin{aligned}
 r(x_1x_2 | 4, \ell) &= r(x_1x_2y_1y_2 | 4, \ell), \\
 r(x_1x_2x_3 | 4, \ell) &= r(x_1y_1y_2 | 4, \ell), \\
 r(x_1x_2x_3y_1 | 4, \ell) &= r(x_1y_1 | 4, \ell),
 \end{aligned}$$

so $S(4, \ell) = 0$ for $\ell = 0, 1, 2, 3$. For $k \geq 5$, Lemma 10 (1) implies $S(k, 0) = 0$.

For $k \geq 5, k > \ell \geq 1$, using Lemmas 10 and 14 we find

$$\begin{aligned}
 S(k, \ell) &= \frac{1}{\binom{k}{2}} \left(\binom{k-3}{2} S(k-1, \ell-1) - (k-3)U(k-1, \ell-1) + kR(k-1, \ell-1) \right. \\
 &\quad \left. + 1 + 2r(x_1y_1 | k-1, \ell-1) + r(x_1y_1y_2 | k-1, \ell-1) \right).
 \end{aligned}$$

Using an inductive hypothesis, Lemmas 15, and 12 and nonnegativity of probabilities, this implies

$$S(k, \ell) > \frac{1}{\binom{k}{2}} \left(\binom{k-3}{2} \cdot 0 - (k-3) \frac{1}{k-3} + k \cdot 0 + 1 + 2 \cdot 0 + 0 \right) = 0.$$

□

Proof of Proposition 8 On the species tree $((T, (a_1, a_2)), (b_1, b_2))$ let ρ denote the root, v the MRCA of the taxa on T and the a_i , and let c be a taxon on T . We first show that since the edge above v has positive length, then

$$\mathbb{P}(Sp(a_1c)) + \mathbb{P}(Sp(a_1a_2c)) + \mathbb{P}(Sp(a_1b_2c)) + \mathbb{P}(Sp(a_1a_2b_2c)) - \mathbb{P}(Sp(b_1c)) - \mathbb{P}(Sp(b_1a_2c)) - \mathbb{P}(Sp(b_1b_2c)) - \mathbb{P}(Sp(b_1a_2b_2c)) > 0. \quad (15)$$

To establish this, it is enough to show it holds when conditioned on whether or not the a_1 and a_2 lineages have coalesced before reaching v , and whether or not the b_1 and b_2 lineages have coalesced before reaching ρ . If both pairs have coalesced in this way, then the events $Sp(a_1c)$, $Sp(a_1b_2c)$, $Sp(a_1a_2b_2c)$, $Sp(b_1c)$, $Sp(b_1a_2c)$, and $Sp(b_1a_2b_2c)$ all have probability zero. Using a for a_1a_2 and b for b_1b_2 , we need only show

$$\mathbb{P}(Sp(ac)) - \mathbb{P}(Sp(bc)) > 0.$$

This follows immediately from Proposition 6. Similarly, the cases in which exactly one of the pairs of a_1, a_2 lineages or b_1, b_2 lineages have coalesced in the population immediately ancestral to their respective MRCAs follow from Proposition 7.

We henceforth condition on the event that the a_i lineages are distinct at v and the b_i lineages are distinct at ρ . Noticing that all eight probabilities in the expression of interest are 0 if the c lineage coalesces with any lineage below v , we further condition on the c lineage being distinct at v (so there are $k \geq 4$ lineages in total entering the population above v) and ℓ coalescent events occur between v and ρ .

Then, with $\mathcal{C} = \mathcal{C}(k, \ell)$ denoting the event that these conditioning requirements are met,

$$\begin{aligned} \mathbb{P}(Sp(a_1c) \mid \mathcal{C}) &= r(x_1x_2 \mid k, \ell), \\ \mathbb{P}(Sp(a_1a_2c) \mid \mathcal{C}) &= r(x_1x_2x_3 \mid k, \ell), \\ \mathbb{P}(Sp(a_1b_2c) \mid \mathcal{C}) &= r(x_1x_2y_1 \mid k, \ell), \\ \mathbb{P}(Sp(a_1a_2b_2c) \mid \mathcal{C}) &= r(x_1x_2x_3y_1 \mid k, \ell), \\ \mathbb{P}(Sp(b_1c) \mid \mathcal{C}) &= r(x_1y_1 \mid k, \ell), \\ \mathbb{P}(Sp(b_1a_2c) \mid \mathcal{C}) &= r(x_1x_2y_1 \mid k, \ell), \\ \mathbb{P}(Sp(b_1b_2c) \mid \mathcal{C}) &= r(x_1y_1y_2 \mid k, \ell), \\ \mathbb{P}(Sp(b_1a_2b_2c) \mid \mathcal{C}) &= r(x_1x_2y_1y_2 \mid k, \ell). \end{aligned}$$

After substituting these in to the expression in (15), from Lemma 16 we see that when conditioned on \mathcal{C} it is strictly positive for $k \geq 5$, $k \geq \ell \geq 1$ and zero for $k = 4$, $\ell = 0, 1, 2, 3$ and for $k \geq 5$, $\ell = 0$. Thus weighting the conditioned expressions by the probabilities of the \mathcal{C} and summing over all relevant k and ℓ , we see the unconditioned inequality (15) holds since T has at least 3 taxa so summands with $k \geq 5$, $\ell \geq 1$ are present.

Interchanging the a_i and b_i in inequality (15) shows the negativity of the expression on the tree $((T, (b_1, b_2)), (a_1, a_2))$. Since its vanishing on the tree $(T, ((a_1, a_2), (b_1, b_2)))$ was shown in Theorem 2, the proof is complete. \square

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