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 more than 5 taxa, with one possible 6-taxon exception.

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 a number of 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 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 [HD10] and Mr.Bayes/BEST [LP07] [RTvdM+12] perform simultaneous gene tree and species tree inference under a combined coalescent and sequence evolution model. The SVDquartets method [CK15] bypasses inference of individual gene trees, yet still gives a statistically consistent estimate of the species tree from sequence data. Alternatively, gene trees inferred by traditional phylogenetic methods can be used as input for a subsequent inference.

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of a species tree, in methods such as Rooted triple Consensus \[EESvH08\], STEM \[KCK09\], STAR \[LYPE09\], NJ\(_st\)/U-STAR/ASTRID \[LY11,ADR17,VW15\], MP-EST \[LYE10\], BUCKy \[LKDA10\] and ASTRAL-II \[MW15\]. 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 time scale used in the multispecies coalescent model on a species tree to those in sequence evolution models used on gene trees. The coalescent time scale 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 \[ADR13\] and \[ADR17\].) These methods all use topological summaries of a sample of gene trees, rather than the full gene 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\textit{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 \cite{PBL+11} for a recent discussion), and allow rooting even when no appropriate outgroup is available. The method by which we show the species tree root is identifiable depends on certain linear relationships between split probabilities, and this simple form gives hope that it can be developed into a well-founded statistical test for root location.

After setting notation in Section 2, we begin our study of split probabilities under the multispecies coalescent in Section 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. Detailed arguments appear in Appendix A.

Since split probabilities are complicated expressions that are difficult to compute for trees with more than 6 taxa, in Section 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 \cite{ADR17} on relationships between the split probabilities and the NJ\textit{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 \cite{ADR11a}, 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, higher degree ones also exist. Unfortunately a computational determination of them was successful only for 5-taxon trees, and their structure remains mysterious, but we report them in Appendix B.
In Section 5 we build on the results on linear invariants from Section 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 \textit{identifiability} result, Theorem 5.10 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 C.

2. Notation

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

By a species tree $\sigma = (\psi, \lambda)$ on $X$ we mean a rooted topological phylogenetic tree $\psi$, with leaves bijectively labelled by $X$, together with an assignment of edge weights $\lambda$ to its internal edges. These edge weights are specified in coalescent units, so that the multispecies coalescent model on $\sigma$ leads to a probability distribution on rooted gene trees with leaves labelled by $X_g$. (For more on the multispecies coalescent model as we use it, see \cite{ADR11b}.) 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 \cite{ADR11a} 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 $X_g$, though by marginalization over edge lengths and root locations, this probability distribution also leads to one 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 $\sigma$ is denoted by $P_\sigma(T)$, or simply $P(T)$ when $\sigma$ is clear from context.

A \textit{split} of a set of taxa $X$ is a bipartition $A \sqcup B$ into nonempty subsets, denoted $A|B = B|A = Sp(A) = Sp(B)$. If $\sigma$ is a species tree on $X$ then by a split on $\sigma$ we mean a split of $X$ formed by deleting a single edge of $\psi$ and grouping taxa according to the connected components of the resulting graph.
We similarly refer to splits of $X_g$, and splits of $X_g$ 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 $Sp(ac) = Sp(\{a, c\})$.

A \textit{trivial} split is one with one of the partition blocks a singleton set. Trivial splits for taxa $X$ appear on every phylogenetic tree on $X$. For $A \subset X$ we will denote the complementary set of $A$ by $\overline{A} = X \setminus A$, so that $A|\overline{A}$ is a split when $\emptyset \not= A \subsetneq X$.

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

\begin{equation}
\mathbb{P}_\sigma(A|B) = \sum_T \mathbb{P}_\sigma(T) \delta_{A|B}(T)
\end{equation}

where $\delta_{A|B}(T)$ is 1 if $A_y|B_y$ is a split on $T$, and 0 otherwise, and the sum runs over all binary unrooted topological phylogenetic trees on $X_g$. 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 \textit{clade} is simply a subset $A \subseteq X$. A clade $A$ is \textit{on} the species tree $\sigma$ if it equals the set of all leaf-descendants of some node in the tree. A \textit{quartet} is a 4-element subset of $X$ partitioned into 2-element sets, denoted as $ab|cd$, with $a, b, c, d \in X$. A quartet $ab|cd$ is \textit{on} $\sigma$ if the unrooted tree with leaves labeled $a, b, c, d$ induced from $\sigma$ has an edge separating $a, b$ from $c, d$.

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 equation (1), one first must compute probabilities of each of the $(2^n - 3)!! = 1 \cdot 3 \cdots (2n - 3)$ unrooted topological gene trees. This can be accomplished by work of [DS05] or [Wu12] 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 [VW15, ADR17] 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 [ADR11a] for clade probabilities, we have the following.
Lemma 3.1. 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,

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

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

$$= \sum_T \mathbb{P}_\sigma(T)(2n - 3) = 2n - 3.$$  

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 [ADR11a], is the content of the next Proposition. T. Warnow first asked if this might hold, and C. Ané independently provided a proof [Ané].

Proposition 3.2. Let $\sigma$ be a binary species tree on $\mathcal{X}$, with internal edge lengths $\lambda_i > \epsilon \geq 0$, and $A|B$ a split of $\mathcal{X}$. Then under the multispecies coalescent model if

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

then $A|B$ is a split on $\sigma$.

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 $\sigma$ with branch lengths $\lambda_i > \epsilon \geq 0$ and a split $A|B$ of $\mathcal{X}$ not displayed on $\sigma$ with $\mathbb{P}_\sigma(A|B) > \alpha$.

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

Now consider a non-trivial split $A|B$ not displayed on $\sigma$. Then there exist $a_1, a_2 \in A$, $b_1, b_2 \in B$ so the quartet $a_1a_2|b_1b_2$ is not displayed on $\sigma$. Thus, by [ADR11b] Section 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 $\sigma$ that form the central edge in the induced quartet tree on $a_1, a_2, b_1, b_2$. But since displaying the split $A|B$ is a subevent of displaying $A_1A_2|B_1B_2$, this implies that if $A|B$ is not displayed on $\sigma$ then

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

establishing the first claim.

For the second claim, we construct an example. For any non-trivial split $A|B$, pick $a \in A$, $b \in B$, and let $A' = A \setminus \{a\}$, $B' = B \setminus \{b\}$. Pick any
binary rooted tree $\sigma_1$ on $A'$, and any binary rooted tree $\sigma_2$ on $B'$, with
internal branch lengths greater than $\epsilon$, and consider the tree

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

Note $A|B$ is not a split on this tree, yet if $\lambda_2, \lambda_4$ are sufficiently large,
so that $A'_g$ and $B'_g$ are almost certainly clades on a gene tree, then the
probability of a gene tree displaying the split $A'_g|B'_g$ 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 $P_{\sigma}(A|B) > \alpha$.

**Corollary 3.3.** Suppose $\sigma$ is a binary species tree on $X$, with positive edge
lengths, and $A|B$ a split of $X$. Then under the multispecies coalescent model if

$$P_{\sigma}(A|B) \geq 1/3$$
	hen $A|B$ is a split on $\sigma$.

**Proof.** Set $\epsilon = 0$ in the preceding theorem. □

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:

- balanced tree $\sigma_{bal} = (((ab):x, c):y, (de):z)$
- pseudocaterpillar tree $\sigma_{ps} = (((ab):x, (de):y):z, c)$
- caterpillar tree $\sigma_{cat} = (((ab):x, c):y, d):z, e)$

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 trans-
formed 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.

**Proposition 3.4.** For the 5-taxon balanced and pseudocaterpillar species trees, \( \sigma = \sigma_{bal}, \sigma_{ps} \), with positive branch lengths,
\[
P_\sigma(Sp(ab)), P_\sigma(Sp(de)) > P_\sigma(S)
\]
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 3.5.** For the 5-taxon caterpillar species tree \( \sigma = \sigma_{cat} \) with positive branch lengths,
\[
P_\sigma(Sp(ab)) > P_\sigma(S) \quad \text{for all non-trivial splits } S \neq Sp(de), \text{ and } P_\sigma(Sp(de)) > P_\sigma(Sp(ce)).
\]

If \( P_\sigma(Sp(de)) > P_\sigma(Sp(cd)) \) 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 \( P_\sigma(Sp(de)) < P_\sigma(Sp(cd)) \), 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
\[
18 + XY^3Z^6 + 2XY^3 - 3XY - 18Y < 0. \quad (2)
\]

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.

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 ([Deg13], equation (4))
\[
18 + XY^3Z^6 + 2XY^3 + 9XY - 12X - 18Y < 0. \quad (3)
\]

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 Figure 1 occurs when \( X = 1, Y \approx 0.93498735, \) and \( Z = 0, \) so \( x = \infty, y \approx 0.06722228, z = 0. \) For values of \( y \) larger than this, regardless of the
Figure 1. The boundary of the too-greedy zone for the 5-taxon caterpillar tree $\sigma_{cat}$. 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 $((a,b),e,(c,d))$.

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 $\sigma_{cat}$, the too-greedy zone for splits coincides exactly with the too-greedy zone for clade consensus on the 4-taxon tree $(((a,b),c),d)$ [DDBR09]. This is as expected, since placing 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], the shape of the surface in Figure 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 $\infty$, the too-greedy zone in the $xy$ plane becomes larger, as shown in Figure 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 $\sigma_{cat}$, 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 \cite{DDBR09} matches precisely that for \(z = \infty\) in Figure 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 to 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 to an $n$-taxon species tree topology $\psi$ is a multivariate polynomial in $2^n - 1$ indeterminates (one for every split) which evaluates to zero at any vector of split probabilities $P_\sigma(A|B)$ arising from $\sigma = (\psi, \lambda)$, regardless of the values of $\lambda$. 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 $\psi$, is

$$n - 3 - \sum_{\text{non-trivial splits } A|B} P_\sigma(A|B).$$

That this evaluates to 0 when the split probabilities arise from the multispecies coalescent on some species tree is established by Lemma 3.1.

### 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 [ADR17]. 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 [ADR17], we know the expected U-STAR distance on a species tree $\sigma$ under the multispecies coalescent can be expressed as

$$D(a,b) = \sum_{\text{splits } A|B \text{ separating } a,b} P_\sigma(A|B),$$

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 $\sigma$.

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 $A|B$ of $X$ separates two non-empty disjoint subsets $Y_0, Y_1 \subset X$ provided $Y_i \subseteq A$ and $Y_{1-i} \subseteq B$ for one of $i = 0, 1$.

**Theorem 4.1.** Suppose $\sigma$ is an $n$-taxon species tree on $X$, and $a, b, c, d \in X$ are any four taxa for which $\sigma$ induces the quartet tree $ab|cd$. Then

$$\sum_{\text{splits } A|B \text{ separating } ac,bd} P_\sigma(A|B) = \sum_{\text{splits } A|B \text{ separating } ad,bc} P_\sigma(A|B) \leq \sum_{\text{splits } A|B \text{ separating } ab,cd} P_\sigma(A|B).$$

**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 [SS03] states that

$$d(a, c) + d(b, d) = d(a, d) + d(b, c) \geq d(a, b) + d(c, d).$$
The equality of the 4-point condition applied to the U-STAR expected distance gives

\[
\sum_{\text{splits } A|B \text{ separating } a,c} P_\sigma(A|B) + \sum_{\text{splits } A|B \text{ separating } b,d} P_\sigma(A|B) =
\sum_{\text{splits } A|B \text{ separating } a,d} P_\sigma(A|B) + \sum_{\text{splits } A|B \text{ separating } b,c} P_\sigma(A|B).
\]

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 cancelled. 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 cancelled. 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.

The inequality of the 4-point condition is

\[
\sum_{\text{splits } A|B \text{ separating } a,d} P_\sigma(A|B) + \sum_{\text{splits } A|B \text{ separating } b,c} P_\sigma(A|B) \geq
\sum_{\text{splits } A|B \text{ separating } a,b} P_\sigma(A|B) + \sum_{\text{splits } A|B \text{ separating } c,d} P_\sigma(A|B).
\]

By reasoning similar to before, only probabilities of splits separating two of \(a, b, c, d\) from the others remain after cancellation. Those separating \(ac\) and \(bd\) occur in all four sums, and hence can be cancelled. Those separating \(ab\) and \(cd\) occur twice on the left side, and those separating \(ad\) and \(bc\) occur twice on the right. After all cancellation and division by 2 we have

\[
\sum_{\text{splits } A|B \text{ separating } ab,cd} P_\sigma(A|B) \geq \sum_{\text{splits } A|B \text{ separating } ad,bc} P_\sigma(A|B),
\]

as claimed. \(\square\)

Since the invariants of Theorem 4.1 depend only on displayed quartets, they hold for all rooted versions of a fixed unrooted species topology.

**Example 4.2.** We apply the theorem to a species tree with unrooted topology \(((a, b), c, (d, e))\). With

\[s_1 = P(Sp(ab)), \quad s_2 = P(Sp(ac)), \quad s_3 = P(Sp(ad)), \quad s_4 = P(Sp(ac)), \quad s_5 = P(Sp(bc)), \quad s_6 = P(Sp(bd)), \quad s_7 = P(Sp(be)), \quad s_8 = P(Sp(cd)), \quad s_9 = P(Sp(ce)), \quad s_{10} = P(Sp(de)),\]
by Theorem 4.1 for each quartet on the species tree we obtain an equality and inequality:

\[
\begin{align*}
ab|cd &: s_3 + s_5 = s_2 + s_6 \leq s_1 + s_8, \\
ab|ce &: s_4 + s_5 = s_2 + s_7 \leq s_1 + s_9, \\
ab|de &: s_3 + s_7 = s_4 + s_6 \leq s_1 + s_{10}, \\
ac|de &: s_3 + s_9 = s_4 + s_8 \leq s_2 + s_{10}, \\
bc|de &: s_6 + s_9 = s_7 + s_8 \leq s_5 + s_{10}.
\end{align*}
\]

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 [DGPS16], by computing and intersecting the ideals of invariants for the 7 rooted versions of the tree. Doing so shows that the invariants given here span the full space of linear invariants for the unrooted tree. There is also a quadratic invariant in a Gröbner basis for the ideal, 

\[
(s_2 - s_5)(s_3 - s_4) = 0,
\]

which reflects the fact that for each of the rooted trees either \( s_2 = s_5 \) or \( s_3 = s_4 \). 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.

**Example 4.3.** 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 given by Theorem 4.1:

\[
s_{ace|bdf} - s_{acf|bde} + s_{ade|bcf} - s_{adf|bce}.
\]

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 to each non-trivial clade on the species tree. The existence of these *clade-induced split invariants*, as given in Theorem 4.4 below, will form the basis of arguments in Section 5 that the root of the species tree can be identified from split probabilities.
Theorem 4.4. Let $\mathcal{A} \subset \mathcal{X}$ be a subset of taxa with $|\mathcal{A}|, |\mathcal{A}| \geq 2$. Choose $\emptyset \neq \mathcal{C} \subset \mathcal{A}$, and distinct $a, b \in \mathcal{A}$. Let $\mathcal{A}' = \mathcal{A} \setminus \{a, b\}$.

Then if $\mathcal{A}$ is a clade on $\sigma$, (5) 
\[
\sum_{S \subseteq \mathcal{A}'} P_\sigma(Sp(S \cup \{a\} \cup \mathcal{C})) - \sum_{S \subseteq \mathcal{A}'} P_\sigma(Sp(S \cup \{b\} \cup \mathcal{C})) = 0.
\]

We note that this theorem applies to any species tree, including non-binary ones. Moreover, since a non-binary species tree $\sigma$ 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 $\sigma$ 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 $\sigma$’ can be replaced with ‘if $\mathcal{A}$ is a clade on a binary resolution of $\sigma$.’

Proof of Theorem 4.4. We derive this result in part using ideas developed for the construction of invariants for clade probabilities in [ADR11a].

Let $\text{Cl}(\mathcal{A})$ represent the event that $\mathcal{A}$ is a clade on an observed gene tree. Then note that 
\[
P_\sigma(Sp(\mathcal{A})) = P_\sigma(\text{Cl}(\mathcal{A})) + P_\sigma(\text{Cl}(\mathcal{A}), \text{Cl}(\bar{\mathcal{A}})) - P_\sigma(\text{Cl}(\mathcal{A}), \text{Cl}(\text{Cl}(\bar{\mathcal{A}}))).
\]

Thus equation (5) will follow from establishing the three equalities:

(6) 
\[
\sum_{S \subseteq \mathcal{A}'} P_\sigma(\text{Cl}(S \cup \{a\} \cup \mathcal{C})) - \sum_{S \subseteq \mathcal{A}'} P_\sigma(\text{Cl}(S \cup \{b\} \cup \mathcal{C})) = 0,
\]

(7) 
\[
\sum_{S \subseteq \mathcal{A}'} P_\sigma(\text{Cl}(\bar{S} \cup \{a\} \cup \mathcal{C})) - \sum_{S \subseteq \mathcal{A}'} P_\sigma(\text{Cl}(\bar{S} \cup \{b\} \cup \mathcal{C})) = 0,
\]

(8) 
\[
\sum_{S \subseteq \mathcal{A}'} P_\sigma(\text{Cl}(S \cup \{a\} \cup \mathcal{C}), \text{Cl}(\bar{S} \cup \{a\} \cup \mathcal{C}))
\]
\[
- \sum_{S \subseteq \mathcal{A}'} P_\sigma(\text{Cl}(S \cup \{b\} \cup \mathcal{C}), \text{Cl}(\bar{S} \cup \{b\} \cup \mathcal{C})) = 0,
\]

That equation (6) holds is Theorem 6 of [ADR11a]. To establish equation (7), for any $S \subseteq \mathcal{A}'$, let $\tilde{S} = \mathcal{A}' \setminus S$, and $\tilde{\mathcal{C}} = (\mathcal{A}) \setminus \mathcal{C}$. With this notation
\[
\bar{S} \cup \{a\} \cup \mathcal{C} = \tilde{S} \cup \{b\} \cup \tilde{\mathcal{C}},
\]
where $\tilde{S} \subseteq \mathcal{A}'$ and $\emptyset \neq \tilde{\mathcal{C}} \subset \mathcal{X} \setminus \mathcal{A}$. We thus see equation (7) is another instance of the equation (6). (It is essential here that $\mathcal{C}$ be a proper subset of $\mathcal{A}$, so that $\tilde{\mathcal{C}}$ is nonempty: this is why $\mathcal{A}$ must exclude at least 2 taxa.)

Establishing equation (8) requires more argument. Using the above notation, it can be restated as
\[
\sum_{S \subseteq \mathcal{A}'} P_\sigma(\text{Cl}(S \cup \{a\} \cup \mathcal{C}), \text{Cl}(\tilde{S} \cup \{b\} \cup \tilde{\mathcal{C}}))
\]
\[
- \sum_{S \subseteq \mathcal{A}'} P_\sigma(\text{Cl}(S \cup \{b\} \cup \mathcal{C}), \text{Cl}(\tilde{S} \cup \{a\} \cup \tilde{\mathcal{C}})) = 0.
\]
We establish this by showing a version of it conditioned on the partition of \( A \) corresponding to lineages present at the MRCA(\( A \)) in the species tree. Now consider any realization of the remainder of the coalescent process (i.e., on \( \sigma \) with edges below MRCA(\( A \)) removed) which displays the clades \( S_g \cup \{ A \} \cup \bar{C}_g \) and \( \hat{S}_g \cup \{ B \} \cup \bar{C}_g \). First note that \( A \) and \( B \) are in different partition sets at MRCA(\( A \)), 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 \( \hat{S}_g \cup \{ B \} \cup \bar{C}_g \) and \( \hat{S}_g \cup \{ A \} \cup \bar{C}_g \). Here \( \hat{S} \) is still a subset of \( A' \), but generally differs from \( S \) because some elements of \( S \) are in the partition sets with \( A \) and \( B \) at MRCA(\( A \)). 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 \( A \), weighted by their probabilities, gives the unconditioned equation (8).

**Example 4.5.** Here we explicitly give the clade-induced split invariants of Theorem 4.4 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 \( A = \{ a,b \} \), so \( A' = \emptyset \). With \( \mathcal{C} = \{ c \} \), equation (5) becomes

\[
\mathbb{P}_\sigma(Sp(ac)) - \mathbb{P}_\sigma(Sp(bc)) = 0.
\]

For other choices of singleton \( \mathcal{C} \) we find

\[
\mathbb{P}_\sigma(Sp(ad)) - \mathbb{P}_\sigma(Sp(bd)) = 0,
\]
\[
\mathbb{P}_\sigma(Sp(ae)) - \mathbb{P}_\sigma(Sp(be)) = 0.
\]

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

\[
\mathbb{P}_\sigma(Sp(ad)) + \mathbb{P}_\sigma(Sp(acd)) - \mathbb{P}_\sigma(Sp(bd)) - \mathbb{P}_\sigma(Sp(bcd)) = 0,
\]
\[
\mathbb{P}_\sigma(Sp(ae)) + \mathbb{P}_\sigma(Sp(ace)) - \mathbb{P}_\sigma(Sp(be)) - \mathbb{P}_\sigma(Sp(bce)) = 0,
\]
both of which were already implied by the cherry-swapping invariants. But using \( a \) and \( c \) as the single taxa we get

\[
\mathbb{P}_\sigma(Sp(ad)) + \mathbb{P}_\sigma(Sp(abd)) - \mathbb{P}_\sigma(Sp(cd)) - \mathbb{P}_\sigma(Sp(bcd)) = 0,
\]
\[
\mathbb{P}_\sigma(Sp(ae)) + \mathbb{P}_\sigma(Sp(abe)) - \mathbb{P}_\sigma(Sp(ce)) - \mathbb{P}_\sigma(Sp(bce)) = 0.
\]

However, these are the same, up to a sign. There is an additional invariant with \( a \) and \( b \) interchanged from this last one, which is obtained with \( b, c \) chosen as the singletons. Alternately, it follows from the last one using the “cherry-swapping” exchangeability of lineages for \( a \) and \( b \).
A computation with the algebra software Singular shows these 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 5.5 (a) below.

**Balanced tree** (((((a, b), c), e), d)): 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,
\[ \mathbb{P}_\sigma(\text{Sp}(ad)) - \mathbb{P}_\sigma(\text{Sp}(ae)) = 0. \]
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} From the first of these clades we obtain the invariants
\[ \mathbb{P}_\sigma(\text{Sp}(ac)) - \mathbb{P}_\sigma(\text{Sp}(bc)) = 0, \]
\[ \mathbb{P}_\sigma(\text{Sp}(ad)) - \mathbb{P}_\sigma(\text{Sp}(bd)) = 0, \]
\[ \mathbb{P}_\sigma(\text{Sp}(ae)) - \mathbb{P}_\sigma(\text{Sp}(be)) = 0, \]
and for the second
\[ \mathbb{P}_\sigma(\text{Sp}(ad)) - \mathbb{P}_\sigma(\text{Sp}(ae)) = 0, \]
\[ \mathbb{P}_\sigma(\text{Sp}(bd)) - \mathbb{P}_\sigma(\text{Sp}(be)) = 0, \]
\[ \mathbb{P}_\sigma(\text{Sp}(cd)) - \mathbb{P}_\sigma(\text{Sp}(ce)) = 0. \]
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.

For the 5-taxon pseudocaterpillar, a computation with Singular produces a 6-dimensional space of linear invariants, with
\[ \mathbb{P}_\sigma(\text{Sp}(ab)) + 2\mathbb{P}_\sigma(\text{Sp}(bc)) - 2\mathbb{P}_\sigma(\text{Sp}(ce)) - \mathbb{P}_\sigma(\text{Sp}(de)) \]
as the additional generator. Note that neither of Theorems 4.1 and 4.4 give equalities involving \( \mathbb{P}_\sigma(\text{Sp}(ab)) \) or \( \mathbb{P}_\sigma(\text{Sp}(de)) \) for this tree, so they cannot provide an explanation for this invariant. One will be given in Proposition 5.6 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 the ones above imply all higher degree invariants. For the caterpillar and balanced trees there were additional non-linear invariants in the basis. These are given in Appendix B. However, we have no theoretical understanding of them.
Table 1. Dimensions of the space of non-trivial linear invariants for 6-taxon species trees. These are defined on a space of $(2^5 - 6 - 1) = 25$ non-trivial split probabilities.

Example 4.6. 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 4.4 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 non-linear 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 equation (4), the space the linear invariants define is of dimension 24 minus the dimension 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.

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 5.5 that the rooted topological species tree is recoverable from split probabilities in most circumstances.

The following lemma is key to our argument.

Lemma 5.1. Let $\psi$ be a binary rooted topological species tree on $X$, and $X = A \sqcup D$ a disjoint union of subsets with $|A|, |D| \geq 2$. Suppose

1. $A$ is not a clade on $\psi$,
2. $D$ is not a 2-clade on $\psi$.

Then there exists some $\emptyset \neq C \subseteq D$, $a, b \in A$, and some choice of edge lengths $\lambda$ on $\psi$ such that the clade-induced split invariant of Theorem 4.4, equation

...
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 \( D \) is a clade displayed on \( \psi \).

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

Choose \( a \in \text{desc}_X(w_1) \subseteq A \), \( b \in A \setminus \text{desc}_X(v) \), and \( \emptyset \neq C = \text{desc}_X(w_2) \subseteq D \). Let all edge lengths on \( \psi \) below \( v \) have length (near) zero, edge \((u,v)\) have length (near) infinity, and the remaining edges have any finite positive length. Then a gene tree arising from the coalescent model will have (near) zero probability of displaying \( \text{Sp}(S \cup \{b\} \cup C) \) for \( S \subseteq A' \), since any displayed split (of non-negligible probability) with \( \{B\} \cup C \) in a partition set is (near) certain to contain all of \( \text{desc}_X(v) \), and hence \( A \), in that set as well. Thus all negative terms in equation (5) are negligible. On the other hand there is a positive term in that equation for \( \mathbb{P}(\text{Sp}(\text{desc}_X(v))) \), which has value (near) 1. Thus equation (5) does not hold.

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

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

\[ ((((((d_1, d_2), d_3), a), c_1), \ldots), c_n), b) \]

with at least 5 taxa, \( A = \{a, b, c_1, \ldots, c_n\} \) with \( n \geq 0, a, b \) chosen as shown. Let \( w = \text{MRCA}(D) \), and \( v \) its parent, so \( v \) is also the parent of \( a \in A \). Let \( C = \{d_3\} \). Choose all internal edge lengths of \( \psi \) to be (near) infinite, except for those below \( v \) which we choose to be (near) zero. Then all rooted gene trees realizable with non-negligible probability will be formed by \( D_g \cup \{A\} \) coalescing into some rooted gene tree in the branch above \( v \), with this subtree then joining to the remaining taxa in \( A \) in a tree that otherwise exactly matches the caterpillar structure of \( \psi \). Thus the event \( \text{Sp}(S \cup \{a\} \cup C) \) is non-negligibly realizable only for \( S = \emptyset \), by gene trees with the rooted subtree on \( D_g \cup \{A\} \) having \( \{D_3, A\} \) as a clade. But the probability of this 2-clade forming is (near) \( 4/18 = 2/9 \), since of the 18 ranked rooted trees on 4 taxa, four have any given 2-clade. On the other hand, the event \( \text{Sp}(S \cup \{b\} \cup C) \) is non-negligibly realizable only for \( S = \{c_1, \ldots, c_n\} \), by gene
trees where the 4-taxon rooted subtree on $D_4 \cup \{ A \}$ has $D_3$ as an outgroup. Such gene trees occur with probability $3/18 = 1/6$. Thus the invariant of equation (5) evaluates (near) to $2/9 - 1/6$, and is thus not zero.

Now for the general case, in which $D$ is a clade with 3 or more elements, by picking some internal edges of $\psi$ within the subtree on $D$ to have (near) infinite and zero lengths we can ensure that with probability (near) 1 that $D$ coalesces into exactly 3 lineages by MRCA($D$). Similarly by picking (near) infinite edge lengths for those edges leading off of the path between MRCA($D$) and the root of $\psi$ to groups of elements in $A$, 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. □

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

**Proposition 5.2.** Let $\psi$ be a species tree topology on $X$, and $X = A \sqcup D$ a disjoint union of subsets with $|D| = 2$. Then if $D$ is a clade on $\psi$, the polynomials defined for $A$ by equation (5) in Theorem 4.4 all vanish, regardless of whether $A$ is a clade on $\psi$.

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

$$P_\sigma(Sp(S \cup \{ a \} \cup \{ d_1 \})) = P_\sigma(Sp(S \cup \{ a \} \cup \{ d_2 \})) = P_\sigma(Sp(S \cup \{ b \} \cup \{ d_1 \})),$$

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

From Theorem 4.4 we obtain the following.

**Corollary 5.3.** Let $\psi$ be a rooted binary species tree topology on at least 5 taxa $X$, where $X = A \sqcup D$ is a disjoint union of subsets with $|A|, |D| \geq 2$. If $A$ is not a clade on $\psi$ and $D$ is not a 2-clade, then for generic choices of internal edge lengths $\lambda$ (i.e., all except those in some set of measure zero) there exists some $\emptyset \neq C \subseteq D$, $a, b \in A$, such that the corresponding clade-induced split invariant of equation (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 $\sigma$ 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 5.1 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.

**Corollary 5.4.** Let \(\psi\) be a rooted binary species tree topology on a set \(X\) of at least 5 taxa. For generic edge lengths \(\lambda\), all clades on \(\psi\) 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 \(\psi\) excluding exactly two taxa can similarly be identified if their complement is not a 2-clade.

**Proof.** For any subset of \(A \subseteq X\) excluding at least three taxa, if we find any invariant given by Theorem 4.4 that fails to vanish on the split probabilities for \(\sigma = (\psi, \lambda)\), then \(A\) is not a clade on \(\psi\). If all such invariants vanish, then by Corollary 5.3 either \(A\) is a clade on \(\psi\), or \(\lambda\) lies in a set of measure zero (which is dependent on \(A, C, a,\) and \(b\) used in defining the invariant).

Thus, considering all such \(A\), we can determine all clades excluding at least three taxa, unless the edge lengths \(\lambda\) 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 \(A\) excludes only two taxa, with complement \(D\). Then for generic edge lengths the non-vanishing of an appropriate invariant can detect whether \(D\) is a 2-clade. If it is not, then using this knowledge, the vanishing of all clade split invariants associated to \(A\) will identify it as a clade.

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 5.10 below by also using some inequalities, equalities alone lead to the following result.

**Corollary 5.5.** A binary rooted species tree topology can be identified from split probabilities via the clade-induced split invariants of equation (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 Figure 3.

**Proof.** Given all split probabilities computed from a species trees with generic edge lengths, we may test every subset of \(X^\prime\) omitting 3 or more taxa to see
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 5.5.

if it is a clade on \( \psi \), using Corollary 5.4. We then form a list of all such clades (including trivial ones) on \( \psi \).

If two clades on this list form a bipartition of \( \mathcal{X} \), then we have determined all clades on \( \psi \), 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 3 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 5. 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 \( \psi \) 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 \(|B| \geq 2\), then every pairwise union of the four clades except \(A \cup B\) would have been tested, so \(A \cup B\) must be a clade. As \(C \cup D\) is not a clade, this also falls into case \([1]\). 

The identifiability results of Corollary \([5.5]\) are based solely on the use of clade-induced linear invariants, that is on certain linear equalities. For-tuitously, by considering other linear invariants and inequalities, we can strengthen these results. For example, those trees in case \([4]\) of Corollary \([5.5]\) 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 \([9]\), 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 5.6.** The expression

\[
(10) \quad \mathbb{P}_\sigma(\text{Sp}(ab)) + \mathbb{P}_\sigma(\text{Sp}(ac)) + \mathbb{P}_\sigma(\text{Sp}(bc)) - \mathbb{P}_\sigma(\text{Sp}(de)) - \mathbb{P}_\sigma(\text{Sp}(cd)) - \mathbb{P}_\sigma(\text{Sp}(ce))
\]

evaluates to 0 for the species tree \(((a,b), (d,e), c))\). Assuming all species tree edge lengths are finite and positive, expression \((10)\) is positive for the species tree \(((a,b), (c, (d,e)))\) and negative for the species tree \(((a,b), (d,e)))\).

**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\((a,b, c))\) in the species tree. To show expression \((10)\) evaluates to 0, it is enough to show this conditioned on disjoint and exhaustive events. To this end, we compute \((10)\) 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 \(\text{Sp}(ab)\) and \(\text{Sp}(de)\) are equal by exchangeability. Similarly, the other 4 split probabilities appearing in formula \((10)\) are all equal. Thus all terms cancel.

Given that coalescences occurs on both \(e_1\) and \(e_2\), then the probabilities of \(\text{Sp}(ab)\) and \(\text{Sp}(de)\) 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 \(\text{Sp}(ab)\) has probability 1, while \(\text{Sp}(ac)\) and \(\text{Sp}(bc)\) have probability 0. The next coalescent event produces the only other non-trivial split of the gene tree, which must be one of \(\text{Sp}(CD), \text{Sp}(CE), \text{Sp}(DE)\). Thus \(\mathbb{P}(\text{Sp}(cd)) + \mathbb{P}(\text{Sp}(ce)) + \mathbb{P}(\text{Sp}(de)) = 1\), 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 \((10)\) is always non-negative, 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 \([10]\) 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 \([10]\) is 1. If $Sp(AB)$ formed, then exactly one of $Sp(DE), Sp(CD), Sp(CE)$ forms, and the expression in \([10]\) is zero. If $Sp(AB)$ does not form, say instead $Sp(AC)$ does, then neither $Sp(DE)$ 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 \([10]\) is positive. Similarly, if $Sp(BC)$ forms, then the expression is positive.

If there are no coalescences on $e_1, e_2, e_3$, 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.

To address root identifiability in case 1 of Corollary 5.5, we have the following.

**Proposition 5.7.** 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 $\psi$ 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.\[\text{C}\]

For case 2 we follow a similar tack, focusing on a split invariant for the clade ab1b2 on the tree (T, (a, (b1, b2))). The proof of the following is also in Appendix.\[\text{C}\]

**Proposition 5.8.** 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 \(\psi\) is one of the species trees \(((T, (a, b_2)), (T, (b_1, b_2)), a), (T, (a, (b_1, b_2))),\) and \(\sigma = (\psi, \lambda)\) has positive length edges incident to the root. Then

\[
\Pr(\text{Sp}(ac)) + \Pr(\text{Sp}(ab_2c)) - \Pr(\text{Sp}(b_1c)) - \Pr(\text{Sp}(b_1b_2c))
\]

\[
\begin{aligned}
&> 0 \text{ if and only if } \psi = (T, (a, (b_1, b_2))) \\
&= 0 \text{ if and only if } \psi = (T, (a, (b_1, b_2))) \\
&< 0 \text{ if and only if } \psi = (T, (a, (b_1, b_2)))
\end{aligned}
\]

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

**Proposition 5.9.** 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 \(\psi\) 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,

\[
\Pr(\text{Sp}(a_1c)) + \Pr(\text{Sp}(a_1a_2c)) + \Pr(\text{Sp}(a_1b_2c)) + \Pr(\text{Sp}(a_1a_2b_2c))
\]

\[
- \Pr(\text{Sp}(b_1c)) - \Pr(\text{Sp}(b_1a_2c)) - \Pr(\text{Sp}(b_1b_2c)) - \Pr(\text{Sp}(b_1a_2b_2c))
\]

\[
\begin{aligned}
&> 0 \text{ if and only if } \psi = ((T, (a_1, a_2)), (b_1, b_2)) \\
&= 0 \text{ if and only if } \psi = (T, ((a_1, a_2), (b_1, b_2))) \\
&< 0 \text{ if and only if } \psi = (T, (b_1, b_2)), (a_1, a_2)
\end{aligned}
\]

We summarize these results with the following.

**Theorem 5.10.** 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 Theorem 5.5, 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, non-linear 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.
SPLIT PROBABILITIES

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

|    | \( \sigma_{bal} \)                                      | \( \sigma_{pc} \)                                      |
|----|--------------------------------------------------------|--------------------------------------------------------|
| \( s_1 \) | 1 - \( \frac{4}{15}XZ^3 - \frac{2}{3}X \)          | 1 - \( \frac{1}{15}XYZ^6 - \frac{1}{5}XY - \frac{2}{3}X \) |
| \( s_2, s_5 \) | \( \frac{1}{15}XY^3Z + \frac{1}{3}X \)             | \( \frac{1}{15}XYZ^6 - \frac{1}{5}XY + \frac{2}{3}X \) |
| \( s_3, s_4, s_6, s_7 \) | \( \frac{1}{15}XY^3Z + \frac{1}{3}XYZ \)      | \( -\frac{1}{15}XYZ^6 + \frac{2}{3}XY \)               |
| \( s_8, s_9 \) | 1 - \( \frac{1}{5}XY^3Z - \frac{1}{3}XYZ + \frac{1}{3}YZ \) | 1 - \( \frac{1}{15}XY^3Z^6 - \frac{1}{5}XY + \frac{3}{3}Y \) |
| \( s_{10} \) | 1 + \( \frac{1}{5}XY^3Z - \frac{3}{5}XYZ - \frac{2}{5}Y \) | 1 - \( \frac{1}{5}XY^3Z^6 - \frac{1}{5}XY - \frac{2}{3}Y \) |

\[ \sigma_{cat} \]

|    | \( \sigma_{cat} \)                                      |
|----|--------------------------------------------------------|
| \( s_1 \) | 1 - \( \frac{1}{15}XY^3Z^6 - \frac{1}{5}XY^2 - \frac{4}{3}X \) |
| \( s_2, s_5 \) | \( -\frac{1}{15}XY^3Z^6 - \frac{1}{5}XY^2 + \frac{1}{3}X \) |
| \( s_3, s_6 \) | \( -\frac{1}{15}XY^3Z^6 + \frac{1}{5}XY^2 + \frac{3}{5}X \) |
| \( s_4, s_7 \) | \( \frac{1}{15}XY^3Z^6 + \frac{1}{5}XY \)               |
| \( s_8 \) | \( -\frac{1}{15}XY^3Z^6 + \frac{1}{5}XY^2 - \frac{1}{5}XY + \frac{1}{3}Y \) |
| \( s_9 \) | \( \frac{1}{15}XY^3Z^6 - \frac{1}{5}XY + \frac{1}{3}Y \) |
| \( s_{10} \) | 1 + \( \frac{1}{5}XY^3Z^6 + \frac{1}{5}XY^2 - \frac{1}{3}XY - \frac{2}{3}Y \) |

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

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Appendix A. Greedy split consensus on 5-taxon trees: proofs

Here we prove Propositions 3.4 and 3.5 from Section 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 the enumeration of splits and their probabilities given in Example 4.2. Computations, assisted by the software COAL [DS05], produce the formulas in Table 2 for these split probabilities on the 3 species tree shapes, \( \sigma_{bal} \), \( \sigma_{pc} \), and \( \sigma_{cat} \).

Proof of Proposition 3.4. Given the equalities of split probabilities in Table 2 we need only show that \( s_1, s_{10} \geq s_2, s_3, s_8 \). Note that positive branch lengths imply \( 0 < X, Y, Z < 1 \).

Then, for \( \sigma_{bal} \), one finds \( s_1 - s_2 = 1 - X > 0 \).
\[ s_1 - s_3 = 1 - \frac{1}{6}XY^3Z - \frac{2}{3}X - \frac{1}{6}XYZ > 1 - \frac{1}{3} - \frac{1}{6} = 0, \]
\[ s_1 - s_8 = 1 - \frac{1}{6}XY^3Z - \frac{2}{3}X + \frac{1}{6}XYZ - \frac{1}{2}YZ \\
= 1 + \frac{1}{6}XYZ(1 - Y^2) - \frac{2}{3}X - \frac{1}{3}YZ \\
> 1 + 0 - \frac{2}{3} - \frac{1}{3} = 0, \]
\[ s_{10} - s_2 = 1 + \frac{1}{3}XY^3Z - \frac{1}{3}XYZ - \frac{1}{3}X + \frac{2}{3}YZ \\
= 1 + \frac{Z}{3} (XY^3 - XY - 2Y) - \frac{1}{3}X > 1 - \frac{2}{3} - \frac{1}{3} = 0 \\
\text{since } XY^3 - XY - 2Y \text{ has minimum } -2 \text{ on the unit square,} \]
\[ s_{10} - s_3 = 1 + \frac{1}{6}XY^3Z - \frac{1}{2}XYZ - \frac{2}{3}YZ \\
= 1 + \frac{1}{6}XZ(Y^3 - 3Y) - \frac{2}{3}YZ > 1 + \frac{1}{6}(-2) - \frac{2}{3} = 0 \\
\text{since the minimum of } Y^3 - 3Y \text{ is } -2 \text{ on } [0, 1], \]
\[ s_{10} - s_8 = 1 + \frac{1}{6}XY^3Z - \frac{1}{6}XYZ - YZ \\
= 1 + \frac{Z}{6} (XY^3 - XY - 6Y) > 1 + \frac{1}{6}(-6) = 0, \\
\text{since } XY^3 - XY - 6Y \text{ has minimum } -6 \text{ on the unit square when } Y = 1. \]

For \( \sigma_{ps} \),

\[ s_1 - s_2 = 1 + \frac{1}{18}XY + \frac{1}{18}XYZ^6 - X \\
= 1 + \frac{1}{18}XY (1 - Z^6) - X > 1 + 0 - 1 = 0, \]
\[ s_1 - s_3 = 1 - \frac{1}{3}XY - \frac{2}{3}X > 1 - \frac{1}{3} - \frac{2}{3} = 0, \]
\[ s_1 - s_8 = 1 + \frac{1}{18}XY - \frac{1}{18}XYZ^6 - \frac{2}{3}X - \frac{1}{3}Y \\
= 1 + \frac{1}{18}XY (1 - Z^6) - \frac{2}{3}X - \frac{1}{3}Y > 1 + 0 - \frac{2}{3} - \frac{1}{3} = 0, \]
\[ s_{10} - s_2 = 1 + \frac{1}{18}XY - \frac{1}{18}XYZ^6 - \frac{1}{3}X - \frac{2}{3}Y \\
= 1 + \frac{1}{18}XY (1 - Z^6) - \frac{1}{3}X - \frac{2}{3}Y > 1 + 0 - \frac{1}{3} - \frac{2}{3} = 0, \]
\[ s_{10} - s_3 = 1 - \frac{1}{3}XY - \frac{2}{3}Y > 1 - \frac{1}{3} - \frac{2}{3} = 0, \]
\[ s_{10} - s_8 = 1 + \frac{1}{18}XY - \frac{1}{18}XYZ - Y \]
\[ = 1 + \frac{1}{18}XY(1 - Z) - Y > 1 + 0 - 1 = 0. \]

\[ \Box \]

**Proof of Proposition 3.5.** We begin by showing that \( s_1 > s_i \) for \( i = 2, \ldots, 9 \), using \( 0 < X, Y, Z < 1 \). One need only check that

\[
\begin{align*}
\text{s}_1 - s_2 &= 1 - X > 0, \\
\text{s}_1 - s_3 &= 1 - \frac{1}{6}XY^3 - \frac{1}{6}XY - \frac{2}{3}X > 0, \\
\text{s}_1 - s_4 &= 1 - \frac{1}{18}XY^3Z^6 - \frac{1}{9}XY^3 - \frac{1}{6}XY - \frac{2}{3}X > 0, \\
\text{s}_1 - s_8 &= 1 - \frac{1}{6}XY^3 + \frac{1}{6}XY - \frac{2}{3}X - \frac{1}{3}Y \\
&= 1 + \frac{1}{6}XY(1 - Y^2) - \frac{2}{3}X - \frac{1}{3}Y > 0, \\
\text{s}_1 - s_9 &= 1 - \frac{1}{18}XY^3Z^6 - \frac{1}{9}XY^3 + \frac{1}{6}XY - \frac{2}{3}X - \frac{1}{3}Y \\
&= 1 + \frac{1}{18}XY(3 - Y^2Z^6 - 2Y^2) - \frac{2}{3}X - \frac{1}{3}Y > 0
\end{align*}
\]

since \( 3 - Y^2Z^6 - 2Y^2 > 0 \).

Suppose now that \( s_{10} \geq s_1 \). Then since \( s_1 \) 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 \( s_1 > s_{10} \), so \( s_1 \) is the strict maximum of the split probabilities. Under the greedy consensus algorithm, splits incompatible with \( Sp(ab) \) are discarded and only the splits \( s_8, s_9, \) and \( s_{10} \) remain as candidate splits for acceptance by the algorithm.

Noting that

\[
\begin{align*}
\text{s}_{10} - s_9 &= 1 + \frac{1}{6}XY^3 - \frac{1}{6}XY - Y \\
&= (1 - Y)
\left(1 - \frac{1}{6}XY(1 + Y)\right),
\end{align*}
\]

and that \( (1 - \frac{1}{6}XY(1 + Y)) > \frac{2}{3} \), it follows that \( s_{10} > s_9 \).

Consider now

\[
\text{s}_{10} - s_8 = 1 + \frac{1}{18}XY^3Z^6 + \frac{1}{9}XY^3 - \frac{1}{6}XY - Y = F(X,Y,Z).
\]

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 \(((a,b), e, (c,d))\). \[ \Box \]
APPENDIX B. NON-LINEAR SPLIT INVARIANTS FOR 5-TAXON TREES

While non-linear split invariants exist for species trees with 5 or more taxa, using Singular we were only able to compute them for 5-taxon trees. We record results here, using the enumeration given in Example 4.2.

For the caterpillar species tree (((((a, b), c), d), e), the ideal of invariants for non-trivial split probabilities is generated by the trivial invariant and the following eight polynomials:

\[
\begin{align*}
    s_2 - s_5, & & s_3 - s_6, \\
    s_4 - s_7, & & s_6 - s_7 - s_8 + s_9,
\end{align*}
\]

\[
\begin{align*}
    s_2^2 + s_1 s_5 - 2 s_5^2 + 14 s_1 s_7 + 4 s_5 s_7 + 24 s_7^2 + 9 s_1 s_8 - 9 s_5 s_8 + 18 s_7 s_8 - 5 s_1 s_9 - 13 s_5 s_9 - 30 s_7 s_9 \\
    - 18 s_8 s_9 + 6 s_2 - s_1 s_{10} + s_5 s_{10} + 6 s_7 s_{10} - 6 s_9 s_{10},
\end{align*}
\]

\[
\begin{align*}
    3780 s_2^2 s_7 - 18163 s_1 s_2^2 - 40466 s_5 s_7^2 - 36060 s_2^3 - 1512 s_1 s_5 s_8 + 1512 s_2^2 s_8 - 27516 s_1 s_7 s_8 \\
    + 9444 s_5 s_7 s_8 - 54363 s_2 s_8 - 10296 s_1 s_8^2 + 10296 s_5 s_8^2 - 21132 s_7 s_8^2 - 1008 s_1 s_9 s_8 - 2772 s_5^2 s_9 \\
    + 14362 s_1 s_7 s_9 + 20768 s_5 s_7 s_9 + 65529 s_2 s_9 + 10356 s_1 s_8 s_9 + 7716 s_5 s_8 s_9 + 75246 s_7 s_8 s_9 \\
    + 21132 s_2 s_9^2 - 3063 s_1 s_5^2 - 985 s_8 s_5^2 - 35226 s_7 s_5^2 - 20883 s_8 s_5^2 + 5757 s_3^3 + 3620 s_1 s_7 s_{10} \\
    - 1340 s_5 s_7 s_{10} - 8397 s_3^2 s_{10} + 2424 s_1 s_8 s_{10} - 2424 s_5 s_8 s_{10} - 6744 s_7 s_8 s_{10} - 2004 s_1 s_9 s_{10} \\
    - 276 s_5 s_9 s_{10} + 12978 s_7 s_9 s_{10} + 6744 s_8 s_9 s_{10} - 4581 s_2 s_{10} + 420 s_7 s_{10} - 420 s_9 s_{10}^2,
\end{align*}
\]

\[
\begin{align*}
    63 s_1 s_5 s_7 + 126 s_2^2 s_7 - 506 s_1 s_2^2 - 28 s_5 s_7^2 - 984 s_2^3 - 771 s_1 s_7 s_8 + 321 s_5 s_7 s_8 - 1518 s_7 s_8 \\
    - 288 s_1 s_5^2 + 288 s_5 s_5^2 - 603 s_7 s_8^2 - 63 s_1 s_5 s_9 - 126 s_2^2 s_9 + 401 s_1 s_7 s_9 + 505 s_5 s_7 s_9 + 1818 s_2 s_9 \\
    + 291 s_1 s_8 s_9 + 159 s_5 s_8 s_9 + 2118 s_7 s_8 s_9 + 603 s_8 s_9 - 87 s_1 s_9^2 - 258 s_5 s_9^2 - 999 s_7 s_9^2 - 600 s_8 s_9^2 \\
    + 165 s_9^3 + 139 s_1 s_8 s_{10} - 25 s_5 s_7 s_{10} - 234 s_2 s_{10} + 96 s_1 s_8 s_{10} - 96 s_5 s_8 s_{10} - 186 s_7 s_{10} \\
    - 75 s_1 s_9 s_{10} - 38 s_5 s_9 s_{10} + 378 s_7 s_9 s_{10} + 186 s_8 s_9 s_{10} - 144 s_2 s_{10} + 21 s_7 s_{10} - 21 s_9 s_{10}^2,
\end{align*}
\]

\[
\begin{align*}
    19845 s_1 s_5^2 + 39690 s_5^2 + 209186 s_1 s_7^2 - 49028 s_5 s_7^2 + 467400 s_2^3 + 16254 s_1 s_5 s_8 + 20601 s_5 s_8 \\
    + 310092 s_1 s_7 s_8 - 257748 s_5 s_7 s_8 + 670146 s_2 s_8 + 112797 s_1 s_8^2 - 183672 s_5 s_8^2 + 212904 s_7 s_8 \\
    - 25515 s_2^3 + 25326 s_5 s_9 + 102249 s_2^2 s_9 - 158744 s_1 s_7 s_9 + 20084 s_5 s_7 s_9 - 729018 s_7 s_9 \\
    - 112962 s_1 s_8 s_9 + 100308 s_5 s_8 s_9 - 785172 s_7 s_8 s_9 - 198729 s_8 s_9 + 33891 s_1 s_9^2 + 55176 s_5 s_9 \\
    + 381852 s_7 s_9^2 + 219921 s_8 s_9 - 63129 s_9^3 + 11970 s_1 s_8 s_{10} + 4095 s_5 s_8 s_{10} - 52900 s_7 s_{10} \\
    - 93860 s_5 s_7 s_{10} - 127746 s_7 s_{10} - 35898 s_8 s_{10} - 49152 s_5 s_8 s_{10} - 236352 s_7 s_{10} \\
    - 112455 s_5 s_{10} + 32538 s_1 s_{10} + 89652 s_5 s_{9} s_{10} + 62244 s_7 s_{9} s_{10} + 71922 s_8 s_{9} s_{10} \\
    + 13527 s_5 s_{10} - 520 s_1 s_{10} - 560 s_5 s_{10}^2 - 31080 s_7 s_{10}^2 - 21105 s_8 s_{10}^2 + 2095 s_9 s_{10}^2 \\
    - 2205 s_{10}^2,
\end{align*}
\]
The four linear invariants here are all given by theorems in the text, but we have no theoretical explanation for the form of the quadratic and 3 cubics.

For the species tree (((a,b),c),(d,e)), the ideal of invariants for non-trivial split probabilities is generated by the trivial invariant and the following six polynomials:

\[
\begin{align*}
    s_2 - s_5, & \quad s_3 - s_6, & \quad s_3 - s_4 \\
    s_6 - s_7, & \quad s_8 - s_9 \\
    s_1^2 + s_1 s_5 - 2 s_5^2 + 14 s_1 s_7 + 4 s_5 s_7 + 24 s_7^2 + 4 s_1 s_9 - 22 s_5 s_9 - 12 s_7 s_9 - 12 s_5^2 - s_1 s_{10} + s_5 s_{10} \\
    & \quad + 6 s_7 s_{10} - 6 s_9 s_{10}
\end{align*}
\]

The five linear polynomials all arise from clade-induced constructions in the text, but the quadratic has not been explained.

For the species tree (((a,b),(d,e)),c), the ideal of invariants for non-trivial split probabilities is generated by the trivial invariant and the following six polynomials:

\[
\begin{align*}
    s_2 - s_5, & \quad s_3 - s_6, & \quad s_4 - s_7 \\
    s_6 - s_7, & \quad s_8 - s_9, & \quad s_1 + 2 s_5 - 2 s_9 - s_{10}
\end{align*}
\]

Note all are linear, with the first five given by the general clade-induced construction, and the last being explained by Proposition 5.6.

**Appendix C. Additional Proofs**

The proofs we give of Propositions 5.7, 5.8, and 5.9 depend on a careful analysis of probabilities under the coalescent. That of Proposition 5.7 is the simplest, and serves as a model for the others.

**C.1. Proof of Proposition 5.7**

The proof of 5.7 depends on several lemmas.

We begin with a definition. Consider a non-binary rooted species tree (((x_1,x_2,...,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 - ℓ 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 \( A \subseteq \mathcal{X} = \{x_1,x_2,...,x_k\}, \) we denote the probability that a resulting gene tree displays a split Sp(\( A,y \)) as

\[
p(A \mid k,\ell).
\]

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

\textbf{Lemma C.1.}

\begin{enumerate}
\item \(p(x_1x_2 \mid k, 0) = p(x_1y \mid k, 0)\) for \(k \geq 2\),
\item \(p(x_1x_2 \mid 3, \ell) = p(x_1y \mid 3, \ell) = \frac{1}{3}\) for \(\ell = 0, 1, 2\),
\item \(p(x_1y \mid k, 0) = \frac{1}{k+1} + \frac{k-1}{k+1}p(x_1y \mid k-1, 0)\) for \(k \geq 3\),
\item \(p(x_1x_2 \mid k, \ell) = \frac{1}{k} + \frac{k-2}{k}p(x_1x_2 \mid k-1, \ell-1)\), for \(k \geq 4\), \(k > \ell \geq 1\),
\item \(p(x_1y \mid k, \ell) = \frac{k-2}{k}p(x_1y \mid k-1, \ell-1)\) for \(k \geq 3\), \(k > \ell \geq 1\).
\end{enumerate}

\textit{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 \(1/(k+1)\), or be between \(x_i\) lineages with \(i \neq 1, 2\), which occurs with probability \((k-2)/k\), with the split forming subsequently.

We next establish some probability bounds.

\textbf{Lemma C.2.} For \(k \geq 4\), \(k > \ell \geq 0\), \(p(x_1y \mid k, \ell) < \frac{1}{k}\).

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

\[p(x_1y \mid k, 0) < \frac{1}{k+1} + \frac{k-1}{k+1} \frac{1}{k-1} = \frac{1}{k+1} < \frac{1}{k}.\]

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

\[p(x_1y \mid k, \ell) = \frac{k-1}{k} \frac{k-2}{k} \cdots \frac{3}{2} p(x_1y \mid 3, \ell - k + 3)\]

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

If instead \(k - \ell \geq 4\), Lemma C.1 (5) and what has already been established imply

\[p(x_1y \mid k, \ell) \leq \frac{(k-\ell)(k-\ell-1)}{k(k-1)} \frac{1}{k-\ell} < \frac{1}{k}.\]
Next, we obtain a key inequality.

**Lemma C.3.** For $k = 3$, $\ell = 0, 1, 2$ and for $k > 3$, $\ell = 0$,

$$p(x_1 x_2 \mid k, \ell) - p(x_1 y \mid k, \ell) = 0.$$ 

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

$$p(x_1 x_2 \mid k, \ell) - p(x_1 y \mid k, \ell) > 0.$$ 

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

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

$$p(x_1 x_2 \mid k, \ell) - p(x_1 y \mid k, \ell) > 0.$$ 

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

Finally, equation (11), an inductive hypothesis, and Lemma C.2 show that for $\ell \geq 2$

$$p(x_1 x_2 \mid k, \ell) - p(x_1 y \mid k, \ell) > 0.$$ 

□

**Proof of Proposition 5.7.** That the equality holds for species tree $(T, (a, b))$ is an instance of Theorem 4.4. 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 $\ell$ 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 $k, \ell$, let $\mathcal{C} = \mathcal{C}(k, \ell)$ denote the event that $k - 1$ agglomerated lineages from $T$ reach $v$, one of which is the lineage from $c$ alone, and that $\ell$ coalescent events occur in the population immediately ancestral to $v$. Fixing $\mathcal{C} = \mathcal{C}(k, \ell)$, with $y = b$, $x_1 = c$, $x_2 = a$ we have

$$\mathbb{P}(Sp(ac) \mid \mathcal{C}) = p(x_1 x_2 \mid k, \ell),$$

$$\mathbb{P}(Sp(bc) \mid \mathcal{C}) = p(x_1 y \mid k, \ell).$$

Lemma C.3 thus shows $\mathbb{P}(Sp(ac) \mid \mathcal{C}) - \mathbb{P}(Sp(bc) \mid \mathcal{C})$ is positive for $k \geq 4, k > \ell \geq 1$, and zero for other relevant cases. Multiplying by the probabilities of each $\mathcal{C} = \mathcal{C}(k, \ell)$ 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.

C.2. Proof of Proposition 5.8

While the proof of Proposition 5.8 follows the same line of reasoning as that of Proposition 5.7, 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 C.4.

1. $p(A \mid k, 0) = p(B \mid k, 0)$ for $|A| = |B|$,
2. $p(x_1 x_2 x_3 \mid k, 0) = \left(\frac{3}{2}\right)p(x_1 x_2 \mid k - 1, 0) + \left(\frac{k-2}{4}\right)p(x_1 x_2 x_3 \mid k - 1, 0)$
   for $k \geq 4$,
3. $p(x_1 x_2 x_3 \mid 3, \ell) = p(x_1 x_2 y \mid 3, \ell) = 1$, for $\ell = 0, 1, 2$,
4. $p(x_1 x_2 x_3 \mid 4, \ell) \frac{1}{2}p(x_1 x_2 \mid 3, \ell - 1)$ for $\ell = 1, 2, 3$,
5. $p(x_1 x_2 x_3 \mid k, \ell) = \left(\frac{3}{2}\right)p(x_1 x_2 \mid k - 1, \ell - 1) + \left(\frac{k-3}{4}\right)p(x_1 x_2 x_3 \mid k - 1, \ell - 1)$
   for $k \geq 5, k > \ell \geq 1$,
6. $p(x_1 x_2 y \mid k, \ell) = \left(\frac{3}{2}\right)p(x_1 y \mid k - 1, \ell - 1) + \left(\frac{k-2}{4}\right)p(x_1 x_2 y \mid k - 1, \ell - 1)$
   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 $Sp(X_1 X_2 X_3)$ 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
Lemma C.5. For \( k \geq 4, k > \ell \geq 0 \),

\[
p(x_{1y} | k, \ell) + p(x_{1x2y} | k, \ell) < \frac{1}{k - 2}.
\]

Proof. We first show the inequality for \( \ell = 0 \), by induction on \( k \). From Lemmas C.1 and C.4, \( p(x_{1y} | 4, 0) + p(x_{1x2y} | 4, 0) = \frac{2}{7} \), establishing the base case of \( k = 4 \).

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

\[
p(x_{1y} | k, 0) + p(x_{1x2y} | k, 0)
= \frac{1}{k+1} \left( 1 + (k+1)p(x_{1y} | k - 1, 0) + \binom{k-2}{2}(p(x_{1y} | k - 1, 0) + p(x_{1x2y} | k - 1, 0)) \right)
< \frac{1}{k+1} \left( 1 + \frac{k+1}{k-1} + \binom{k-2}{2} \frac{1}{k-3} \right)
= \frac{k^2 + k + 2}{(k+1)k(k-1)} < \frac{1}{k - 2}.
\]

Next observe that for \( \ell = 1, 2, 3 \), Lemmas C.1 and C.4 imply

\[
p(x_{1y} | 4, \ell) + p(x_{1x2y} | 4, \ell) = \frac{7}{18} < \frac{1}{4 - 2}.
\]

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 C.1 (5), Lemma C.4 (1), Lemma C.2 and an inductive hypothesis,

\[
p(x_{1y} | k, \ell) + p(x_{1x2y} | k, \ell)
= \frac{k-1}{2} \frac{1}{k-1} + \binom{k-2}{2} \frac{1}{k-3} < \frac{1}{k - 2}.
\]

Lemma C.6. Let

\[
P(k, \ell) = p(x_{1x2} | k, \ell) + p(x_{1x2x3} | k, \ell) - p(x_{1y} | k, \ell) - p(x_{1x2y} | k, \ell).
\]

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
\[
\begin{align*}
p(x_1x_2 \mid 4, \ell) &= p(x_1x_2y \mid 4, \ell), \\
p(x_1x_2x_3 \mid k, \ell) &= p(x_1y \mid k, \ell).
\end{align*}
\]
Thus \( P(4, \ell) = 0 \) for \( \ell = 0, 1, 2, 3 \). For \( k \geq 5 \), Lemma \( \text{C.4} \) implies \( P(k, 0) = 0 \).

For \( k \geq 5 \), \( \ell \geq 1 \), by Lemmas \( \text{C.1} \) \( (1), (3) \) and \( \text{C.4} \) \( (5), (6) \) we find
\[
P(k, \ell) = \frac{1}{\binom{k}{2}} \left[ 1 + (k - 2) (p(x_1x_2 \mid k - 1, \ell - 1) - p(x_1y \mid k - 1, \ell - 1)) \right.
\]
\[
- (k - 2) (p(x_1y \mid k - 1, \ell - 1) + p(x_1x_2y \mid k - 1, \ell - 1))
\]
\[
+ 2p(x_1x_2 \mid k - 1, \ell - 1) + p(x_1x_2y \mid k - 1, \ell - 1) + \binom{k - 3}{2} P(k - 1, \ell - 1) \right].
\]
Using Lemmas \( \text{C.3} \) and \( \text{C.5} \) the non-negativity of probabilities, and an inductive hypothesis that \( P(k - 1, \ell - 1) \geq 0 \), it follows that
\[
P(k, \ell) > \frac{1}{\binom{k}{2}} \left( 1 + (k - 2) \cdot 0 - (k - 2) \cdot \frac{1}{k - 2} + 2 \cdot 0 + 0 + \binom{k - 3}{2} 0 \right) = 0.
\]

\( \square \)

Lemma C.7. 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 \( \text{C.4} \).

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 \( \ell \) coalescent events occuring between \( v \) and the root.

Then, with \( C = C(k, \ell) \) denoting the events we condition on,
\[
\mathbb{P}(Sp(ac) \mid C) = p(x_1y \mid k, \ell),
\]
$$P(\text{Sp}(ab_2c) \mid C) = p(x_1x_2y \mid k, \ell),$$
$$P(\text{Sp}(b_1c) \mid C) = p(x_1x_2 \mid k, \ell),$$
$$P(\text{Sp}(b_2c) \mid C) = p(x_1x_2x_3 \mid k, \ell).$$

From Lemma C.6 we find conditioned on $C$ 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 $C$ 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.

To handle the species tree $((T, a), (b_1, b_2))$ we proceed analogously, but consider a rooted species tree $((x_1, x_2, \ldots x_k) : L, y_1, y_2)$ 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 $\ell$ 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 $X = \{x_1, \ldots x_k, y_1, y_2\}$ and $A \subset X$, let

$$r(A \mid k, \ell) = P(\text{Sp}(A) \mid k, \ell)$$

for this species tree. By exchangeability of lineages in the coalescent model, $r(A \mid k, \ell)$ depends on $A$ 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 C.8.**

\begin{enumerate}
  \item $r(A \mid k, 0) = r(B \mid k, 0)$ for $|A| = |B|$, \hfill \text{(1)}
  \item $r(x_1x_2 \mid 3, 0) = \frac{1}{2}, \  r(x_1x_2 \mid 3, \ell) = \frac{1}{\ell}$ for $\ell = 1, 2$, \hfill \text{(2)}
  \item $r(x_1x_2 \mid k, 0) = \frac{1}{\binom{k}{2}} + \frac{\binom{k}{2}}{\binom{k}{2}} r(x_1x_2 \mid k - 1, 0)$ for $k \geq 3$, \hfill \text{(3)}
  \item $r(x_1x_2 \mid k, \ell) = \frac{1}{\binom{k}{2}} + \frac{k-2}{\binom{k}{2}} r(x_1x_2 \mid k - 1, \ell - 1)$ for $k \geq 4$, $k > \ell \geq 1$, \hfill \text{(4)}
  \item $r(x_1y_1) \mid 2, 0 = \frac{1}{2}, \ r(x_1y_1) \mid 2, 1 = 0$, \hfill \text{(5)}
  \item $r(x_1y_1) \mid k, \ell) = \frac{k-1}{\binom{k}{2}} r(x_1y_1 \mid k - 1, \ell - 1)$ for $k \geq 3$, $k > \ell \geq 1$, \hfill \text{(6)}
  \item $r(x_1x_2x_3) \mid 4, \ell) = \frac{3}{2} r(x_1x_2 \mid 3, \ell - 1)$ for $\ell = 1, 2, 3$, \hfill \text{(7)}
  \item $r(x_1x_2x_3) \mid k, \ell) = \frac{3}{\binom{k}{2}} r(x_1x_2 \mid k - 1, \ell - 1) + \frac{k-3}{\binom{k}{2}} r(x_1x_2x_3 \mid k - 1, \ell - 1)$ for $k \geq 5$, $k > \ell \geq 1$, \hfill \text{(8)}
  \item $r(x_1x_2y_1) \mid 2, 0) = r(x_1x_2y_1) \mid 2, 1) = 1, \ r(x_1x_2y_1) \mid 3, 1) = \frac{1}{5}$, \hfill \text{(9)}
  \item $r(x_1x_2y_1) \mid 3, 2) = 0$, \hfill \text{(9)}
  \item $r(x_1x_2y_1) \mid k, 0) = \frac{3}{\binom{k}{2}} r(x_1x_2 \mid k - 1, 0) + \frac{k-1}{\binom{k}{2}} r(x_1x_2y_1 \mid k - 1, 0)$ for $k \geq 3$, \hfill \text{(10)}
\end{enumerate}
(11) \( r(x_1 x_2 y_1 \mid k, \ell) = \frac{1}{(k^2)} r(x_1 y_1 \mid k-1, \ell-1) + \frac{(k-2)}{(k^2)} r(x_1 x_2 y_1 \mid k-1, \ell-1) \) for \( k \geq 4 \), \( k > \ell \geq 1 \).

(12) \( r(x_1 y_1 y_2 \mid 2, 0) = r(x_1 y_1 y_2 \mid 2, 1) = 1 \).

(13) \( r(x_1 y_1 y_2 \mid k, \ell) = \frac{r(x_1 y_1 y_2 \mid k-1, \ell-1)}{k^2} r(x_1 y_1 y_2 \mid k-1, \ell-1) \) for \( k \geq 3 \), \( k > \ell \geq 1 \).

Lemma C.9.

1. \( r(x_1 y_1 \mid k, 0) \leq \frac{1}{k + 2} \) for \( k \geq 3 \).
2. \( r(x_1 y_1 \mid k, \ell) + r(x_1 y_1 y_2 \mid k, \ell) < \frac{1}{k-1} \) 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 C.8 [1] and (2) establish the \( k = 3 \) case. Then using Lemma C.8 (3) one sees inductively that for \( k > 3 \),

\[
\begin{align*}
r(x_1 y_1 \mid k, 0) & \leq \left( \frac{1}{k + 2} + \frac{(k-1)}{(k+2)} \right) \frac{1}{k + 1} = \frac{k^2 + k + 2}{(k+2)(k+1)^2} \\
& \leq \frac{1}{(k+2)(k+1)^2} = \frac{1}{k+2}.
\end{align*}
\]

For claim [2] when \( \ell = 0 \), note that by Lemma C.8 (1), (3), and (10),

\[
r(x_1 y_1 \mid 3, 0) + r(x_1 y_1 y_2 \mid 3, 0) = \frac{1}{5} + \frac{3}{10} \cdot \frac{1}{3} + \frac{1}{10} \cdot 1 = \frac{2}{5} < \frac{1}{2},
\]

so the base case of \( k = 3 \) holds. Then for \( k > 3 \), using Lemma C.8 [1], (3), and (10) we have

\[
r(x_1 y_1 \mid k, 0) + r(x_1 y_1 y_2 \mid k, 0)
\]

\[
= \frac{1}{(k+2)^2} + \frac{(k-1)}{(k+2)^2} r(x_1 y_1 \mid k-1, 0) + \frac{(k-1)}{(k+2)^2} r(x_1 y_1 y_2 \mid k-1, 0)
\]

\[
= \frac{1}{(k+2)^2} + \frac{k+2}{(k+2)^2} r(x_1 y_1 \mid k-1, 0) + \frac{(k-1)}{(k+2)^2} \left( r(x_1 y_1 \mid k-1, 0) + r(x_1 y_1 y_2 \mid k-1, 0) \right).
\]

Using an inductive hypothesis and claim [1] of this proposition yields

\[
r(x_1 y_1 \mid k, 0) + r(x_1 y_1 y_2 \mid k, 0)
\]

\[
< \frac{2}{(k+2)(k+1)} + \frac{2}{k+1} \cdot \frac{1}{k+1} + \frac{(k-1)(k-2)}{(k+2)(k+1)} \cdot 1
\]

\[
= \frac{1}{k+2} + \frac{2}{(k+1)^2} < \frac{1}{k+1} + \frac{2}{(k+1)^2} < \frac{1}{k+1}.
\]

Assume now \( k \geq 4 \) and \( k > \ell \geq 1 \), and consider first the case that \( k-\ell = 1 \) or 2. Applying Lemma C.8 [6] and [13] repeatedly we have

\[
r(x_1 y_1 \mid k, \ell) + r(x_1 y_1 y_2 \mid k, \ell) = \frac{3}{k^2} \left( r(x_1 y_1 \mid 3, \ell-1) + r(x_1 y_1 y_2 \mid 3, \ell-1) \right).
\]
From Lemma C.8
\[ r(x_1y_1 \mid 3, 1) + r(x_1y_1y_2 \mid 3, 1) = \frac{1}{9} + \frac{1}{3} = \frac{4}{9}, \]
\[ r(x_1y_1 \mid 3, 2) + r(x_1y_1y_2 \mid 3, 2) = 0 + \frac{1}{3} = \frac{1}{3}, \]
so for \( k \geq 4, \)
\[ r(x_1y_1 \mid k, \ell) + r(x_1y_1y_2 \mid k, \ell) < \frac{6}{k(k-1)} \cdot \frac{4}{9} < \frac{1}{k-1}. \]
If \( k - \ell \geq 3, \) then applying Lemma C.8 (6) and (13) repeatedly gives
\[ r(x_1y_1 \mid k, \ell) + r(x_1y_1y_2 \mid k, \ell) = \left(\frac{k-\ell}{2}\right) \left(\frac{r(x_1y_1 \mid k - \ell, 0) + r(x_1y_1y_2 \mid k - \ell, 0)}{r}\right). \]
Using what we proved above, this shows
\[ r(x_1y_1 \mid k, \ell) + r(x_1y_1y_2 \mid k, \ell) < \left(\frac{k-\ell}{2}\right) \cdot \frac{1}{k-\ell-1} < \frac{1}{k-1}. \]

Lemma C.10. Let
\[ R(k, \ell) = r(x_1x_2 \mid k, \ell) + r(x_1x_2y_1 \mid k, \ell) - r(x_1y_1 \mid k, \ell) - r(x_1y_1y_2 \mid k, \ell). \]
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 exchangability of the \( x_i \) and of the \( y_i \) lineages
\[ r(x_1x_2 \mid k, \ell) = r(x_1y_1y_2 \mid k, \ell), \]
\[ r(x_1x_2y_1 \mid k, \ell) = r(x_1y_1 \mid k, \ell). \]
Thus \( R(3, \ell) = 0 \) for \( \ell = 0, 1, 2. \) For \( k \geq 3, \) Lemma C.8 (1) implies \( R(k, 0) = 0. \)
Now consider \( k \geq 4, k > \ell \geq 1. \) By Lemma C.8 (4), (6), (11), and (13) we find
\[ R(k, \ell) = \frac{1}{\binom{k}{2}} \left(1 + r(x_1y_1 \mid k - 1, \ell - 1) + \binom{k-2}{2} R(k - 1, \ell - 1) \right. \]
\[ - (k-2) \left(r(x_1y_1 \mid k - 1, \ell - 1) + r(x_1y_1y_2 \mid k - 1, \ell - 1) \right). \]
An inductive hypothesis that \( R(k - 1, \ell - 1) \geq 0, \) Lemma C.9 and the positivity of \( r(x_1y_1 \mid k - 1, \ell - 1) \) then show
\[ R(k, \ell) > \frac{1}{\binom{k}{2}} \left(1 + 0 + \binom{k-2}{2} 0 - (k-2) \frac{1}{k-2} \right) = 0. \]
\[ \Box \]
Lemma C.11. 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 \(\rho\) 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 \(\rho\) or not. If they have coalesced below \(\rho\) 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 5.7.

We henceforth condition on the event that the lineages from \(b_1\) and \(b_2\) are distinct at \(\rho\). 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 \(\ell\) coalescent events occur on the edge above \(v\). Calling this event \(C = C(k, \ell)\),
\[
\mathbb{P}(Sp(ac) \mid C) = r(x_1x_2 \mid k, \ell),
\]
\[
\mathbb{P}(Sp(ab_2c) \mid C) = r(x_1x_2y_1 \mid k, \ell),
\]
\[
\mathbb{P}(Sp(b_1c) \mid C) = r(x_1y_1 \mid k, \ell),
\]
\[
\mathbb{P}(Sp(b_1b_2c) \mid C) = r(x_1y_1y_2 \mid k, \ell).
\]
From Lemma [C.10] we find that conditioned on \(C\) 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 \(C\) 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 \(C(k, \ell)\) with \(k \geq 4\), \(k > \ell \geq 1\); thus the full expression is positive. \(\square\)

Finally, Proposition 5.8 follows from Theorem 4.4, Lemma C.7 and Lemma C.11.

C.3. Proof of Proposition 5.9.

To establish Proposition 5.9 we first extend the results of Lemma C.8 and those that follow it, to splits of size 4.

A proof of the following is left to the reader.

**Lemma C.12.**

1. \(r(x_1x_2x_3y_1 \mid 3, 0) = 1\),
2. \(r(x_1x_2x_3y_1 \mid k, 0) = \frac{6}{(k+2)}r(x_1x_2y_1 \mid k-1, 0) + \binom{k-2}{k-1}r(x_1x_2x_3y_1 \mid k-1, 0)\) for \(k \geq 4\),
In particular, since we see using Lemma C.9 (2), we have
\[ \frac{k-1}{2} r(x_1 x_2 y_1 | k - 1, \ell - 1) \]
for \( k \geq 5 \), \( k > \ell \geq 1 \).

Let \( U \) be defined as
\[ U(k, \ell) = \frac{1}{r(x_1 y_1 | k, \ell) + r(x_1 x_2 y_1 | k, \ell) + r(x_1 y_1 y_2 | k, \ell) + r(x_1 x_2 y_1 y_2 | k, \ell)}. \]

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 C.8 and C.12 that for \( k \geq 4 \),
\[ U(k, 0) = \frac{1}{(k+1)^2} \left[ 1 + (2k+3) \left( r(x_1 y_1 | k - 1, 0) + r(x_1 y_1 y_2 | k - 1, 0) \right) - r(x_1 y_2 | k - 1, 1) + \left( \frac{k-2}{2} \right) U(k-1, 0) \right]. \]

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 C.9 (2), we have
\[ U(k, 0) < \frac{1}{(k+1)^2} \left[ \left( 1 + (2k+3) \left( \frac{1}{k-2} - 0 + \left( \frac{k-2}{2} \right) \frac{1}{k-3} \right) \right) \frac{k^2 + 2k + 6}{k^2 + 3k + 2} \cdot \frac{1}{k-2} < \frac{1}{k-2}. \]

For \( \ell > 0 \), if \( k \geq 4 \), \( k > \ell \geq 1 \), Lemmas C.8 and C.12 show
\[ U(k, \ell) = \frac{1}{(k-2)^2} \left( \left( \frac{k}{2} \right) U(k-1, \ell) \right. \]
\[ + \left( k - 1 \right) \left( r(x_1 y_1 | k - 1, \ell - 1) + r(x_1 y_1 y_2 | k - 1, \ell - 1) \right) \).

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

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 equation (12), Lemma C.9 (2), and an inductive hypothesis that
\[ U(k, \ell) < \frac{1}{k} \left( \binom{k-2}{2} \frac{1}{k-3} + (k-1) \frac{1}{k-2} \right) \]
\[ = \frac{k^2 - 2k + 2}{k(k-1)(k-2)} < \frac{1}{k-2}. \]

\[ \square \]

**Lemma C.14.** Let
\[ S(k, \ell) = r(x_1x_2 \mid k, \ell) + r(x_1x_2x_3 \mid k, \ell) + r(x_1x_2x_3y_1 \mid k, \ell) \]
\[ - r(x_1y_1 \mid k, \ell) - r(x_1y_1y_2 \mid k, \ell) - r(x_1x_2y_1y_2 \mid k, \ell). \]

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
\[ r(x_1x_2 \mid 4, \ell) = r(x_1x_2y_1y_2 \mid 4, \ell), \]
\[ r(x_1x_2x_3 \mid 4, \ell) = r(x_1y_1y_2 \mid 4, \ell), \]
\[ r(x_1x_2x_3y_1 \mid 4, \ell) = r(x_1y_1 \mid 4, \ell), \]

so \( S(4, \ell) = 0 \) for \( \ell = 0, 1, 2, 3 \). For \( k \geq 5 \), Lemma C.13 (1) implies \( S(k, 0) = 0 \).

For \( k \geq 5, k > \ell \geq 1 \), using Lemmas C.8 and C.12 we find
\[ S(k, \ell) = \frac{1}{k^2} \left( \binom{k-3}{2} S(k-1, \ell - 1) - (k-3)U(k-1, \ell - 1) + kR(k-1, \ell - 1) \right. \]
\[ + 1 + 2r(x_1y_1 \mid k-1, \ell - 1) + r(x_1y_1y_2 \mid k-1, \ell - 1)) \].

Using an inductive hypothesis, Lemmas C.13 and C.10 and non-negativity of probabilities, this implies
\[ S(k, \ell) > \frac{1}{k^2} \left( \binom{k-3}{2} \cdot 0 - (k-3) \frac{1}{k-3} + k \cdot 0 + 1 + 2 \cdot 0 + 0 \right) = 0. \]

\[ \square \]
Proof of Proposition 5.9. On the species tree \((T, (a_1, a_2), (b_1, b_2))\) let \(\rho\) 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

\[
\begin{align*}
\mathbb{P}(Sp(a_1c)) + \mathbb{P}(Sp(a_1a_2c)) + \mathbb{P}(Sp(a_1b_2c)) + \mathbb{P}(Sp(a_1a_2b_2c)) \\
- \mathbb{P}(Sp(b_1c)) - \mathbb{P}(Sp(b_1a_2c)) - \mathbb{P}(Sp(b_1b_2c)) - \mathbb{P}(Sp(b_1a_2b_2c)) > 0.
\end{align*}
\]

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 \(\rho\). 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 5.7. 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 MRCA\(s\) follow from Proposition 5.8.

We henceforth condition on the event that the \(a_i\) lineages are distinct at \(v\) and the \(b_i\) lineages are distinct at \(\rho\). 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 \(\ell\) coalescent events occur between \(v\) and \(\rho\).

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

\[
\begin{align*}
\mathbb{P}(Sp(a_1c) | C) &= r(x_1x_2 | k, \ell), \\
\mathbb{P}(Sp(a_1a_2c) | C) &= r(x_1x_2x_3 | k, \ell), \\
\mathbb{P}(Sp(a_1b_2c) | C) &= r(x_1x_2y_1 | k, \ell), \\
\mathbb{P}(Sp(a_1a_2b_2c) | C) &= r(x_1x_2x_3y_1 | k, \ell), \\
\mathbb{P}(Sp(b_1c) | C) &= r(x_1y_1 | k, \ell), \\
\mathbb{P}(Sp(b_1a_2c) | C) &= r(x_1x_2y_1 | k, \ell), \\
\mathbb{P}(Sp(b_1b_2c) | C) &= r(x_1y_1y_2 | k, \ell), \\
\mathbb{P}(Sp(b_1a_2b_2c) | C) &= r(x_1x_2y_1y_2 | k, \ell).
\end{align*}
\]

After substituting these in to the expression in (13), from Lemma C.14 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 \(\ell\), we see the unconditioned inequality (13) 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 (13) 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 4.4, the proof is complete. □

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