

## Phylogenetic Mixtures on a Single Tree Can Mimic a Tree of Another Topology

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**Abstract.**—Phylogenetic mixtures model the inhomogeneous molecular evolution commonly observed in data. The performance of phylogenetic reconstruction methods where the underlying data are generated by a mixture model has stimulated considerable recent debate. Much of the controversy stems from simulations of mixture model data on a given tree topology for which reconstruction algorithms output a tree of a different topology; these findings were held up to show the shortcomings of particular tree reconstruction methods. In so doing, the underlying assumption was that mixture model data on one topology can be distinguished from data evolved on an unmixed tree of another topology given enough data and the “correct” method. Here we show that this assumption can be false. For biologists, our results imply that, for example, the combined data from two genes whose phylogenetic trees differ only in terms of branch lengths can perfectly fit a tree of a different topology. [Mixture model; model identifiability; phylogenetics; sequence evolution.]

It is now well known that molecular evolution is heterogeneous; i.e., that it varies across time and position (Simon et al., 1996). A classic example is stems and loops of ribosomal RNA: the evolution of one side of a stem is strongly constrained to match the complementary side, whereas for loops different constraints exist (Springer and Douzery, 1996). Heterogeneous evolution between genes is also widespread, where even the general features of evolutionary history for neighboring genes may differ wildly (Ochman et al., 2000). Presently it is not uncommon to use concatenated sequence data from many genes for phylogenetic inference (Phillips et al., 2004), which can lead to very high levels of apparent heterogeneity (Baldauf et al., 2000). Furthermore, empirical evidence using the covarion model shows that sometimes more subtle partitions of the data can exist, for which separate analysis is difficult (Wang et al., 2007).

This heterogeneity is typically formulated as a mixture model (Pagel and Meade, 2004). Mathematically, a phylogenetic mixture model is simply a weighted average of site pattern frequencies derived from a number of phylogenetic trees, which may be of the same or different topologies. Even though many phylogenetics programs accept aligned sequences as input, the only data actually used in the vast majority of phylogenetic algorithms are the derived site pattern frequencies. Thus, in these algorithms, any record of position is lost and heterogeneous evolution appears identical to homogeneous evolution under an appropriate phylogenetic mixture model. For simplicity, we call a mixture of site pattern frequencies from two trees (which may be of the same or different topology) a *mixture of two trees*; when the two trees have the same underlying topology, the mixture will be called a *mixture of branch length sets on a tree*.

Mixture models have proven difficult for phylogenetic reconstruction methods, which have historically sought to find a single process explaining the data. For example, it has been shown that mixtures of two different tree topologies can mislead MCMC-based tree reconstruction (Mossel and Vigoda, 2005). It is also known that there exist mixtures of branch length sets on one tree that are indistinguishable from mixtures of branch length

sets on a tree of a different topology (Steel et al., 1994; Štefankovič and Vigoda, 2007a, 2007b). Recently, simulations of mixture models from “heterotachous” (changing rates through time) evolution have been shown to cause reconstruction methods to fail (Ruano-Rubio and Fares, 2007).

The motivation for our work is the observation that both theory and simulations have shown that in certain parameter regimes, phylogenetic reconstruction methods return a tree topology different from the one used to generate the mixture data. The parameter regime in this class of examples is similar to that shown in Figure 1, with two neighboring pendant edges that alternate being long and short. After mixing and reconstruction, these edges may no longer be adjacent on the reconstructed tree. We call this *mixed branch repulsion*. This phenomenon has been observed extensively in simulation (Kolaczowski and Thornton, 2004; Spencer et al., 2005; Philippe et al., 2005; Gadakar and Kumar, 2005) and it has been proved that certain distance and maximum likelihood methods are susceptible to this effect (Chang, 1996; Štefankovič and Vigoda, 2007a, 2007b). Up to this point such results have been interpreted as pathological behavior of the reconstruction algorithms, which has led to a heated debate about which reconstruction methods perform best in this situation (Steel, 2005; Thornton and Kolaczowski, 2005). Implicit in this debate is the assumption that a mixture of trees on one topology gives different site pattern frequencies than that of an unmixed tree of a different topology. This leads to the natural question of how similar these two site pattern frequencies can be.

Here we demonstrate that mixtures of two sets of branch lengths on a tree of one topology can exactly mimic the different (expected) site pattern frequencies of a tree of a different topology under the two-state symmetric model. In fact, there is a precisely characterizable (codimension two) region of parameter space where such mixtures exist. Consider two quartet trees of topology 12|34, as shown in Figure 1. Label the pendant branches 1 through 4 according to the taxon labels, and label the internal edge with 5. The first branch length set will be written  $t_1, \dots, t_5$  and the second  $s_1, \dots, s_5$ . Now, if  $k_1, \dots, k_4$

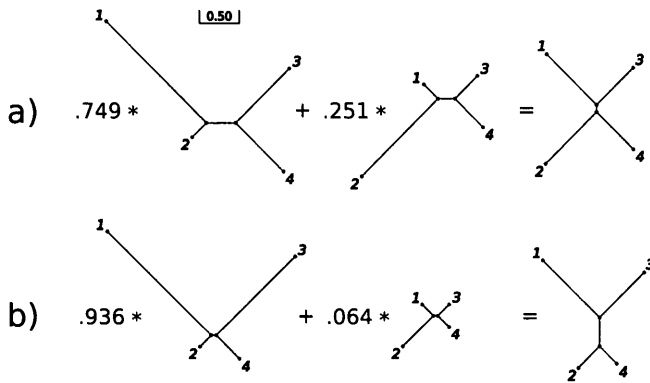


FIGURE 1. Mixtures of two sets of branch lengths on a tree of a given topology can give exactly the same expected site pattern frequencies as a tree of a different topology under the two-state symmetric model. The notation in the diagram showing  $x \times T_1 + (1 - x) \times T_1' = T_2$  means that the indicated mixture of the two branch lengths sets  $T_1$  and  $T_1'$  shown in the diagram gives the same expected site pattern frequencies as the tree  $T_2$ . The diagrams show two examples of this "mixed branch repulsion"; the general criteria for such mixtures is explained in the text. The branch length scale in the diagrams is given by the line segment indicating the length of a branch with 0.5 substitutions per site. Note that the mixing weights in this example have been rounded.

satisfy the following system of inequalities,

$$k_1 > k_3 > k_4 > 1 > k_2,$$

$$\frac{1 - k_1^2}{k_1} \frac{1 - k_4^2}{k_4} + \frac{1 - k_2^2}{k_2} \frac{1 - k_3^2}{k_3} > 0,$$

$$\frac{k_1 + k_4}{1 + k_1 k_4} \times \frac{k_2 + k_3}{1 + k_2 k_3} > 1$$

then they specify a class of examples of mixed branch repulsion. More precisely, then there exist nonzero internal branch lengths  $t_5$  and  $s_5$ , mixing weights, and positive numbers  $\ell_1, \dots, \ell_4$  such that if for  $i = 1, \dots, 4$ ,  $k_i = \exp[-2(t_i - s_i)]$  and  $t_i \geq \ell_i$ , the corresponding mixture of two 12|34 trees will have the same site pattern frequencies as a single tree of the 13|24 topology. We have illustrated two examples of branch length sets satisfying these criteria in Figure 1 and provided the corresponding branch lengths in Table 1.

TABLE 1. Rounded branch lengths for the examples in Figure 1. The top division of the table is example (a); the bottom is example (b). The top two lines in each example are the branch lengths forming the mixture and the third line gives the branch lengths for the unmixed tree.

Weight	Pendant 1	Pendant 2	Pendant 3	Pendant 4	Internal
Example (a)					
0.748646	1.772261	0.25	0.949306	0.846574	0.366516
0.251354	0.25	1.353637	0.4	0.5	0.213387
1.	0.888101	0.905792	0.648625	0.654236	0.086051
Example (b)					
0.936064	1.838398	0.2	1.397309	0.411489	0.062429
0.063936	0.2	0.543932	0.2	0.2	0.055312
1.	1.011471	0.375718	0.794529	0.305338	0.360827

The exact zone for mixed branch repulsion is described above and detailed in Proposition 6; here we present some simple necessary criteria for mixed branch repulsion to occur. First, note that except for the internal edge and a (typically small) lower bound on pendant branch lengths, the relevant parameters are differences of branch lengths between sets rather than absolute branch lengths themselves. Given two branch length sets with edges numbered as above, let  $d_i$  denote the difference between the branch lengths for edge  $i$ ; i.e.,  $t_i - s_i$ . Then (perhaps after changing the arbitrary numbering of the taxa) either  $d_1 > d_3 > d_4 > 0 > d_2$  or  $d_1 > 0 > d_3 > d_4 > d_2$  must be satisfied in order for mixed branch repulsion to occur. Thus, for example, in one set of branch lengths the pendant edge for taxa 1 should be long and the pendant edge for taxa 2 should be short, whereas in the other set of branch lengths these roles should be reversed. On the other hand, the branch lengths for taxa 3 and 4 should be both long for one set and both short for the other. Additionally, at least one of the two internal branch lengths needs to be relatively short. There are other more complex criteria, but the above is necessary for exact mixed branch repulsion to occur. However, as noted below, exact mixed branch repulsion is not necessary to "fool" model based methods.

We believe that this similarity between site pattern frequencies generated by mixtures of branch lengths on one tree and corresponding unmixed frequencies on a different tree is what is leading to the mixed branch repulsion observed in theory and simulation. Furthermore, it is possible that even the simple case presented here is directly relevant to reconstructions from data. First, it is not uncommon to simplify the genetic code from the four standard bases to two (pyrimidines versus purines) in order to reduce the effect of compositional bias when working with genome-scale data on deep phylogenetic relationships (Phillips et al., 2004). Second, when working on such relationships, concatenation of genes is common (Baldauf et al., 2000), for which a phylogenetic mixture is the expected result. Finally, the region of parameter space bringing about mixed branch repulsion may become more extensive as the number of concatenated genes increases. Therefore, in concatenated gene analysis, it may be worthwhile considering incongruence in terms of branch lengths and not just in terms of topology (Rokas et al., 2003; Jeffroy et al., 2006), as highly incongruent branch lengths may produce artificial results upon concatenation. Other methods may be useful in this setting, such as gene order data, gene presence/absence, or coalescent-based methods to infer the most likely species tree from a collection of gene trees.

Mixed branch repulsion may be more difficult to detect than the usual model misspecification issues; in the cases presented here, the misspecified single tree model fits the data perfectly. In contrast, although using the wrong mutation model for reconstruction using maximum likelihood can lead to incorrect tree topologies (Goremykin et al., 2005), the resulting model misspecification can be seen from a poor likelihood score. In the mixtures presented here, there is no way of telling when one is

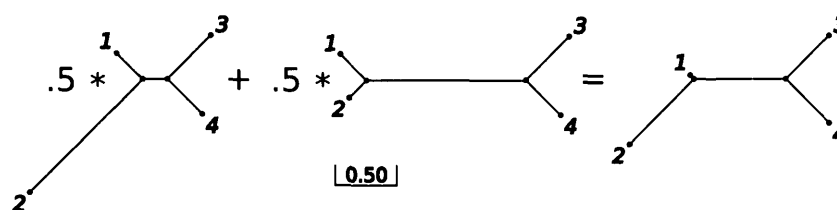


FIGURE 2. Mixtures of two sets of branch lengths on a tree of a given topology can give exactly the same expected site pattern frequencies as a tree of the same topology under the two-state symmetric model. The criterion for the occurrence of this phenomenon is explained in the text and an example is shown in the figure. Note in particular that the branch lengths need not average: for example, the branch length for the pendant edge leading to taxon 1 virtually disappears after mixing.

in the mixed regime on one topology or an unmixed regime on another topology. Furthermore, any model selection technique (including likelihood-ratio tests, the Akaike information criterion, and the Bayesian Information Criterion), which chooses a simple model given equal likelihood scores, would, in this case, choose a simple unmixed model. Thereby it would select a tree that is different from the historically correct tree if the true process was generated by a mixture model.

The derivation of the zone resulting in mixed branch repulsion is a conceptually simple application of two of the pillars of theoretical phylogenetics: the Hadamard transform and phylogenetic invariants (Hendy and Penny, 1989; Semple and Steel, 2003; Felsenstein, 2004). The Hadamard transform is a closed form invertible transformation (expressed in terms of the discrete Fourier transform) for gaining the expected site pattern frequencies from the branch lengths and topology of a tree or vice versa. Phylogenetic invariants characterize when a set of site pattern frequencies could be the expected site pattern frequencies for a tree of a given topology. They are identities in terms of the discrete Fourier transform of the site pattern frequencies. Therefore, to derive the above equations, we simply insert the Hadamard formulae for the Fourier transform of pattern probabilities into the phylogenetic invariants, then check to make sure the resulting branch lengths are positive.

Similar considerations lead to an understanding of when it is possible to mix two branch length sets on a tree to reproduce the site pattern frequencies of a tree of the same topology (Proposition 3 of Appendix 1). For a quartet, two cases are possible. First, a pair of neighboring pendant branch lengths can be equal between the two branch length sets of the mixture. Alternatively, the sum of one pair of neighboring pendant branch lengths and the difference of the other pair can be equal. For trees larger than quartets, the allowable mixtures are determined by these restrictions on the quartets (results to appear elsewhere). For pairs of branch lengths satisfying these criteria, any choice of mixing weights will produce site pattern frequencies satisfying the phylogenetic invariants.

Intuitively, one might expect that when two sets of branch lengths mix to mimic a tree of the same topology, some sort of averaging property would hold for the branch lengths. This is true for pairwise distances in the tree but need not be the case for individual branches, as

demonstrated by Figure 2. In fact, it is possible to mix two sets of branch lengths on a tree to mimic a tree of the same topology such that a resulting pendant branch length is arbitrarily small whereas the corresponding branch length in either of the branch length sets being mixed stays above some arbitrarily large fixed value.

The results in this paper shed some light on the geometry of phylogenetic mixtures (Kim, 2000). As is well known, the set of phylogenetic trees of a given topology forms a compact subvariety of the space of site pattern frequencies (Sturmfels and Sullivan, 2005). The first part of our work demonstrates that there are pairs of points in one such subvariety such that a line between those two points intersects a distinct subvariety (Fig. 3). Therefore, the convex hull of one subvariety has a region of intersection with distinct subvarieties. This is stronger than the recently derived result by Štefankovič and Vigoda (2007a, 2007b) that the convex hulls of the varieties intersect. The second part of our work shows that there exist pairs of points in a subvariety such that the line between those points intersects the subvariety. Furthermore, it demonstrates that when such a line between two points intersects the subvariety in a third point, then a subinterval of the line is contained in the subvariety.

This geometric perspective can aid in understanding practical problems of phylogenetic estimation. The question of when maximum likelihood selects the “wrong” topology given mixture data was initiated by Chang (1996) who found a one-parameter space of such examples under the two-state symmetric (CFN) model. Recently Štefankovič and Vigoda (2007a) found a two-parameter space of such examples for the CFN model, and a one-dimensional space of examples for the Jukes-Cantor DNA (JC) and Kimura two- and three-parameter (K2P, K3P) models. A potential criticism of these previous results is that because the set of examples has lower dimension than the ambient parameter space, one is unlikely to encounter them in practice.

However, a simple geometric argument can show that the dimension of the set of all such pathological examples is equal to the dimension of the parameter space for all four of these models. To see why this holds, we first recall the definition of the Kullback-Leibler divergence of probability distribution  $q$  from a second distribution  $p$ :

$$\delta_{KL}(p, q) = \sum_i p_i \log \frac{p_i}{q_i}.$$

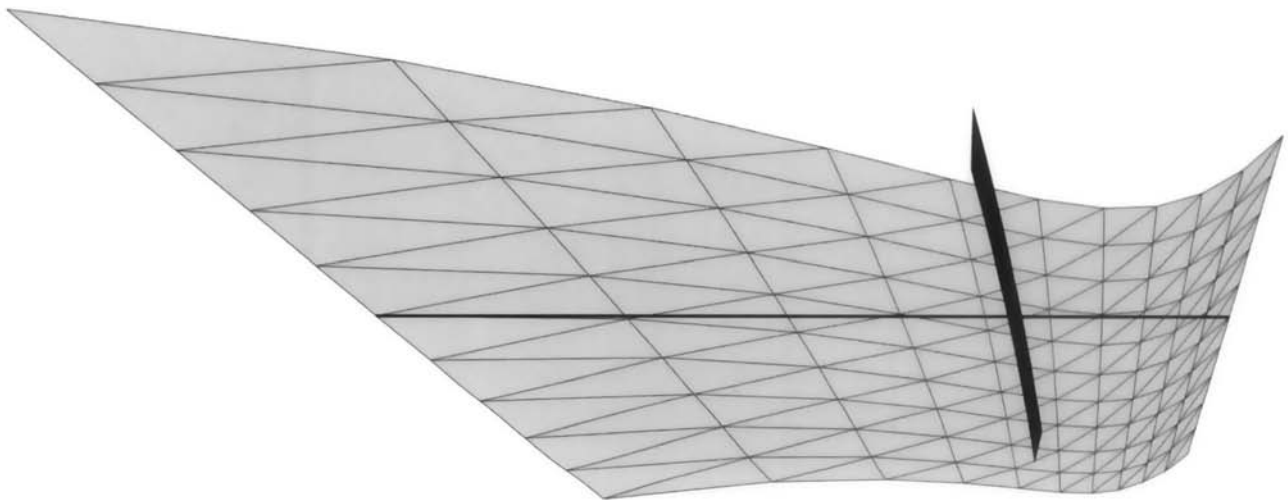


FIGURE 3. A geometric depiction of the main result. The ambient space is a projection of the seven-dimensional probability simplex of site pattern frequencies for trees on four leaves. The gray sheet is a subset of a two-dimensional subvariety of the site pattern frequencies for trees of the 12|34 topology, whereas the black sheet is an analogous subset for the 13|24 topology. The horizontal line represents the possible mixtures for the two sets of branch lengths for the 12|34 topology in Figure 1a. The fact that these two sets of branch lengths can mix to make a tree of topology 13|24 is shown here by the fact that the horizontal line intersects the black sheet.

The  $p$  vector is typically thought of as a data vector and the  $q$  vector is typically the model data. Maximum likelihood seeks to find the model data vector  $q$  which minimizes  $\delta_{KL}(p, q)$ . Let  $V_{12|34}$  be the set of all data vectors that correspond exactly to trees of topology 12|34, and similarly for  $V_{13|24}$ . For  $V = V_{12|34}$  or  $V_{13|24}$ , let  $\delta_{KL}(p, V)$  denote the divergence of  $p$  from the “closest” point in  $V$ ; i.e., the minimum of  $\delta_{KL}(p, v)$  where  $v$  ranges over  $V$ . We show in Lemma 8 that this function exists and is continuous across the set of probability vectors  $p$  with all components positive.

Now, pick any of the above group-based models, and let  $y$  be a corresponding pathological mixture on 12|34 for that model supplied by Theorem 2 of Štefankovič and Vigoda (2007a). Maximum likelihood chooses topology 13|24 over 12|34 for a data vector  $p$  exactly when  $\delta_{KL}(p, V_{13|24})$  is less than  $\delta_{KL}(p, V_{12|34})$ ; therefore,  $\delta_{KL}(y, V_{13|24}) < \delta_{KL}(y, V_{12|34})$ . By the properties of continuous functions, this inequality also holds for all probability vectors  $y'$  close to  $y$  that also have all components positive. Therefore, ML will choose 13|24 over 12|34 for all such  $y'$ . Because the transformation taking branch length and mixing weight parameters to expected site pattern frequencies is continuous, one can change branch lengths and mixing weight arbitrarily by a small amount and still have ML choose 13|24 for the resulting data. This gives the required full-dimensional space of examples.

We now indicate how our results fit into previous work on identifiability and discuss prospects for generalization. For four-state models with extra symmetries such as the Jukes-Cantor DNA model and the Kimura two-parameter model, it is known that there exist linear phylogenetic invariants that imply identifiability of the topology for mixture model data (Štefankovič and Vigoda 2007a). The topology is also identifiable for phy-

logenetic mixtures in which each underlying process is described by an infinite state model (Mossel and Steel, 2004, 2005)—such processes may be relevant to data involving rare (homoplasy-free) genomic changes. Therefore, the pathologies observed here could not occur for those models. Furthermore, Allman and Rhodes (2006) have shown generic identifiability (i.e., identifiability for “almost all” parameter regimes) when the number of states exceeds the number of mixture classes. As stated above, the dimension of the set of examples presented here is of dimension two less than the ambient space (even though the conditions of the Allman and Rhodes work is not satisfied). However, we note that even when tree topology is generically identifiable (but not globally identifiable) for some model, arguments similar to the above can show that there exist positive-volume regions where the data are closer to those from a tree of a different topology than a tree of the same topology.

A related though distinct question concerns identifiability under mixture models when the data partitions are known. For example, we may have a number of independent sequence data sets for the same set of taxa, perhaps corresponding to different genes. In this setting it may be reasonable to assume that the sequence sites *within* each data set evolve under the same branch lengths (perhaps subject to some i.i.d. rates-across-sites distribution), but that the branch lengths *between* the data sets may vary. The underlying tree topology may be the same or different across the data sets; however, let us first consider the case where there is a common underlying topology. In the case where each data set consists of sequences of length 1 we are back in the setting of phylogenetic mixtures considered above. However, for longer blocks of sequences, we might hope to exploit the knowledge that the sequences within each block have evolved under a common mechanism. If the sequence length within

any one data set becomes large, we will be able to infer the underlying tree for that data set correctly, so the interesting question is what happens when the data sets provide only “mild” support for their particular reconstructed tree. Assume that all (or nearly all) of the data sets contain sufficiently many sites so that the tree reconstruction method  $M$  positively favors the true tree over any particular alternative tree. By this we mean that  $M$  returns the true tree with a probability that is greater by a factor of at least  $1 + \epsilon$  (with  $\epsilon > 0$ ) than the probability that  $M$  returns each particular different tree. Then it is easily shown that a majority-rule selection procedure applied to the reconstructed trees across the  $k$  independent data sets will correctly return the true underlying tree topology with a probability that goes to 1 as  $k$  grows. Note that this claim holds generally, not just for the two-state symmetric model. Of course it is also possible that the underlying tree may differ across data sets—in the case of genes perhaps due to lineage sorting (Degnan and Rosenberg, 2006)—in which case the reconstruction question becomes more complex.

In a forthcoming article (Matsen et al., 2007), we further investigate identifiability of mixture models. Using geometric methods, we make some progress towards understanding how “common” nonidentifiable mixtures should be for the symmetric and nonsymmetric two-state models; for mixtures of many trees they appear to be quite common. A new combinatorial theorem implies identifiability for certain types of mixture models when branch lengths are clock-like. A simple argument shows identifiability for rates-across-sites models. We also investigate mixed branch repulsion for larger trees.

Many interesting questions remain. First of all, is exact mixed branch repulsion an issue for any nontrivial model on four states? Also, what is the zone of parameter space for which a mixture of branch lengths on a tree is closer (in some meaningful way) to the expected site pattern frequencies of a tree of different topology than to those for a tree of the original topology? How often does mixed branch repulsion present itself given “random” branch lengths? Considering the rapid pace of development in this field, we do not expect these questions to be open for long.

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## APPENDIX 1

In this appendix we provide more precise statements and proofs of the propositions in the text. The proofs will be presented in the reverse order than they were stated in the main text—first the fact that it is possible to mix two branch lengths on a tree to mimic a tree of the same topology, then that it is possible to mix branch lengths to mimic a tree of a distinct topology.

As stated in the main text, the general strategy of the proofs is simple: use the Hadamard transform to calculate Fourier transforms of site pattern probabilities and then insert these formulas into the phylogenetic invariants. These steps would become very messy except for a number of simplifications: First, because the discrete Fourier transform is linear, a transform of a mixture is simply a mixture of the corresponding transforms. Second, the fact that the original trees satisfy a set of phylogenetic invariants reduces the complexity of the mixed invariants. Finally, the product of the exponentials of the branch lengths appear in all formulas, and division leads to a substantial simplification.

First, we remind the reader of the main tools and fix notation. Note that for the entire paper we will be working with the two-state symmetric (also known as Cavender-Farris-Neyman) model.

### The Hadamard Transform and Phylogenetic Invariants

For a given edge  $e$  of branch length  $\gamma(e)$  we will denote

$$\theta(e) = \exp[-2\gamma(e)] \quad (1)$$

which ranges between 0 and 1 for positive branch lengths. We call this number the “fidelity” of the edge, as it quantifies the quality of transmission of the ancestral state across the edge. For  $A \subseteq \{1, \dots, n\}$  of even order, let  $q_A = (H_{n-1} \bar{p})_A$  be the Fourier transform of the split probabilities, where  $H_n$  is the  $n$  by  $n$  Hadamard matrix (Semple and Steel, 2003).

Quartet trees will be designated by their splits; i.e., 13|24 refers to a quartet with taxa labeled 1 and 3 on one side of the quartet and taxa 2 and 4 on the other.

By the first identity in the proof of theorem 8.6.3 of Semple and Steel (2003), one can express the Fourier transform of the split probabilities in terms of products of fidelities. That is, for any subset  $A \subseteq \{1, \dots, n\}$  of even order,

$$q_A = \prod_{e \in \mathcal{P}(T, A)} \theta(e) \quad (2)$$

where  $\mathcal{P}(T, A)$  is the set of edges that lie in the set of edge-disjoint paths connecting the taxa in  $A$  to each other. This set is uniquely defined (again, see Semple and Steel, 2003).

From this equation, we can derive values for the fidelities from the Fourier transforms of the split probabilities. In particular, it is simple to write out the fidelity of a pendant edge on a quartet. For example,

$$\theta_1 = \sqrt{\frac{\theta_1 \theta_5 \theta_4 \times \theta_1 \theta_2}{\theta_2 \theta_5 \theta_4}} = \sqrt{\frac{q_{14} q_{12}}{q_{24}}}$$

for a tree of topology 12|34. In general, we have the following lemma:

**Lemma 1.** *If  $a$ ,  $b$ , and  $c$  are distinct pendant edge labels on a quartet such that  $a$  and  $b$  are adjacent, then the fidelity of a pendant edge  $a$  is*

$$\sqrt{\frac{q_{ab} q_{ac}}{q_{bc}}}.$$

A similar calculation leads to an analogous lemma for the internal edge:

**Lemma 2.** *The fidelity of the internal edge of an  $ab|cd$  quartet tree is*

$$\sqrt{\frac{q_{ac} q_{bd}}{q_{ab} q_{cd}}}.$$

This paper will also make extensive use of the method of phylogenetic invariants. These are polynomial identities in the Fourier transform of the split probabilities that are satisfied for a given tree topology. Invariants are understood in a very general setting (see Sturmfels and Sullivan, 2005); however, here we only require invariants for the simplest case: a quartet tree with the two-state symmetric model. In particular, for the quartet tree  $ab|cd$ , the two phylogenetic invariants are

$$q_{abcd} - q_{ab} q_{cd} = 0 \quad (3)$$

$$q_{ac} q_{bd} - q_{ad} q_{bc} = 0. \quad (4)$$

A  $q$ -vector mimics the Fourier transforms of site pattern frequencies of a nontrivial tree exactly when they satisfy the phylogenetic invariants and have corresponding edge fidelities (given by Lemmas 1 and 2) between 0 and 1.

This paper is primarily concerned with the following situation: a mixture of two sets of branch lengths on a quartet tree that mimics the site pattern frequencies of an unmixed tree. We fix the following notation: the two branch length sets will be called  $t_i$  and  $s_i$ , the corresponding fidelities will be called  $\theta_i$  and  $\psi_i$ , and the Fourier transforms of the site pattern frequencies will be labeled with  $q$  and  $r$ , respectively. The internal edge of the quartet will carry the label  $i = 5$ , and the pendant edges are labeled according to their terminal taxa (e.g.,  $i = 2$  is the edge terminating in the second taxon). The mixing weight will be written  $\alpha$ , and we make the convention that the mixture will take the  $t_i$  branch length set with probability  $\alpha$  time and  $s_i$  with probability  $1 - \alpha$ .

### Mixtures Mimicking a Tree of the Same Topology

In this section we describe conditions on mixtures such that a nontrivial mixture of two branch lengths on 12|34 can give the same probability distribution as a single tree of the same topology.

Mixing two branch length sets on a 12|34 quartet tree with the above notation leads to the following form of Invariant (3) for a resulting tree also of topology 12|34:

$$(\alpha + 1 - \alpha)[\alpha q_{1234} + (1 - \alpha) r_{1234}] - [\alpha q_{12} + (1 - \alpha) r_{12}][\alpha q_{34} + (1 - \alpha) r_{34}] = 0. \quad (5)$$

Multiplying out terms then collecting, there will be a  $\alpha^2(q_{1234} - q_{12}q_{34})$  term, which is 0 by the phylogenetic invariants for the 12|34 topology. Similarly, the terms with  $(1 - \alpha)^2$  vanish. Dividing by  $\alpha(1 - \alpha)$ , which we assume to be nonzero, Equation (5) becomes

$$q_{1234} + r_{1234} - (q_{12}r_{34} + r_{12}q_{34}) = 0.$$

Applying Invariant (3) for the 12|34 topology and simplifying leads to the following equivalent form of (5):

$$(q_{12} - r_{12})(q_{34} - r_{34}) = 0. \quad (6)$$

The same sorts of moves lead to the second invariant of the mixed tree:

$$q_{13}r_{24} + r_{13}q_{24} - (q_{14}r_{23} + r_{14}q_{23}) = 0. \quad (7)$$

The fact that  $\alpha$  doesn't appear in these equations already delivers an interesting fact: if a mixture of two branch lengths in this setting satisfy the phylogenetic invariants for a single  $\alpha$ , then they do so for all  $\alpha$ . Geometrically, this means if the line between two points on the subvariety cut out by the phylogenetic invariants intersects the subvariety nontrivially, then it sits entirely in the subvariety.

We can gain more insight by considering these equations in terms of fidelities. Direct substitution using (2) into (6) gives

$$(\theta_1\theta_2 - \psi_1\psi_2)(\theta_3\theta_4 - \psi_3\psi_4) = 0.$$

This equation will be satisfied exactly when the branch lengths satisfy

$$t_1 + t_2 = s_1 + s_2 \text{ or } t_3 + t_4 = s_3 + s_4. \quad (8)$$

The corresponding substitution into (7) and then division by  $\theta_2\theta_5\theta_4\psi_2\psi_5\psi_4$  gives, after simplification,

$$\left(\frac{\theta_1}{\theta_2} - \frac{\psi_1}{\psi_2}\right)\left(\frac{\theta_3}{\theta_4} - \frac{\psi_3}{\psi_4}\right) = 0$$

This equation will be satisfied exactly when the branch lengths satisfy

$$t_1 - t_2 = s_1 - s_2 \text{ or } t_3 - t_4 = s_3 - s_4. \quad (9)$$

To summarize,

**Proposition 3.** *The mixture of two 12|34 quartet trees with pendant branch lengths  $t_i$  and  $s_i$  satisfies the 12|34 phylogenetic invariants for the binary symmetric model exactly (up to renumbering) when either  $t_1 = s_1$  and  $t_2 = s_2$ , or  $t_1 + t_2 = s_1 + s_2$  and  $t_3 - t_4 = s_3 - s_4$ .*

As described above this proposition makes no reference to the mixing weight  $\alpha$ .

In quartets where  $t_1 = s_1$  and  $t_2 = s_2$ , the resulting tree will also have pendant branch lengths  $t_1$  and  $t_2$ :

**Proposition 4.** *A mixture of two 12|34 quartet trees with branch lengths  $t_i$  and  $s_i$  which satisfies  $t_1 = s_1$  and  $t_2 = s_2$  will have resulting pendant branch lengths for the first and second taxa equal to  $t_1$  and  $t_2$ , respectively.*

*Proof.* Let the fidelity of the edges leading to taxon one and two be denoted  $\mu_1$  and  $\mu_2$ . We have by Lemma 1 with  $a = 1$ ,  $b = 2$  and  $c = 3$ ,

$$\mu_1 = \sqrt{\frac{[\alpha\theta_1\theta_2 + (1-\alpha)\psi_1\psi_2] \cdot [\alpha\theta_1\theta_5\theta_3 + (1-\alpha)\psi_1\psi_5\psi_3]}{\alpha\theta_2\theta_5\theta_3 + (1-\alpha)\psi_2\psi_5\psi_3}}$$

This fraction is equal to  $\theta_1$  after substituting  $\psi_1 = \theta_1$  and  $\psi_2 = \theta_2$ , which are implied by the hypothesis. The same calculation implies that  $\mu_2 = \theta_2$ .

In the rest of this section we note that anomalous branch lengths can emerge from mixtures of trees mimicking a tree of the same topology.

**Proposition 5.** *It is possible to mix two sets of branch lengths on a tree to mimic a tree of the same topology such that one resulting pendant branch length is arbitrarily small while the corresponding branch length in either of the branch length sets being mixed stays above some arbitrarily large fixed value.*

*Proof.* To get such an anomalous mixture, set  $\theta_1 = \psi_1$ ,  $\theta_3 = \psi_3$ ,  $\theta_4 = \psi_4$ ,  $\theta_2 = \psi_5$ ,  $\theta_5 = \psi_2$ , and  $\alpha = .5$ . Equations (8) and (9) are satisfied because  $\theta_3 = \psi_3$  and  $\theta_4 = \psi_4$ , and therefore  $t_3 = s_3$  and  $t_4 = s_4$ . This implies that the mixture will indeed satisfy the phylogenetic invariants.

Now, because again the Fourier transform of a mixture is the mixture of the Fourier transform, using Lemma 1 and simplifying gives

$$\mu_1 = \frac{\theta_1|\theta_2 + \theta_5|}{\sqrt{\theta_2\theta_5}} \quad (10)$$

Now note that by making the ratio  $\theta_2/\theta_5$  small, it is possible to have  $\mu_1$  be close to 1, although  $\theta_1$  can be small. This setting corresponds (via (1)) to the case of the first branch length of the resulting tree to be going to 0, although the trees used to make the mixture may have long first branch lengths. It can be checked by calculations analogous to (10) that the other fidelities of the tree resulting from mixing will be, in order,  $\sqrt{\theta_2\theta_5}$ ,  $\theta_3$ ,  $\theta_4$ ,  $\sqrt{\theta_2\theta_5}$ . These are clearly strictly between 0 and 1, so the resulting tree will have positive branch lengths.

### Mixtures Mimicking a Tree of a Different Topology

In this section we answer the question of what branch lengths on a quartet can mix to mimic a quartet of a different topology.

**Proposition 6.** *Let  $k_1, \dots, k_4$  satisfy the following inequalities:*

$$k_1 > k_3 > k_4 > 1 > k_2 > 0, \quad (11)$$

$$\frac{1 - k_1^2}{k_1} \frac{1 - k_4^2}{k_4} + \frac{1 - k_2^2}{k_2} \frac{1 - k_3^2}{k_3} > 0, \quad (12)$$

$$\frac{k_1 + k_4}{1 + k_1k_4} \times \frac{k_2 + k_3}{1 + k_2k_3} > 1. \quad (13)$$

*Then there exists  $\pi_5$  such that for any  $\pi_5 < k_5 < \pi_5^{-1}$  sufficiently close to either  $\pi_5$  or  $\pi_5^{-1}$  there exists a mixing weight such that for any  $t_1, \dots, t_5$  and  $s_1, \dots, s_5$  satisfying  $\pi_5 = \exp(-2(t_5 + s_5))$  and  $k_i = \exp(-2(t_i - s_i))$  for  $i = 1, \dots, 5$ , the corresponding mixture of two 12|34 trees will satisfy the phylogenetic invariants for a single tree of the 13|24 topology. The resulting internal branch length is guaranteed to be positive, and the pendant branch lengths will be positive as long as the pendant branch lengths being mixed are sufficiently large.*

*Proof.* Let  $m$  denote the Fourier transform vector of the site pattern frequencies of the mixture. The invariants for a tree of topology 13|24 are (by (3) and (4))

$$m_{1234} - m_{13}m_{24} = 0 \quad (14)$$

$$m_{12}m_{34} - m_{14}m_{23} = 0. \quad (15)$$

As before, we insert the mixture of the Fourier transforms of the pattern frequencies into the invariants. For the first invariant,

$$(\alpha + 1 - \alpha)[\alpha q_{1234} + (1 - \alpha)r_{1234}] - [\alpha q_{13} + (1 - \alpha)r_{13}][\alpha q_{24} + (1 - \alpha)r_{24}] = 0.$$

Multiplying, this is equivalent to

$$\begin{aligned} &\alpha^2(q_{1234} - q_{13}q_{24}) \\ &+ \alpha(1 - \alpha)[q_{1234} + r_{1234} - (q_{13}r_{24} + r_{13}q_{24})] \\ &+ (1 - \alpha)^2(r_{1234} - r_{13}r_{24}) = 0. \end{aligned} \quad (16)$$

A similar calculation with the second invariant leads to

$$\begin{aligned} &\alpha^2(q_{12}q_{34} - q_{14}q_{23}) \\ &+ \alpha(1 - \alpha)[q_{12}r_{34} + r_{12}q_{34} - (q_{14}r_{23} + r_{14}q_{23})] \\ &+ (1 - \alpha)^2(r_{12}r_{34} - r_{14}r_{23}) = 0. \end{aligned} \quad (17)$$



Rather than (16) and (17) themselves, we can take (16) and the difference of (16) and (17). Because the  $q$  and  $r$  vectors come from a tree with topology 12|34, they satisfy  $q_{1234} = q_{12}q_{34}$  and  $q_{13}q_{24} = q_{14}q_{23}$  and the equivalent equations for the  $r$ . Thus the difference of (16) and (17) can be simplified to (assuming  $\alpha(1 - \alpha) \neq 0$ )

$$q_{1234} + r_{1234} - (q_{12}r_{34} + r_{12}q_{34}) \\ = q_{13}r_{24} + r_{13}q_{24} - (q_{14}r_{23} + r_{14}q_{23}). \quad (18)$$

We would like to ensure that the tree coming from the mixture has nonzero internal branch length. By Lemma 2 this is equivalent to showing that

$$m_{13} m_{24} > m_{14} m_{23}. \quad (19)$$

Substituting in for the mixture fidelities and simplifying results in

$$\alpha^2(q_{13}q_{24} - q_{14}q_{23}) \\ + \alpha(1 - \alpha)[q_{13}r_{24} + r_{13}q_{24} - (q_{14}r_{23} + r_{14}q_{23})] \\ + (1 - \alpha)^2(r_{13}r_{24} - r_{14}q_{23}) > 0.$$

The first and last terms of this expression vanish because the  $q$  and  $r$  satisfy the 12|34 phylogenetic invariants coming from (3) and (4). Simplifying leads to

$$q_{13}r_{24} + r_{13}q_{24} > q_{14}r_{23} + r_{14}q_{23}. \quad (20)$$

Define  $k_i = \psi_i/\theta_i$  for  $i = 1, \dots, 5$  and  $\rho = \alpha/(1 - \alpha)$ . Note that

$$0 < \theta_i < \min(k_i^{-1}, 1) \text{ and } 0 < k_i < \infty \quad (21)$$

is equivalent to  $0 < \theta_i < 1$  and  $0 < \psi_i < 1$ . Define

$$\chi_{12} = k_1 k_2 + k_3 k_4 \quad \chi_{13} = k_1 k_3 + k_2 k_4 \\ \chi_{14} = k_1 k_4 + k_2 k_3 \quad \chi_{1234} = 1 + k_1 k_2 k_3 k_4.$$

Later we will make use of the fact that the  $\chi$  are invariant under the action of the Klein 4 group.

Using these definitions, direct substitution using (2) into (16), (18), and (20) and some simplification shows that the set of equations

$$\rho^2(1 - \theta_5^2) + \rho(\chi_{1234} - \theta_5 \psi_5 \chi_{13}) \\ + (1 - \psi_5^2)(\chi_{1234} - 1) = 0 \quad (22)$$

$$\chi_{1234} - \chi_{12} = \theta_5 \psi_5 (\chi_{13} - \chi_{14}) \quad (23)$$

$$\chi_{13} > \chi_{14} \quad (24)$$

is equivalent to Equations (14), (15), and (19).

Equation (23) is simply satisfied by setting

$$\theta_5 \psi_5 = \frac{\chi_{1234} - \chi_{12}}{\chi_{13} - \chi_{14}}. \quad (25)$$

However, in doing so, we must require that this ratio is strictly between 0 and 1. The fact that it must be less than one can be written

$$\chi_{14} + \chi_{1234} < \chi_{12} + \chi_{13} \quad (26)$$

which by a short calculation is equivalent to (13). Later it will be shown that other equations imply that (25) is greater than 0.

Assign variables  $A$ ,  $B$ , and  $C$  in the standard way such that (22) can be written  $A\rho^2 + B\rho + C$ . The  $A$  and  $C$  terms are strictly positive, thus the existence of a  $0 < \rho < \infty$  satisfying this equation implies

$$B < 0 \text{ and } B^2 - 4AC > 0. \quad (27)$$

On the other hand, (27) implies the existence of a  $0 < \rho < \infty$  satisfying (22).

Note that using (25),  $B < 0$  is equivalent to

$$\chi_{1234} - \frac{\chi_{1234} - \chi_{12}}{\chi_{13} - \chi_{14}} \chi_{13} < 0.$$

Multiplying by  $\chi_{13} - \chi_{14}$ , which is positive by (24), this equation is equivalent to

$$\chi_{12} \chi_{13} < \chi_{1234} \chi_{14} \quad (28)$$

which by a short calculation is equivalent to (12). The conclusion then is that the existence of a  $\rho \geq 0$  satisfying (22) is equivalent to (12) and  $B^2 - 4AC > 0$  given the rest of the invariants.

Now, (24) and (28) imply that  $\chi_{12} < \chi_{1234}$ . Therefore, according to (25), the product  $\theta_5 \psi_5$  is greater than 0, given (24). For convenience, set  $\pi_5 = \theta_5 \psi_5$ , which as described is determined by  $k_1, \dots, k_4$ . Now,  $\theta_5$  being less than 1 and  $\psi_5$  being less than 1 are equivalent to

$$\pi_5 < k_5 < \pi_5^{-1}. \quad (29)$$

In summary, the problem of finding branch lengths and a mixing parameter such that the derived variables satisfy (14), (15), and (19) is equivalent to finding  $k_i$  and  $\theta_i$  satisfying (12), (13), (21), (24), (25), (29), and  $B^2 - 4AC > 0$ , which can be written

$$(\chi_{1234} - \pi_5 \chi_{13})^2 - 4(1 - \pi_5/k_5)(1 - \pi_5 k_5)(\chi_{1234} - 1) > 0. \quad (30)$$

Note that  $\chi_{1234} = \pi_5 \chi_{13}$  is impossible using (23) and (28). Therefore, (30) can be satisfied while fixing the other variables by taking  $k_5$  close to  $\pi_5$  or  $\pi_5^{-1}$  while satisfying (29).

Now we show that (possibly after relabeling) Equation (11) is equivalent to (24) in the presence of the other inequalities. Recall that the  $\chi$  are invariant under the action of the Klein group acting on the indices of  $k_i$ . Because the invariants are equivalent to equations that can be expressed in terms of the  $\chi$  with  $\theta_5$  and  $\psi_5$ , we can assume that  $k_1 \geq k_2$  and  $k_1 \geq k_3$  by renumbering via an element of the Klein group.

Now, subtract  $\chi_{12} \chi_{14}$  from (28) to find

$$\chi_{12}(\chi_{13} - \chi_{14}) < (\chi_{1234} - \chi_{12})\chi_{14}.$$

Rearranging (26), it is clear that this implies that

$$\chi_{12} < \chi_{14}. \quad (31)$$

Inserting the definition of the  $\chi$  into (24) and (31) shows that these equations are equivalent to

$$0 < (k_1 - k_2)(k_3 - k_4) \text{ and } 0 < (k_1 - k_3)(k_4 - k_2). \quad (32)$$

We have assumed by symmetry that  $k_1 \geq k_2$  and  $k_1 \geq k_3$ ; now (32) shows that  $k_1$  can't be equal to either  $k_2$  or  $k_3$ . Also, (32) shows that  $k_3 > k_4$  and  $k_4 > k_2$ . All of these inequalities put together imply that  $k_1 > k_3 > k_4 > k_2$ , which directly implies (24).

Furthermore, another rearrangement of (26) using the inequality (31) leads to  $\chi_{1234} < \chi_{13}$ . This after substitution gives  $(1 - k_1 k_3)(1 - k_2 k_4) < 0$ , which implies that it is impossible for all of the  $k_i$  to be either less than or greater than 1.



Note that (12) excludes the case  $k_1 > k_3 > 1 > k_4 > k_2$ ; this leaves  $k_1 > 1 > k_3 > k_4 > k_2$  and  $k_1 > k_3 > k_4 > 1 > k_2$ . We can assume the latter without loss of generality by exchanging the  $\theta_i$  and the  $\psi_i$  (which corresponds to replacing  $k_i$  with  $k_i^{-1}$ ) and renumbering.

So far we have described how to find values for the branch lengths so that the Invariants (3) and (4) and the internal branch length inequality (19) are satisfied. However, we also need to check that the resulting pendant branch lengths for the tree are positive. Here we describe how this can be achieved by taking a lower bound on the values of  $t_i$ .

Assume edges  $a$  and  $b$  are adjacent on the 12|34 trees being mixed, and  $a$  and  $c$  are adjacent on the resulting 13|24 tree. Then, by Lemma 1 and (2), the fidelity of the pendant  $a$  edge is

$$\sqrt{\frac{(\alpha\theta_a\theta_b + (1-\alpha)\psi_a\psi_b)[\alpha\theta_a\theta_5\theta_c + (1-\alpha)\psi_a\psi_5\psi_c]}{\alpha\theta_b\theta_5\theta_c + (1-\alpha)\psi_b\psi_5\psi_c}}.$$

In order to assure that the resulting pendant branch length for edge  $a$  is positive, we must show that the above fidelity is less than 1. This is equivalent to showing that  $\theta_a$  must satisfy

$$\theta_a < \sqrt{\frac{\alpha + (1-\alpha)k_b k_5 k_c}{[\alpha + (1-\alpha)k_a k_b][\alpha + (1-\alpha)k_a k_5 k_c]}} \quad (33)$$

for all such  $a, b, c$  triples. Thus this equation along with (21) imply upper bounds for  $\theta_a$ ; by the definition of fidelities these translate to lower bounds for  $t_a$ . This concludes the proof.

Note that the proof actually completely characterizes (up to relabeling) the set of branch lengths and mixing weights such that the resulting mixture mimics a tree of different topology.

**Proposition 7.** *If two sets of branch lengths on the 12|34 tree mix to mimic a tree of the topology 13|24, then up to relabeling the associated  $k_i$  must satisfy the inequalities (11), (12), (13), and (29); the  $\theta_i$  must satisfy the inequalities (21) and (33). The two required equalities are that the product  $\theta_5\psi_5$  must satisfy (25), and the associated  $\rho$  must satisfy (22).*

#### Kullback-Leibler Lemma

**Lemma 8.** *Assume some group-based model  $G$  and let  $\Delta$  be the probability simplex for distributions on four taxa under  $G$ . Let  $V \subset \Delta$  be the set of all*

*site-pattern frequencies for some quartet tree under  $G$ . Then*

$$\delta_{KL}(p, V) := \min_{v \in V} \delta_{KL}(p, v)$$

*exists and is continuous for all  $p$  in the interior of  $\Delta$ .*

*Proof.* Note that  $\delta_{KL}(p, q)$  is a continuous function when probability distributions  $p$  and  $q$  have no components 0; i.e., they sit in the interior  $\Delta^\circ$  of the probability simplex  $\Delta$ . We will show that for any  $p \in \Delta^\circ$ , there exists an open neighborhood  $U$  of  $p$  such that  $\delta_{KL}(p', V)$  exists and is continuous for all  $p' \in U$ . Given  $p$ , let  $p_{\min}$  be the smallest component  $p_i$  of  $p$ . Let  $U = \{p' \in \Delta^\circ : p'_i > p_{\min}/2\}$ . Then choose  $\varepsilon > 0$  such that

$$\log(p_{\min}/2) + \frac{1}{2} p_{\min} \log(1/\varepsilon) > \sup_{p' \in U} \inf_{q \in V} \delta_{KL}(p', q).$$

The right-hand side of this equation is finite (because it is bounded above by  $\sup_{p' \in U} \delta_{KL}(p', q^*)$  for any point  $q^* \in V$  with no components 0).

Let  $B = \{q \in V : q_i \geq \varepsilon \text{ for all } i\}$ .  $V$ , being the continuous image of a closed unit cube, is a compact set (Moulton and Steel, 2004); therefore,  $B \subset \Delta^\circ$  is compact as well. Now for any  $p' \in U$  and  $q' \in V - B$

$$\begin{aligned} \delta_{KL}(p', q') &= \sum_i p'_i \log p'_i + \sum_i p'_i \log(1/q'_i) \\ &> \log(p_{\min}/2) + \frac{1}{2} p_{\min} \log(1/\varepsilon) \\ &> \inf_{q \in V} \delta_{KL}(p', q) \end{aligned}$$

so the infimum cannot be achieved outside  $B$ . Consequently,

$$\inf_{q \in V} \delta_{KL}(p', q) = \min_{q \in B} \delta_{KL}(p', q)$$

for all  $p' \in U$ . Thus the right-hand side exists; continuity follows from standard analytic arguments.