Invertibility of the TKF model of sequence evolution *

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Abstract

We consider character sequences evolving on a phylogenetic tree under the TKF91 model. We show that as the sequence lengths tend to infinity the topology of the phylogenetic tree and the edge lengths are determined by any one of (a) the alignment of sequences (b) the collection of sequence lengths. We also show that the probability of any homology structure on a collection of sequences related by a TKF91 process on a tree is independent of the root location.

Keywords: phylogenetics, DNA sequence evolution models, identifiability, alignment

1 Introduction

One of the important mathematical problems in phylogenetics is formulated as follows. Suppose a collection of sequences (of nucleotides or amino acids) has evolved from a common ancestral sequence under a stochastic process of evolution on a phylogenetic tree. The process of evolution allows substitution of characters, insertion or deletions of segments of the sequence, and possibly other types of mutations. A natural question is: given current sequences (that is, sequences at the leaves of the tree), can we reconstruct the evolutionary relationship between them unambiguously? Here evolutionary relationship means the topology of the underlying phylogenetic tree and edge lengths, as well as other parameters that define the stochastic process, and possibly more information such as the homology relationships between individual characters.

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One hopes that given long enough sequences, the evolutionary relationship between sequences is obtained accurately.

There are several reasons for studying such models. Such models are a useful compromise between biological reality and computational tractability. They allow the use of maximum likelihood methods for estimating evolutionary relationships, and maximum likelihood methods can be proved to be consistent for many such models.

Several earlier studies focused on pure substitution models only, that is, models that didn’t consider insertion-deletion events. In one of the earlier papers by Felsenstein [1], an algorithm was proposed to compute the likelihood of a tree (given sequences) for a class of reversible substitution models. Many invertibility (unique identifiability of the underlying tree) results have already been known for classes of Markov substitution models. For example, in [2] and [3] it was shown that the tree topology and edge lengths are identifiable from the joint distribution of character states at pairs of terminal nodes under some mild conditions on the Markov transition matrices on the edges of the tree. This result was proved using the following result due to Hakimi and Patrinos [4] about additive functions on trees.

**Lemma 1.** Let \( T \) be a tree on the vertex set \( V \). Let \( f \) be a non-zero real valued function defined on the set of subsets of \( V \) of cardinality 2, satisfying the additivity condition

\[
f(\{x, y\}) = \sum_{i=0}^{r-1} f(\{x_i, x_{i+1}\})
\]

where \( x_0, x_1, \ldots, x_r \) is the unique path in \( T \) connecting \( x = x_0 \) and \( y = x_r \). Then the value of \( f \) on all pairs of leaf nodes of \( T \) determines uniquely the tree \( T \) and the function \( f \).

A restricted version of the above result for positive weights was proved by Zaretskii [5] and Buneman [6].

General identifiability results in case of four-state characters, which are of particular interest for the phylogenetic analysis of DNA sequences, were proved using a mathematical technique known as Hadamard Conjugation, for example, see [3, 7-8, 9]. However, these results are based on certain symmetry assumptions on transition matrices. Strong results for general 12 parameter 4x4 Markov transition matrices have been proved by Chang [10]. He has proved that full reconstruction (under some mild restrictions on the Markov models) is possible by looking at character distributions at triples of leaf nodes.

The above mentioned results do not consider insertion-deletion events. In 1991 and 1992, Thorne, Kishino and Felsenstein [11, 12] introduced models that allowed insertion-deletion events in addition to character substitution events. We refer to the
models by TKF91 and TKF92, respectively. The models consider sequence evolution as a birth-death process. In the TKF91 model, a birth event inserts a single character in the sequence, and a death event deletes a single character from the sequence. In the TKF92 model, a birth event inserts a segment of characters in the sequence, and a death event deletes a segment of characters from the sequence. They proposed dynamic programming alignment algorithms for a pair of sequences. Recently the models have been extensively studied, and many intricate algorithms for sequence alignment and phylogenetic tree reconstruction have been proposed. Although the algorithms based on the TKF framework are difficult to describe and to implement, they have many advantages, see for example, [13, 14, 15]. It is therefore important to analyse the TKF models mathematically in the same way the symmetric and asymmetric substitution models were analysed by Hendy, Penny, Steel and Chang. In particular, it is worthwhile to know if these models are invertible and if the algorithms based on them are consistent.

In this paper, we prove two invertibility results for the TKF91 model. We prove that an alignment of sufficiently long multiple sequences, (to be precise a homology structure on them,) contains enough statistical information to estimate the parameters of the birth-death process as well as the tree topology and edge lengths. We also show how to estimate the topology and model parameters from only the sequence lengths provided the sequences are sufficiently long. In the appendix, we prove that the probability of a general homology structure on a collection of sequences does not depend on the root location. This result is of theoretical as well as practical importance. It has been used (without proof) to test implementations of algorithms in [14]. Thus this paper gives a rigorous mathematical analysis of the TKF91 model rather than proposing new algorithms.

The paper is structured as follows. In Section 2 we give an overview of the TKF91 model, and demonstrate the main idea of this paper for a pair of sequences. In Section 3 we show the invertibility based on the homology structure, and in Section 4 we prove the invertibility based on sequence lengths. In the last section we show the independence of the root location and the likelihood of the homology structure, and also discuss future directions.

2 The TKF91 model

Let a DNA or an amino acid sequence be represented by an alternating string of links and characters. The characters are denoted by # throughout, since the actual base or an amino acid is not important in any discussion in this paper. At the left end of the string is an immortal link. All links undergo a birth process at a rate $\lambda$. When a birth event occurs, a new character is placed to the right of the link, and a new link is
placed to the right of the new character. All characters undergo a death process at a rate $\mu$. When a character dies, the character and the link to its right are deleted from the string. The new character born in a birth event is chosen from a distribution. While a character is alive, it undergoes a substitution process at a rate $s$, and the substituted character is chosen from a distribution. The substitution model and the model for the choice of the new character in a birth event are not relevant here, since the homology structure (information as to which characters are homologous to which others) is what interests us in this paper. Suppose that a sequence $A$ evolves to a sequence $B$ in time $t$. Since all characters at all times are assumed to be evolving independently of all other characters, we look at the evolution of a single character in $A$. We describe the model by three coupled differential equations and their solutions. We refer the reader to [11] for details.

Let $p^H_n(t)$ be the probability that a character survives for time $t$, and at time $t$, it has $n$ descendents (counting the survived character as one of the descendents). Here superscript $H$ stands for “homologous”. By definition, $p^H_n(t) = 0$ for $n \leq 0$. The differential equation for $p^H_n(t)$ is

$$\frac{dp^H_n}{dt} = \lambda (n - 1) p^H_{n-1} + \mu n p^H_{n+1} - (\lambda + \mu) n p^H_n$$  \hspace{1cm} (1)

with the initial conditions

$$p^H_1(t = 0) = 1$$
$$p^H_n(t = 0) = 0 \text{ for } n > 1$$  \hspace{1cm} (2)

Let $p^N_n(t)$ be the probability that the character in $A$ dies before time $t$, but leaves $n$ descendents in $B$. Here superscript $N$ stands for “non-homologous”. By convention, $p^N_n(t) = 0$ for $n < 0$. The differential equation for $p^N_n(t)$ is

$$\frac{dp^N_n}{dt} = \lambda(n + 1) p^N_{n+1} + \mu n p^N_{n+1} + \mu p^H_{n+1} - (\lambda + \mu) n p^N_n$$  \hspace{1cm} (3)

with the initial condition

$$p^N_n(t = 0) = 0 \text{ for all } n$$  \hspace{1cm} (4)

Let $p^I_n(t)$ be the probability that the immortal link has $n$ descendents at time $t$. Here superscript $I$ stands for “immortal”. By convention, $p^I_n(t) = 0$ for $n < 0$. The differential equation for $p^I_n(t)$ is

$$\frac{dp^I_n}{dt} = \lambda n p^I_{n-1} + \mu (n + 1) p^I_{n+1} - \lambda(n + 1) p^I_n - \mu n p^I_n \text{ for } n > 0$$  \hspace{1cm} (5)

with the initial condition

$$p^I_n(t = 0) = 0 \text{ for all } n > 0$$  \hspace{1cm} (6)
Lemma 2. Solutions to the above differential equations are given by

\[
\begin{align*}
p_n^H(t) &= e^{-\mu t}(1 - \lambda \beta(t))((\lambda \beta(t))^{n-1} \quad \text{for } n > 0 \\
p_n^N &= \mu \beta(t) \quad \text{for } n = 0 \\
p_n^I &= (1 - e^{-\mu t} - \mu \beta(t))(1 - \lambda \beta(t))((\lambda \beta(t))^{n-1} \quad \text{for } n > 0 \\
p_n^I &= (1 - \lambda \beta(t))((\lambda \beta(t))^{n} \quad \text{for } n \geq 0
\end{align*}
\] (7)

where

\[
\beta(t) = \frac{1 - e^{(\lambda - \mu)t}}{\mu - \lambda e^{(\lambda - \mu)t}}
\] (8)

The main idea of this paper is the following observation about the solutions.

Corollary 3. Let A and B be two sufficiently long sequences related by the TKF91 model, A being the ancestor of B. Then model parameters (scaled time and the ratio of birth and death rates) are uniquely determined by the true alignment of A and B.

Proof. Observe that \(e^{-\mu t}\) is simply the probability that a character in A survives in B, \(e^{-\mu t} \lambda \beta(t)\) is \(\sum_{n \geq 2} p_n^H\), and \(1 - e^{-\mu t} - \mu \beta(t)\) is \(\sum_{n \geq 1} p_n^N\). This allows us to accurately estimate \(\mu t\), \(\lambda \beta(t)\) and \(\mu \beta(t)\), and therefore \(\lambda/\mu\), from an alignment of sufficiently long sequences.

In the next section, this idea, together with Lemma \(\text{Lemma 2}\) is applied to an alignment of several sequences. One has to show that probabilities of certain patterns in the alignment do not depend on the position of the root on the tree. Since not all patterns are required for estimating the pairwise distances between leaves, the independence of the probability of a pattern and the root position for general patterns is presented in the Appendix.

3 Multiple sequences

The main result of this section is the following.

Theorem 4. A multiple sequence alignment of sufficiently long sequences that have evolved under a TKF91 process on a phylogenetic tree uniquely determines the phylogenetic tree and the (scaled) edge lengths.

Before presenting the full proof, it will be useful to look at the main ideas behind the proof.

1. To be able to apply Lemma \(\text{Lemma 2}\) to construct the tree, we would like to compute the distance between each pair of vertices. Here the distance between a pair of vertices is the (scaled) divergence time between the corresponding taxa.
2. The distance between a pair of vertices is estimated by the method of Corollary 3. This is done by restricting the multiple alignment to the two sequences under consideration and then equating the observed pattern frequencies with those expected from the solutions of the TKF model.

3. Before we can equate the observed frequencies of patterns and the expected frequencies, we have to solve one important problem: in the discussion of the TKF91 model, we assumed that the sequence $A$ is the ancestor of the sequence $B$. But if the sequences $A$ and $B$ evolve from a common ancestor $C$ that is not $A$ or $B$, do we expect the same pattern frequencies? The bulk of the proof is devoted to answering this question by showing that the pattern probabilities do not depend on the location of $C$.

Let $S_i; 1 \leq i \leq k$ be the sequences at the leaves of a phylogenetic tree $T$ with root vertex $v$. The sequences are related by a TKF91 process on the tree. The vertices of the tree are labelled $v_i$ such that the first $k$ of them are leaf vertices respectively associated with the sequences $S_i$. Each vertex $v_i$ has associated with it a time parameter $t_i$, which is the duration for which the TKF91 process operates on the edge leading to $v_i$ from its immediate ancestor $a(v_i)$.

Consider any two sequences, say $S_1$ and $S_2$, and let $v_i$ be their most recent common ancestor. That is, the ancestral sequence $S$ at the root $v$ evolves into a sequence $S_i$ along the path from $v$ to $v_i$, and then two copies of $S_i$ evolve independently to sequences $S_1$ and $S_2$, respectively, along paths from $v_i$ to $v_1$ and $v_i$ to $v_2$. It is assumed that the unknown ancestral sequence at the root has already evolved for an infinite duration. It implies that the sequence $S_i$ has evolved for an infinite duration. Therefore, the homology relationship between $S_1$ and $S_2$ does not depend on the location of $v$, but perhaps depends on the location of $v_i$ on the path between $v_1$ and $v_2$. Therefore, the analysis of the homology structure of $S_1$ and $S_2$ may be performed by assuming that $v_i$ is the same as the root $v$. In other words, we will first analyse only two sequences evolving from their most recent common ancestor, which is assumed to have evolved for infinite time.

**The restricted alignment and its blocks**

Consider the alignment of $S_1$ and $S_2$ that is obtained by discarding all other rows in the multiple alignment (sequences $S_3$ to $S_k$), and then discarding the columns containing only gaps in the first two rows (rows for $S_1$ and $S_2$). The most recent common ancestor of $v_1$ and $v_2$ is $v$. Since we have discarded other sequences, and pruned the tree, we can assume that $v$ and $v_1$ are separated by time $t_1$, and $v$ and $v_2$ are separated by time $t_2$. We are interested in showing that the probabilities of the
patterns of interest are functions of $t_1 + t_2$, that is, they do not depend individually on $t_1$ or $t_2$.

An alignment of two sequences can be thought of as a sequence of blocks where a block consists of a pair of homologous characters followed by non-homologous characters, and terminated by a pair of homologous characters of the next block. For example, a possible alignment of $S_1$ and $S_2$, ignoring the beginning and end is

$$\begin{pmatrix} S_1 \# \# \# \# \# \# - \# - \# \# \\ S_2 \# \# - \# - \# \# \# \# - \# \end{pmatrix}$$

The alignment shows four types of blocks.

$$A \equiv \begin{pmatrix} \# & \# \\ \# & \# \end{pmatrix} \quad B \equiv \begin{pmatrix} \# & \# & \# \\ \# & - & \# \end{pmatrix} \quad C \equiv \begin{pmatrix} \# & \# & - & \# \\ \# & - & \# & \# \end{pmatrix} \quad D \equiv \begin{pmatrix} \# & - & \# & \# \\ \# & \# & - & \# \end{pmatrix}$$

Homologous characters in a column imply that there was a character in ancestral sequence $S$ that survived in both $S_1$ and $S_2$. We are interested in computing the probabilities $p(A)$, $p(B)$, $p(C)$ and $p(D)$ of observing, respectively, blocks of types $A$, $B$, $C$ and $D$.

**The classes of events that result in the blocks of types $A$, $B$, $C$ and $D$.**

Computing the probabilities involves summing over infinitely many possible events in the history that result in a block of a certain type. For example, a block of type $A$ is a result of historical events of the type

$$\begin{pmatrix} S_1 \# - \# \\ S \# \# \# i \# \\ S_2 \# - \# \end{pmatrix}; i \geq 0$$

Here we have $i$ characters in the ancestral sequence $S$ that died along the edges $v - v_1$ and $v - v_2$. Of course, we do not know $S$. So looking at the alignment of $S_1$ and $S_2$, we do not know how many characters died resulting in a block of type $A$. So the probability of observing $A$ would be the sum of probabilities over all non-negative values of $i$.

Similarly, there are two types of events that result in a block of type $B$. They are

$$E^1_B \equiv \begin{pmatrix} S_1 \# - \# - \# \\ S \# \# i \# \# j \# \\ S_2 \# - \# - \# \end{pmatrix} \quad \text{and} \quad E^2_B \equiv \begin{pmatrix} S_1 \# - \# - \# \\ S \# \# i \# - \# j \# \\ S_2 \# - \# - \# \end{pmatrix}$$
where $i \geq 0, j \geq 0$. Therefore, we write $p(B) = p(E^1_B) + p(E^2_B)$. Here $E^1_B$ is the possibility that the middle character in $S_1$ was homologous to a character in $S$, but along the branch from $v$ to $v_2$, the character in $S$ died. It is also possible that the middle character in $S_1$ in block $B$ was created in a birth event during the evolution from $S$ to $S_1$. This is the possibility $E^2_B$.

The following four types of events result in a block of type $C$.

$$E^1_C \equiv \begin{pmatrix} S_1 & \# - \# - \# - \# - \# \\ S & \# - \#^i \# - \#^j \# - \#^k \# \\ S_2 & \# - \# - \# - \# - \# \end{pmatrix}; i \geq 0, j \geq 0, k \geq 0$$

$$E^2_C \equiv \begin{pmatrix} S_1 & \# - \# - \# - \# - \# \\ S & \# - \#^i \# - \#^j - \#^k \# \\ S_2 & \# - \# - \# - \# - \# \end{pmatrix}; i \geq 0, j \geq 0, k \geq 0$$

$$E^3_C \equiv \begin{pmatrix} S_1 & \# - \# - \# - \# - \# \\ S & \# - \#^i - \#^j \# - \#^k \# \\ S_2 & \# - \# - \# - \# - \# \end{pmatrix}; i \geq 0, j \geq 0, k \geq 0$$

$$E^4_C \equiv \begin{pmatrix} S_1 & \# - \# - \# - \# - \# \\ S & \# - \#^i - \#^j - \#^k \# \\ S_2 & \# - \# - \# - \# - \# \end{pmatrix}; i \geq 0, j \geq 0, k \geq 0$$

Observe that they are disjoint classes of events, so we write

$$p(C) = p(E^1_C) + p(E^2_C) + p(E^3_C) + p(E^4_C)$$

Classes of events resulting in blocks of types $D$, are similar to those that produce the pattern $C$. But there is no distinction between types $C$ and $D$ when $j = 0$ in $E^4_C$. In the language of [14], $C$ and $D$ have the same homology structure. Therefore, we compute

$$p(C \lor D) = p(C) + p(D) - p(C; j = 0)$$

The probability computations involve summations over $i, j, k$ varying over all non-negative integers, but the summations can be easily computed since they happen to be geometric series.

**The Markov structure along the sequences**

How do we compute the probability of a class of events contributing to a block? The probability of an alignment of sequences on a tree is the product of the probabilities of the pairwise alignments on the edges of the tree. Also, an alignment of two sequences has a Markov structure along the sequences provided one of the sequences is the
ancestor of the other, see [15] for details. We use this idea to compute probabilities of classes of events using the Markov structure on \( v - v_1 \) and \( v - v_2 \).

The transition matrix for the Markov chain for a two sequence alignment, assuming one of the sequences to be the ancestor, is given by

\[
\begin{pmatrix}
H & D & I & \text{end} \\
(1 - \lambda \beta)\left(\frac{\lambda}{\mu}\right)e^{-\mu t} & (1 - \lambda \beta)\left(\frac{\lambda}{\mu}\right)(1 - e^{-\mu t}) & \lambda \beta & (1 - \lambda \beta)(1 - \frac{\lambda}{\mu}) \\
(1 - \kappa)\left(\frac{\lambda}{\mu}\right)e^{-\mu t} & (1 - \kappa)\left(\frac{\lambda}{\mu}\right)(1 - e^{-\mu t}) & \kappa & (1 - \kappa)(1 - \frac{\lambda}{\mu}) \\
(1 - \lambda \beta)\left(\frac{\lambda}{\mu}\right)e^{-\mu t} & (1 - \lambda \beta)\left(\frac{\lambda}{\mu}\right)(1 - e^{-\mu t}) & \lambda \beta & (1 - \lambda \beta)(1 - \frac{\lambda}{\mu}) \\
\end{pmatrix}
\]

where \( H \) (homology), \( D \) (deletion) and \( I \) (insertion) denote the three states \((\#\#)\), \((\#\ -)\) and \((\-\#)\), respectively, and

\[
\kappa(t) = \kappa = 1 - \frac{\mu \beta}{1 - e^{-\mu t}}
\]

Now on we denote \( \beta(t_i) \) by \( \beta_i \), \( e^{-\mu t_i} \) by \( X_i \), \( \kappa(t_i) \) by \( \kappa_i \); for \( i \in \{1, 2\} \), and \( \beta(t) = \beta(t_1 + t_2) \) by \( \beta \), \( e^{-\mu t} \) by \( X = X_1X_2 \), and \( \kappa(t) = \kappa(t_1 + t_2) \) by \( \kappa \).

**Computing \( p(A) \)**

Using the transition matrix in (9), we can write

\[
p(A) = (1 - \lambda \beta_1)(1 - \lambda \beta_2) \left(\frac{\lambda}{\mu}\right) X_1X_2 \\
+ (1 - \lambda \beta_1)(1 - \lambda \beta_2) \left(\frac{\lambda}{\mu}\right)(1 - X_1)(1 - X_2) \\
\times \sum_{i \geq 1} \left( \left(\frac{\lambda}{\mu}\right)(1 - \kappa_1)(1 - \kappa_2)(1 - X_1)(1 - X_2) \right)^{i-1} \\
\times (1 - \kappa_1)(1 - \kappa_2) \left(\frac{\lambda}{\mu}\right) X_1X_2 \\
\times \frac{(1 - \lambda \beta_1)(1 - \lambda \beta_2)(\frac{\lambda}{\mu})e^{-\mu(t_1+t_2)}}{1 - (\frac{\lambda}{\mu})(1 - \kappa_1)(1 - \kappa_2)(1 - X_1)(1 - X_2)}
\]

(10)
where the first term corresponds to $i = 0$.

One can verify that

\[
\frac{(1 - \lambda \beta_1)(1 - \lambda \beta_2)}{1 - \left(\frac{\lambda}{\mu}\right)(1 - \kappa_1)(1 - \kappa_2)(1 - X_1)(1 - X_2)} = \frac{(1 - \lambda \beta_1)(1 - \lambda \beta_2)}{1 - \lambda \mu \beta_1 \beta_2} = 1 - \lambda \beta
\]

Therefore,

\[
p(A) = (1 - \lambda \beta) \left(\frac{\lambda}{\mu}\right) e^{-\mu t}
\]

Thus, as required, $p(A)$ depends only on $t = t_1 + t_2$ but not on $t_1$ and $t_2$ individually.

**Computing $p(B)$**

We compute $p(E_B^1)$ as a product two factors $F_1$ and $F_2$ which, respectively, correspond to the transitions shown below.

\[
\begin{pmatrix}
1 & \# & - & \# \\
v & \# & \#^i & \# \\
2 & \# & - & -
\end{pmatrix}; i \geq 0 \text{ and } \begin{pmatrix}
1 & \# & - & \# \\
v & \# & \#^j & \# \\
2 & \# & - & -
\end{pmatrix}; j \geq 0
\]

Therefore,

\[
F_1 = (1 - \lambda \beta_1)(1 - \lambda \beta_2) \left(\frac{\lambda}{\mu}\right) X_1(1 - X_2)
\]

\[
+ (1 - \lambda \beta_1)(1 - \lambda \beta_2) \left(\frac{\lambda}{\mu}\right)(1 - X_1)(1 - X_2)
\]

\[
\times \sum_{i \geq 1} \left((1 - \kappa_1)(1 - \kappa_2)\left(\frac{\lambda}{\mu}\right)(1 - X_1)(1 - X_2)\right)^{i-1}
\]

\[
\times (1 - \kappa_1)(1 - \kappa_2) \left(\frac{\lambda}{\mu}\right) X_1(1 - X_2)
\]

\[
= (1 - \lambda \beta) \left(\frac{\lambda}{\mu}\right) X_1(1 - X_2)
\]

\[
\text{and,}
\]

\[
F_2 = \frac{(1 - \lambda \beta_1)(1 - \kappa_2)\left(\frac{\lambda}{\mu}\right) X_1 X_2}{1 - \lambda \mu \beta_1 \beta_2}
\]
Therefore,
\[
p(E^1_B) = \frac{(1 - \lambda \beta)(1 - \lambda \beta_1)(1 - \kappa_2)(\frac{\lambda}{\mu})^2 X_1^2 X_2 (1 - X_2)}{1 - \lambda \mu \beta_1 \beta_2} \quad (15)
\]

Next, the contribution from the class of events \( E^2_B \) is computed.
\[
p(E^2_B) = \left( \frac{\lambda \beta_1 (1 - \lambda \beta_2)}{1 - \lambda \mu \beta_1 \beta_2} + \frac{(1 - \lambda \beta_1)(1 - \lambda \beta_2)(\frac{\lambda}{\mu})(1 - X_1)(1 - X_2) \kappa_1 (1 - \kappa_2)}{1 - \lambda \mu \beta_1 \beta_2} \right) \times \frac{(1 - \lambda \beta_1)(\frac{\lambda}{\mu}) X_1 X_2}{1 - \lambda \mu \beta_1 \beta_2} \quad (16)
\]

Adding \( p(E^1_B) \) and \( p(E^2_B) \), and after simplification, we have
\[
p(B) = (1 - \lambda \beta) \lambda \beta \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \quad (17)
\]

Again, \( p(B) \) depends only on \( t = t_1 + t_2 \).

**Computing \( p(C \lor D) \)**

This computation is performed on similar lines as the computation for \( p(A) \) and \( p(B) \) except that the events in \( E^4_C \) when \( j = 0 \) also contribute to \( p(E^4_D) \). Therefore,
\[
p(C \lor D) = p(E^1_C) + p(E^1_D) + p(E^2_C) + p(E^2_D) + p(E^3_C) + p(E^3_D) + p(E^4_C - E^4_D) \quad (18)
\]

Skipping the details, we state below different terms that contribute to \( p(C \lor D) \).
\[
p(E^1_C) + p(E^1_D) = 2(1 - \lambda \beta)^2 \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \frac{\lambda \mu \beta_1 \beta_2}{1 - \lambda \mu \beta_1 \beta_2} \quad (19)
\]
\[
p(E^2_C) + p(E^2_D) = (1 - \lambda \beta)^2 \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \left( \frac{\lambda}{\mu} \right) \left( \frac{X_1 (1 - X_2) \kappa_1 + X_2 (1 - X_1) \kappa_2}{1 - \lambda \mu \beta_1 \beta_2} \right) \quad (20)
\]
\[
p(E^3_C) + p(E^3_D) \\
= (1 - \lambda \beta)^2 \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \left( \frac{\lambda}{\mu} \right) \left( (1 - X_1) X_2 \kappa_1 + (1 - X_2) X_1 \kappa_2 \right) \left( \frac{\lambda \mu \beta_1 \beta_2}{1 - \lambda \mu \beta_1 \beta_2} \right) + (1 - \lambda \beta) \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \left( \frac{\lambda}{\mu} \right) \frac{\lambda \mu \beta_1 \beta_2 X_2 (1 - \lambda \beta_2) + \lambda \mu \beta_1 \beta_2 X_1 (1 - \lambda \beta_1)}{1 - \lambda \mu \beta_1 \beta_2} \quad (21)
\]
\[
p(E_C^4) + p(E_B^4) - p(E_C^4; j = 0)
= (1 - \lambda\beta)^2 \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \left( \frac{\lambda}{\mu} \right) (1 - X_1)(1 - X_2)\kappa_1\kappa_2
+ (1 - \lambda\beta) \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \left( \frac{\lambda}{\mu} \right) \lambda\mu\beta_1\beta_2
+ 2(1 - \lambda\beta)^2 \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \left( \frac{\lambda}{\mu} \right) (1 - X_1)(1 - X_2) \times \kappa_1\kappa_2 \left( \frac{\lambda\mu\beta_1\beta_2}{1 - \lambda\mu\beta_1\beta_2} \right)
+ (1 - \lambda\beta) \left( \frac{\lambda}{\mu} \right) e^{-\mu t} \left( \frac{\lambda}{\mu} \right) (1 - X_1)(1 - X_2)
\times \frac{\lambda\beta_1(1 - \lambda\beta_2)(1 - \kappa_1)\kappa_2 + \lambda\beta_2(1 - \lambda\beta_1)(1 - \kappa_2)\kappa_1}{1 - \lambda\mu\beta_1\beta_2}
\]

Adding Equations (19), (20), (21), and (22), and simplifying, we get
\[
p(C \lor D) = (1 - \lambda\beta)^2 \left( \frac{\lambda}{\mu} \right) (1 - e^{-\mu t} - \mu\beta) \left( \frac{\lambda}{\mu} \right) e^{-\mu t}
+ \lambda\beta(1 - \lambda\beta) \left( \frac{\lambda}{\mu} \right) \mu\beta \left( \frac{\lambda}{\mu} \right) e^{-\mu t}
= (1 - \lambda\beta)^2 \left( \frac{\lambda}{\mu} - \frac{\lambda}{\mu} e^{-\mu t} - \lambda\beta \right) \left( \frac{\lambda}{\mu} \right) e^{-\mu t} + (\lambda\beta)^2(1 - \lambda\beta) \left( \frac{\lambda}{\mu} \right) e^{-\mu t}
\]

Identifying \( T, \lambda t_i \) and \( \mu t_i \)

The pattern probabilities obtained above are sufficient for the estimation of model parameters and the scaled edge lengths. From Equations (12) and (17) we can write:
\[
\lambda\beta = \frac{p(B)}{p(A)}
\left( \frac{\lambda}{\mu} \right) e^{-\mu t} = \frac{p(A)}{1 - \lambda\beta} = \frac{p(A)^2}{p(A) - p(B)}
\]
Substituting $\lambda \beta$ and $\left( \frac{\lambda}{\mu} \right) e^{-\mu t}$ in Equation (23) we can express $\left( \frac{\lambda}{\mu} \right)$ and $e^{-\mu t}$ (and hence $\mu t$ and $\lambda t$) in terms of $p(A)$, $p(B)$ and $p(C \lor D)$.

Repeating this exercise for all pairs of leaves and then applying Lemma 11 we see that the tree topology and the scaled time parameters on all edges are a function of the probabilities of blocks of type $A$, $B$ and $C \lor D$ in all pairwise alignments. This completes the proof of Theorem 4.

4 Invertibility based on sequence lengths

The main result of this section is

**Theorem 5.** Given a collection of sequences that have evolved under a TKF91 process with parameters $\lambda$ and $\mu$ on a phylogenetic tree, the phylogenetic tree and the scaled edge lengths are uniquely determined by the collection of sequence lengths provided the sequences are sufficiently long.

The idea of the proof is that if a sequence of length $X_0$ evolves under a TKF91 model into a sequence of length $X$ in time $t$, then assuming $X$ to be the expected length of the sequence at time $t$ allows us to estimate $\mu t$. To justify this, we demonstrate that the relative standard deviation of the length distribution goes to zero as the initial length tends to infinity.

Let $P(X, t)$ be the probability that a sequence evolving under the TKF91 model has length $X$ at time $t$. The differential equation for $P(X, t)$ is

$$\frac{dP(X, t)}{dt} = \lambda XP(X-1, t) + \mu (X+1)P(X+1, t) - \lambda(X+1)P(X, t) - \mu XP(X, t)$$

with the initial conditions $P(X = X_0, 0) = 1$ and $P(X \neq X_0, 0) = 0$. Observe that this is slightly different from the standard equation for the simple birth-death process because of the immortal link, which has 0 death-rate. Let $M_1(t)$ and $M_2(t)$ denote the first and the second moments of $P(X, t)$. They are calculated below.

Multiplying both sides of Equation (24) by $X$, and summing over $X$, we get

$$\frac{dM_1}{dt} = (\lambda - \mu)M_1 + \lambda$$

with the initial condition

$$M_1(0) = X_0$$

This has the solution

$$M_1(t) = \frac{(\lambda - \mu)X_0 + \lambda}{\lambda - \mu} e^{(\lambda - \mu)t} - \lambda$$

$$= \frac{\lambda/\mu}{1 - \lambda/\mu} + \left( X_0 - \frac{\lambda/\mu}{1 - \lambda/\mu} \right) e^{-\mu(1-\lambda/\mu)}$$
Multiplying both sides of Equation (24) by $X^2$ and summing over $X$, we get the differential equation for $M_2(t)$.

$$ \frac{dM_2}{dt} = 2(\lambda - \mu)M_2 + (3\lambda + \mu)M_1 + \lambda $$

with the initial condition

$$ M_2(0) = X_0^2 $$

This has the solution

$$ M_2(t) = \frac{(\lambda + \mu)\lambda}{(\lambda - \mu)^2} \frac{((\lambda - \mu)X_0 + \lambda)(3\lambda + \mu)}{(\lambda - \mu)^2} e^{(\lambda - \mu)t} + \left(X_0^2 + \frac{((\lambda - \mu)X_0 + \lambda)(3\lambda + \mu)}{(\lambda - \mu)^2} - \frac{(\lambda + \mu)\lambda}{(\lambda - \mu)^2} \right) e^{2(\lambda - \mu)t} $$

Therefore, the variance is given by

$$ \sigma^2 = M_2 - M_1^2 = \frac{(\lambda + \mu)(\lambda - \mu)X_0 + \lambda^2}{(\lambda - \mu)^2} e^{2(\lambda - \mu)t} - \frac{(\lambda + \mu)((\lambda - \mu)X_0 + \lambda)}{(\lambda - \mu)^2} e^{(\lambda - \mu)t} + \frac{\lambda \mu}{(\lambda - \mu)^2} $$

The expected length $\bar{L}$ at equilibrium is given by $\frac{\lambda / \mu}{1 - \lambda / \mu}$. So we calculate $\sigma^2 / \bar{L}^2$ as follows.

$$ \frac{\sigma^2}{\bar{L}^2} = \frac{(\lambda + \mu)(\lambda - \mu)X_0}{\lambda^2} \left(e^{2(\lambda - \mu)t} - e^{(\lambda - \mu)t}\right) + \left(e^{2(\lambda - \mu)t} - \frac{\lambda + \mu}{\lambda} e^{(\lambda - \mu)t} + \frac{\mu}{\lambda}\right) $$

This tends to 0 as $\lambda / \mu$ tends to 1. Therefore, for sufficiently long sequences the tree and scaled time parameters can be estimated as follows.

First we estimate $\frac{\lambda}{\mu}$.

$$ \frac{\lambda}{\mu} = \frac{\bar{L}}{L + 1} $$

Now for any two sequences $S_1$ and $S_2$, with lengths $X_1$ and $X_2$, respectively, $\mu t = \mu(t_1 + t_2)$ (which is the scaled time separating $S_1$ and $S_2$) is estimated from Equation (27) by substituting $X_0 = X_1$ and $M_1(t) = X_2$. Repeating this for all pairs of sequences, and applying Lemma 1, the tree $T$ and the scaled time parameters $\lambda t_i$ and $\mu t_i$ are uniquely recovered.

**Remark.** A weaker form of the results in the appendix is implicitly assumed here: for every pair of sequences, we treated one of them to be the ancestor of the other.
5 Discussion

This paper gives a mathematical analysis of the TKF91 model on a tree. We present two uniqueness results for sufficiently long sequences: the first one establishes a correspondence between the distribution of certain patterns in a multiple alignment and the phylogenetic tree and scaled time parameters associated with edges of the tree; the second result gives a correspondence between sequence lengths and the phylogenetic tree along with other model parameters. Results presented here give us consistency criteria that the alignments and trees constructed under the TKF91 model from a set of sequences must meet.

It is not likely that the idea of the proofs presented here will be useful as it is for building trees. The limiting situation of sequence lengths going to infinity arises simultaneously with the ratio $\lambda/\mu$ going to one, and we do not know if the real sequences can be modelled with the ratio close to one. Nevertheless, the correspondence between the homology structures and the trees has useful algorithmic implications. Lunter et al. [14] mention that a good method of sampling multiple alignments under a fixed tree is currently missing. The correspondence between the homology structures and the trees could be useful in an alignment sampling procedure. Many alignments could be discarded purely on the basis of pattern probabilities without actually evaluating the alignment likelihoods. There are two more directions in which future progress may be made. For substitution models, Erdős, et al. [17] have studied the problem of finding a lower bound on sequence lengths that would be sufficient for the correct reconstruction of the tree with a high probability. Interestingly this bound is not large: for $n$ taxa, sequence lengths of the order of a power of $\log n$ are sufficient. It is conceivable that such a result exists for TKF type processes. Another direction is to prove similar results for the variants of TKF model, such as the model proposed by Metzler et al. [18] in which $\lambda$ and $\mu$ are the same and the variants in which longer segments are inserted or deleted, (for example, the TKF92 model and the model of [16]).

Appendix: General Homology Structures

A purpose of this appendix is to demonstrate that the likelihood of a general homology structure on sequences related by a TKF91 process on a tree does not depend on the location of the root on the tree.

Let $[i,j]$ denote the set of integers from $i$ to $j$. A rooted phylogenetic $X$-tree $T$ is a rooted tree with leaf set $X = \{x_i; i \in [1,p]\}$, root vertex $x_0$ of degree at least 2, and all other vertices (denoted by $x_i; i \in [p+1,p+q]$) of degree at least 3. Let $V$ and $E$ denote, respectively, the vertex set and the edge set of $T$. The edges of
$T$ are directed away from the root. Associated with each vertex $x_i$ is a sequence $S_i$ of length $L_i + 1$ over a finite alphabet $\Sigma$. The sequences are related to each other by a TKF91 process with parameters $\lambda$, $\mu$ and time parameter $t_e$ for each edge $e$ of $T$. The sequences $S_i; i \in [1, p]$ are observed sequences, and remaining sequences are unobserved.

Let $s_{ij}$ denote the $j$-th character in $S_i$. A segment of $S_i$ from $s_{ij}$ to $s_{ik}$ is denoted by $S_i[j, k]$. The characters $s_{ij}$ and $s_{kl}$ are said to be homologous (denoted by $s_{ij} \sim s_{kl}$) if $x_i$ and $x_k$ have a common ancestor $x_h$ such that a character in $S_h$ survives as characters $s_{ij}$ and $s_{kl}$ under the TKF91 process. For notational convenience we think of the immortal link as the zeroth character in all sequences, and write $s_{i0} \sim s_{j0}$ for all $i$ and $j$. Thus each $S_i$ has $L_i$ characters other than the immortal link. By definition, each character in each sequence is homologous to itself. Therefore, $\sim$ is an equivalence relation on $\{s_{ij}; i \in [0, p + q]\}$. The collection of equivalence classes (or their restriction to a set of sequences $\{S_i; i \in I \subseteq [0, p + q]\}$) is called a homology structure on the sequences $\{S_i; i \in I \subseteq [0, p + q]\}$, and is denoted by $\mathcal{H}^*$ (or $\mathcal{H}^I$).

For a character ‘-’ not in $\Sigma$ (called the gap character), let $\Sigma' = \Sigma \cup \{-\}$. An alignment of $S_i; i \in I \subseteq [0, p + q]$ is a collection $\{R_i = (r_{ij}); i \in I\}$ of sequences over $\Sigma'$ such that

1. deleting gap characters from $R_i$ gives $S_i$
2. all $R_i$ have the same length
3. for each $j$, there is an $i$ such that $r_{ij}$ is a non-gap character
4. for each $j$, non-gap characters $r_{ij}$ constitute an equivalence class of $\mathcal{H}^I$.

Each alignment corresponds to a unique homology structure, so each homology structure is represented by a representative alignment.

For a directed edge $(x_i, x_j)$ of $T$ let $\mathcal{H}^{ij}$ denote the homology structure obtained by restricting $\mathcal{H}^*$ to the sequences $S_i$ and $S_j$. Let $P(\mathcal{H}^*)$ denote the probability of $\mathcal{H}^*$.

**Proposition 6.**

$$P(\mathcal{H}^*) = P(L_0) \prod_{(x_i, x_j) \in E} P(\mathcal{H}^{ij}|L_i)$$

where $P(L_0)$ is the equilibrium probability of $L_0$ given by

$$p(L_0) = \left(1 - \frac{\lambda}{\mu}\right)^{L_0} \left(\frac{\lambda}{\mu}\right)^{L_0}$$
Proof. This follows from the fact that the sequences evolve independently along the edges of the tree.

Let $H^{obs}$ denote a homology structure on the observed sequences. Its probability $P(H^{obs})$ is given by the following

**Proposition 7.**

$$P(H^{obs}) = \sum P(H^*)$$ (35)

where the summation is over all homology structures $H^*$ on $S_i; i \in [0, p + q]$ whose restriction on $S_i; i \in [1, p]$ is $H^{obs}$.

Proof. The R.H.S. is a sum of the probabilities of all possible histories that result in the homology structure $H^{obs}$.

In this appendix we want to prove that $P(H^{obs})$ does not depend on the location of $x_0$. The idea is to apply Felsenstein’s pulley principle [1]. But to be able to do that, we first have to prove the pulley principle when there are just two observed sequences.

Let $S_i; i \in [0, 2]$ be three sequences, $S_0$ being the ancestor that evolves to $S_i$ in time $t_i$, for $i \in [1, 2]$, and let $t = t_1 + t_2$.

A homology structure on two sequences can be decomposed into blocks of characters bounded on the left by homologous characters. In the case of the leftmost block, the immortal links constitute the left boundary. This is formally defined below.

We introduce the term related only for the analysis of $S_0, S_1$ and $S_2$: two characters $s_{ia}$ and $s_{jb}$ are said to be related if they are descendents of the same character in $S_0$. By definition, each character in $S_0$ is its own descendent and parent. A set of segments $\{S_0[a, a + u], S_1[b, b + v], S_2[c, c + w]\}$ is called a block of $H^*$ if the following conditions hold

1. $s_{0a} \sim s_{1b} \sim s_{2c}$ (Note: by convention, $s_{i0} \sim s_{j0}$.)

2. together the three segments are closed under relatedness.

A two element subset $\{S_i[a, a + u], S_j[b, b + v]\}$ of a block is called a block of $H^{ij}$. The event that the set $\{S_0[a, a + u], S_1[b, b + v], S_2[c, c + w]\}$ is a block is denoted by $B^*(a, u, b, v, c, w)$. The event that the set $\{S_i[a, a + u], S_j[b, b + v]\}$ is a block is denoted by $B^{ij}(a, u, b, v)$. Probabilities of these events are computed by summing over the probabilities of all possible contributing events.
Lemma 8. For \( i \in [1, 2] \),
\[
P(B^0_i(a, m, b, n) | s_{ib} \sim s_{0a}, L_0 \geq a + m)
\]
\[
= \sum_{e=0}^{m} \binom{m}{e} (\mu \beta_i)^e \left( \frac{n}{m-e} \right) (1 - \mu \beta_i)^{m-e} (\lambda \beta_i)^{n-m+e} (1 - \lambda \beta_i)^{m-e+1}
\]
(36)

Proof. This follows from the solutions of the TKF91 model given in Lemma 2. A term in the above summation gives a contribution to the probability when \( e \) characters in \( S_0[a, a + m] \) have no descendents in \( S_i \). The remaining \( m - e + 1 \) characters in \( S_0[a, a + m] \) have \( n + 1 \) descendents in \( S_i \) (counting \( s_{ib} \), which is a descendent of \( s_{0a} \)). Therefore, there are \( m - e + 1 \) possible groups of siblings in \( S_i \), and \( \binom{n}{m-e} \) ways of making groups. Each group contributes a factor \( 1 - \lambda \beta_i \). The probability that a character in \( S_0 \) has at least one descendent in \( S_i \) is \( 1 - \mu \beta_i \). Therefore, all groups, except the first group that corresponds to the descendents of \( S_{0a} \), contribute a factor \( 1 - \mu \beta_i \). A group of \( k > 0 \) siblings contributes a factor \( (\lambda \beta_i)^{k-1} \). Thus all groups of siblings together contribute the factor \( (\lambda \beta_i)^{n-m+e} \). Note that there is no change in the formula even when \( a = b = 0 \).

The above probability does not depend on \( a \) and \( b \). It is simply the probability that \( n+1 \) characters in the ancestral sequence have \( m+1 \) descendents after time \( t_i \), with the first character in the ancestor surviving after time \( t_i \). Let this probability be denoted by \( P(n \rightarrow m, t_i) \).

Corollary 9. For \( i \in \{1, 2\} \),
\[
(\lambda/\mu)^n P(n \rightarrow m, t_i) = (\lambda/\mu)^m P(m \rightarrow n, t_i)
\]
(37)

Proof. When the probabilities in the above equation are written using Lemma 8, the coefficients of \( (1 - \mu \beta_i)^k (1 - \lambda \beta_i)^k \) on the two sides of the above equation are equal for each \( k \).
Corollary 10.

\[ \sum_{n=0}^{\infty} \left( \frac{\lambda}{\mu} \right)^n P(n \rightarrow m_1 \rightarrow t_1) P(n \rightarrow m_2 \rightarrow t_2) \]

\[ = \sum_{n=0}^{\infty} \left( \frac{\lambda}{\mu} \right)^m P(m_1 \rightarrow n \rightarrow t_1) P(n \rightarrow m_2 \rightarrow t_2) \]

\[ = \left( \frac{\lambda}{\mu} \right)^m P(m_1 \rightarrow m_2, t_1 + t_2) \]

\[ = \left( \frac{\lambda}{\mu} \right)^m \sum_{e=0}^{m_1} \left( \frac{m_1}{e} \right) \left( \frac{m_2}{m_1 - e} \right) (\mu \beta)^e (1 - \mu \beta)^{m_1 - e} (\lambda \beta)^{m_2 - m_1 + e} (1 - \lambda \beta)^{m_1 - e+1} \]

\[ = \left( \frac{\lambda}{\mu} \right)^m P(B^{12}(b, m_1, c, m_2) | s_{1b} \sim s_{2c}, L_1 \geq b + m_1) \] (38)

Proof. The second and the third lines follow from Corollary 9. The fourth line follows from Lemma 8 and the fact that the expression in the third line is a function of \( t_1 + t_2 \). If \( s_{1b} \sim s_{2c} \) then there is some character \( s_{0a} \) in \( S_0 \) such that \( s_{1b} \sim s_{0a} \sim s_{2c} \). This implies the last line.

Let \( N^{ij}(a, m, b, n) \) denote the event that \( B^{ij}(a, m, b, n) \) AND \( s_{ip} \sim s_{jq} \) for \( a < p < a + m + 1 \) and \( b < q < b + n + 1 \). Informally speaking, it is an event that represents a block with only one pair of homologous characters.

The following lemma relates the probabilities of \( B \)-type and \( N \)-type events.

Lemma 11.

\[ P(B^{12}(b_0, m_0, c_0, n)|s_{1b_0} \sim s_{2c_0}, L_1 \geq b_0 + m) \]

\[ = P(N^{12}(b_0, m_0, c_0, n)|s_{1b_0} \sim s_{2c_0}, L_1 \geq b_0 + m) \]

\[ + \sum_{1 \leq k \leq \min(n, m)} X^k \prod_{i=0}^{k} P(N^{12}(b_i, m_i, c_i, n_i)|s_{1b_i} \sim s_{2c_i}, L_1 \geq b_i + m_i) \] (39)

where \( c_i = c_{i-1} + n_{i-1} + 1 \) and \( b_i = b_{i-1} + m_{i-1} + 1 \) for \( i \geq 1 \).
Proof. We have considered all possible ways in which a $B(\ldots)$-type event is partitioned into $N(\ldots)$-type events. The index $k$ denotes the number of pairs of homologous characters in $S_1[b_0, b_0 + m]$ and $S_2[c_0, c_0 + n]$ other than the homologous pair $s_{1b_0} \sim s_{2c_0}$. Therefore, each value of $k$ partitions the $B(\ldots)$-type block in $N(\ldots)$-type blocks. Histories contributing to each $N(\ldots)$-type block are independent. The term corresponding to the $k = 0$ case is written separately as the first term because that is what we would like to compute.

Lemma 12.

\[
P(N^{12}(b, m, c, n)|s_{1b} \sim s_{2c}, L_1 \geq b + m)
\]
\[
= \sum_{e=0}^{m} \binom{m}{e} \binom{n}{m-e} (\mu\beta)^e(1 - X - \mu\beta)^{m-e}(\lambda\beta)^{n-m+e}(1 - \lambda\beta)^{m-e+1}
\]

(40)

Proof. It follows from Corollary 10 that the LHS of Equation (39) is a function of $t_1 + t_2$. We prove by induction on $m + n$ that $P(N^{12}(b_0, m, c_0, n)|s_{1b_0} \sim s_{2c_0}, L_1 \geq b_0 + m)$ is a function of $t_1 + t_2$. Probabilities $p(A)$, $p(B)$ and $p(C \lor D)$ computed in Section 3 are functions of $t_1 + t_2$, and correspond to $m + n = 0, 1$ and 2, respectively, and are used as the base case of induction. Let $P(N^{12}(b_0, m, c_0, n)|s_{1b_0} \sim s_{2c_0}, L_1 \geq b_0 + m)$ be a function of $t_1 + t_2$ for $m + n < r$, where $r > 2$. Let $m + n = r$ in Equation (39). Since $m_i + n_i < r$ for all terms in the summation, and the L.H.S. of Equation (39) is a function of $t_1 + t_2$, the only remaining term (the first term on the R.H.S. of Equation (39)) is a function of $t_1 + t_2$.

Once the L.H.S. of Equation (40) is proved to be independent of the root location, the R.H.S. is written by treating $x_1$ as the ancestor and arguing in the same manner as in Lemma 8.

Lemma 13. Let $H^{12}$ be a homology structure on $S_1$ and $S_2$. Then $p(H^{12})$ is a function of $t_1 + t_2$.

Proof. A homology structure has a natural decomposition into blocks each of which has only one pair of homologous characters. Events in distinct blocks are independent. Thus the result follows from Lemma 12.

Theorem 14. $p(H^{\text{obs}})$ is independent of the root location.

Proof. This follows from Propositions 6 and 7 and Lemma 13.
Remark The proofs presented here (in particular the proof that $P(N^{12}(\ldots))$ is a function of $t_1 + t_2$) appear somewhat convoluted. We could have written results similar to Lemma 8 and Corollary 9 for the probabilities of the $N(\ldots)$ type events. For example, the following corollary is similar to Corollary 9.

**Corollary 15.** For $i \in \{1, 2\}$,

$$
(\lambda/\mu)^nP(N^{0i}(a, n, b, m)|s_{ib} \sim s_{0a}, L_0 \geq a + n) = (\lambda/\mu)^mP(N^{0i}(a, m, b, n)|s_{ib} \sim s_{0a}, L_0 \geq a + m)
$$

But this is not useful to claim that $P(N^{12}(\ldots))$ is a function of $t_1 + t_2$. For example, we cannot prove something like Corollary 10 for $P(N^{12}(\ldots))$ because there may be a situation such as $s_{1i} \sim s_{2j}$, $s_{1i} \sim s_{0k}$ and $s_{0k} \sim s_{2j}$. As a result the second step of Corollary 10 (derivation of the 3rd line from the 2nd) fails for $P(N^{12}(\ldots))$. In fact, Equation (41) cannot be regarded as a “detailed balance condition” even if it looks like one. If a Markov process has transition probabilities given by $P_{ij}$ and if $\pi_i$ is the stationary distribution, then the process is reversible if and only if $\pi_i P_{ij} = \pi_j P_{ji}$. But in our problem, $P(N^{12}(\ldots))$ is not a probability of transition from state 1 to state 2. It is merely a probability of observing certain configuration that is defined by parameters that take values 1 and 2. A similar condition has been used as a detailed balance condition in [16] (see Equation (30) on p539).

Theorem 14 has been believed to be true by researchers, and has been used in [14] to test correctness of implementation of algorithms. In general such a result should not be taken for granted as a consequence of reversibility of the model and the Pulley Principle of Felsenstein. In fact, a result such as Theorem 14 implies the pulley principle.

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