

THE SHAPE OF THE ONE-DIMENSIONAL PHYLOGENETIC LIKELIHOOD FUNCTION¹

BY VU DINH AND FREDERICK A. MATSEN IV

Fred Hutchinson Cancer Research Center

By fixing all parameters in a phylogenetic likelihood model except for one branch length, one obtains a one-dimensional likelihood function. In this work, we introduce a mathematical framework to characterize the shapes of such one-dimensional phylogenetic likelihood functions. This framework is based on analyses of algebraic structures on the space of all frequency patterns with respect to a polynomial representation of the likelihood functions. Using this framework, we provide conditions under which the one-dimensional phylogenetic likelihood functions are guaranteed to have at most one stationary point, and this point is the maximum likelihood branch length. These conditions are satisfied by common simple models including all binary models, the Jukes–Cantor model and the Felsenstein 1981 model.

We then prove that for the simplest model that does not satisfy our conditions, namely, the Kimura 2-parameter model, the one-dimensional likelihood functions may have multiple stationary points. As a proof of concept, we construct a nondegenerate example in which the phylogenetic likelihood function has two local maxima and a local minimum. To construct such examples, we derive a general method of constructing a tree and sequence data with a specified frequency pattern at the root. We then extend the result to prove that the space of all rescaled and translated one-dimensional phylogenetic likelihood functions under the Kimura 2-parameter model is dense in the space of all nonnegative continuous functions on $[0, \infty)$ with finite limits. These results indicate that one-dimensional likelihood functions under advanced evolutionary models can be more complex than it is typically assumed by phylogenetic inference algorithms; however, these complexities can be effectively captured by the Kimura 2-parameter model.

1. Introduction. The likelihood of a phylogenetic model is a function of the parameters of continuous time Markov chains (CTMCs) used to model sequence evolution along each branch. It is common to assume a single rate matrix and stationary frequency for the CTMCs but allow the branch lengths to vary, representing a single evolutionary process but differing amounts of evolution along each branch. Commonly used maximum-likelihood phylogeny programs improve likelihood by modifying branch lengths iteratively and one at a time [2]. The general

Received July 2015; revised July 2016.

¹Supported by NSF Grants DMS-1223057 and CISE-1564137 and NIH Grant U54GM111274.
MSC2010 subject classifications. Primary 05C05, 92B10; secondary 05C25, 92D15.

Key words and phrases. Evolutionary model, molecular evolution, phylogenetics, likelihood model, characteristic polynomial, algebraic representation, multimodality, universal model.

approach for numerical maximization of the one-dimensional likelihood function given by fixing every parameter except for one branch length is to iteratively sample the function at a number of points, use surrogate functions to fit simple curves to those points, and use those fits as approximations to locate the maximum branch length. For example, programs often employ Newton's method, in which the intuitive idea is to use first and second derivatives to approximate the likelihood function (varying along that branch) by a surrogate quadratic function. Since evaluations of the likelihoods (and their derivatives) are computationally expensive, many approaches have been tried to improve the efficiency of this optimization procedure [2].

Such approaches, however, rely on the assumptions that one-dimensional phylogenetic likelihood functions belong to some class of simple functions, and that the surrogate model can, at least, capture the shape of the functions. While there has been a considerable amount of work on finding multiple maxima of the multidimensional likelihood surfaces parameterized by all branch lengths for a tree [3, 12, 13], little has been done about the shapes of one-dimensional phylogenetic likelihood functions. The only attempt to investigate the shape of the one-dimensional phylogenetic likelihood functions has been [7], which provided a proof of uniqueness of the stationary points for one-dimensional phylogenetic likelihood functions in the case of the one parameter model of nucleotide substitution. Based on this proof, the authors of [7] asserted that there is at most one stationary point of the full likelihood surface. This claim was later disproved by [13], although the proof for the one-dimensional case still holds. However, the result has not been examined for the more complex models used in practice.

In this work, we introduce a mathematical framework to characterize the shapes of such one-dimensional phylogenetic likelihood functions. This framework is based on analyses of algebraic structures on the space of all frequency patterns with respect to a polynomial representation of the likelihood functions. Specifically, we introduce the new concept of *logarithmic relative frequency patterns* and analyze algebraic structures on the space of such patterns. These structures, along with the *characteristic polynomial representations* of one-dimensional phylogenetic likelihood functions, open a new way to explore the space of all possible likelihood functions. Moreover, by composing these structures, we are able to tackle the inverse problem of constructing a phylogenetic tree that has a given frequency pattern at the root. This enables us to construct phylogenetic trees that approximate any given likelihood function with arbitrary precision.

Using this framework, we provide conditions under which the one-dimensional phylogenetic likelihood functions are guaranteed to have at most one stationary point, and this point is the maximum of the one-dimensional function. These conditions are satisfied by common simple models including all binary models, the Jukes–Cantor model [10] and the Felsenstein 1981 model [5]. We then prove that for the simplest model that does not satisfy our conditions, namely, the Kimura

2-parameter model [11], the one-dimensional likelihood functions may have multiple stationary points. As a proof of concept, we construct a nondegenerate example in which the phylogenetic likelihood function has two local maxima and a local minimum.

We then extend the result to prove that the space of all rescaled and translated one-dimensional phylogenetic likelihood functions under the Kimura 2-parameter model is dense in the set of all nonnegative continuous functions on $[0, \infty)$ with finite limits. These results indicates that one-dimensional likelihood functions under advanced evolutionary models can be more complex than it is typically assumed by phylogenetic inference algorithms; however, these complexities can be effectively captured by the Kimura 2-parameter model.

2. Background and definitions.

2.1. *Markov models of sequence evolution.* Our setting is the standard IID setting for likelihood-based phylogenetics with a finite number of sites; we review the basics here but refer the reader to [6] for more details. Let Ω denote the set of states and let $r = |\Omega|$. For convenience, we assume that the states have indices 1 to r .

For an unrooted tree T with N taxa, we use $E(T)$ and $V(T)$ to denote the set of edges and vertices of T , respectively. On each edge $e \in E(T)$, we assume that the mutation events occur according to a continuous time Markov chain on states Ω with instantaneous rate matrix Q_e (Figure 1). This rate matrix Q_e and the branch length t_e on the edge e define the transition matrix $P^e = e^{Q_e t_e}$ on edge e , where $P_{ij}^e(t_e)$ denotes the probability of mutating from state i to state j across the edge e (with length t_e).

We further assume that for all edges $e \in E(T)$, the Markov chains that describe the mutation events are ergodic and time-reversible with respect to a fixed stationary distribution π , that is,

$$\lim_{t \rightarrow \infty} P_{ij}^e(t) = \pi_j,$$

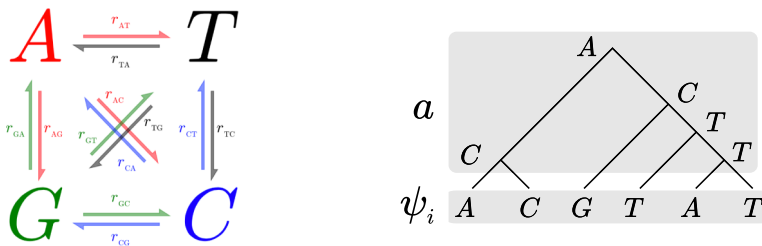


FIG. 1. [Left:] A general Markov model of DNA evolution along a tree edge. [Right:] An extension a of the labeling ψ_i (corresponds to site i in the sequences) of the leaves of a simple tree τ to its inner nodes.

and

$$\pi_i P_{ij}^e(t) = \pi_j P_{ji}^e(t) \quad \forall t,$$

for all $i, j \in \Omega$ and $e \in E(T)$.

The phylogenetic likelihood is computed as follows given a set of (aligned) observed sequences $\psi = (\psi_1, \psi_2, \dots, \psi_S) \in \Omega^{N \times S}$ of length S over N taxa of a tree τ . First, orient the edges of τ away from an arbitrarily chosen root ρ of the tree. (We can choose the root arbitrarily since each P_e is reversible with respect to π .) Each site i in the sequences determines a labeling ψ_i of each leaf by a state in Ω . An extension a of a labeling ψ_i is an assignment of states to all of the nodes in the tree that agrees with ψ on the leaves (Figure 1).

The probability of an extension a given the vector of branch lengths $\mathbf{t} = (t_e)_{e \in E(T)}$ is defined to be the probability of the state at the root (given by the stationary distribution) multiplied by the probabilities of all the state transitions (including self-transitions) across each branch in the tree

$$P(a|\mathbf{t}) = \pi(a_\rho) \prod_{(u,v) \in E(T)} P_{a_u a_v}^{u v}(t_{uv}),$$

where a_u denotes the assigned state of node u by a .

The likelihood of the data at site i is then the marginal probability over all the extensions

$$P(\psi_i|\mathbf{t}) = \sum_{a \text{ extends } \psi} P(a|\mathbf{t}).$$

We further assume, as is standard, that evolution is independent between sites. This implies that the likelihood of a set of sequences evolving is just the product of the probabilities for the individual sites

$$L(\psi|\mathbf{t}) = \prod_{s=1}^S P(\psi_s|\mathbf{t}).$$

In summary, the likelihood of observing ψ given the tree topology τ and the vector of branch lengths $\mathbf{t} = (t_e)_{e \in E(T)}$ has the form

$$L(\psi|\mathbf{t}) = \prod_{s=1}^S \sum_a \pi(a_\rho) \prod_{(u,v) \in E(T)} P_{a_u a_v}^{u v}(t_{uv}),$$

where a ranges over all extensions of ψ to the internal nodes of T and a_u denotes the assigned state of node u by a .

For readers familiar with the theory of probabilistic inference on graphical models, the likelihood functions studied in this paper can be alternatively described as follows. Consider a tree T and let $\{X_v : v \in V(T)\}$ be a collection of random variables indexed by the nodes of the tree. For each edge $(u, v) \in E(T)$, we define the nonnegative potential function:

$$k_{(u,v)}(i, j, t) := P_{ij}^{uv}(t).$$

We assume that the joint probability distribution $p(x_{V(T)})$ factorizes over the tree edges:

$$p(x_{V(T)}) \sim \prod_{(u,v) \in E(T)} k_{(u,v)}(x_u, x_v, t_{uv}).$$

The likelihood functions of interest may then be represented as the marginal probability of the observation ψ on the leaves of the tree T . This formulation allows us to study the phylogenetic likelihood functions beyond the reversible Markov framework. We will investigate partial extensions to this more general case in Section 7, but for the next several sections we will focus on the standard phylogenetic setting (in which we can prove the strongest results).

2.2. One-dimensional phylogenetic likelihood functions. To investigate the one-dimensional likelihood function on one branch e_0 , we fix all other branches, partition the set of all extensions of ψ according to their labels at the end points of e_0 , and split $E(T)$ into two sets of edges E_{left} and E_{right} corresponding to the location of the edges with respect to e_0 . The likelihood function can be rewritten as a univariate function of t , the branch length of e_0 :

$$L(\psi|t) = \prod_{s=1}^S \sum_{ij} \sum_{a \in \mathcal{A}_{ij}} \pi(a_\rho) \left(\prod_{e \in E_{\text{left}}} P_{a_u a_v}^e(t_{uv}) \right) \times P_{ij}^{e_0}(t) \times \left(\prod_{e \in E_{\text{right}}} P_{a_u a_v}^e(t_{uv}) \right),$$

where \mathcal{A}_{ij} denotes the set of all extensions of ψ for which the labels at the left end point and the right end point of e_0 are i and j , respectively. We note that some \mathcal{A}_{ij} may be empty if e_0 is a pendant edge and the observed value on the corresponding leaf is not i .

By grouping the products over E_{left} and E_{right} as well as the sum over a in a single term b_{ij}^s , we can define the one-dimensional log-likelihood function as

$$\ell_{e_0}(t) = \log L(\psi|t) = \sum_{s=1}^S \log \left(\sum_{ij} b_{ij}^s P_{ij}^{e_0}(t) \right).$$

Such $\ell_{e_0}(t)$ are the object of study of this paper.

For convenience, we will assume that e_0 has been chosen and will drop the index e_0 hereafter.

2.3. Evolutionary models. Throughout the paper, we use the term *evolutionary model* on state set Ω to refer to a collection \mathcal{H} of (Q, π) pairs, where π is a vector of stationary frequencies and Q is a rate matrix on Ω that is reversible with respect to π . If at every edge of the tree τ , the matrix-frequency pair (Q_e, π) belongs to \mathcal{H} , we say that τ is a tree under evolutionary model \mathcal{H} .

We will consider a number of different evolutionary models of DNA sequences. These DNA substitution models differ in terms of the parameters used to describe the rates at which one state replaces another during evolution and the stationary frequencies:

- Jukes–Cantor model [10]: this model assumes equal stationary frequencies ($\pi_A = \pi_G = \pi_T = \pi_C = 1/4$) and equal mutation rates.
- Felsenstein 1981 model [5]: this is an extension of the Jukes–Cantor model in which stationary frequencies are allowed to vary.
- Kimura 2-parameter model [11]: this model assumes equal stationary frequencies, but distinguishes between the rates of transitions ($A \leftrightarrow G$, that is, from purine to purine, or $C \leftrightarrow T$, that is, from pyrimidine to pyrimidine) and transversions (from purine to pyrimidine or vice versa).

Following common usage, we use κ to denote the transition/transversion rate ratio and write the rate matrix for this model as

$$Q_\kappa = \frac{1}{2(\kappa + 1)} \begin{pmatrix} -(\kappa + 2) & \kappa & 1 & 1 \\ \kappa & -(\kappa + 2) & 1 & 1 \\ 1 & 1 & -(\kappa + 2) & \kappa \\ 1 & 1 & \kappa & -(\kappa + 2) \end{pmatrix}.$$

The special case $\kappa = 3$ will play a central role in the analysis of this paper. Note that the single κ parameter in the Kimura 2-parameter model determines a rate matrix that is shared across the tree, while this paper primarily concerns the effect of changing a single branch length parameter.

While the focus here is on DNA models, we emphasize that our theoretical framework is capable of analyzing any time-reversible evolutionary model on any state space. In fact, we do not assume a uniform molecular clock, or even a single evolutionary model along the edges of the tree.

2.4. *Characteristic polynomials of one-dimensional phylogenetic likelihood functions.* We will frequently use the following assumption.

ASSUMPTION 2.1. The eigenvalues of the rate matrix Q are equal to

$$0 = d_0\gamma \geq -d_1\gamma \geq -d_2\gamma \geq \cdots \geq -d_{r-1}\gamma$$

for some positive number γ and nonnegative integers d_1, \dots, d_{r-1} .

The following remark, the proof of which is provided in the [Appendix](#), guarantees that Assumption 2.1 does not affect the generality of our analyses up to an arbitrarily small approximation error.

REMARK 2.1. The set of rate matrices Q for a given evolutionary model that satisfy Assumption 2.1 is dense in the set of rate matrices under the same evolutionary model.

Under Assumption 2.1, if we denote the entries of the diagonalizing matrix M and N of Q by m_{ij} and n_{ij} , respectively, then the transition probabilities can be computed as

$$P_{ij}(t) = \sum_k m_{ik} e^{-d_k \gamma t} n_{kj}.$$

By reparametrizing with $x := e^{-\gamma t}$, we can represent these transition probabilities as polynomial functions

$$P_{ij}(x) = \sum_k m_{ik} x^{d_k} n_{kj}.$$

Similarly, the log-likelihood function can be rewritten as

$$\ell(x) = \sum_{s=1}^S \log(\lambda_s(x)) \quad \text{where } \lambda_s(x) = \sum_{ij} b_{ij}^s P_{ij}(x).$$

Hereafter, we will refer to $P_{ij}(x)$ and $\lambda_s(x)$ as the *transition polynomials* of the evolutionary model and the *characteristic polynomials* of the one-dimensional phylogenetic likelihood function, respectively.

As we will see in later sections, this polynomial representation will enable us to exploit many algebraic and analytic properties of the likelihood functions. The most noticeable feature is that one can use the fundamental theorem of algebra to factorize $\lambda_s(x)$ as products of linear and quadratic polynomials. As a result, the log-likelihood function can be written in the form

$$\begin{aligned} \ell(x) = & \sum_{s=1}^S \sum_{i=1}^{i_{s,1}} \log(\alpha_{s,i} + \beta_{s,i} x) \\ & + \sum_{s=1}^S \sum_{i=1}^{i_{s,2}} \log(\mu_{s,i} + \nu_{s,i} x + \omega_{s,i} x^2), \end{aligned}$$

where $\mu_{s,i}$, $\nu_{s,i}$, $\omega_{s,i}$ are the (real) coefficients of the quadratic polynomials in the decomposition of λ_s , while $\alpha_{s,i}$, $\beta_{s,i}$ are coefficients of the linear terms in the decomposition.

This enables us to decompose a complicated evolutionary model into smaller modules, each of which can be approximated either by a “linear” model (like the binary symmetric model) or by a “quadratic” model (like the Kimura 2-parameter model). In Section 3, we use this formulation to prove that if the phylogenetic log-likelihood function is essentially linear (i.e., there are no quadratic terms in the expression), its shape resembles those generated by binary models, with a unique stationary point that is also the maximum point. In Section 5, we illustrate that this property does not hold for quadratic models by constructing a counterexample with the Kimura 2-parameter model. Finally, in Section 6, we use this formulation

once again to prove that the space of all rescaled and translated one-dimensional phylogenetic likelihood functions under the Kimura 2-parameter model is dense in the space of all continuous functions on $[0, \infty)$ with finite limits.

3. Uniqueness of the stationary point. In this section, we discuss a condition under which the uniqueness of the stationary branch length is guaranteed.

The analyses in this section stem from two observations:

1. If for every site index s , the characteristic polynomial λ_s has no nonreal root, then the likelihood function can be decomposed into smaller modules, each of which resemble a binary model.
2. The likelihood functions of binary models and summations of such models are *incave*.

DEFINITION 3.1 (Hanson [8]). A vector-valued function f is said to be *incave* in \mathbb{R}^n if there exists a vector-valued function $\phi(t, u)$ such that

$$f(t) - f(u) \leq \phi(t, u) \cdot \nabla f(u) \quad \forall t, u \in \mathbb{R}^n,$$

where ∇f denotes the gradient of f .

Incave functions were introduced in the optimization literature as a generalization of concave functions [8]. It can be proven that a function is *incave* if and only if every stationary point is a global maximum [1]. We are interested in the case of functions of a single real argument, for which the following result also holds.

LEMMA 3.1. *If f is a real-valued incave function with a finite number of stationary points, then f has at most one stationary point. Moreover, if such a point exists, it is also a global maximum.*

PROOF. Denote $A = \{t \in [0, \infty) : f'(t) = 0\}$ and assume that A has more than one element. Since A is finite, we can choose two elements t_1 and t_2 in A such that the interval $(t_1, t_2) \subset \mathbb{R} - A$. Since f is *incave*, every stationary point of f is a global maximum. We deduce that t_1 and t_2 are both global maxima of f and $f(t_1) = f(t_2)$. Using the mean value theorem, there exists $t \in (t_1, t_2)$ such that $f'(t) = 0$. This is a contradiction. \square

This enables us to prove the following theorem.

THEOREM 3.1. *If for every site index s , the polynomial λ_s has only real roots, then ℓ has at most one stationary point. Moreover, if such a point exists, it is also a global maximum.*

PROOF. Since λ_s has only real roots, it can be written as product of linear functions

$$\lambda_s(x) = \prod_{i=1}^{d_p} (\alpha_{s,i} + \beta_{s,i}x),$$

where d_p , defined in Assumption 2.1, is the degree of the polynomial λ_s .

The log-likelihood function ℓ can be computed as

$$\begin{aligned} \ell(t) &= \sum_{s=1}^S \log(\lambda_s(e^{-\gamma t})) \\ &= \sum_{s=1}^S \log\left(\prod_{i=1}^{d_p} (\alpha_{s,i} + \beta_{s,i}e^{-\gamma t})\right) \\ &= \sum_{s=1}^S \sum_{i=1}^{d_p} \log(\alpha_{s,i} + \beta_{s,i}e^{-\gamma t}). \end{aligned}$$

For any $t, u > 0$, we have

$$\begin{aligned} \ell(t) - \ell(u) &= \sum_{s=1}^S \sum_{i=1}^{d_p} \log\left(\frac{\alpha_{s,i} + \beta_{s,i}e^{-\gamma t}}{\alpha_{s,i} + \beta_{s,i}e^{-\gamma u}}\right) \\ &\leq \sum_{s=1}^S \sum_{i=1}^{d_p} \left(\frac{\alpha_{s,i} + \beta_{s,i}e^{-\gamma t}}{\alpha_{s,i} + \beta_{s,i}e^{-\gamma u}} - 1\right) \\ &= \sum_{s=1}^S \sum_{i=1}^{d_p} \left(\frac{\beta_{s,i}(e^{-\gamma t} - e^{-\gamma u})}{\alpha_{s,i} + \beta_{s,i}e^{-\gamma u}}\right) \\ &= \frac{1}{\gamma}(1 - e^{-\gamma(t-u)}) \sum_{s=1}^S \sum_{i=1}^{d_p} \left(\frac{-\beta_{s,i}\gamma e^{-\gamma u}}{\alpha_{s,i} + \beta_{s,i}e^{-\gamma u}}\right) \\ &= \frac{1}{\gamma}(1 - e^{-\gamma(t-u)})\ell'(u). \end{aligned}$$

Hence, ℓ is an incave function.

Furthermore, since λ_s are polynomial and $e^{-\gamma t}$ is a bijective map from $[0, \infty)$ to $(0, 1]$, we deduce that $\ell(t)$ only has a finite number of stationary points. Using Lemma 3.1, we conclude that ℓ has at most one stationary point; moreover, if such a point exists, it is also a global maximum. \square

We note that Theorem 3.1 imposes a condition on the characteristic polynomials rather than the evolutionary model, and can be applied to assess the uniqueness of

the stationary point of any time-reversible evolutionary model satisfying Assumption 2.1. In fact, Theorem 3.1 does not assume a uniform molecular clock, or even a single evolutionary model along the edges of the tree. However, it is worth noting that for the class of models on which the rate matrices have only one nonzero eigenvalue, the result automatically holds.

COROLLARY 3.1. *For binary, Jukes–Cantor and Felsenstein 1981 models, the one-dimensional likelihood function has at most one stationary point; if such point exists, it is the global maximum.*

We also note that the results in previous studies about the number of maxima of likelihood surfaces [3, 12, 13] are derived for binary models. Theorem 3.1 complements those results in the sense that while the likelihood surfaces considered in those work may have multiple (or even a continuum of) local maxima, the stationary points of one-dimensional likelihood functions are still unique.

This corollary also extends and clarifies a result from the first attempt to investigate the shape of the one-dimensional phylogenetic likelihood functions [7]. By studying the location of the solutions of phylogenetic likelihood functions, the paper proves that one-dimensional phylogenetic likelihood functions have unique stationary points under the same model assumptions as Corollary 3.1.

This result also provides a full characterization of one-dimensional likelihood functions of binary models (and those considered by Corollary 3.1). Indeed, since the derivatives of log-likelihood functions are continuous with at most one zero, this result implies that:

1. If there is no stationary point, then $\ell(t)$ is a monotonic function (either strictly decreasing or strictly increasing).
2. If the stationary point t_0 exists and is unique, then the function is increasing in the interval $(0, t_0)$ and is decreasing in (t_0, ∞) .

This simplicity of the shapes of phylogenetic likelihood functions provides a strong theoretical foundation for the use of simple optimization methods to locate the maximum likelihood branch length. However, we emphasize that these results are only about one-dimensional phylogenetic likelihood functions and do not mean that there is a unique (multivariate) stationary point of the likelihood surface or that simple hill-climbing methods will find this optima.

4. Algebraic structures on the space of all logarithmic relative frequency patterns under the Kimura 2-parameter model. While Section 3 provides a uniqueness result for the maximum likelihood branch lengths under three simple models, the result does not extend to more general models. In fact, as we will illustrate in the next section, the shapes of likelihood functions under the Kimura 2-parameter model [11] can be quite complicated, for example, with multiple local and global maxima.

In order to enable theoretical analyses of phylogenetic likelihood functions under more complex evolutionary models, here we introduce the concept of conditional logarithmic frequency patterns and study the algebraic structures on the space of such patterns.

DEFINITION 4.1. Given a rooted tree τ with root ρ and N taxa, some labelings $\psi=(\psi_1, \dots, \psi_S) \in \Omega^{N \times S}$ of its taxa and a vector of real constants (c_1, \dots, c_S) we define the *logarithmic relative frequency pattern* $\phi(\tau, \psi, c)$ as the $r \times S$ matrix with entries

$$\phi_{i,s} = c_s + \log \sum_{a \in \mathcal{Z}_{i,s}} \pi(i) \prod_{(u,v) \in E(\tau)} P_{a_u a_v}^{uv}(t_{uv})$$

for $i \in \Omega, s = 1, \dots, S$ and $\mathcal{Z}_{i,s}$ being the set of all extensions a of ψ_s to all the nodes of τ such that $a(\rho) = i$.

For convenience, we will use the shorter term *frequency pattern* to refer to a logarithmic relative frequency pattern.

In probabilistic terms, for a fixed site index s , the (i, s) -entry of a logarithmic relative frequency pattern $\phi(\tau, \psi, c)$ is (up to a constant c_s) the logarithm of the likelihood of observing state i at the root of the tree, given leaf states ψ_s . This definition is directly related to the formulation of the characteristic polynomials λ_s , whose coefficients b_{ij}^s are the product of the probabilities of observing state i and j at the two end points of an edge, given that the labeling ψ_s is observed at the taxa. It is straightforward to verify that for models with uniform stationary distribution on a fixed tree, we have

$$\log b_{ij}^s = \phi_{i,s}(\tau_1) + \phi_{j,s}(\tau_2) + \tilde{c}_s$$

for all i, j, s , where \tilde{c}_s is a constant depending only on s , and τ_1 and τ_2 are the trees obtained by removing the edge e_0 from the tree τ and rooting the newly created trees at the endpoints of e_0 (see the proof of Theorem 4.1 in the Appendix for more details).

Hence, to characterize the space of all phylogenetic characteristic polynomials under a given evolutionary model, we just need to characterize the space of all possible logarithmic relative frequency patterns under that model.

DEFINITION 4.2. We denote the space of all possible logarithmic relative frequency patterns under the Kimura 2-parameter model by

$$G = \{ \phi(\tau, \psi, c) : \tau \in \mathcal{T}, \psi \in \Psi_\tau^S, c \in \mathbb{R}^S \},$$

where \mathcal{T} denotes the set of all rooted trees and Ψ_τ^S denotes the set of all tuples (ψ_1, \dots, ψ_S) of S labelings of the taxa of τ .

The goal of this section is to establish that for any sequence of S column vectors v_1, v_2, \dots, v_S in \mathbb{R}^4 , there exists a tree τ under the Kimura 2-parameter model, labelings $\psi = (\psi_1, \psi_2, \dots, \psi_S)$ of its taxa and a vector of real constants c such that

$$\phi(\tau, \psi, c) = [v_1 \ v_2 \ \dots \ v_S].$$

The existence of such tree is guaranteed indirectly by proving that under the Kimura 2-parameter model:

1. G is an algebraic subgroup of $(\mathbb{R}^{4 \times S}, +)$.
2. G is a linear subspace of $\mathbb{R}^{4 \times S}$.
3. G is equal to $\mathbb{R}^{4 \times S}$ itself.

Noting again that the stationary distribution of the Kimura 2-parameter model is the uniform distribution across states $\pi = (1/4, 1/4, 1/4, 1/4)$, the first two steps are confirmed by the following theorem.

THEOREM 4.1. *If the stationary frequency of the evolutionary model is the same for every state, then the following properties hold:*

1. $(G, +)$ is a subgroup of $(\mathbb{R}^{4 \times S}, +)$.
2. G is path-connected.
3. G is a linear subspace of $\mathbb{R}^{4 \times S}$.

SKETCH OF PROOF. A detailed proof of this theorem is provided in the [Appendix](#), but the main arguments can be simply illustrated. The fact that G is closed under addition follows because we can add two frequency patterns just by gluing the roots of the two corresponding trees, labeling the taxa of τ correspondingly and taking the pattern at the new root (Figure 2). Similarly, we can create the inverse of a pattern by gluing all permuted versions of its corresponding tree (with an appropriate vector of real constants).

To prove that G is path-connected, given two arbitrary trees with roots ρ_1, ρ_2 , we create a new tree by adding a new root ρ , joining ρ_1, ρ_2 with ρ by two new edges of length t and $1/t$, respectively, and making ρ the root of τ (Figure 3). By varying t continuously from zero to infinity, we can make a continuous path in G that connects the two frequency patterns. Since any path-connected subgroup of \mathbb{R}^n is a linear subspace [9], so is G . \square

We note that although the aforementioned arguments are made for the Kimura 2-parameter model, which describes a model of DNA evolution ($r = 4$), Theorem 4.1 only requires that the stationary frequency of the evolutionary model is the same for every state. Hence, this result also extends to models with more parameters.

Similarly, the fact that $(G, +)$ is a subgroup of $(\mathbb{R}^{r \times S}, +)$ can be established under the assumption that the root distribution π is uniform, without assuming that

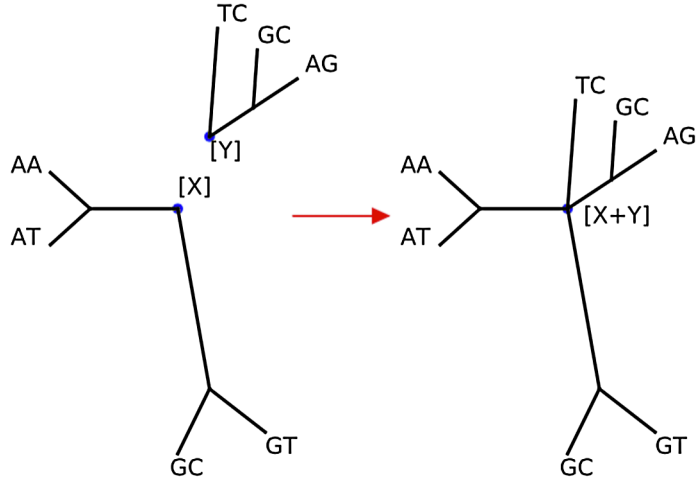


FIG. 2. G is closed under addition: we can add two frequency patterns $[X]$ and $[Y]$ just by gluing the roots of the two corresponding trees, labeling the taxa of τ correspondingly and taking the pattern at the new root.

it is the stationary distribution of the evolutionary process. However, our current approach requires the uniform root distribution to be the stationary distribution for the proof of path-connectivity of G , and an alternative approach to the proof of path-connectivity will be needed if we want to extend the analyses to a more general framework.

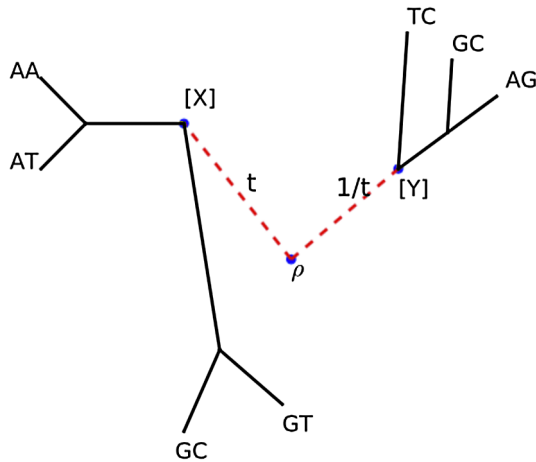


FIG. 3. G is path-connected: We can connect any two patterns $[X]$ and $[Y]$ by adding a new root ρ , joining it with the roots of the two corresponding trees with two new edges of length t and $1/t$, respectively, and making ρ the root of τ .

Recalling that the Kimura 2-parameter model corresponds to the uniform stationary distribution and a family of rate matrices Q_κ indexed by κ , the transition/transversion rate ratio, we then establish that when $\kappa = 3$, the space of all frequency pattern $G = \mathbb{R}^{4 \times S}$. The proof is done through proving by induction that G contains $4 \times S$ independent frequency patterns (also proven in the [Appendix](#)).

THEOREM 4.2. *The set of all possible logarithmic conditional frequency patterns with S sites under the Kimura 2-parameter model with $\kappa = 3$ is equal to $\mathbb{R}^{4 \times S}$.*

With those results, we finally can establish the main theorems of the section.

THEOREM 4.3. *For any sequence of column vectors v_1, v_2, \dots, v_S in \mathbb{R}^4 , there exists a rooted tree τ under the Kimura 2-parameter model with $\kappa = 3$, S labelings $\psi_1, \psi_2, \dots, \psi_S$ of its taxa, and a vector of real constants c such that*

$$\phi(\tau, \psi, c) = [v_1 \quad v_2 \quad \dots \quad v_S].$$

While [Theorem 4.3](#) provides a theoretical guarantee about the existence of a tree under the Kimura model with a given frequency patterns, the proof is not constructive. This raises some concerns about the practicality of the approach. For example, one cannot derive an estimation of the number of edges required to produce a given frequency pattern. Those concerns are addressed by the following theorem.

THEOREM 4.4. *A tree as in [Theorem 4.3](#) can be constructed with at most $64S$ edges.*

Not only does the theorem provide an upper bound on the number of edges required to construct a tree with a given frequency pattern, its proof also provides a simple algorithm to construct such a tree.

PROOF OF THEOREM 4.4. The main steps of the proof are as follows:

Step 1. As shown in the [Appendix](#), any frequency pattern of the form $[x, 0, 0, 0]^t$ can be produced (up to a real constant c_1) by a tree τ with 4 edges and some labeling ψ of its taxa.

Step 2. Using τ from Step 1, we create a tree τ' of 16 edges by gluing the roots of 4 different versions $\tau_1, \tau_2, \tau_3, \tau_4$ of τ together and define S labelings of τ' as follows.

- For $s = 1$, we copy the labeling of τ onto τ' .

$$\psi_1(a) = \psi(a)$$

for each taxon a of $\tau_1, \tau_2, \tau_3, \tau_4$.

- For all $s \geq 2$, the labelings are defined as follows:

$$\psi_s(a) = \sigma^j(\psi(a)) \quad \text{if } a \text{ is a taxon of } \tau_j,$$

where σ is the permutation $(A \ G \ T \ C)$ in cycle notation.

The construction of τ' is similar to the construction of the inverse of elements in the group G in the proof of Theorem 4.1. Because of symmetry, for $s \geq 2$, the frequency pattern corresponding to site s at the root of the newly created tree will be the same for every state while for $s = 1$, the frequency pattern of τ' is obtained by multiplying the frequency pattern of τ by a factor of 4.

We deduce that the pattern created by $(\tau', \{\psi_i\})$ is

$$\begin{pmatrix} 4x & 0 & \dots & 0 \\ 0 & 0 & \dots & 0 \\ 0 & 0 & \dots & 0 \\ 0 & 0 & \dots & 0 \end{pmatrix} + \begin{pmatrix} c_1 & c_2 & \dots & c_S \\ c_1 & c_2 & \dots & c_S \\ c_1 & c_2 & \dots & c_S \\ c_1 & c_2 & \dots & c_S \end{pmatrix}$$

for some real constants c_1, c_2, \dots, c_S .

Step 3. By similar arguments, for any $i = 1, 2, 3, 4$ and $s = 1, 2, \dots, S$, we can construct a tree of 16 edges for any patterns with S sites whose only nonzero entry is at the (i, s) -position. Hence, it takes $16 \times 4S = 64S$ edges to construct a tree with an arbitrary given frequency pattern. □

5. Nonuniqueness of stationary points: Kimura 2-parameter model. In this section, we provide an example for which there are multiple stationary points of the likelihood function. To construct such an example, we find two polynomials $p_1(x)$ and $p_2(x)$ with coefficients b_1, b_2 such that the product $p_1 p_2$ has 2 local maxima in $[0, 1]$, and p_1 and p_2 can be expressed as positive linear combination of the basis polynomial functions P_i derived from an evolutionary model (as will be carefully described in this section). This gives a counterexample with $S = 2$ sites.

Consider the Kimura 2-parameter model with $\kappa = 3$ which has the rate matrix

$$(5.1) \quad Q = \begin{pmatrix} -5/8 & 3/8 & 1/8 & 1/8 \\ 3/8 & -5/8 & 1/8 & 1/8 \\ 1/8 & 1/8 & -5/8 & 3/8 \\ 1/8 & 1/8 & 3/8 & -5/8 \end{pmatrix}.$$

This matrix has eigenvalues $0 > -\gamma > -2\gamma$ where $\gamma = 0.5$. The transition probabilities under this evolutionary model can be computed explicitly by

$$\begin{aligned} P_1(t) &= 0.25 + 0.25 \exp(-0.5t) + 0.5 \exp(-t), \\ P_2(t) &= 0.25 + 0.25 \exp(-0.5t) - 0.5 \exp(-t), \\ P_3(t) &= P_4(t) = 0.25 - 0.25 \exp(-0.5t), \end{aligned}$$

where $P_1(t)$, $P_2(t)$, $P_3(t)$, $P_4(t)$ are the probabilities of transitioning from state A to state A , T , G , C , respectively. This simple model is “universal” in an appropriate sense as shown in the end of the paper.

This leads to a representation of the likelihood as the product of two different linear combinations of the transition polynomials

$$(5.2) \quad \begin{aligned} P_1(x) &= 0.25 + 0.25x + 0.5x^2, \\ P_2(x) &= 0.25 + 0.25x - 0.5x^2, \\ P_3(x) &= P_4(x) = 0.25 - 0.25x, \end{aligned}$$

where $x = \exp(-0.5t)$.

We assume that the likelihood is computed by observing two sites s_1 and s_2 , and that the edge of interest e is a pendant edge with the observed values at that tip being A for both sites. Assume further that the state observation probabilities at the inner node of the edge e are provided by

$$b^1 = [0.24977275, 0.34067358, 0.2051904, 0.20436327]$$

and

$$b^2 = [0.25, 0.16087344, 0.29328435, 0.29584221].$$

As discussed earlier, the log-likelihood function can be computed as

$$(5.3) \quad \ell(t) = \log(\lambda_1(t)) + \log(\lambda_2(t)),$$

where

$$\lambda_s(t) = \sum_{i=1}^4 b^s(i) P_i(t).$$

Plots of the log-likelihood function ℓ and its perturbations (by varying the coefficients slightly) in terms of x and t are provided in Figure 4 and Figure 5, respectively. The figures show that ℓ has three stationary points (two local maxima at $t_1 < t_2$ and one local minimum), all in the interval $[0, 1]$. The fact that $\ell(t_1) > \ell(t_2)$ for some cases and $\ell(t_1) < \ell(t_2)$ for some others indicates that there exist some values of b_i^s such that $\ell(t_1) = \ell(t_2)$, that is, the smoothly varying likelihood function can even have two global maxima.

We note that these examples can be achieved under the assumption that given any positive coefficients b_i^s of the inner node, we can find some trees under the Kimura 2-parameter model with these precise coefficients. This assumption is confirmed by the following result, proven in the [Appendix](#).

Log-likelihood functions with multiple stationary points

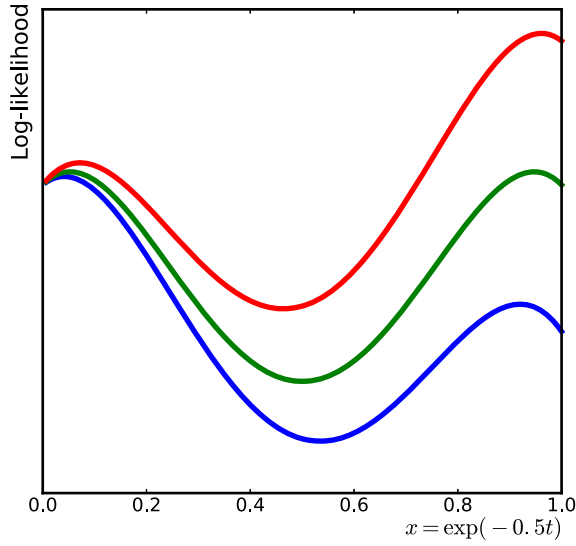


FIG. 4. The log-likelihood (5.3) as a function of $x = \exp(-0.5t)$ for various values of the coefficients of the characteristic polynomial.

Log-likelihood functions with multiple stationary points

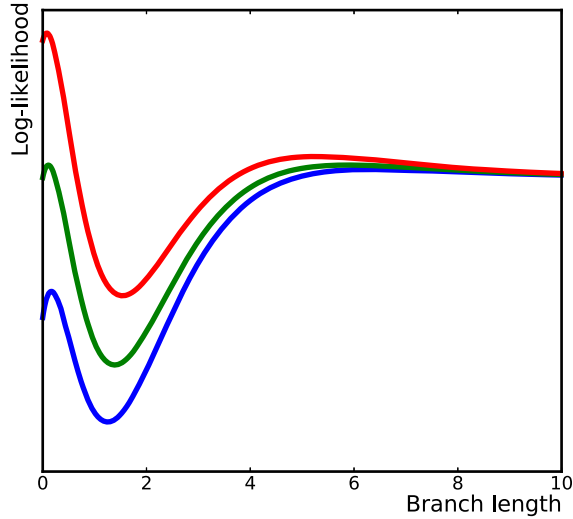


FIG. 5. The log-likelihood (5.3) as a function of branch length t for various values of the coefficients of the characteristic polynomial.

THEOREM 5.1. *For every set of positive coefficients η_i^s , there exist a phylogenetic tree τ and S labelings $\psi_1, \psi_2, \dots, \psi_S$ of the taxa such that for some edge e in τ , the one-dimensional likelihood function on e under the Kimura 2-parameter model with $\kappa = 3$ satisfies*

$$\ell(\tau, t) = C_0 + \sum_{s=1}^S \log \left(\sum_i \eta_i^s P_i(t) \right),$$

where $P_i(t)$ is the probability of transition from state A to state i and C_0 is a constant. Moreover, such a tree τ can be constructed with at most $64S + 1$ edges.

In our examples, the upper bound on the number of edges to produce the given frequency pattern is $64 \times 2 + 1 = 129$ edges.

REMARK 5.1. While the algorithm to construct a tree given a frequency pattern given by Theorem 4.4 always outputs a star-tree (a tree without internal edges), we note that:

1. We can approximate any star tree by resolved trees with arbitrary precision.
2. The maximum number of stationary points of a polynomial of degree four is 3, hence small perturbations on the coefficient of a polynomial of degree four with three stationary points do not change the number of stationary points.

We deduce that there are resolved trees for which the one-dimensional likelihood function on certain edges have multiple maxima.

Since a resolved tree with n taxa has $2n - 3$ edges, the upper bound on the number of edges of a resolved tree for which the one-dimensional likelihood function on certain edges has multiple maxima is $2 \times 129 - 3 = 255$ edges.

6. Universality and complexity of the Kimura 2-parameter model. As we discussed earlier in the paper, the main idea behind the results in Section 3 and Section 4 is that by using the fundamental theorem of algebra, we can decompose a complicated evolutionary model into smaller modules, each of which can be approximated either by a “linear” model or by a “quadratic” model. This paradigm focuses on the branch lengths of the tree and is independent of the state space Ω of the evolutionary model, which provides a way to represent advanced evolutionary models (amino-acid models, codon models) by simple ones (nucleotide models).

This motivates the problem of constructing a complete characterization of one-dimensional likelihood functions. The main question is: does there exist an evolutionary model that can represent all one-dimensional likelihood functions of any time-reversible evolutionary model?

Such a model \mathcal{M} , if it exists, and which we will refer to as a universal model, needs to satisfy the following two conditions:

1. All one-dimensional likelihood functions under any reversible evolutionary model can be written as a product of polynomials, each of which is a positive linear combination of the transition polynomials of \mathcal{M} .

2. For every set of positive coefficients b_{ij}^s , there exists a phylogenetic tree τ and S labelings $\psi_1, \psi_2, \dots, \psi_S$ of the taxa such that for some edge e in τ , the one-dimensional likelihood function on e under the \mathcal{M} satisfies

$$\ell(\tau, t) = C_1 + \sum_{s=1}^S \log \left(\sum_{ij} b_{ij}^s P_{ij}(t) \right)$$

for some constant C_1 .

In this section, we will prove that the Kimura 2-parameter model with $\kappa = 3$ is, in fact, a universal model. The key components of the proof are Theorem 5.1, the fundamental theorem of algebra and the fact that the transition polynomials of the Kimura 2-parameter model effectively span a large class of linear and quadratic polynomials.

6.1. *Universality of the Kimura 2-parameter model.* We first make the following observation, proven in the [Appendix](#).

LEMMA 6.1. *If f is a real-coefficient polynomial that satisfies:*

1. *f is positive on $[0, 1]$,*
2. *$\deg f = 1$ or f is a quadratic polynomial with no real root,*

then f can be written as positive linear combination of the transition polynomials of the Kimura 2-parameter model if and only if:

1. *$\deg f = 1$ and $f(-1) > 0$,*
or
2. *$\deg f = 2$ and f has no root inside the set*

$$(6.1) \quad B = \{z \in \mathbb{C} : |z + 1| \leq 1 \text{ or } |z - 1| \leq \sqrt{2}\}.$$

This enables us to establish the universality of the Kimura 2-parameter model.

THEOREM 6.1 (Universality). *If L is a one-dimensional phylogenetic likelihood function of a tree under an arbitrary time-reversible model that satisfies Assumption 2.1, then up to translation and rescaling, L is equal to a one-dimensional likelihood under the Kimura 2-parameter model.*

That is, there exist $c_1, c_2, c_3 > 0$ such that

$$L(t) = c_2 L_{\text{K2P}}(\tau, \psi, c_3 t) - c_1 \quad \forall t \in [0, \infty),$$

where $L_{\text{K2P}}(\tau, \psi, \cdot)$ is the one-dimensional likelihood function under the Kimura 2-parameter model on some edge of a tree τ with labeling ψ .

PROOF. Assumption 2.1 implies that the function

$$\mathcal{L}(x) := L\left(-\frac{1}{\gamma} \log x\right)$$

is a polynomial in x for some $\gamma > 0$. Since \mathcal{L} is continuous and the set B defined by (6.1) is compact, if we define

$$c_1 = 1 + \sup_{z \in B} |\mathcal{L}(z)|,$$

then by the triangle inequality, the polynomial $\mathcal{L}(x) + c_1$ has no root in B .

By the fundamental theorem of algebra, the polynomial $\mathcal{L}(x) + c_1$ can be written as

$$\mathcal{L}(x) + c_1 = \prod_{s=1}^S g_s(x),$$

where each g_s is either a quadratic polynomial with no real root, or a polynomial of degree 1. Moreover, each g_s is positive on $[0, 1]$ and has no root in B [which also implies $g_s(-1) > 0$ if $\deg g_s = 1$]. Lemma 6.1 implies that each g_s can be written as a positive linear combination of the transition polynomials of the Kimura 2-parameter model

$$g_s(x) = \sum_{ij} b_{ij}^s P_{ij}(x).$$

We deduce that

$$\log(\mathcal{L}(x) + c_1) = \sum_{s=1}^S \log\left(\sum_{ij} b_{ij}^s P_{ij}(x)\right).$$

We recall that the Kimura 2-parameter model has symmetries such that any transition probability $P_{ij}(t)$ is in fact equal to $P_{Al}(t) = P_l(t)$ for some l . Therefore, by grouping

$$\eta_l^s := \sum_{i,j:P_{ij}=P_{Al}} b_{ij}^s,$$

we have

$$\log(\mathcal{L}(x) + c_1) = \sum_{s=1}^S \log\left(\sum_l \eta_l^s P_l(x)\right).$$

Also, the characteristic polynomial for the Kimura 2-parameter model (5.1) with $\kappa = 3$ is parameterized by $x = \exp(-0.5t)$ such that the one-dimensional likelihood $L_{K2P}(\tau, \psi, t)$ satisfies

$$L_{K2P}(\tau, \psi, t) = \mathcal{L}_{K2P}(\tau, \psi, \exp(-0.5t)).$$

Now, Theorem 5.1 guarantees that there exists a tuple (τ, ψ) under the Kimura 2-parameter model on an edge of the tree such that

$$\log \mathcal{L}_{\text{K2P}}(\tau, \psi, x) = -\log c_2 + \sum_{s=1}^S \log \left(\sum_l \eta_l^s P_l(x) \right)$$

for some positive constant c_2 .

In other words, we have

$$\mathcal{L}(x) = c_2 \mathcal{L}_{\text{K2P}}(\tau, \psi, x) - c_1 \quad \forall x \in (0, 1].$$

Hence,

$$L\left(-\frac{1}{\gamma} \log x\right) = c_2 L_{\text{K2P}}(\tau, \psi, -2 \log x) - c_1 \quad \forall x \in (0, 1]$$

or

$$L(t) = c_2 L_{\text{K2P}}(\tau, \psi, c_3 t) - c_1, \quad c_3 = \gamma/2 \quad \forall t \in [0, \infty).$$

That is, up to translation and rescaling, L is equal to a one-dimensional phylogenetic likelihood function under the Kimura 2-parameter model. \square

Since the set of rate matrices for a given evolutionary model that satisfy Assumption 2.1 is dense in the set of all possible rate matrices under the same evolutionary model (Remark 2.1), we also have the following corollary.

COROLLARY 6.1. *Any one-dimensional phylogenetic likelihood function under an arbitrary time-reversible evolutionary model can be uniformly approximated with arbitrary precision by (rescaled and translated) one-dimensional phylogenetic likelihood functions under the Kimura 2-parameter model.*

We also note that the rescaling and translation constants in the statements of Theorem 6.1 cannot be removed: Lemma 6.1 indicates that some polynomial function cannot be represented exactly as a Kimura 2-parameter likelihood function. For example, one of the transition polynomials of the Jukes–Cantor model is

$$J(x) = 0.25 + 0.75x$$

which has $J(-1) < 0$. For this reason, some likelihood functions under the Jukes–Cantor model may not be represented exactly by the Kimura 2-parameter model without adjusting by an additive constant.

6.2. *Complexity of the Kimura 2-parameter model.* The universality results in the previous section can be adapted easily to analyze the set of all one-dimensional phylogenetic likelihood functions under the Kimura 2-parameter model. The following complexity results imply that one-dimensional likelihood functions under advanced evolutionary models can be more complex than it is typically assumed by phylogenetic inference algorithms.

First, it is straightforward to check that Theorem 6.1 still holds (without changing the proof) if we replace the one-dimensional phylogenetic likelihood function L with an arbitrary polynomial P in $x = \exp(-\gamma t)$ for some $\gamma > 0$ and relax Assumption 2.1. Moreover, if P is of degree n , then by Theorem 5.1, it can be represented by a one-dimensional likelihood function of a tree with at most $(64n + 1)$ edges with respect to some n -site labeling of its taxa.

COROLLARY 6.2. *Given an arbitrary polynomial P of degree n and $\gamma > 0$, then up to translation and rescaling, $P(\exp(-\gamma t))$ is equal to a one-dimensional likelihood under the Kimura 2-parameter model on a phylogeny with at most $64n + 1$ edges.*

This corollary indicates that by increasing the number of sites and the size of the tree, we can obtain likelihood functions shaped like an arbitrary polynomial in the interval $[0, 1]$. For example, given an arbitrary finite sequence $t_1, t_2, \dots, t_k \in (0, \infty)$, we can construct a polynomial P_k that peaks precisely at $x_k = \exp(-0.5t_k)$ and use Corollary 6.2 to obtain the following result.

COROLLARY 6.3. *Given an arbitrary finite sequence $t_1, t_2, \dots, t_k \in (0, \infty)$, there exists a phylogenetic tree τ and some labeling of its taxa such that for some edge of the tree, the one-dimensional likelihood function under the Kimura 2-parameter model peaks precisely at t_1, t_2, \dots, t_k .*

Furthermore, since rescaling and translation do not change the relative order of the likelihood values at the stationary points, we can make any of the t_i 's (or all of them) the function's global maxima.

Finally, we can replace the phylogenetic likelihood functions in Corollary 6.1 by an arbitrary continuous function f with finite limit to obtain the following density result.

COROLLARY 6.4. *The space of all rescaled and translated one-dimensional phylogenetic likelihood functions under the Kimura 2-parameter model is dense in the space of all nonnegative continuous functions on $[0, \infty)$ with finite limits.*

PROOF. Let f be a continuous function on $[0, \infty)$ with finite limit. Define

$$g(x) = f(-\log(x)) \quad \forall x \in (0, 1],$$

then $g(x)$ can be extended continuously to $[0, 1]$. By Weierstrass’s theorem [4], there exists a sequence of positive polynomials $\{P_n\}$ such that

$$\sup_{x \in [0,1]} |P_n(x) - g(x)| \rightarrow 0.$$

This implies that

$$\sup_{t \in [0, \infty)} |P_n(\exp(-t)) - f(t)| \rightarrow 0.$$

On the other hand, we deduce from Corollary 6.1 that $P_n(\exp(-t))$ is, up to rescaling and translation, a one-dimensional likelihood under the Kimura 2-parameter model. This completes the proof. \square

7. Nonreversible Markov models of evolution. As we mentioned in Section 2, the analyses in the previous sections can be described in the more general framework of probabilistic inference for graphical models. In this framework, the likelihood function can be defined as the marginal distribution on the leaf nodes of a joint probability distribution that factorizes over the edges of the tree via the nonnegative kernels (also referred to as potential functions) $k_e(i, j, t)$. The one-dimensional phylogenetic likelihood functions can be obtained by fixing all but one branch length.

In this section, we briefly analyze the extent to which our analyses of one-dimensional likelihood functions are valid in this more general setting. As we illustrate below, the results in this section do not assume the reversibility of the kernels, and thus apply for nonreversible models of evolution. However, we need to modify our assumptions accordingly.

Several parts of our analysis rely on the core assumption that the kernel functions need to be polynomials of $x = \exp(-\gamma t)$ for some $\gamma > 0$. Thus, we require the following assumption, which is the equivalent of Assumption 2.1 but in a more general setting.

ASSUMPTION 7.1 (Polynomial representation). There exists a constant $\gamma_e > 0$ and polynomials $p_e^{ij}(x)$ such that

$$k_e(i, j, t) = p_e^{ij}(\exp(-\gamma_e t)) \quad \forall t,$$

for all $i, j \in \Omega$ and $e \in E(T)$.

This assumption implies that the limit of $k_e(i, j, t)$ for large t exists, that is, the kernels are stationary. We also note that using the density results for Bernstein’s polynomial approximation (see, e.g., [4]), any nonnegative continuous function on $[0, \infty)$ with finite limit can be approximated with arbitrary precision by some kernels that satisfy Assumption 7.1.

Under this assumption, the characteristic polynomials $\lambda_s(x)$ can be defined in a similar manner and the result in Section 3 (Theorem 3.1) is still valid.

THEOREM 7.1. *Under Assumption 7.1, if for every site index s , the polynomial λ_s has only real roots, then one-dimensional likelihood function has at most one stationary point. Moreover, if such a point exists, it is also a global maximum.*

While Section 4 is specifically developed to analyze the Kimura 2-parameter model, the logarithmic relative frequency pattern can be extended easily by replacing the transition probabilities across the edge e by the kernel k_e and by setting the distribution of the root by the uniform distribution. Building upon this concept, we can study the algebraic structure of the space of all frequency patterns and obtain a partial extension of Theorem 4.1 in a more general setting as described below. However, the proofs of Theorems 4.2 and 4.3 are tailor-made for the Kimura 2-parameter model and are not easily extended to the general case. We leave their extension as open problems.

We do obtain the following partial extension of Theorem 4.1 in the more general setting.

THEOREM 7.2. *The following properties hold:*

1. $(G, +)$ is a subgroup of $(\mathbb{R}^{r \times S}, +)$.
2. Assume

$$\lim_{t \rightarrow \infty} k_e(i, j, t) = p_e^{ij}(0) = \frac{1}{r} \quad \text{and} \quad \lim_{t \rightarrow 0} k_e(i, j, t) = p_e^{ij}(1) = \delta_{ij}$$

for all $i, j \in \Omega$ and $e \in E(T)$, where $\delta_{ij} = 1$ if $i = j$ and $\delta_{ij} = 0$ otherwise. Then G is a linear subspace of $\mathbb{R}^{r \times S}$.

We recall that the frequency patterns can be defined without the polynomial representation of the likelihood. Thus, in Theorem 7.2, Assumption 7.1 is not required. We further note that for part (1) of the theorem to be valid, we do not need to assume that the kernels are stationary. In fact, the only condition required is that the distribution at the root is uniform. To provide a proof for path-connectivity of G , however, the conditions about the behavior of the kernels at 0 and ∞ are necessary.

Polynomial representation of likelihood functions (Assumption 7.1) is needed to extend the results of Section 6. By the same arguments as in the proof of Theorem 6.1, we obtain an equivalent result.

THEOREM 7.3. *If L is a one-dimensional phylogenetic likelihood function of a tree under a model whose kernels satisfy Assumption 7.1, then up to translation and rescaling, L is equal to a one-dimensional likelihood under the Kimura 2-parameter model.*

8. Conclusions and discussion. In this work, we investigate the problem of characterizing the shape of one-dimensional phylogenetic likelihood functions. Our results classify all evolutionary models into two categories:

1. For binary, the Jukes–Cantor and Felsenstein 1981 models: the one-dimensional likelihood function has at most one stationary point.
2. For the Kimura 2-parameter model and more advanced evolutionary models: the shape of the one-dimensional likelihood function can be much more complex. In fact, the space of all rescaled and translated one-dimensional phylogenetic likelihood functions under such a model is dense in the set of all nonnegative continuous functions on $[0, \infty)$ with finite limits.

Despite the complexity of the one-dimensional likelihood functions under advanced evolutionary models, we prove that all one-dimensional phylogenetic likelihood function are essentially Kimura 2-parameter likelihood functions. This result establishes a strong foundation for the use of the Kimura 2-parameter as the building block of all evolutionary models.

Our results are based on two novel techniques. First, we introduce and use *characteristic polynomial representations* of one-dimensional phylogenetic likelihood functions and the fundamental theorem of algebra to decompose any evolutionary models into smaller modules, each of which resembles the Kimura 2-parameter model. Second, we introduce the new concept of *logarithmic relative frequency patterns* and analyze algebraic structures on the space of such patterns. These structures open a new way to explore the space of all possible likelihood functions. Moreover, by analyzing these structures, we are able to tackle the inverse problem of constructing a phylogenetic tree that has a given frequency pattern at the root. This enables us to construct phylogenetic trees that approximate any given likelihood function with arbitrary precision.

There are several avenues for improvement. First, while we know that the shape of one-dimensional likelihood function can be very complex, it is not clear how frequently multimodality might be encountered in practice and to which degree it affects the accuracy of phylogenetic algorithms. Since the space of high degree polynomials are dominated by multimodal functions, one might expect that as the number of sites and the size of the tree increase, multimodality becomes more likely. However, since the space of phylogenies is known to possess considerable hidden structure which sometimes lead to counterintuitive properties, careful analysis of the space of all rescaled and translated one-dimensional phylogenetic likelihood functions under the Kimura 2-parameter model are required to evaluate this hypothesis. Second, although the focus of this work is on one-dimensional phylogenetic likelihood functions, it is possible to utilize the framework we propose to study full phylogenetic likelihood functions. This will be a subject for future work.

APPENDIX

PROOF OF REMARK 2.1. If we denote the entries of the diagonalizing matrix M and N of Q by m_{ij} and n_{ij} , respectively, then

$$Q_{ij} = \sum_k m_{ik} e^{-r_k} n_{kj},$$

where $-r_k$ are the eigenvalues of Q . (The eigenvalues are known to be nonpositive, so r_k are nonnegative.)

Since the set of rational numbers \mathbb{Q} is dense in \mathbb{R}^+ , we can find $r(k, l) \in \mathbb{Q}^+$ such that for all k , $r(k, l) \rightarrow r_k$ as l approaches infinity. If we define

$$Q^l_{ij} = \sum_k m_{ik} e^{-r(k,l)} n_{kj},$$

then $Q^l \rightarrow Q$ element-wise as l approaches infinity. Since $r(k, l)$ are all rational the matrices are of fixed finite dimension, we can also find $\gamma_l > 0$ and $d(k, l) \in \mathbb{N}$ such that $r(k, l) = d(k, l)\gamma_l$. \square

PROOF OF THEOREM 4.1. We define the equivalence relation \sim on $\mathbb{R}^{4 \times S}$ as follows: $u \sim v$ if and only if there exists a vector of real constants $c = (c_1, \dots, c_S)$ such that for all $i = 1, 2, 3, 4$ and $s = 1, \dots, S$, we have

$$u_{i,s} = v_{i,s} + c_s.$$

If we define

$$[h(\tau, \psi)]_{i,s} = \log \sum_{a \in \mathcal{Z}_{i,s}} \prod_{(u,v) \in E(\tau)} P_{a_u a_v}^{uv}(t_{uv})$$

for $i \in \Omega$, $s = 1, \dots, S$ and $\mathcal{Z}_{i,s}$ being the set of all extensions a of ψ_s to all the nodes of τ such that $a(\rho) = i$.

Recall that the Kimura 2-parameter model has a uniform stationary distribution, for all τ, ψ, c , we have $\phi(\tau, \psi, c) \sim h(\tau, \psi)$.

1. (Addition): Consider any two elements $x_1, x_2 \in G$. By the definition of G and since the stationary frequency of the evolutionary model is the same for every state, there exist trees τ_1, τ_2 with n_1, n_2 taxa and labelings ψ_1, ψ_2 such that

$$x_i \sim h(\tau_i, \psi_i), \quad i = 1, 2.$$

If we construct a new tree τ from τ_1 and τ_2 by gluing the roots ρ_1, ρ_2 and label the taxa of τ corresponding to ψ_1, ψ_2 , then we have

$$[h(\tau, \psi)]_{i,s} = \log \sum_{a \in \mathcal{Z}_{i,s}} \prod_{(u,v) \in E(\tau_1)} P_{a^1_u a^2_v}^{uv}(t_{uv}) \prod_{(u,v) \in E(\tau_2)} P_{a^2_u a^1_v}^{uv}(t_{uv}),$$

where each term a in the sum corresponds uniquely to a pair of extensions (a^1, a^2) of ψ_1^s, ψ_2^s to the internal nodes of τ_1, τ_2 , respectively, such that $a^1(\rho) = a^2(\rho) = i$.

Therefore,

$$\begin{aligned} [h(\tau, \psi)]_{i,s} &= \log \sum_{a^1} \prod_{(u,v) \in E(\tau_1)} P_{a_u^1 a_v^1}^{uv}(t_{uv}) + \log \sum_{a^2} \prod_{(u,v) \in E(\tau_2)} P_{a_u^2 a_v^2}^{uv}(t_{uv}) \\ &= [h(\tau_1, \psi_1)]_{i,s} + [h(\tau_2, \psi_2)]_{i,s} \end{aligned}$$

for all $i \in \Omega$ and $s = 1, \dots, S$.

Therefore,

$$h(\tau, \psi) \sim h(\tau_1, \psi_1) + h(\tau_2, \psi_2)$$

and

$$x_1 + x_2 \sim h(\tau, \psi) \in G$$

which implies that G is closed under addition.

2. (Inverse): Consider any element $x \in G$ and its corresponding representative tree τ and labeling ψ . For any permutation σ of the states, we define the labeling ψ_σ as

$$\psi_\sigma(\omega) = \sigma(\psi(\omega))$$

for every taxon ω of T . For example, if σ is the permutation $(A \ G \ T \ C)$ in cycle notation, then ψ_σ is obtained from ψ by replacing A by G , G by T , T by C and C by A .

Now let σ_0 be a permutation of order r on the state space Ω , create r identical copies $\tau_1, \tau_2, \dots, \tau_r$ of the tree τ with labelings $\psi_{\sigma_0}, \psi_{\sigma_0^2}, \dots, \psi_{\sigma_0^r}$ and glue the root of all the trees together with taxon labeling γ corresponding to the labelings of $\tau_1, \tau_2, \dots, \tau_r$. Then because of symmetry, the frequency pattern f at the root of the newly created tree μ will be the same for every state, that is, $f \sim 0$. We deduce that $0 \in G$ and for every $x \in G$, there exists $y \in G$ such that $x + y = 0$.

This property and the fact that G is closed under addition prove that $(G, +)$ is a subgroup of $(\mathbb{R}^{r \times S}, +)$.

3. (Connectedness): Consider any two elements $x_1, x_2 \in G$ and their corresponding trees τ_1, τ_2 , labelings ψ_1, ψ_2 and vectors of real constants c_1, c_2 . For any $\alpha \in (0, 1)$, we create a new tree $\tau(\alpha)$ by adding a new root ρ , joining ρ_1, ρ_2 by new edges of length $t_1 = \tan(\frac{\pi}{2}\alpha), t_2 = 1/t_1$, respectively. We make ρ the root of τ and label the taxa of τ according to ψ_1, ψ_2 .

Now we note that when $\alpha \rightarrow 0$, we have

$$h(\tau(\alpha), \psi) \rightarrow h(\tau_1, \psi_1) + \log \frac{1}{r}$$

since the contribution of τ_2 becomes stationary (the stationary frequency is $1/r$ because of the model's symmetry). Similarly, when $\alpha \rightarrow 1$, we have

$$h(\tau(\alpha), \psi) \rightarrow h(\tau_2, \psi_2) + \log \frac{1}{r}$$

Therefore, the function $g(\alpha) = \phi(\tau(\alpha), \psi)$ can be extended continuously to the closed interval $[0, 1]$. By changing c continuously from c_1 to $\log(1/r)$, varying α continuously from 0 to 1, then changing c continuously from $\log(1/r)$ to c_2 , we can make a path in G that connects x_1 and x_2 .

4. Since any path-connected subgroup of \mathbb{R}^n is a linear subspace [9], so is G . \square

PROOF OF THEOREM 4.2. Denote by \mathcal{H} the set of all rooted trees with one edge (which have varying branch lengths) and

$$H = \{\phi(\tau, \psi, c) : \tau \in \mathcal{H}, \psi = (\psi_1, \psi_2, \dots, \psi_S) \in \mathbb{R}^S, c \in \mathbb{R}^S\}.$$

Note that in the context of this paper, trees with different branch lengths (or in other words, different values of x) are considered as different trees. Thus, the set H defined here is nontrivial.

We have

$$(A.1) \quad [h(\tau, \psi)]_{j,s} = \log P_{\psi_s, j}(x),$$

where $x = \exp(-0.5t)$, $j = A, G, T, C$, and t is the length of the unique edge of τ .

Let $x_1 = 1/4$, $x_2 = 1/2$, $x_3 = 3/4$. We will prove, by induction on S , that H contains $4 \times S$ independent frequency patterns.

For $S = 1$, by considering the 4 different patterns (A) , (G) , (T) , (C) at the only leaf and the 3 values of x (corresponding to different branch lengths) described above, we can create a set of $4 \times 3 = 12$ different pairs (τ, ψ) . A quick check by computer shows that the corresponding frequency patterns generated by those pairs span the whole vector space $\mathbb{R}^{4 \times 1}$. We can achieve similar result for $S = 2$ with the patterns (A, G) , (G, T) , (T, C) , (C, A) .

Now assume that for $S = n$, H contains $4 \times n$ independent frequency patterns of the form (A.1).

For $l = A, G, T, C$ and $x \in [0, 1]$, we define the building blocks

$$R_l(x) := [\log P_{lA}(x) \quad \log P_{lG}(x) \quad \log P_{lT}(x) \quad \log P_{lC}(x)],$$

$$W_l(x) := \begin{pmatrix} R_l(x_1) \\ R_l(x_2) \\ R_l(x_3) \end{pmatrix}.$$

The induction hypothesis implies that there exist $4 \times n$ independent frequency patterns of the form (A.1). This means that for some labelings $\psi_1, \psi_2, \dots, \psi_{4n}$, the block matrix

$$J = \begin{pmatrix} B_1 \\ B_2 \\ \dots \\ B_{4n} \end{pmatrix}$$

has maximal rank $4n$, where

$$B_s := \begin{pmatrix} R_{\psi_s^1}(x_1) \cdots R_{\psi_s^n}(x_1) \\ R_{\psi_s^1}(x_2) \cdots R_{\psi_s^n}(x_2) \\ R_{\psi_s^1}(x_3) \cdots R_{\psi_s^n}(x_3) \end{pmatrix}.$$

For $s = 1, \dots, 4n$, we consider all the labelings obtained by appending ψ_s with one of the four nucleotides A, G, T, C . By doing so, we create a set of $48n$ different frequency patterns. We want to prove that the block matrix

$$C = \begin{pmatrix} B_1 & W_A(x) \\ B_2 & W_A(x) \\ \dots & \dots \\ B_{4n} & W_A(x) \\ B_1 & W_G(x) \\ B_2 & W_G(x) \\ \dots & \dots \\ B_{4n} & W_G(x) \\ B_1 & W_T(x) \\ B_2 & W_T(x) \\ \dots & \dots \\ B_{4n} & W_T(x) \\ B_1 & W_C(x) \\ B_2 & W_C(x) \\ \dots & \dots \\ B_{4n} & W_C(x) \end{pmatrix}$$

has maximal rank $4n + 4$.

Note that this matrix is row-equivalent to

$$\begin{pmatrix} J & U \\ 0 & V \end{pmatrix},$$

where each row of V is of the form $R_i(x_k) - R_A(x_k)$ for $i = G, T, C$. [This is done by subtracting the blocks $(B_s \ R_i(x))$ by the block $(B_s \ R_A(x))$ then rearranging the row to obtain the sub-matrix J at the top-left corner.]

On the other hand, from the case $S = 1$, we have

$$\text{rank} \begin{pmatrix} W_A(x) \\ W_G(x) \\ W_T(x) \\ W_C(x) \end{pmatrix} = 4,$$

which implies that $\text{rank}(V) = 4$. Hence, $\text{rank}(C) = \text{rank}(J) + \text{rank}(V) = 4n + 4$.

We deduce that for every S , the set G of all possible logarithmic conditional frequency patterns with S sites under the Kimura 2-parameter model is a linear

subspace of $\mathbb{R}^{4 \times S}$ (Theorem 4.1) that contains $4S$ linearly independent vectors. This implies that $G = \mathbb{R}^{4 \times S}$. \square

PROOF OF THEOREM 4.4 (STEP 1). (Any pattern of the form $v = [x \ 0 \ 0 \ 0]$ can be produced by a tree τ with four edges.)

Denote

$$\begin{aligned} x_1(t) &= P_{AA}(t), & x_2(t) &= P_{AG}(t), \\ x_3(t) &= P_{AT}(t), & x_4(t) &= P_{AC}(t) \end{aligned}$$

we note that in the Kimura 2-parameter model, $x_3(t) = x_4(t)$.

Now consider two trees τ_1 and τ_2 , each with one edge, whose branch lengths are t and s , respectively. We label the only nodes of τ_1 and τ_2 by the patterns $\psi_1 = (A)$ and $\psi_2 = (G)$, and obtain the frequency patterns $f_1(t)$ and $f_2(s)$, respectively. By gluing the roots of τ_1 (1 edge) and the “inverse” of the tree τ_2 (3 edges), we obtain a tree $T(t, s)$ with 4 edges whose frequency pattern is equivalent to

$$f_1(t) - f_2(s) \sim \left[\log \frac{x_1(t)x_4(s)}{x_4(t)x_2(s)}, \log \frac{x_2(t)x_4(s)}{x_4(t)x_1(s)}, 0, 0 \right].$$

On the other hand, we note that for the Kimura 2-parameter model (5.1),

$$\frac{x_2(t)}{x_4(t)} = 1 + 2 \exp(-0.5t)$$

only admits values in the interval $[1, 3]$, while $x_1(s)/x_4(s)$ is a continuous decreasing function in s that admits all values in the interval $[1, \infty)$. Hence, for every $t > 0$, there exists a unique $k(t) > 0$ such that

$$\frac{x_2(t)x_4(k(t))}{x_4(t)x_1(k(t))} = 1.$$

Moreover, $k(t)$ is a continuous function in t and

$$\lim_{t \rightarrow \infty} k(t) = \infty, \quad \lim_{t \rightarrow 0} k(t) = k_0,$$

where k_0 satisfies $x_1(k_0)/x_4(k_0) = 3$.

Now if we denote

$$g(t) = \frac{x_1(t)x_4(k(t))}{x_4(t)x_2(k(t))}$$

then $g(t)$ is a continuous function that satisfies

$$\lim_{t \rightarrow \infty} g(t) = 1, \quad \lim_{t \rightarrow 0} g(t) = \infty.$$

We deduce that for a range of t

$$f_1(t) - f_2(k(t)) \sim [\log g(t), 0, 0, 0]$$

which admits every patterns of the form $[x, 0, 0, 0]$ with $x > 0$. Similarly,

$$f_2(k(t)) - f_1(t) \sim [-\log g(t), 0, 0, 0]$$

admits every patterns of the form $[x, 0, 0, 0]$ with $x < 0$. This completes the proof. \square

PROOF OF THEOREM 5.1. From Theorem 4.1, there exists a rooted tree τ , a labeling ψ and a vector of real constants $c = (c_1, \dots, c_S)$ such that

$$c_s + \log \sum_{a \in \mathcal{Z}_{i,s}} \pi(i) \prod_{(u,v) \in E(\tau)} P_{a_u a_v}^{uv}(t_{uv}) = \log(\eta_i^s).$$

[Recall that $\mathcal{Z}_{i,s}$ is the set of all extensions a of ψ_s to all the nodes of τ such that $a(\rho) = i$.] For any $t > 0$, we create a new tree $\tau(t)$ by adding an edge e of length t to the root ρ and labeling the additional taxon by the constant vector (A, A, \dots, A) . The log-likelihood function on e of $\tau(t)$ given this taxon labeling is

$$\begin{aligned} \ell(t) &= \sum_{s=1}^S \log \left(\sum_i \sum_{a \in \mathcal{Z}_{i,s}} \pi(i) \prod_{(u,v) \in E(\tau)} P_{a_u a_v}^{uv}(t_{uv}) P_{iA}(t) \right) \\ &= - \sum_{s=1}^S c_s + \sum_{s=1}^S \log \left(\sum_i \eta_i^s P_i(t) \right). \end{aligned}$$

Theorem 4.4 implies that the tree τ can be constructed with at most $64S$ edges. Hence, $\tau(t)$ has at most $(64S + 1)$ edges. \square

PROOF OF LEMMA 6.1. We first consider the case of linear functions. Assume that $f(x) = ax + b$ such that f is positive in $[0, 1]$. We deduce that $b + a = f(1) > 0$. Hence, f can be written as

$$\begin{aligned} f(x) &= ax + b \\ &= 2(b - a) \left(\frac{1}{4} - \frac{1}{4}x \right) + (b + a) \left(\frac{1}{4} + \frac{1}{4}x - \frac{1}{2}x^2 \right) \\ &\quad + (b + a) \left(\frac{1}{4} + \frac{1}{4}x + \frac{1}{2}x^2 \right) \\ &= 2(b - a) P_3(x) + (b + a) P_2(x) + (b + a) P_1(x) \end{aligned}$$

using the transition polynomials $P_i(x)$ from equation (5.2).

Since $\{P_1, P_2, P_3\}$ are linearly independent, we deduce that f can be expressed as positive linear combination of P_1, P_2, P_3 if and only if $f(-1) = b - a > 0$.

If $f(x)$ is a monic polynomial of degree 2 with no real roots, then f can be written as

$$\begin{aligned} f(x) &= x^2 - 2ax + a^2 + b^2 \\ &= [(a - 1)^2 + b^2 - 1]P_1(x) \\ &\quad + [(a - 1)^2 + b^2 - 2]P_2(x) \\ &\quad + 2[(a + 1)^2 + b^2 - 1]P_3(x). \end{aligned}$$

The coefficients are positive if and only if $a \pm bi$ do not belong to B . \square

Acknowledgments. We are grateful to Connor McCoy and Brian Claywell for their work on surrogate functions for likelihood computation, which motivated this research.

REFERENCES

- [1] BEN-ISRAEL, A. and MOND, B. (1986). What is invexity? *J. Aust. Math. Soc. A* **28** 1–9. [MR0846778](#)
- [2] BRYANT, D., GALTIER, N. and POURSAT, M.-A. (2005). Likelihood calculation in molecular phylogenetics. In *Mathematics of Evolution and Phylogeny* 33–62.
- [3] CHOR, B., HENDY, M. D., HOLLAND, B. R. and PENNY, D. (2000). Multiple maxima of likelihood in phylogenetic trees: An analytic approach. *Mol. Biol. Evol.* **17** 1529–1541.
- [4] FAROUKI, R. T. (2012). The Bernstein polynomial basis: A centennial retrospective. *Comput. Aided Geom. Design* **29** 379–419. [MR2921860](#)
- [5] FELSENSTEIN, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* **17** 368–376.
- [6] FELSENSTEIN, J. (2004). *Inferring Phylogenies*. Sinauer Associates, Sunderland.
- [7] FUKAMI, K. and TATENO, Y. (1989). On the maximum likelihood method for estimating molecular trees: Uniqueness of the likelihood point. *J. Mol. Evol.* **28** 460–464.
- [8] HANSON, M. A. (1981). On sufficiency of the Kuhn–Tucker conditions. *J. Math. Anal. Appl.* **80** 545–550. [MR0614849](#)
- [9] HAYASHIDA, T. (1949). Arc-wise connected subgroup of a vector group. *Kodai Math. Semin. Rep.* **1** 16–16.
- [10] JUKES, T. H. and CANTOR, C. R. (1969). Evolution of protein molecules. *Mammalian Protein Metabolism* **3** 21–132.
- [11] KIMURA, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16** 111–120.
- [12] ROGERS, J. S. and SWOFFORD, D. L. (1999). Multiple local maxima for likelihoods of phylogenetic trees: A simulation study. *Mol. Biol. Evol.* **16** 1079–1085.
- [13] STEEL, M. (1994). The maximum likelihood point for a phylogenetic tree is not unique. *Syst. Biol.* **43** 560–564.

PROGRAM IN COMPUTATIONAL BIOLOGY
 FRED HUTCHINSON CANCER RESEARCH CENTER
 SEATTLE, WASHINGTON 98109
 USA
 E-MAIL: vdinh@fredhutch.org
matsen@fredhutch.org