The Mathematics of Phylogenomics

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“The lack of real contact between mathematics and biology is either a tragedy, a scandal or a challenge, it is hard to decide which.” – Gian-Carlo Rota, [34, p. 2]

1 Introduction

The grand challenges in biology today are being shaped by powerful high-throughput technologies that have revealed the genomes of many organisms, global expression patterns of genes and detailed information about variation within populations. We are therefore able to ask, for the first time, fundamental questions about the evolution of genomes, the structure of genes and their regulation, and the connections between genotypes and phenotypes of individuals. The answers to these questions are all predicated on progress in a variety of computational, statistical, and mathematical fields [35].

The rapid growth in the characterization of genomes has led to the advancement of a new discipline called Phylogenomics. This discipline, whose scope and potential was first outlined in [22], results from the combination of two major fields in the life sciences: Genomics, i.e., the study of the function and structure of genes and genomes; and Molecular Phylogenetics, i.e., the study of the hierarchical evolutionary relationships among organisms and their genomes. The objective of this article is to offer mathematicians a first introduction to this emerging field, and to discuss specific problems and developments arising from phylogenomics.

The mathematical tools to be highlighted in this paper are statistics, probability, combinatorics and – last but not least – algebraic geometry.
Emphasis is placed on the use of Algebraic Statistics, which is the study of statistical models for discrete data using algebraic methods. See [44] §1 for details. Several models which are relevant for phylogenomics are shown to be algebraic varieties in certain high-dimensional spaces of probability distributions. This interplay between statistics and algebraic geometry offers a conceptual framework for (understanding existing and developing new) combinatorial algorithms for biological sequence analysis. It is our hope that this will contribute to some “real contact” between mathematics and biology.

This paper is organized as follows. In Section 2 we begin by reviewing the organization and structure of genomes. This section is meant as a brief tutorial, aimed at readers who have a little or no background in molecular biology. It offers definitions of the relevant biological terminology.

Section 3 describes a very simple example of a statistical model for inferring information about the genetic code. The point of this example is to explain the philosophy of algebraic statistics: model means algebraic variety.

A more realistic model, which is widely used in computational biology, is the hidden Markov model. In Section 4 we explain this model and discuss its applications to the problem of identifying genes in a genome. Another key problem is the alignment of biological sequences. Section 5 reviews the statistical models and combinatorial algorithms for sequence alignment. We also discuss the relevance of parametric inference in this context.

In Section 6 we present statistical models for the evolution of biological sequences. These models are algebraic varieties associated with phylogenetic trees, and they play a key role in inferring the ancestral relationships among organisms and in identifying regions in genomes that are under selection.

Section 7 gives an introduction to the field of Phylogenetic Combinatorics, which is concerned with the combinatorics and geometry of finite metric spaces, and their application to data analysis in the life sciences. We shall discuss the space of all trees, the neighbor-joining algorithm for projecting metrics onto this space, and several natural generalizations of these concepts.

In Section 8 we go back to the data. We explain how one obtains and studies DNA sequences generated by genome sequencing centers, and we illustrate the mathematical models by estimating the probability that the DNA sequence in Conjecture occurred by chance in ten vertebrate genomes.
2 The Genome

Every living organism has a genome, made up of deoxyribonucleic acids (DNA) arranged in a double helix \[59\], which encodes (in a way to be made precise) the fundamental ingredients of life. Organisms are divided into two major classes: eukaryotes (organisms whose cells contain a nucleus) and prokaryotes (for example bacteria). In our discussion we focus on genomes of eukaryotes, and, in particular, the human genome \[38, 57\].

Eukaryotic genomes are divided into chromosomes. The human genome has two copies of each chromosome. There are 23 pairs of chromosomes: 22 autosomes (two copies each in both men and women) and two sex chromosomes, which are denoted X and Y. Women have two X chromosomes, while men have one X and one Y chromosome. Parents pass on a mosaic of their pair of chromosomes to their children.

The sequence of DNA molecules in a genome is typically represented as a sequence of letters, partitioned into chromosomes, from the four letter alphabet \( \Omega = \{A, C, G, T\} \). These letters correspond to the bases in the double helix, that is, the nucleotides Adenine, Cytosine, Guanine and Thymine. Since every base is paired with an opposite base (\( A \) with \( T \), and \( C \) with \( G \) in the other half of the double helix), in order to describe a genome it suffices to list the bases in only one strand. However, it is important to note that the two strands have a directionality which is indicated by the numbers 5′ and 3′ on the ends (corresponding to carbon atoms in the helix backbone). The convention is to represent DNA in the 5′ → 3′ direction. The human genome consists of approximately 2.8 billion bases, and has been obtained using high throughput sequencing technologies that can be used to read the sequence of short DNA fragments hundreds of bases long. Sequence assembly algorithms are then used to piece together these fragments \[39\]. See also \[44, \S4\].

Despite the tendency to abstract genomes as strings over the alphabet \( \Omega \), one must not forget that they are highly structured: for example, certain subsequences within a genome correspond to genes. These subsequences play the important role of encoding proteins. Proteins are polymers made of twenty different types of amino acids. Within a gene, triplets of DNA, known as codons, encode the amino acids for the proteins. This is known as the genetic code. Table 1 shows the 64 possible codons, and the twenty amino acids they code for. Each amino acid is represented by a three letter identifier (“Phe” = Phenylalanine, “Leu” = Leucin, ...). The three codons \( TAA, TAG \) and \( TGA \) are special: instead of coding for an amino acid, they
Table 1: The genetic code.

|   | T | C | A | G |
|---|---|---|---|---|
| T | TTT → Phe | TCT → Ser | TAT → Tyr | TGT → Cys |
|   | TTC → Phe | TCC → Ser | TAC → Tyr | TGC → Cys |
|   | TTA → Leu | TCA → Ser | TAA → stop | TGA → stop |
|   | TTG → Leu | TCG → Ser | TAG → stop | TGG → Trp |
| C | CTT → Leu | CCT → Pro | CAT → His | CGT → Arg |
|   | CTC → Leu | CCC → Pro | CAC → His | CGC → Arg |
|   | CTA → Leu | CCA → Pro | CAA → Gln | CGA → Arg |
|   | CTG → Leu | CCG → Pro | CAG → Gln | CGG → Arg |
| A | ATT → Ile | ACT → Thr | AAT → Asn | AGT → Ser |
|   | ATC → Ile | ACC → Thr | AAC → Asn | AGC → Ser |
|   | ATA → Ile | ACA → Thr | AAA → Lys | AGA → Arg |
|   | ATG → Met | ACG → Thr | AAG → Lys | AGG → Arg |
| G | GTT → Val | GCT → Ala | GAT → Asp | GGT → Gly |
|   | GTC → Val | GCC → Ala | GAC → Asp | GGC → Gly |
|   | GTA → Val | GCA → Ala | GAA → Glu | GGA → Gly |
|   | GTG → Val | GCG → Ala | GAG → Glu | GGG → Gly |

are used to indicate that the protein ends.

In order to make protein, DNA is first copied into a similar molecule called messenger RNA (abbreviated mRNA) in a process called transcription. It is the RNA that is translated into protein. The entire process is referred to as expression. Proteins can be structural elements, or perform complex tasks (such as regulation of expression) by interacting with the many molecules and complexes in cells. Thus, the genome is a blueprint for life. An understanding of the genes, the function of their proteins, and their expression patterns is fundamental to biology.

The human genome contains approximately 25,000 genes, although the exact number has still not been determined. While there are experimental methods for validating and discovering genes, there is still no known high throughput technology for accurately identifying all the genes in a genome. The computational problem of identifying genes, the gene finding problem, is an active area of research. One of the main difficulties lies in the fact that only a small portion of any genome is genic. For instance, less than 5% of the human genome is known to be functional. In Section 4 we discuss this problem, and the role of probabilistic models in formulating statistically sound methods for distinguishing genes from non-genic sequence. The models of
choice, hidden Markov models, allow for the integration of diverse biological information (such as the genetic code and the structure of genes) and yet are suitable for designing efficient algorithms. By virtue of being algebraic varieties, they provide a key example for the link between algebra, statistics and genomics. Nevertheless, the current understanding of genes is not sufficient to allow for the ab-initio identification of all the genes in a genome, and it is through comparison with other genomes that the genes are revealed [3].

The differences between the genomes of individuals in a population are small and are primarily due to recombination events (part of the process by which two copies of parental chromosomes are merged in the offspring). On the other hand, the genomes of different species (classes of organisms that can produce offspring together) tend to be much more divergent. Genome differences between species can be explained by many biological events including:

- **Genome rearrangement** – comparing chromosomes of related species reveals large segments that have been reversed and flipped (inversions), segments that have been moved (transpositions), fusions of chromosomes, and other large scale events. The underlying biological mechanisms are poorly understood [45, 48].

- **Duplications and loss** – some genomes have undergone whole genome duplications. This process was recently demonstrated for yeast [36]. Individual chromosomes or genes may also be duplicated. Duplication events are often accompanied by gene loss, as redundant genes slowly lose or adapt their function over time [23].

- **Parasitic expansion** – large sections of genomes are repetitive, consisting of elements which can duplicate and re-integrate into a genome.

- **Point mutation, insertion and deletion** – DNA sequences mutate, and in non-functional regions these mutations accumulate over time. Such regions are also likely to exhibit deletions; for example, strand slippage during replication can lead to an incorrect copy number for repeated bases.

Accurate mathematical models for sequence alignment and evolution, our topics in Sections 5–7, have to take these processes into consideration.
Two distinct DNA bases that share a common ancestor are called homologous. Homologous bases can be related via speciation and duplication events, and are therefore divided into two classes: orthologous and paralogous. Orthologous bases are descendant from a single base in an ancestral genome that underwent a speciation event, whereas two paralogous bases correspond to two distinct bases in a single ancestral genome that are related via a duplication. Because we cannot sequence ancestral genomes, it is never possible to formally prove that two DNA bases are homologous. However, statistical arguments can show that it is extremely likely that two bases are homologous, or even orthologous. The problem of identifying homologous bases between genomes of related species is known as the alignment problem. We shall discuss this in Section 5.

The alignment of genomes is the first step in identifying highly conserved sequences that point to the small fraction of the genome that is under selection, and therefore likely to be functional. Although the problem of sequence alignment is mathematically and computationally challenging, proposed homologous sequences can be rapidly and independently validated (it is easy to check whether two sequences align once they have been identified), and the regions can often be tested in a molecular biology laboratory to determine their function. In other words, sequence alignment reveals concrete verifiable evidence for evolutionary selection and often results in testable hypotheses.

As a focal point for our discussion, we present a specific DNA sequence of length 42. This sequence was found in April 2004 as a byproduct of computational work conducted by Lior Pachter’s group at Berkeley [10]. Whole genome alignments were found and analyzed of the human (hs), chimpanzee (pt), mouse (mm), rat (rn), dog (cf), chicken (gg), frog (xt), zebra-fish (dr), fugu-fish (tr) and tetraodon (tn) genomes. The abbreviations refer to the Latin names of these organisms. They will be used in Table 3 and Figure 4. From alignments of the ten genomes, the following hypothesis was derived, which we state in the form of a mathematical conjecture.

**Conjecture 1.** (The “Meaning of Life”) The sequence of 42 bases

\[
\text{T}T\text{T}A\text{A}T\text{G}\text{A} \text{A}\text{A} \text{A}\text{G} \text{G}\text{T} \text{A}\text{T} \text{G} \text{A} \text{A}\text{A}\text{A}\text{T} \text{G}\text{A} \text{A}\text{T} \text{C} \text{A} \text{A}\text{T} \text{A}\text{A}\text{G}
\]

\[\text{(1)}\]

was present in the genome of the ancestor of all vertebrates, and it has been completely conserved to the present time (i.e., none of the bases have been mutated, nor have there been any insertions or deletions).
The identification of such a sequence requires a highly non-trivial computation: the alignment of ten genomes (including mammalian genomes close to 3 billion bases in length) and subsequent analysis to identify conserved orthologous regions within the alignment \[^{[60]}\]. Using the tools described in Section 8, one checks that the sequence \(\mathtt{1}\) is present in all ten genomes. For instance, in the human genome (May 2004 version), the sequence occurs on chromosome 7 in positions 156501197–156501238. By examining the alignment, one verifies that, with very high probability, the regions containing this sequence in all ten genomes are orthologous. Furthermore, the implied claim that \(\mathtt{1}\) occurs in all present-day vertebrates can, in principle, be tested.

Identifying and analyzing sequences such as \(\mathtt{1}\) is important because they are highly conserved yet often non-genic \[^{[7]}\]. One of the ongoing mysteries in biology is to unravel the function of the parts of the genome that is non-genic and yet very conserved. The extent of conservation points to the possibility of critical functions within the genome. It may be a coincidence that the segment above contains two copies of the motif \(\mathtt{TTAATTGAA}\), but this motif may also have some function (for example, it may be bound by a protein). Indeed, the identification of such elements is the first step towards understanding the complex regulatory code of the genome. Back in 2003, we were amused to find that 42 was the length of the longest such sequence. In light of \(\mathtt{1}\), it was decided to name this DNA-sequence “The Meaning of Life”.

The conjecture was formulated in the spring of 2004 and it was circulated in the first arXiv version of this paper. In the fall of 2004, Drton, Eriksson and Leung \[^{[21]}\] conducted a new study based on improved alignments. Their work led to even longer sequences with similar properties. So, the Meaning of Life sequence no longer holds the record in terms of length. However, since Conjecture \(\mathtt{1}\) has been inspiration for our group, and it still remains open today, we decided to stick with this example. It needs to be emphasized that disproving Conjecture \(\mathtt{1}\) would not invalidate any of the methodology presented in this article. For a biological perspective we refer to \[^{[21]}\].

3 Codons

Because of the genetic code, the set \(\Omega^3\) of all three-letter words over the alphabet \(\Omega = \{A, C, G, T\}\) plays a special role in molecular biology. As was discussed in Section 2, these words are called codons, with each triplet coding for one of 20 amino acids (Table 1). The map from 64 codons to
20 amino acids is not injective, and so multiple codons code for the same amino acid. Such codons are called *synonymous*. Eight amino acids have the property that the synonymous codons that code for them all agree in the first two positions. The third positions of such codons are called *four-fold degenerate*. The translation of a series of codons in a gene (typically a few hundred) results in a three-dimensional folded protein.

A *model for codons* is a statistical model whose state space is the 64-element set $\Omega^3$. Selecting a model means specifying a family of probability distributions $p = (p_{IJK})$ on $\Omega^3$. Each probability distribution $p$ is a $4 \times 4 \times 4$-table of non-negative real numbers which sum to one. Geometrically, a distribution on codons is a point $p$ in the 63-dimensional probability simplex

$$\Delta_{63} = \{ p \in \mathbb{R}^{\Omega^3} : \sum_{IJK \in \Omega^3} p_{IJK} = 1 \text{ and } p_{IJK} \geq 0 \text{ for all } IJK \in \Omega^3 \}.$$ 

A model for codons is hence nothing but a subset $\mathcal{M}$ of the simplex $\Delta_{63}$. Statistically meaningful models are usually given in parametric form. If the number of parameters is $d$, then there is a set $\mathcal{P} \subset \mathbb{R}^d$ of allowed parameters, and the model $\mathcal{M}$ is the image of a map $\phi$ from $\mathcal{P}$ into $\Delta_{63}$. We illustrate this statistical point of view by means of a very simple independence model.

Models for codons have played a prominent role in the work of Samuel Karlin, who was one of the mathematical pioneers in this field. One instance of this is the *genome signature* in [13]. We refer to [44, Example 4.3] for a discussion of this model and more recent work on codon usage in genomes.

Consider a DNA sequence of length $3m$ which has been grouped into $m$ consecutive codons. Let $u_{IJK}$ denote the number of occurrences of a particular codon $IJK$. Then our data is the $4 \times 4 \times 4$-table $u = (u_{IJK})$. The entries of this table are non-negative integers, and if we divide each entry by $m$ then we get a new table $\frac{1}{m} \cdot u$ which is a point in the probability simplex $\Delta_{63}$. This table is the *empirical distribution of codons* in the given sequence.

Let $\mathcal{M}$ be the statistical model which stipulates that, for the sequence under consideration, the first two positions in a codon are independent from the third position. We may wish to test whether this independence model fits our data $u$. This question makes sense in molecular biology because many of the amino acids are uniquely specified by the first two positions in any codon which represents that particular amino acid (see Table 1). Therefore, third positions in synonymous codons tend to be independent of the first two.

Our independence model $\mathcal{M}$ has 18 free parameters. The set of allowed
parameters is an 18-dimensional convex polytope, namely, it is the product

$$\mathcal{P} = \Delta_{15} \times \Delta_3.$$  

Here $\Delta_{15}$ is the 15-dimensional simplex consisting of probability distributions $\alpha = (\alpha_{IJ})$ on $\Omega^2$, and $\Delta_3$ is the tetrahedron consisting of probability distributions $\beta = (\beta_K)$ on $\Omega$. Our model $\mathcal{M}$ is parameterized by the map

$$\phi : \mathcal{P} \to \Delta_{63}, \quad \phi((\alpha, \beta))_{IJK} = \alpha_{IJ} \cdot \beta_K.$$  

Hence $\mathcal{M} = \text{image}(\phi)$ is an 18-dimensional algebraic subset inside the 63-dimensional simplex. To test whether a given $4 \times 4 \times 4$-table $p$ lies in $\mathcal{M}$, we write that table as a two-dimensional matrix with 16 rows and 4 columns:

$$p' = \begin{pmatrix}
  p_{AAA} & p_{AAC} & p_{AAG} & p_{AAT} \\
  p_{ACA} & p_{ACC} & p_{ACG} & p_{ACT} \\
  p_{AGA} & p_{AGC} & p_{AGG} & p_{AGT} \\
  p_{ATA} & p_{ATC} & p_{ATG} & p_{ATT} \\
  p_{CAA} & p_{CAC} & p_{CAG} & p_{CAT} \\
  \vdots & \vdots & \vdots & \vdots \\
  p_{TTA} & p_{TTC} & p_{TTG} & p_{TTT}
\end{pmatrix}.$$  

Linear algebra furnishes the following characterizations of our model:

**Proposition 2.** For a point $p \in \Delta_{63}$, the following conditions are equivalent:

1. The distribution $p$ lies in the model $\mathcal{M}$.
2. The $16 \times 4$ matrix $p'$ has rank one.
3. All $2 \times 2$-minors of the matrix $p'$ are zero.
4. $p_{IJK} \cdot p_{LMN} = p_{IJN} \cdot p_{LMK}$ for all nucleotides $I, J, K, L, M, N$.

In the language of algebraic geometry, the model $\mathcal{M}$ is known as the Segre variety. More precisely, $\mathcal{M}$ is the set of non-negative real points on the Segre embedding of $\mathbb{P}^{15} \times \mathbb{P}^3$ in $\mathbb{P}^{63}$. Here and throughout, the symbol $\mathbb{P}^m$ denotes the complex projective space of dimension $m$. One of the points argued in this paper is that many of the more advanced statistical models, such as graphical models \[11\, \S 1.5\], actually used in practice by computational biologists are also algebraic varieties with a special combinatorial structure.
Returning to our original biological motivation, we are faced with the following statistics problem. The DNA sequence under consideration is summarized in the data $u$, and we wish to test whether or not the model $M$ fits the data. The geometric idea of such a test is to determine whether or not the empirical distribution $\frac{1}{m} \cdot u$ lies close to the Segre variety $M$. Statisticians have devised a wide range of such tests, each representing a statistically meaningful notion of “proximity to $M$”. These include the $\chi^2$-test, the $G^2$-test, Fisher’s exact test, and others, as explained in standard statistics texts such as [8] or [28]. A useful tool of numerical linear algebra for measuring the distance of a point to the Segre variety is the singular value decomposition of the matrix $p'$. Indeed, $p'$ lies on $M$ if and only if the second singular value of $p'$ is zero. Singular values provide a good notion of distance between a given matrix and various determinantal varieties such as $M$.

One key ingredient in statistical tests is maximum likelihood estimation. The basic idea is to find those model parameters $\alpha_{IJ}$ and $\beta_K$ which would best explain the observed data. If we consider all possible genome sequences of length $3m$, then the likelihood of observing our particular data $u$ equals

$$
\gamma \cdot \prod_{IJK \in \Omega^3} p_{IJK}^{u_{IJK}},
$$

where $\gamma$ is a combinatorial constant. This expression is a function of $(\alpha, \beta)$, called the likelihood function. We wish to find the point in our parameter domain $\mathcal{P} = \Delta_{15} \times \Delta_3$ which maximizes this function. The solution $(\hat{\alpha}, \hat{\beta})$ to this non-linear optimization problem is said to be the maximum likelihood estimate for the data $u$. In our independence model, the likelihood function is convex, and it is easy to write down the global maximum explicitly:

$$
\hat{\alpha}_{IJ} = \frac{1}{m} \sum_{K \in \Omega} u_{IJK} \quad \text{and} \quad \hat{\beta}_K = \frac{1}{m} \sum_{IJ \in \Omega^2} u_{IJK}.
$$

In general, the likelihood function of a statistical model will not be convex, and there is no easy formula for writing the maximum likelihood estimate as a function of the data. In practice, numerical hill-climbing methods are used to solve this optimization problem, but, of course, there is no guarantee that a local maximum found by such methods is actually the global maximum.
4 Gene Finding

In order to find genes in DNA sequences, it is necessary to identify structural features and sequence characteristics that distinguish genic sequence from non-genic sequence. We begin by describing more of the detail of gene structure which is essential in developing probabilistic models.

Genes are not contiguous subsequences of the genome, but rather split into pieces called *introns* and *exons*. After transcription, introns are spliced out and only the remaining exons are used in translation (Figure 1). Not all of the sequence in the exons is translated; the initial and terminal exons may consist of *untranslated regions* (indicated in grey in the figure). Since the genetic code is in (non-overlapping) triplets, it follows that the lengths of the translated portions of the exons must sum to 0 mod 3. In addition to

![Figure 1: Structure of a gene.](image)

the exon-intron structure of genes, there are known sequence signals. The codon *ATG* initiates translation, and thus is the first codon following the untranslated portion of the initial exons. The final codon in a gene must be one of *TAG*, *TAA* or *TGA*, as indicated in Table 1. These codons signal
the translation machinery to stop. There are also sequence signals at the intron-exon boundaries: GT at the 5′ end of an intron and AG at the 3′ end.

A hidden Markov model (HMM) is a probabilistic model that allows for simultaneous modeling of the bases in a DNA sequence of length $n$ and the structural features associated with that sequence. The HMM consists of $n$ observed random variables $Y_1, \ldots, Y_n$ taking on $l$ possible states, and $n$ hidden random variables $X_1, \ldots, X_n$ taking on $k$ possible states. In the context of phylogenomics, the observed variables $Y_i$ usually have $l = 4$ states, namely $\Omega = \{A, C, G, T\}$. The hidden random variables $X_i$ serve to model features associated with the sequence which is generated by $Y_1, Y_2, \ldots, Y_n$. A simple scenario is $k = 2$, with the set of hidden states being $\Theta = \{exon, intron\}$.

The characteristic property of an HMM is that the distributions of the $Y_i$ depend on the $X_i$, while the $X_i$ form a Markov chain. This is illustrated for $n = 3$ in Figure 2, where the unshaded circles represent the hidden variables $X_1, X_2, X_3$ and the shaded circles represent the observed variables $Y_1, Y_2, Y_3$.

![Figure 2: The hidden Markov model of length three.](image)

Computational biologists use HMMs to annotate DNA sequences. The basic idea is this: it is postulated that the bases are instances of the random variables $Y_1, \ldots, Y_n$, and the problem is to identify the most likely assignments of states to $X_1, \ldots, X_n$ that could be associated with the observations. In gene finding, homogeneous HMMs are used. This means that all transition probabilities $X_i \rightarrow X_{i+1}$ are given by the same $k \times k$-matrix $S = (s_{ij})$, and all the transitions $X_i \rightarrow Y_i$ are given by another $k \times 4$-matrix $T = (t_{ij})$. Here $s_{ij}$ represents the probability of transitioning from hidden state $i$ to hidden state $j$; for instance, if $k = 2$ then $i, j \in \Theta = \{exon, intron\}$. The parameter $t_{ij}$ represents the probability that state $i \in \Theta$ outputs letter $j \in \Omega$. 

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In practice, the parameters $s_{ij}$ and $t_{ij}$ range over real numbers satisfying
\begin{equation}
 s_{ij}, t_{ij} \geq 0 \quad \text{and} \quad \sum_{j \in \Theta} s_{1j} = \sum_{j \in \Omega} t_{1j} = 1. \tag{2}
\end{equation}
However, just like in our discussion of the Segre variety in Section 3, we may relax the requirements (2) and allow the parameters to be arbitrary complex numbers. This leads to the following algebraic representation [42, §2].

**Proposition 3.** The homogeneous HMM is the image of a map $\phi : \mathbb{C}^{k(k+l)} \to \mathbb{C}^n$, where each coordinate of $\phi$ is a bi-homogeneous polynomial of degree $n-1$ in the transition probabilities $s_{ij}$ and degree $n$ in the output probabilities $t_{ij}$.

The coordinate $\phi_{\sigma}$ of the map $\phi$ indexed by a particular DNA sequence $\sigma \in \Omega^n$ represents the probability that the HMM generates the sequence $\sigma$. The following explicit formula for that probability establishes Proposition 3:
\begin{equation}
\phi_{\sigma} = \sum_{i_1 \in \Theta} t_{i_1\sigma_1} \left( \sum_{i_2 \in \Theta} s_{i_1i_2} t_{i_2\sigma_2} \left( \sum_{i_3 \in \Theta} s_{i_2i_3} t_{i_3\sigma_3} \left( \cdots \sum_{i_n \in \Theta} s_{i_{n-1}i_n} t_{i_n\sigma_n} \right) \right) \right) \tag{3}
\end{equation}

The expansion of this polynomial has $k^n$ terms
\begin{equation}
t_{i_1\sigma_1} s_{i_1i_2} t_{i_2\sigma_2} s_{i_2i_3} t_{i_3\sigma_3} \cdots s_{i_{n-1}i_n} t_{i_n\sigma_n}. \tag{4}
\end{equation}
For any fixed parameters as in [2], one wishes to determine a string $\hat{i} = (i_1, i_2, \ldots, i_n) \in \Theta^n$ which indexes a term (4) of largest numerical value among all $k^n$ terms of $\phi_{\sigma}$. (If there is more than one string with maximum value then we break ties lexicographically). We call $\hat{i}$ the *explanation* of the observation $\sigma$. In our example ($k = 2, l = 4$), the explanation $\hat{i}$ of a DNA sequence $\sigma$ is an element of $\Theta^n = \{ \text{exon, intron} \}^n$. It reveals the crucial information of Figure 1, namely, the location of the exons and introns. In summary, the DNA sequence to be annotated by an HMM corresponds to the observation $\sigma \in \Omega^n$, and the explanation $\hat{i}$ is the gene prediction. Thus *gene finding* means nothing but computing the output $\hat{i}$ from the input $\sigma$.

In real-world applications, the integer $n$ may be quite large. It is not uncommon to annotate DNA sequences of length $n \geq 1,000,000$. The size $k^n$ of the search space for finding the explanation is enormous (exponential in $n$). Fortunately, the recursive decomposition in (3), reminiscent of *Horner’s Rule*, allows us to evaluate a multivariate polynomial with exponentially many
terms in linear time (in \( n \)). In other words, for given numerical parameters \( s_{ij} \) and \( t_{ij} \), we can compute the probability \( \phi_\sigma(s_{ij}, t_{ij}) \) quite efficiently.

Similarly, the explanation \( \hat{i} \) of an observed DNA sequence \( \sigma \) can be computed in linear time. This is done using the Viterbi algorithm, which evaluates

\[
\max_{i_1 \in \Theta} T_{i_1} \sigma_1 + \left( \max_{i_2 \in \Theta} S_{i_1} + T_{i_2} \sigma_2 + \left( \max_{i_3 \in \Theta} S_{i_2} + T_{i_3} \sigma_3 + \left( \max_{i_4 \in \Theta} S_{i_3} + T_{i_4} \sigma_4 + (\cdots) \right) \right) \right)
\]

where \( S_{ij} = \log(s_{ij}) \) and \( T_{ij} = \log(t_{ij}) \). This expression is a piecewise linear convex function on \( \mathbb{R}^{k(k+l)} \), known as the tropicalization of the polynomial \( \phi_\sigma \). Indeed, evaluating this expression requires exactly the same operations as evaluating \( \phi_\sigma \), with the only difference that we are replacing ordinary arithmetic by the tropical semiring. The tropical semiring (also known as the max-plus algebra) consists of the real numbers \( \mathbb{R} \) together with an extra element \( \infty \), where the arithmetic operations of addition and multiplication are redefined to be \( \max \) (or equivalently \( \min \)) and \( \text{plus} \) respectively. The tropical semiring and its use in dynamic programming optimizations is explained in [44, §2.1].

Every choice of parameters \((s_{ij}, t_{ij})\) specifies a gene finding function

\[
\Omega^n \rightarrow \Theta^n, \quad \sigma \mapsto \hat{i}
\]

which takes a sequence \( \sigma \) to its explanation \( \hat{i} \). The number of all functions from \( \Omega^n \) to \( \Theta^n \) equals \( 2^n \cdot 4^n \) and hence grows double-exponentially in \( n \). However, the vast majority of these functions are not gene finding functions. The following remarkable complexity result was proved by Elizalde [24]:

**Theorem 4.** The number of gene finding functions grows at most polynomially in the sequence length \( n \).

As an illustration consider the \( n = 3 \) example visualized in Figure 2. There are \( 8^6 = 6.277 \cdot 10^{57} \) functions \( \{A, C, G, T\}^3 \rightarrow \{\text{exon}, \text{intron}\}^3 \) but only a tiny fraction of these are gene finding functions. (It would be interesting to determine the exact number). It is an open problem to give a combinatorial characterization of gene finding functions, and to come up with accurate lower and upper bounds for their number as \( n \) grows.

For gene finding HMMs, it is always the case that \( l \) is small and fixed (usually, \( l = 4 \)), and \( n \) is large. However, the size of \( k \) or structure of the state space for the hidden variables \( X_t \) tends to vary a lot. While the \( k = 2 \) used in our discussion of gene finding functions was meant to be just an
illustration, a biologically meaningful gene finding model could work with just three hidden states: one for introns, one for exons, and a state for intergenic sequence. However, in order to enforce the constraint that the sum of the lengths of the exons is 0 mod 3, a more complicated hidden state space is necessary. Solutions to this problem were given in [12, 37].

We conclude this section with a brief discussion of the important problem of estimating parameters for HMMs. Indeed, so far nothing has been said how the values of the parameters $s_{ij}$ and $t_{ij}$ are to be chosen when running the Viterbi algorithm. Typically, this choice involves a combination of biological and statistical considerations. Let us concentrate on the latter aspect.

Recall that maximum likelihood estimation is concerned with finding parameters for a statistical model which best explain the observed data. As was the case for the codon model (Section 3), the maximum likelihood estimate is an algebraic function of the data. In contrast to what we did at the end of Section 3, it is now prohibitive to locate the global maximum in the polytope $\mathcal{P}$. The expectation-maximization (EM) algorithm is a general technique used by statisticians to find local maxima of the likelihood function [44, §1.3]. For HMMs, this algorithm is also known as the Baum-Welch algorithm. It takes advantage of the recursive decomposition in (3) and it is fast (linear in $n$). The widely used book [18] provides a good introduction to the use of the Baum-Welch algorithm in training HMMs for biological sequence applications. The connection between the EM algorithm and the Baum-Welch algorithm is explained in detail in [33]. In order to understand the performance of EM or to develop more global methods [14], it would be desirable to obtain upper and lower bounds on the algebraic degree [32] of the maximum likelihood estimate.

5 Sequence Alignment

Although tools such as the hidden Markov model are important for modeling and analyzing individual genome sequences, the essence of phylogenomics lies in the power of sequence comparison. Because functional sequences tend to accumulate fewer mutations over time, it is possible, by comparing genomes, to identify and characterize such sequences much more effectively.

In this section we examine models for sequence evolution that allow for insertions, deletions and mutations in the special case of two genomes. These are known as pairwise sequence alignment models. The specific model to be
discussed here is the pair hidden Markov model. In the subsequent section we shall examine phylogenetic models for more than two DNA sequences.

We have already seen two instances of statistical models that are represented by polynomials in the model parameters (the codon model and the hidden Markov model). Models for pairwise sequence alignment are also specified by polynomials, and are in fact close relatives of hidden Markov models. What distinguishes the sequence alignment problem is an extra layer of complexity which arises from a combinatorial explosion in the number of possible alignments between sequences. Here we describe one of the simplest alignment models (for a pair of sequences), with a view towards connections with tree models and algebraic statistics.

Given two sequences \( \sigma_1 = \sigma_1^1 \sigma_2^1 \cdots \sigma_1^n \) and \( \sigma_2 = \sigma_2^1 \sigma_2^2 \cdots \sigma_2^m \) over the alphabet \( \Omega = \{ A, C, G, T \} \), an alignment is a string over the auxiliary alphabet \( \{ M, I, D \} \) such that \( \#M + \#D = n \) and \( \#M + \#I = m \). Here \( \#M, \#I, \#D \) denote the number of characters \( M, I, D \) in the word respectively. An alignment records the “edit steps” from the sequence \( \sigma_1 \) to the sequence \( \sigma_2 \), where edit operations consist of changing characters, preserving them, or inserting/deleting them. An \( I \) in the alignment string corresponds to an insertion from the first sequence to the second, a \( D \) is a deletion from the first sequence to the second, and an \( M \) is either a character change, or lack thereof. The set \( \mathcal{A}_{n,m} \) of all alignments depends only on the integers \( n \) and \( m \), and not on \( \sigma_1 \) and \( \sigma_2 \).

**Proposition 5.** The cardinality of the set \( \mathcal{A}_{n,m} \) of all alignments can be computed as the coefficient of the monomial \( x^m y^n \) in the generating function

\[
\frac{1}{1 - x - y - xy} = 1 + x + y + x^2 + 3xy + y^2 + \cdots + x^5 + 9x^4y + 25x^3y^2 + \cdots
\]

These cardinalities \( |\mathcal{A}_{n,m}| \) are known as Delannoy numbers in combinatorics [53, §6.3]. For instance, there are \( |\mathcal{A}_{2,3}| = 25 \) alignments of two sequences of length two and three. They are listed in Table 2 below.

The pair hidden Markov model is visualized graphically in Figure 3. The hidden random variables (unshaded nodes forming the Markov chain) take on the values \( M, I, D \). Depending on the state at a hidden node, either one or two characters are generated; in this way, pair hidden Markov models differ from standard hidden Markov models. The squares around the observed states (called plates) are used to indicate that the number of characters generated may vary depending on the hidden state. The number of characters
generated is a random variable, indicated by unshaded nodes within the plates (called class nodes). In pair hidden Markov models, the class nodes take on the values 0 or 1 corresponding to whether or not a character is generated. Pair hidden Markov models are therefore HMMs, where the structure of the model depends on the assignments to the hidden states. The graphical model structure of pair HMMs is explained in more detail in [2].

The next proposition gives the algebraic representation of the pair hidden Markov model. For a given alignment \( \mathbf{a} \in \mathcal{A}_{n,m} \), we denote the \( j \)th character in \( \mathbf{a} \) by \( a_j \), we write \( a[i] \) for \( \#M + \#D \) in the prefix \( a_1a_2\ldots a_i \), and we write \( a(j) \) for \( \#M + \#I \) in the prefix \( a_1a_2\ldots a_j \). Let \( \sigma^1 \) and \( \sigma^2 \) be two DNA sequences of lengths \( n, m \) respectively. Then the probability that our model generates these two sequences equals

\[
\phi_{\sigma^1, \sigma^2} = \sum_{\mathbf{a} \in \mathcal{A}_{n,m}} t_{a_1}(\sigma_{a[1]}^1; \sigma_{a(1)}^2) \cdot \prod_{i=2}^{\|\mathbf{a}\|} s_{a_{i-1}a_i} \cdot t_{a_i}(\sigma_{a[i]}^1; \sigma_{a(i)}^2), \tag{5}
\]

where the parameter \( s_{a_{i-1}a_i} \) is the transition probability from state \( a_{i-1} \) to \( a_i \), and the parameter \( t_{a_i}(\sigma_{a[i]}^1; \sigma_{a(i)}^2) \) is the output probability for a given state \( a_i \) and the indicated output characters on the strings \( \sigma^1 \) and \( \sigma^2 \).
Proposition 6. The pair hidden Markov model for sequence alignment is the image of a polynomial map $\phi : \mathbb{C}^{33} \rightarrow \mathbb{C}^{4^n-m}$. The coordinates of the map $\phi$ are the polynomials of degree $\leq 2n + 2m - 1$ which are given in (5).

We need to explain why the number of parameters in our representation of the pair hidden Markov model is 33. First, there are nine parameters

$$S = \begin{pmatrix} s_{MM} & s_{MI} & s_{MD} \\ s_{IM} & s_{II} & s_{ID} \\ s_{DM} & s_{DI} & s_{DD} \end{pmatrix}$$

which play the same role as in Section 4, namely, they represent transition probabilities in the Markov chain. There are 16 parameters $t_{M}(a,b) = t_{Mab}$ for the probability that letter $a$ in $\sigma^1$ is matched with letter $b$ in $\sigma^2$. The insertion parameters $t_{I}(a,b)$ depend only on the letter $b$, and the deletion parameters $t_{D}(a,b)$ depend only on the letter $a$, so there are only 8 of these parameters. Hence the total number of (complex) parameters is $9 + 16 + 8 = 33$. Of course, in our applications, probabilities are non-negative reals that sum to one, so we get a reduction in the number of parameters, just like in (2). In the upcoming example, which explains the algebraic representation of Proposition 6, we use the abbreviations $t_{Ib}$ and $t_{Da}$ for these parameters.

Consider two sequences $\sigma^1 = ij$ and $\sigma^2 = klm$ of length $n = 2$ and $m = 3$ over the alphabet $\Omega = \{A, C, G, T\}$. The number of alignments is $|A_{2,3}| = 25$, and they are listed in Table 2. For instance, the alignment $MIID$, here written $(i \cdots j \ klm \cdots)$, corresponds to $\begin{array}{c} i \end{array} \begin{array}{c} j \end{array} \begin{array}{c} k \end{array} \begin{array}{c} l \end{array} \begin{array}{c} m \end{array}$ in standard genomics notation.

The polynomial $\phi_{\sigma^1, \sigma^2}$ is the sum of the 25 monomials (of degree 9,7,5) in the rightmost column. Thus the pair hidden Markov model presented in Table 2 is nothing but a polynomial map

$$\phi : \mathbb{C}^{33} \rightarrow \mathbb{C}^{1024}.$$ 

Statistics is all about making inferences. We shall now explain how this is done with this model. For any fixed parameters $s\ldots$ and $t\ldots$, one wishes to determine the alignment $\hat{a} \in \mathcal{A}_{n,m}$ which indexes the term of largest numerical value among the Delannoy many terms of the polynomial $\phi_{\sigma^1, \sigma^2}$. (If there is more than one alignment with maximum value then we break ties lexicographically). We call $\hat{a}$ the explanation of the observation $(\sigma^1, \sigma^2)$.

The explanation for a pair of DNA sequences can be computed in polynomial time (in their lengths $n$ and $m$) using a variant of the Viterbi algorithm.

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| IIID | (i . j , k l m .) | \( t_{I} s_{I} I_{I} t_{I} I_{I} t_{I} I_{l} t_{I} m_{I} s_{I} t_{D} I_{D} s_{D} t_{D} t_{D} t_{D} j_{I} \) |
|------|-------------------|------------------------------------------------------------------|
| IIDD | (i . j , k l m .) | \( t_{I} s_{I} I_{I} t_{I} I_{I} t_{I} I_{l} t_{I} m_{I} s_{I} t_{D} I_{D} s_{D} t_{D} \) |
| IDDI | (i . j , k l m .) | \( t_{I} s_{I} I_{I} t_{I} I_{l} s_{I} I_{I} \) |
| IDID | (i . j , k l m .) | \( t_{I} s_{I} I_{I} t_{I} I_{l} s_{I} I_{l} s_{D} t_{D} I_{D} t_{D} s_{I} t_{D} t_{D} I_{m} \) |
| IIDD | (i . j , k l m .) | \( t_{I} s_{I} I_{I} t_{I} I_{l} t_{I} I_{l} s_{I} I_{m} \) |
| DIIH | (i . j , k l m .) | \( t_{D} s_{D} t_{I} I_{m} s_{I} I_{t} I_{l} t_{I} m_{I} t_{I} I_{l} \) |
| DIDI | (i . j , k l m .) | \( t_{D} s_{D} t_{I} I_{l} t_{I} I_{m} s_{I} t_{D} I_{D} \) |
| DIDII | (i . j , k l m .) | \( t_{D} s_{D} t_{I} t_{I} I_{m} s_{I} t_{I} I_{l} \) |
| DDII | (i . j , k l m .) | \( t_{D} s_{D} t_{I} I_{m} s_{I} t_{I} I_{l} \) |
| MID | (i . j , k l m .) | \( t_{M} s_{I} I_{I} t_{I} I_{m} s_{I} t_{I} I_{l} \) |
| MIDI | (i . j , k l m .) | \( t_{M} s_{I} I_{m} s_{D} s_{I} t_{D} I_{D} t_{I} m_{I} t_{D} j_{I} \) |
| MDII | (i . j , k l m .) | \( t_{M} s_{I} I_{m} s_{D} s_{D} t_{I} t_{I} I_{l} \) |
| IMD | (i . j , k l m .) | \( t_{M} s_{I} M_{I} s_{I} I_{m} \) |
| MIDM | (i . j , k l m .) | \( t_{M} s_{I} I_{l} t_{I} I_{m} t_{I} I_{m} s_{I} t_{M} t_{D} I_{D} j_{I} \) |
| IIDI | (i . j , k l m .) | \( t_{I} s_{I} I_{I} t_{I} I_{l} t_{I} I_{l} s_{I} t_{I} m_{I} s_{I} t_{I} t_{I} I_{m} \) |
| IIDM | (i . j , k l m .) | \( t_{I} s_{I} I_{l} t_{I} I_{l} t_{I} I_{m} \) |
| IDMI | (i . j , k l m .) | \( t_{I} s_{I} I_{l} t_{I} I_{m} s_{I} t_{I} I_{l} t_{I} j_{I} \) |
| DID | (i . j , k l m .) | \( t_{D} s_{D} t_{I} t_{I} I_{m} s_{I} t_{I} s_{I} I_{l} t_{I} m_{I} t_{D} j_{I} \) |
| IDM | (i . j , k l m .) | \( t_{I} s_{I} I_{l} t_{I} I_{m} t_{I} I_{m} t_{I} I_{l} \) |

Table 2: Alignments for a pair of sequences of length 2 and 3.
Just like in the previous section, the key idea is to tropicalize the coordinate polynomials (5) of the statistical model in question. Namely, we compute
\[
\max_{a \in A_{n,m}} T_{a_1} (\sigma_{a[1]}, \sigma_{a(1)}) + \sum_{i=2}^{n} S_{a_{i-1}a_i} + T_{a_i} (\sigma_{a[i]}, \sigma_{a(i)})\tag{6}
\]
where \(S_. = \log(s_.)\) and \(T_. = \log(t_.)\). The “arg max” of this piecewise linear convex function is the optimal alignment \(\hat{a}\). Inference in the pair HMM means computing the optimal alignment of two observed DNA sequences. In other words, by inference we mean evaluating the alignment function
\[\Omega^n \times \Omega^m \to A_{n,m}, \quad (\sigma^1, \sigma^2) \mapsto \hat{a}\.\]

There are doubly-exponentially many functions from \(\Omega^n \times \Omega^m\) to \(A_{n,m}\), but, by Elizalde’s Few Inference Functions Theorem [24], at most polynomially many of them are alignment functions. Like for gene finding functions (cf. Theorem 4), it is an open problem to characterize alignment functions.

The function \(\mathbb{R}^{33} \to \mathbb{R}\) given in (6) is the support function of a convex polytope in \(\mathbb{R}^{33}\), namely, the Newton polytope of the polynomial \(\phi_{\sigma^1, \sigma^2}\). The vertices of this polytope correspond to all optimal alignments of the sequences \(\sigma^1, \sigma^2\), with respect to all possible choices of the parameters, and the normal fan of the polytope divides the logarithmic parameter space into regions which yield the same optimal alignment. This can be used for analyzing the sensitivity of alignments to parameters, and for the computation of posterior probabilities of optimal alignments. The process of computing this polytope is called parametric alignment or parametric inference. It is known [27, 43, 58] that parametric inference can be done in polynomial time (in \(m\) and \(n\)).

An important remark is that the formulation of sequence alignment with pair Hidden Markov models is equivalent to combinatorial “scoring schemes” or “generalized edit distances” which can be used to assign weights to alignments [11]. The simplest scoring scheme consists of two parameters: a mismatch score \(mis\), and an indel score \(gap\) [29]. The weight of an alignment is the sum of the scores for all positions in the alignment, where a match gets a score of 1. In the case where \(mis\) and \(gap\) are non-negative, this is equivalent to specializing the 33 logarithmic parameters \(S_. = \log(s_.)\) and \(T_. = \log(t_.)\) of the pair hidden Markov model as follows:
\[
S_{ij} = 0, \quad T_{ij} = T_{Di} = -gap \text{ for all } i, j, \\
T_{Mij} = -1 \text{ if } i = j, \text{ and } T_{Mij} = -mis \text{ if } i \neq j.
\]
The case where the scoring scheme consists of both positive and negative parameters corresponds to a normalized pair hidden Markov model \[18\]. This specialization of the parameters corresponds to projecting the Newton polytope of \(\phi_{\sigma_1, \sigma_2}\) into two dimensions. Parametric alignment means computing the resulting two-dimensional polygon. For two sequences of length \(n\), an upper bound on the number of vertices in the polygon is \(O(n^{2/3})\). We have observed that for biological sequences the number may be much smaller. See \[27\] for a survey from the perspective of computational geometry.

In the strict technical sense, our polynomial formulation (5) is not needed to derive or analyze combinatorial algorithms for sequence alignment. However, the translation from algebraic geometry (5) to discrete optimization (6) offers much more than just aesthetically pleasing formulas. We posit that (tropical) algebraic geometry is a conceptual framework for developing new models and designing new algorithms of practical value for phylogonomics.

6 Models of Evolution

Because organisms from different species cannot produce offspring together, mutations and genome changes that occur within a species are independent of those occurring in another species. There are some exceptions to this statement, such as the known phenomenon of horizontal transfer in bacteria which results in the transfer of genetic material between different species, however we ignore such scenarios in this discussion. We can therefore represent the evolution of species (or phyla) via a tree structure. The study of tree structures in genome evolution is referred to as phylogenetics. A phylogenetic \(X\)-tree is a tree \(T\) with all internal vertices of degree at least 3, and with the leaves labeled by a set \(X\) which consists of different species. In this section, we assume that \(T\) is known and that vertices in \(T\) correspond to known speciation events. We begin by describing statistical models of evolution that are used to identify regions between genomes that are under selection.

Evolutionary models attempt to capture three important aspects of evolving sequences: branch length, substitution and mutation. Consider a single ancestral base \(b\) at the root \(r\) of a phylogenetic tree \(T\), and assume that there are no insertions or deletions over time. Since the ancestral base changes, it is possible that at two leaves \(x, y \in X\) we observe bases \(c_1 \neq c_2\). We say that there has been a substitution between \(x\) and \(y\). In a probabilistic model of evolution, we would like to capture the possibility for change along
internal edges of the tree, with the possibility of back substitutions as well. For example, it is possible that $b \rightarrow c_1 \rightarrow b \rightarrow c_1$ along the path from $r$ to $x$.

**Definition 7.** A *rate matrix* (or *Q-matrix*) is a square matrix $Q = (q_{ij})_{i,j \in \Omega}$ (with rows and columns indexed by the nucleotides) satisfying the properties

$$q_{ij} \geq 0 \quad \text{for} \quad i \neq j,$$

$$\sum_{j \in \Omega} q_{ij} = 0 \quad \text{for all} \quad i \in \Omega,$$

$$q_{ii} < 0 \quad \text{for all} \quad i \in \Omega.$$

Rate matrices capture the notion of *instantaneous rate of mutation*. From a given rate matrix $Q$ one computes the substitution matrices $P(t)$ by exponentiation. The entry of $P(t)$ in row $b$ and column $c$ equals the probability that the substitution $b \rightarrow \cdots \rightarrow c$ occurs in a time interval of length $t$. We recall the following well-known result about continuous-time Markov models.

**Proposition 8.** Let $Q$ be any rate matrix and $P(t) = e^{Qt} = \sum_{i=0}^{\infty} \frac{1}{i!} Q^i t^i$. Then

1. $P(s + t) = P(s) + P(t)$,

2. $P(t)$ is the unique solution to $P'(t) = P(t) \cdot Q, P(0) = 1$ for $t \geq 0$,

3. $P(t)$ is the unique solution to $P'(t) = Q \cdot P(t), P(0) = 1$ for $t \geq 0$.

Furthermore, a matrix $Q$ is a rate matrix if and only if the matrix $P(t) = e^{Qt}$ is a stochastic matrix (nonnegative with row sums equal to one) for every $t$.

The simplest model is the *Jukes-Cantor DNA model*, whose rate matrix is

$$Q = \begin{pmatrix}
-3\alpha & \alpha & \alpha & \alpha \\
\alpha & -3\alpha & \alpha & \alpha \\
\alpha & \alpha & -3\alpha & \alpha \\
\alpha & \alpha & \alpha & -3\alpha
\end{pmatrix},$$

where $\alpha \geq 0$ is a parameter. The corresponding substitution matrix equals

$$P(t) = \frac{1}{4} \begin{pmatrix}
1 + 3e^{-4\alpha t} & 1 - e^{-4\alpha t} & 1 - e^{-4\alpha t} & 1 - e^{-4\alpha t} \\
1 - e^{-4\alpha t} & 1 + 3e^{-4\alpha t} & 1 - e^{-4\alpha t} & 1 - e^{-4\alpha t} \\
1 - e^{-4\alpha t} & 1 - e^{-4\alpha t} & 1 + 3e^{-4\alpha t} & 1 - e^{-4\alpha t} \\
1 - e^{-4\alpha t} & 1 - e^{-4\alpha t} & 1 - e^{-4\alpha t} & 1 + 3e^{-4\alpha t}
\end{pmatrix}.$$
The expected number of substitutions over time $t$ is the quantity

$$3at = -\frac{1}{4} \cdot \text{trace}(Q) \cdot t = -\frac{1}{4} \cdot \log \det(P(t)). \quad (7)$$

This number is called the *branch length*. It can be computed from the substitution matrix $P(t)$ and is used to weight the edges in a phylogenetic $X$-tree.

One way to specify an *evolutionary model* is to give a phylogenetic $X$-tree $T$ together with a rate matrix $Q$ and an initial distribution for the root of $T$ (which we here assume to be the stationary distribution on $\Omega$). The branch lengths of the edges are unknown parameters, and the objective is to estimate these branch lengths from data. Thus if the tree $T$ has $r$ edges, then such a model has $r$ free parameters, and, according to the philosophy of algebraic statistics, we would like to regard it as an $r$-dimensional algebraic variety.

Such an algebraic representation does indeed exist. We shall explain it for the Jukes-Cantor DNA model on an $X$-tree $T$. Suppose that $T$ has $r$ edges and $|X| = n$ leaves. Let $P_i(t)$ denote the substitution matrix associated with the $i$-th edge of the tree. We write $3\alpha_i t_i = -\frac{1}{4} \log \det(P_i(t))$ for the branch length of the $i$-th edge, and we set $\pi_i = \frac{1}{4}(1 - e^{-4\alpha_i t_i})$ and $\theta_i = 1 - 3\pi_i$. Thus

$$P_i(t) = \begin{pmatrix} \theta_i & \pi_i & \pi_i & \pi_i \\ \pi_i & \theta_i & \pi_i & \pi_i \\ \pi_i & \pi_i & \theta_i & \pi_i \\ \pi_i & \pi_i & \pi_i & \theta_i \end{pmatrix}.$$ 

In algebraic geometry, we would regard $\theta_i$ and $\pi_i$ as the homogeneous coordinates of a (complex) projective line $\mathbb{P}^1$, but in phylogenomics we limit our attention to the real segment specified by $\theta_i \geq 0$, $\pi_i \geq 0$ and $\theta_i + 3\pi_i = 1$.

Let $\Delta_{4^n-1}$ denote the set of all probability distributions on $\Omega^n$. Since $\Omega^n$ has $4^n$ elements, namely the DNA sequences of length $n$, the set $\Delta_{4^n-1}$ is a simplex of dimension $4^n - 1$. We identify the $j$-th leaf of our tree $T$ with the $j$-th coordinate of a DNA sequence $(u_1, \ldots, u_n) \in \Omega^n$, and we introduce an unknown $p_{u_1 u_2 \cdots u_n}$ to represent the probability of observing the nucleotides $u_1, u_2, \ldots, u_n$ at the leaves $1, 2, \ldots, n$. The $4^n$ quantities $p_{u_1 u_2 \cdots u_n}$ are the coordinate functions on the simplex $\Delta_{4^n-1}$, or, in the setting of algebraic geometry, on the projective space $\mathbb{P}^{4^n-1}$ obtained by complexifying $\Delta_{4^n-1}$.

**Proposition 9.** In the Jukes-Cantor model on a tree $T$ with $r$ edges, the probability $p_{u_1 u_2 \cdots u_n}$ of making the observation $(u_1, u_2, \ldots, u_n) \in \Omega^n$ at the
leaves is expressed as a multilinear polynomial of degree $r$ in the model parameters $(\theta_1, \pi_1), (\theta_2, \pi_2), \ldots, (\theta_n, \pi_n)$. Equivalently, in more geometric terms, the Jukes-Cantor model on $T$ is the image of a multilinear map

$$\phi : (\mathbb{P}^1)^r \to \mathbb{P}^{4^n-1}. \quad (8)$$

The coordinates of the map $\phi$ are easily derived from the assumption that the substitution processes along different edges of $T$ are independent. It turns out that the $4^n$ coordinates of $\phi$ are not all distinct. To see this, we work out the formulas explicitly for a very simple tree with three leaves.

**Example 10.** Let $n = r = 3$, and let $T$ be the tree with three leaves, labeled by $X = \{1, 2, 3\}$, directly branching off the root of $T$. We consider the Jukes-Cantor DNA model with uniform root distribution on $T$. This model is a three-dimensional algebraic variety, given as the image of a trilinear map

$$\phi : \mathbb{P}^1 \times \mathbb{P}^1 \times \mathbb{P}^1 \to \mathbb{P}^{63}.$$  

The number of states in $\Omega^3$ is $4^3 = 64$ but there are only five distinct polynomials occurring among the coordinates of the map $\phi$. Let $p_{123}$ be the probability of observing the same letter at all three leaves, $p_{ij}$ the probability of observing the same letter at the leaves $i, j$ and a different one at the third leaf, and $p_{\text{dis}}$ the probability of seeing three distinct letters. Then

$$p_{123} = \theta_1 \theta_2 \theta_3 + 3\pi_1 \pi_2 \pi_3,$$

$$p_{\text{dis}} = 6\theta_1 \pi_2 \pi_3 + 6\pi_1 \theta_2 \pi_3 + 6\pi_1 \pi_2 \theta_3 + 6\pi_1 \pi_2 \pi_3,$$

$$p_{12} = 3\theta_1 \theta_2 \pi_3 + 3\pi_1 \pi_2 \theta_3 + 6\pi_1 \pi_2 \pi_3,$$

$$p_{13} = 3\theta_1 \pi_2 \theta_3 + 3\pi_1 \theta_2 \pi_3 + 6\pi_1 \pi_2 \pi_3,$$

$$p_{23} = 3\pi_1 \theta_2 \theta_3 + 3\theta_1 \pi_2 \pi_3 + 6\pi_1 \pi_2 \pi_3.$$

All 64 coordinates of $\phi$ are given by these five trilinear polynomials, namely,

$$p_{AAA} = p_{CCC} = p_{GGG} = p_{TTT} = \frac{1}{4} \cdot p_{123},$$

$$p_{ACG} = p_{ACT} = \cdots = p_{GTC} = \frac{1}{24} \cdot p_{\text{dis}},$$

$$p_{AAC} = p_{AAT} = \cdots = p_{TTG} = \frac{1}{12} \cdot p_{12},$$

$$p_{ACA} = p_{ATA} = \cdots = p_{TGT} = \frac{1}{12} \cdot p_{13},$$

$$p_{CAA} = p_{TAA} = \cdots = p_{GTT} = \frac{1}{12} \cdot p_{23}.$$
This means that our Jukes-Cantor model is the image of the simplified map
\[ \phi' : \mathbb{P}^1 \times \mathbb{P}^1 \times \mathbb{P}^1 \to \mathbb{P}^4, \quad ((\theta_1, \pi_1), (\theta_2, \pi_2), (\theta_3, \pi_3)) \mapsto (p_{123}, p_{dis}, p_{12}, p_{13}, p_{23}). \]

In order to characterize the image of \( \phi' \) algebraically, we perform the following linear change of coordinates:

\[
\begin{align*}
q_{111} &= p_{123} + \frac{1}{3}p_{dis} - \frac{1}{3}p_{12} - \frac{1}{3}p_{13} - \frac{1}{3}p_{23} = (\theta_1 - \pi_1)(\theta_2 - \pi_2)(\theta_3 - \pi_3) \\
q_{110} &= p_{123} - \frac{1}{3}p_{dis} + p_{12} - \frac{1}{3}p_{13} - \frac{1}{3}p_{23} = (\theta_1 - \pi_1)(\theta_2 - \pi_2)(\theta_3 + 3\pi_3) \\
q_{101} &= p_{123} - \frac{1}{3}p_{dis} - \frac{1}{3}p_{12} + p_{13} - \frac{1}{3}p_{23} = (\theta_1 - \pi_1)(\theta_2 + 3\pi_2)(\theta_3 - \pi_3) \\
q_{011} &= p_{123} - \frac{1}{3}p_{dis} - \frac{1}{3}p_{12} - \frac{1}{3}p_{13} + p_{23} = (\theta_1 + 3\pi_1)(\theta_2 - \pi_2)(\theta_3 - \pi_3) \\
q_{000} &= p_{123} + p_{dis} + p_{12} + p_{13} + p_{23} = (\theta_1 + 3\pi_1)(\theta_2 + 3\pi_2)(\theta_3 + 3\pi_3)
\end{align*}
\]

This reveals that our model is the hypersurface in \( \mathbb{P}^4 \) whose ideal equals

\[ I_T = \langle q_{000}q_{111}^2 - q_{011}q_{101}q_{110} \rangle \]

If we set \( \theta_i = 1 - 3\pi_i \) then we get the additional constraint \( q_{000} = 1 \). \( \square \)

The construction in this example generalizes to arbitrary trees \( T \). There exists a change of coordinates, simultaneously on the parameter space \( (\mathbb{P}^1)^r \) and on the probability space \( \mathbb{P}^{4n-1} \), such that the map \( \phi \) in \( [n] \) becomes a monomial map in the new coordinates. This change of coordinates is known as the Fourier transform or as the Hadamard conjugation (see \( [25, 31, 55, 56] \)).

We regard the Jukes-Cantor DNA model on a tree \( T \) with \( n \) leaves and \( r \) edges as an algebraic variety of dimension \( r \) in \( \mathbb{P}^{4n-1} \), namely, it is the image of the map \( [n] \). Its homogeneous prime ideal \( I_T \) is generated by differences of monomials \( q^a - q^b \) in the Fourier coordinates. In the phylogenetics literature (including the books \( [26, 49] \)), the polynomials in the ideal \( I_T \) are known as phylogenetic invariants of the model. The following result was shown in \( [55] \).

**Theorem 11.** The ideal \( I_T \) which defines the Jukes-Cantor model on a binary tree \( T \) is generated by monomial differences \( q^a - q^b \) of degree at most three.

It makes perfect sense to allow arbitrary distinct stochastic matrices \( P(t) \) on the edges of the tree \( T \). The resulting model is the **general Markov model** on the tree \( T \). Allman and Rhodes \( [4, 5] \) determined the complete system of phylogenetic invariants for the general Markov model on a trivalent tree \( T \).

An important problem in phylogenomics is to identify the maximum likelihood branch lengths, given a phylogenetic \( X \)-tree \( T \), a rate matrix \( Q \) and
an alignment of sequences. For the Jukes-Cantor DNA model on three taxa, described in Example [10], the exact “analytic” solution of this optimization problem leads to an algebraic equation of degree 23. See [32, §6] for details.

Let us instead consider the maximum likelihood estimation problem in the much simpler case of the Jukes-Cantor DNA model on two taxa. Here the tree $T$ has only two leaves, labeled by $X = \{1, 2\}$, directly branching off the root of $T$. The model is given by a surjective bilinear map

$$\phi : \mathbb{P}^1 \times \mathbb{P}^1 \to \mathbb{P}^1, \quad ((\theta_1, \pi_1), (\theta_2, \pi_2)) \mapsto (p_{12}, p_{\text{dis}}). \quad (9)$$

The coordinates of the map $\phi$ are

$$p_{12} = \theta_1 \theta_2 + 3 \pi_1 \pi_2,$$
$$p_{\text{dis}} = 3 \theta_1 \pi_2 + 3 \theta_2 \pi_1 + 6 \pi_1 \pi_2.$$

As before, we pass to affine coordinates by setting $\theta_i = 1 - 3 \pi_i$ for $i = 1, 2$.

One crucial difference between the model (9) and Example [10] is that the parameters in (9) are not identifiable. Indeed, the inverse image of any point in $\mathbb{P}^1$ under the map $\phi$ is a curve in $\mathbb{P}^1 \times \mathbb{P}^1$. Suppose we are given data consisting of two aligned DNA sequences of length $n$ where $k$ of the bases are different. The corresponding point in $\mathbb{P}^1$ is $u = (n - k, k)$. The inverse image of $u$ under the map $\phi$ is the curve in the affine plane with the equation

$$12n \pi_1 \pi_2 - 3n \pi_1 - 3n \pi_2 + k = 0.$$

Every point $(\pi_1, \pi_2)$ on this curve is an exact fit for the data $u = (n - k, k)$. Hence this curve equals the set of all maximum likelihood parameters for this model and the given data. We rewrite the equation of the curve as follows:

$$(1 - 4 \pi_1)(1 - 4 \pi_2) = 1 - \frac{4k}{3n}. \quad (10)$$

Recall from [7] that the branch length from the root to leaf $i$ equals

$$3 \alpha_i t_i = -\frac{1}{4} \cdot \log \det(P_i(t)) = -\frac{3}{4} \cdot \log(1 - 4 \pi_i).$$

By taking logarithms on both sides of (10), we see that the curve of all maximum likelihood parameters becomes a line in the branch length coordinates:

$$3 \alpha_1 t_1 + 3 \alpha_2 t_2 = -\frac{3}{4} \cdot \log(1 - \frac{4k}{3n}). \quad (11)$$

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The sum on the left hand side is the distance from leaf 1 to leaf 2 in the tree $T$. Our discussion of the two-taxa model leads to the following formula which is known in evolutionary biology [26] under the name Jukes-Cantor correction:

**Proposition 12.** Given an alignment of two sequences of length $n$, with $k$ differences between the bases, the ML estimate of the branch length equals

$$\delta_{12} = -\frac{3}{4} \cdot \log \left( 1 - \frac{4k}{3n} \right).$$

There has been recent progress on solving the likelihood equations exactly for small trees [15, 16, 32, 47]. We believe that these results will be useful in designing new algorithms for computing maximum likelihood branch lengths, and to better understand the mathematical properties of existing methods (such as fastDNAml [40]) which are widely used by computational biologists.

It may also be the case that $T$ is unknown, in which case the problem is not to select a point on a variety, but to select from (exponentially many) varieties. This problem is discussed in the next section.

The evolutionary models discussed above do not allow for insertion and deletion events. They also assume that sites evolve independently. Although many widely used models are based on these assumptions, biological reality calls for models that include insertion and deletion events [31], site interactions [50], and the flexibility to allow for genome dynamics such as rearrangements. Interested mathematicians will find a cornucopia of fascinating research problems arising from such more refined evolutionary models.

## 7 Phylogenetic Combinatorics

Fix a set $X$ of $n$ taxa. A **dissimilarity map** on $X$ is a function $\delta : X \times X \to \mathbb{R}$ such that $\delta(x, x) = 0$ and $\delta(x, y) = \delta(y, x)$. The set of all dissimilarity maps on $X$ is a real vector space of dimension $\binom{n}{2}$ which we identify with $\mathbb{R}^{\binom{n}{2}}$. A dissimilarity map $\delta$ is called a **metric** on $X$ if the triangle inequality holds:

$$\delta(x, z) \leq \delta(x, y) + \delta(y, z) \quad \text{for } x, y, z \in X.$$

The set of all metrics on $X$ is a full-dimensional convex polyhedral cone in $\mathbb{R}^{\binom{n}{2}}$, called the **metric cone**. Phylogenetic combinatorics is concerned with the study of certain subsets of the metric cone which are relevant for biology.
This field was pioneered in the 1980’s by Andreas Dress and his collaborators; see Dress’ 1998 ICM lecture [19] and the references given there.

Let $T$ be a phylogenetic $X$-tree whose edges have specified lengths. These lengths can be arbitrary non-negative real numbers. The tree $T$ defines a metric $\delta_T$ on $X$ as follows: $\delta_T(x, y)$ equals the sum of the lengths of the edges on the unique path in $T$ between the leaves labeled by $x$ and $y$.

The space of $X$-trees is the following subset of the metric cone:

$$\mathcal{T}_X = \{ \delta_T : T \text{ is a phylogenetic } X\text{-tree}\} \subset \mathbb{R}^{\binom{n}{2}}.$$  \hspace{1cm} (12)

Metric properties of the tree space $\mathcal{T}_X$ and its statistical and biological significance were studied by Billera, Holmes and Vogtmann [9]. The following classical Four Point Condition characterizes membership in the tree space:

**Theorem 13.** A metric $\delta$ on $X$ lies in $\mathcal{T}_X$ if and only if, for any four taxa $u, v, x, y \in X$, $\delta(u, v) + \delta(x, y) \leq \max\{\delta(u, x) + \delta(v, y), \delta(u, y) + \delta(v, x)\}$.

We refer to the book [49] for a proof of this theorem and several variants.

To understand the structure of $\mathcal{T}_X$, let us fix the combinatorial type of a trivalent tree $T$. The number of choices of such trees is the Schröder number $(2n-5)!! = 1 \cdot 3 \cdot 5 \cdot \cdots \cdot (2n-7) \cdot (2n-5).$  \hspace{1cm} (13)

Since $X$ has cardinality $n$, the tree $T$ has $2n-3$ edges, and each of these edges corresponds to a split $(A, B)$ of the set $X$ into two non-empty disjoint subsets $A$ and $B$. Let $\text{Splits}(T)$ denote the collection of all $2n-3$ splits $(A, B)$ arising from $T$.

Each split $(A, B)$ defines a split metric $\delta_{(A,B)}$ on $X$ as follows:

$$\delta_{(A,B)}(x, y) = 0 \quad \text{if} \quad (x \in A \text{ and } y \in A) \text{ or } (x \in B \text{ and } y \in B),$$

$$\delta_{(A,B)}(x, y) = 1 \quad \text{if} \quad (x \in A \text{ and } y \in B) \text{ or } (y \in A \text{ and } x \in B).$$

The vectors $\{\delta_{(A,B)} : (A, B) \in \text{Splits}(T)\}$ are linearly independent in $\mathbb{R}^{\binom{n}{2}}$. Their non-negative span is a cone $\mathcal{C}_T$ isomorphic to the orthant $\mathbb{R}_{\geq 0}^{2n-3}$.

**Proposition 14.** The space $\mathcal{T}_X$ of all $X$-trees is the union of the $(2n-5)!!$ orthants $\mathcal{C}_T$. It is hence a simplicial fan of pure dimension $2n-3$ in $\mathbb{R}^{\binom{n}{2}}$.

The tree space $\mathcal{T}_X$ can be identified combinatorially with a simplicial complex of pure dimension $2n-4$, to be denoted $\tilde{\mathcal{T}}_X$. The vertices of $\tilde{\mathcal{T}}_X$ are the $2^{n-1}-1$ splits of the set $X$. We say that two splits $(A, B)$ and $(A', B')$ are compatible if at least one of the four sets $A \cap A', A \cap B', B \cap A'$ and $B \cap B'$ is the empty set. Here is a combinatorial characterization of the tree space:
Proposition 15. A collection of splits of the set \( X \) forms a face in the simplicial complex \( \tilde{T}_X \) if and only if that collection is pairwise compatible.

The phylogenetics problem is to reconstruct a tree \( T \) from \( n \) aligned sequences. In principle, one can select from evolutionary models for all possible trees in order to find the maximum likelihood fit. Even if the maximum likelihood problem can be solved for each individual tree, this approach becomes infeasible in practice when \( n \) increases, because of the combinatorial explosion in the number \( |\tilde{T}_X| \) of trees. A number of alternative approaches have been suggested that attempt to find evolutionary models which fit summaries of the data. They build on the characterizations of trees given above.

Distance-based methods are based on the observation that trees can be encoded by metrics satisfying the Four Point Condition (Theorem 13). Starting from a multiple sequence alignment, one can produce a dissimilarity map on the set \( X \) of taxa by computing the maximum likelihood distance between every pair of taxa, using Proposition 12. The resulting dissimilarity map \( \delta \) is typically not a tree metric, i.e., it does not actually lie in the tree space \( T_X \). What needs to be done is to replace \( \delta \) by a nearby tree metric \( \delta_T \in T_X \).

The method of choice for most biologists is the neighbor-joining algorithm, which provides an easy-to-compute map from the cone of all metrics onto \( T_X \). The algorithm is based on the following “cherry-picking theorem” [46, 54]:

**Theorem 16.** Let \( \delta \) be a tree metric on \( X \). For every pair \( i, j \in X \) set

\[
Q_\delta(i, j) = (n - 2) \cdot \delta(i, j) - \sum_{k \neq i} \delta(i, k) - \sum_{k \neq j} \delta(j, k).
\]

Then the pair \( x, y \in X \) that minimizes \( Q_\delta(x, y) \) is a cherry in the tree, i.e., \( x \) and \( y \) are separated by only one internal vertex \( z \) in the tree

Neighbor-joining works as follows. Starting from an arbitrary metric \( \delta \) on \( n \) taxa, one sets up the \( n \times n \)-matrix \( Q_\delta \) whose \((i, j)\)-entry is given by the formula (14), and one identifies the minimum off-diagonal entry \( Q_\delta(x, y) \). If \( \delta \) were a tree metric then the internal vertex \( z \) which separates the leaves \( x \) and \( y \) would have the following distance from any other leaf \( k \) in the tree:

\[
\delta(z, k) = \frac{1}{2}(\delta(x, k) + \delta(y, k) - \delta(x, y)).
\]

One now removes the taxa \( x, y \) and replaces them by a new taxon \( z \) whose distance to the remaining \( n - 2 \) taxa is given by (15). This replaces the \( n \times n \) matrix \( Q_\delta \) by an \((n - 1) \times (n - 1)\) matrix, and one iterates the process.
This neighbor-joining algorithm recursively constructs a tree $T$ whose metric $\delta_T$ is reasonably close to the given metric $\delta$. If $\delta$ is a tree metric then the method is guaranteed to reconstruct the correct tree. More generally, instead of estimating pairwise distances, one can attempt to (more accurately) estimate the sum of the branch lengths of subtrees of size $m \geq 3$.

We define an $m$-dissimilarity map on $X$ to be a function $\delta : X^m \to \mathbb{R}$ such that $\delta(i_1, i_2, \ldots, i_m) = \delta(i_{\pi(1)}, i_{\pi(2)}, \ldots, i_{\pi(m)})$ for all permutations $\pi$ on $\{1, \ldots, m\}$ and $\delta(i_1, i_2, \ldots, i_m) = 0$ if the taxa $i_1, i_2, \ldots, i_m$ are not distinct. The set of all $m$-dissimilarity maps on $X$ is a real vector space of dimension $\binom{n}{m}$ which we identify with $\mathbb{R}^{\binom{n}{m}}$. Every $X$-tree $T$ gives rise to an $m$-dissimilarity map $\delta_T$ as follows. We define $\delta_T(i_1, \ldots, i_m)$ to be the sum of all branch lengths in the subtree of $T$ spanned by $i_1, \ldots, i_m \in X$.

The following theorem [17, 41] is a generalization of Theorem 16. It leads to a generalized neighbor-joining algorithm which provides a better approximation of the maximum likelihood tree and parameters:

**Theorem 17.** Let $T$ be an $X$-tree and $m < n = |X|$. For any $i, j \in X$ set

$$Q_T(i, j) = \left( \frac{n-2}{m-1} \right) \sum_{Y \in \binom{X \setminus \{i, j\}}{m-2}} \delta_T(i, j, Y) - \sum_{Y \in \binom{X \setminus \{i\}}{m-1}} \delta_T(i, Y) - \sum_{Y \in \binom{X \setminus \{j\}}{m-1}} \delta_T(j, Y).$$

Then the pair $x, y \in X$ that minimizes $Q_T(x, y)$ is a cherry in the tree $T$.

The subset of $\mathbb{R}^{\binom{n}{m}}$ consisting of all $m$-dissimilarity maps $\delta_T$ arising from trees $T$ is a polyhedral space which is the image of the tree space $\mathcal{T}_X$ under a piecewise-linear map $\mathbb{R}^{\binom{n}{2}} \to \mathbb{R}^{\binom{n}{m}}$. We do not know a simple characterization of this $m$-version of tree-space which extends the Four Point Condition.

Here is another natural generalization of the space of trees. Fix an $m$-dissimilarity map $\delta : X^m \to \mathbb{R}$ and consider any $(m - 2)$-element subset $Y \in \binom{X}{m-2}$. We get an induced dissimilarity map $\delta_Y$ on $X \setminus Y$ by setting

$$\delta_Y(i, j) = \delta(i, j, Y) \quad \text{for all } i, j \in X \setminus Y.$$  

We say that $\delta$ is an $m$-tree if $\delta_Y$ is a tree metric for all $Y \in \binom{X}{m-2}$. Thus, by Theorem [18] an $m$-dissimilarity map $\delta$ on $X$ is an $m$-tree if

$$\delta(i, j, Y) + \delta(k, l, Y) \leq \max\{\delta(i, k, Y) + \delta(j, l, Y), \delta(i, l, Y) + \delta(k, j, Y)\}$$

for all $Y \in \binom{X}{m-2}$ and all $i, j, k, l \in X \setminus Y$.  

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Let $Gr_{m,n}$ denote the subset of $\mathbb{R}^{{n \choose m}}$ consisting of all $m$-trees. The space $Gr_{m,n}$ is a polyhedral fan which is slightly larger than the tropical Grassmannian studied in [52]. For every $m$-tree $\delta \in Gr_{m,n}$ there is an $(m - 1)$-dimensional tree-like space whose “leaves” are the taxa in $X$. This is the tropical linear space defined in [51]. This construction, which is described in [52, §6] and [44, §3.5], specializes to the construction of an $X$-tree $T$ from its metric $\delta_T$ when $m = 2$. The study of $m$-trees and the tropical Grassmannian was anticipated in [19, 20]. The Dress-Wenzel theory of matroids with coefficients [20] contains our $m$-trees as a special case. The space $Gr_{m,n}$ of all $m$-trees is discussed in the context of buildings in [19]. Note that the tree space $T_X$ in (12) is precisely the tropical Grassmannian $Gr_{2,n}$.

It is an open problem to find a natural and easy-to-compute projection from $\mathbb{R}^{{n \choose m}}$ onto $Gr_{m,n}$ which generalizes the neighbor-joining method. Such a variant of neighbor-joining would be likely to have applications for more intricate biological data that are not easily explained by a tree model. We close this section by discussing an example.

**Example 18.** Fix a set of six taxa, $X = \{1, 2, 3, 4, 5, 6\}$, and let $m = 3$. The space of 3-dissimilarity maps on $X$ is identified with $\mathbb{R}^{20}$. An element $\delta \in \mathbb{R}^{20}$ is a 3-tree if $\delta/i$ is a tree metric on $X\{i\}$ for all $i$. Equivalently,

$$\delta(i, j, k) + \delta(i, l, m) \leq \max\{\delta(i, j, l) + \delta(i, k, m), \delta(i, j, m) + \delta(i, k, l)\}$$

for all $i, j, k, l, m \in X$. The set $Gr_{3,6}$ of all 3-trees is a 10-dimensional polyhedral fan. Each cone in this fan contains the 6-dimensional linear space $L$ consisting of all 3-dissimilarity maps of the particular form

$$\delta(i, j, k) = \omega_i + \omega_j + \omega_k$$

for some $\omega \in \mathbb{R}^6$.

The quotient $Gr_{3,6}/L$ is a 4-dimensional fan in the 14-dimensional real vectorspace $\mathbb{R}^{20}/L$. Let $\tilde{Gr}_{3,6}$ denote the three-dimensional polyhedral complex obtained by intersecting $Gr_{3,6}/L$ with a sphere around the origin in $\mathbb{R}^{20}/L$.

It was shown in [52, §5] that $\tilde{Gr}_{3,6}$ is a three-dimensional simplicial complex consisting of 65 vertices, 550 edges, 1395 triangles and 1035 tetrahedra. Each of the 1035 tetrahedra parameterizes six-tuples of tree metrics

$$(\delta/1, \delta/2, \delta/3, \delta/4, \delta/5, \delta/6),$$

where the tree topologies on five taxa are fixed. The homology of the tropical Grassmannian $\tilde{Gr}_{3,6}$ is concentrated in the top dimension and is free abelian:

$$H_3(\tilde{Gr}_{3,6}, \mathbb{Z}) = \mathbb{Z}^{126}.$$
If $T$ is an $X$-tree and $\delta_T$ the corresponding 3-dissimilarity map (as in Theorem 17) then it is easy to check that $\delta_T$ lies in $Gr_{3,6}$. The set of all 3-trees $\delta = \delta_T$ has codimension one in $Gr_{3,6}$. It is the intersection of $Gr_{3,6}$ with the 15-dimensional linear subspace of $\mathbb{R}^{20}$ defined by the equations

\[
\begin{align*}
\delta(123) + \delta(145) + \delta(246) + \delta(356) &= \delta(124) + \delta(135) + \delta(236) + \delta(456), \\
\delta(123) + \delta(145) + \delta(346) + \delta(256) &= \delta(134) + \delta(125) + \delta(236) + \delta(456), \\
\delta(123) + \delta(245) + \delta(146) + \delta(356) &= \delta(124) + \delta(235) + \delta(136) + \delta(456), \\
\delta(123) + \delta(345) + \delta(246) + \delta(156) &= \delta(234) + \delta(135) + \delta(126) + \delta(456), \\
\delta(123) + \delta(345) + \delta(146) + \delta(256) &= \delta(134) + \delta(235) + \delta(126) + \delta(456).
\end{align*}
\]

Working modulo $L$ and intersecting with a suitable sphere, the tree space $\tilde{T}_X$ is a two-dimensional simplicial complex, consisting of $105 = 5!!$ triangles. To be precise, the simplicial complex in Proposition 15 is the join of this triangulated surface with the 5-simplex on $X$. Theorem 17 relates to the following geometric picture: the triangulated surface $\tilde{T}_X$ sits inside the triangulated threefold $Gr_{3,6}$, namely, as the solution set of the five equations.

\[\square\]

8 Back to the Data

In Section 2, a conjecture was proposed based on our finding that the “meaning of life” sequence (1) is present (without mutations, insertions or deletions) in orthologous regions in ten vertebrate genomes. In this section we explain how the various ideas outlined throughout this paper can be used to estimate the probability that such an extraordinary degree of conservation would occur by chance. The mechanics of the calculation also provide a glimpse into the types of processing and analyses that are performed in computational biology. Two research papers dealing with this subject matter are [7, 21].

What we shall compute in this section is the probability under the Jukes-Cantor model that a single ancestral base that is not under selection (and is therefore free to mutate) is identical in the ten present day vertebrates.

Step 1 (the genomes): The National Center for Biotechnology Information (NCBI – [http://www.ncbi.nlm.nih.gov/]) maintains a public database called GENBANK which contains all publicly available genome sequences from around the world. Large sequencing centers that receive public funding are generally required to deposit raw sequences into this database.
within 24 hours of processing by sequencing machines, and thus many automatic pipelines have been set up for generating and depositing sequences. The growth in GENBANK has been spectacular. The database contained only 680,000 base pairs when it was started in 1982, and this number went up to 49 million by 1990. There are currently 44 billion base pairs of DNA in GENBANK.

The ten genomes of interest are not all complete, but are all downloadable from GENBANK, either in pieces mapped to chromosomes (e.g. for human) or as collections of subsequences called contigs (for less complete genomes).

**Step 2 (annotation):** In order to answer our question we need to know where genes are in the genomes. Some genomes have annotations that were derived experimentally, but **all** the genomes are annotated using HMMs (Section 4) shortly after the release of the sequence. These annotations are performed by centers such as at UC Santa Cruz [http://genome.ucsc.edu/](http://genome.ucsc.edu/) as well as by individual authors of programs. It remains an open problem to accurately annotate genomes. But HMM programs are quite good on average. For example, typically 98% of coding bases are predicted correctly to be in genes. On the other hand, boundaries of exons are often misannotated: current state of the art methods only achieve accuracies of about 80% [6].

**Step 3 (alignment):** We start out by performing a genome alignment. Current methods for aligning whole genomes are all based, to varying degrees, on the pair HMM ideas of Section 5. Although in practice it is not possible to align sequences containing billions or even millions of base pairs with hidden Markov models, pair HMMs are subroutines of more complex alignment strategies where smaller regions for alignment are initially identified from the entire genomes by fast string matching algorithms [10]. The ten vertebrate whole genome alignments which gave rise to Conjecture 1 are accessible at [http://bio.math.berkeley.edu/genomes/](http://bio.math.berkeley.edu/genomes/).

**Step 4 (finding neutral DNA):** In order to compute the probability that a certain subsequence is conserved between genomes, it is necessary to estimate the **neutral rate of evolution**. This is done by estimating parameters for an evolutionary model of base pairs in the genome that are not under selection, and are therefore free to mutate. Since neutral regions are difficult to identify a-priori, commonly used surrogates are synonymous substitutions in codons (Section 3). Because synonymous substitutions do not change the amino acids, it is unlikely that they are selected for or against, and various studies have shown that such data provide good estimates for neutral
mutation rates. By searching through the annotations and alignments, we identified $n = 14,202$ four-fold degenerate sites. These can be used for analyzing probabilities of neutral mutations.

**Step 5 (deriving a metric):** We would ideally like to use maximum likelihood techniques to reconstruct a tree $T$ with branch lengths from the alignments of the four-fold degenerate sites. One approach is to try to use a maximum-likelihood approach, but this is difficult to do reliably because of the complexity of the likelihood equations, even for the Jukes-Cantor models with $|X| = 10$. An alternative approach is to estimate pairwise distances between species $i, j$ using the formula in Proposition 12. The resulting metric on the set $X = \{gg, hs, mm, pt, rn, cf, dr, tn, tr, xt\}$ is given in Table 3. For example, the pairwise alignment between human and chicken (extracted from the multiple alignment) has $n = 14202$ positions, of which $k = 7132$ are different. Thus, the Jukes-Cantor distance between the genomes of human and chicken equals

$$-rac{3}{4} \cdot \log \left( 1 - \frac{4k}{3n} \right) = -\frac{3}{4} \cdot \log \left( \frac{14078}{42606} \right) = 0.830536...$$

**Step 6 (building a tree):** From the pairwise distances in Table 3 we construct a phylogenetic $X$-tree using the neighbor joining algorithm (Section 7). The tree with the inferred branch lengths is shown in Figure 4. The tree is drawn such that the branch lengths are consistent with the horizontal distances in the diagram. The root of the tree was added manually in order to properly indicate the ancestral relationships between the species.

|      | gg | hs | mm | pt | rn | cf | dr | tn | tr | xt |
|------|----|--|---|---|---|---|---|---|---|---|
| gg   | -  | 0.831 | 0.928 | 0.831 | 0.925 | 0.847 | 1.321 | 1.326 | 1.314 | 1.121 |
| hs   | -  | -  | 0.414 | 0.013 | 0.411 | 0.275 | 1.296 | 1.274 | 1.290 | 1.166 |
| mm   | -  | -  | -  | 0.413 | 0.176 | 0.441 | 1.256 | 1.233 | 1.264 | 1.218 |
| pt   | -  | -  | -  | -  | 0.411 | 0.275 | 1.291 | 1.267 | 1.288 | 1.160 |
| rn   | -  | -  | -  | -  | -  | 0.443 | 1.255 | 1.233 | 1.258 | 1.212 |
| cf   | -  | -  | -  | -  | -  | -  | 1.300 | 1.251 | 1.269 | 1.154 |
| dr   | -  | -  | -  | -  | -  | -  | -  | 1.056 | 1.067 | 1.348 |
| tn   | -  | -  | -  | -  | -  | -  | -  | -  | 0.315 | 1.456 |
| tr   | -  | -  | -  | -  | -  | -  | -  | -  | -  | 1.437 |

Table 3: Jukes-Cantor pairwise distance estimates.
At this point we wish to add a philosophical remark: The tree in Figure 4 is a point on an algebraic variety! Indeed, that variety is the Jukes-Cantor model (Proposition 9), and the preimage coordinates \((\theta_i, \pi_i)\) of that point are obtained by exponentiating the branch lengths as described in Section 6.

Step 7 (calculating the probability): We are now given a specific point on the variety representing the Jukes-Cantor model on the tree depicted in Figure 4. Recall from Proposition 9 that this variety, and hence our point, lives in a projective space of dimension \(4^{10} - 1 = 1,048,575\). What we are interested in are four specific coordinates of that point, namely, the probabilities that the same nucleotide occurs in every species:

\[
p_{\text{AAAAA}} = p_{\text{CCCCC}} = p_{\text{GGGGG}} = p_{\text{TTTTT}}
\]  

As discussed in Section 6, this expression is a multilinear polynomial in the edge parameters \((\theta_i, \phi_i)\). When we evaluate it at the parameters derived
from the branch lengths in Figure 4 we find that
\[ p_{\text{AAAAAA}} = 0.009651 \ldots \]

Returning to the "meaning of life" sequence (1), this implies the following Proposition 19.

Assuming the probability distribution on \( \Omega^{10} \) given by the Jukes-Cantor model on the tree in Figure 4, the probability of observing a sequence of length 42 unchanged at a given location in the ten vertebrate genomes within a neutrally evolving region equals \( (0.038604)^{42} = 4.3 \cdot 10^{-60} \).

This calculation did not take into account the fact that the "meaning of life" sequence may occur in an arbitrary location of the genome in question. In order to adjust for this, we can multiply the number in Proposition 19 by the length of the genomes. The human genome contains approximately 2.8 billion nucleotides, so it is reasonable to conclude that the probability of observing a sequence of length 42 unchanged somewhere in the ten vertebrate genomes is approximately
\[ 2.8 \cdot 10^9 \times 4.3 \cdot 10^{-60} \simeq 10^{-50}. \]

This probability is a very small number, i.e., it is unlikely that the remarkable properties of the sequence (1) occurred by "chance". Despite the shortcomings of the Jukes-Cantor model discussed at the end of Section 6, we believe that Proposition 19 constitutes a sound argument in support of Conjecture 1.

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