

# **Statistical Topology of Reticulate Evolution**

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Submitted in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy  
in the Graduate School of Arts and Sciences

**COLUMBIA UNIVERSITY**

2016

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# **ABSTRACT**

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This thesis contains results of applying methods from topological data analysis to various problems in genomics and evolution. It primarily details the use of persistent homology as a tool to measure the prevalence and scale of nonvertical evolutionary events, such as reassortments and recombinations. In so doing, various techniques are developed to extract statistical information from the topological complexes that are constructed.



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# Chapter 1

## Introduction

Darwin's *On the Origin of Species* contains a single figure, depicting the ancestry of species as a branching genealogical tree [12] (see Figure 1.1). Since then, the tree structure has been the dominant framework to understand, visualize, and communicate discoveries about evolution. Indeed, a primary focus of evolutionary biology has been to expand the *universal tree of life*, the set of evolutionary relationships among all extant and extinct organisms on Earth [5]. Traditionally, this was the realm of phenotype-derived taxonomies. With the advent of molecular sequence data and computational approaches for tree-inference, molecular phylogenetics has become the standard tool for inferring evolutionary relationships. However, a tree is accurate only if the Darwinian model of descent via reproduction with modification is the sole process driving evolution. It has long been recognized that there exist alternative evolutionary processes that can allow organisms to exchange genetic material through means beyond reproduction [3]. Notable examples include horizontal gene transfer in bacteria, species hybridization in plants, and meiotic recombination in eukaryotes. Collectively, these processes are known as *reticulate evolution*. These stand in contrast to descent with modification, an example of *clonal evolution*.<sup>1</sup> Increasing genomic data, powered by new high-throughput sequencing technologies, has shown that these reticulate processes are more

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<sup>1</sup>Clonal and reticulate evolution are also known by the terms *vertical* and *horizontal* evolution, respectively.

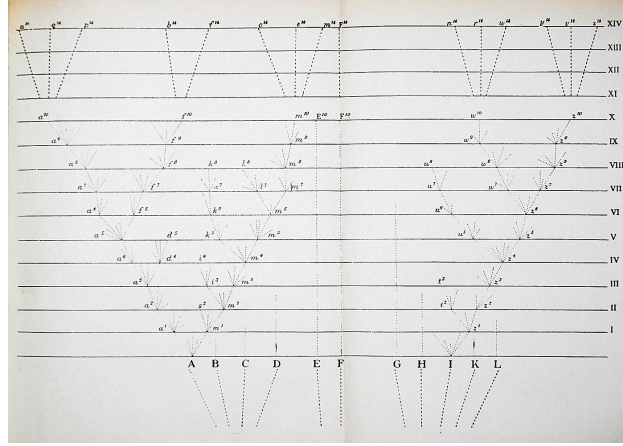


Figure 1.1: The only figure in Darwin’s *On the Origin of Species*. Darwin argued for descent with modification and natural selection as the driving processes underscoring evolution. In this figure, Darwin sketched his idea for how diverging species would result in a tree structure. Reproduced from [12].

prevalent than originally expected. For some, this has called into question the tree of life hypothesis as an organizing principle and prompted the search for new ways of representing evolutionary relationships [14, 33].

This thesis presents a new approach to quantifying and representing reticulate evolutionary processes using recently developed ideas from algebraic and computational topology. The methods we employ fall under the collective heading of *topological data analysis* (henceforth TDA), a new branch of applied topology concerned with inferring structure in high-dimensional data sets. The thesis consists of three aims: (1) introduce the methods of TDA and their application to biological data, (2) develop approaches tailored to the unique problems and assumptions inherent in genomic data, and (3) apply these approaches to a wide range of biological datasets in which reticulate processes play an important role.

In the following brief introduction, we survey salient aspects of molecular evolution, the tree paradigm, and the challenges posed by reticulate processes. We then introduce the idea of representing evolution as a topological space and give a flavor of the results to be discussed.

## 1.1 Molecular Evolution and the Tree Paradigm

The combination of Darwin’s theory of natural selection with Mendelian genetics led to the *modern evolutionary synthesis*, outlined in the first half of the twentieth century in pioneering works by Ronald Fisher, Sewall Wright, JBS Haldane, and others.<sup>2</sup> The modern synthesis was based largely on an analysis of distributions of allele frequencies in distinct populations, the purview of classical population genetics. The field was placed on a molecular foundation with Watson and Crick’s discovery of the DNA double-helix in 1953 [36]. These developments led to the establishment of *molecular evolution*, the analysis of how processes such as mutation, drift, and recombination act to induce changes in populations and species.

The information underlying an organism’s form and function is encoded in its genome, the complete sequence of DNA contained in each cell. The genome can be represented as a string of nucleotides, indexed by position. Embedded within the genome are regions defining the genes which code for functional proteins, as well as non-coding regions which have as-yet unknown function.<sup>3</sup> When an organism reproduces, either sexually or asexually, a complete copy of the genomic information is passed to the offspring. Because the molecular mechanisms that control this copying are not exact, errors in replication are introduced. These errors can take the form of single point mutations (or single nucleotide polymorphisms, SNPs) or small insertions and deletions of a few nucleotides (indels).<sup>4</sup> Under the neutral theory of evolution, the majority of these errors will have very little impact, either positive or negative, on the descendant organism. A small fraction of mutations will result in an appreciable fitness differential compared to other organisms, and it is on these organisms that natural selection will act.

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<sup>2</sup>See [26] and [24] for historical detail.

<sup>3</sup>In humans, only 1.5% of the genome is protein-coding, the rest largely non-functional. [29]. Up to 5-8% of the human genome is believed to consist of endogenous retroviruses, dead viruses which have integrated their genome into the human genome.

<sup>4</sup>Mutation rates vary across species. In humans,  $10^{-8}$  per site per generation. In single cell bacteria,  $10^{-10}$  per site per generation.

While molecular biology has largely focused on the biochemical and biophysical mechanisms underlying these processes, *molecular phylogenetics* has focused on the comparative analysis of macromolecular sequences to infer genealogical and evolutionary relationships. Molecular phylogenetics began with Emile Zuckerkandl and Linus Pauling’s recognition in the early 1960’s that the information encoded in a set of molecular sequences could itself be used as a document of evolutionary history [40, 41]. It became apparent that given two sequenced organisms, counting the differences between their respective sequences could be used as a quantitative measure of the amount of evolutionary divergence between the two. If one has a larger set of sequenced organisms, computing the complete set of pairwise distances gives a *distance matrix* for the organisms. From the distance matrix, one attempts to associate a tree to the data such that pairwise distances along the tree are close to the pairwise sequence distances. Walter Fitch and Emanuel Margolish helped popularized this approach by constructing a weighted least squares approach to fitting phylogenetic trees from distance matrices [20]. Since that time, the development of numerical approaches for inferring evolutionary relationships has evolved into a mature discipline and the use of molecular sequence data to infer phylogeny has become a standard practice across a wide range of biology and ecology. While other approaches to tree inference have been developed, including parsimony, quartet analysis, and Bayesian methods, we will focus on distance matrix methods because of their close connection with the topological ideas we employ later.

One important early result from molecular phylogenetics was Carl Woese’s organization of bacteria, eukarya, and archaea into the three domains of life [38]. Prior to Woese, there were two recognized domains of life: prokaryotes, single-celled organisms lacking a nucleus, and eukaryotes, multi-celled organisms with an enveloped nucleus. Using 16S subunit ribosomal RNA sequencing, Woese discovered that the prokaryotic domain actually split into two evolutionarily distinct groups. One of these, which he termed *archaeobacteria* was more closely related to eukaryotes than were the rest of the prokaryotes. This led to the three-domain system of life (see Figure 1.2).

# Phylogenetic Tree of Life

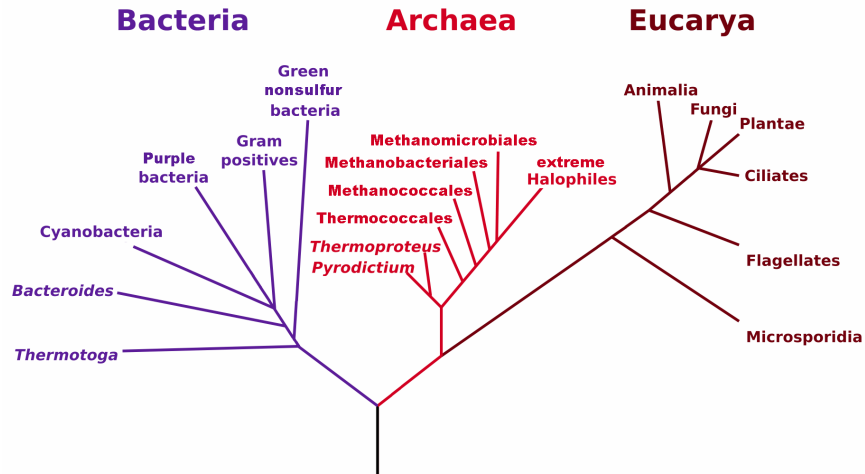


Figure 1.2: Carl Woese's three kingdom tree of life. Using 16S subunit ribosomal RNA, Carl Woese identified archaea as a distinct phylogenetic kingdom. Previously, based on morphological similarity (specifically, unicellular and lacking a nucleus), archaea had been grouped with bacteria. This result was an early success for molecular phylogenetics and the use of conserved gene segments for molecular classification. Figure adapted from [39].

This work had several important consequences. First, it established the use of molecular data to inform about large-scale patterns of evolutionary history. Using only morphological data had led to an inconsistent classification of archaea. Second, it positioned 16S rRNA profiling as the primary source of data for use in comparative genomics. The use of this genomic region was justified on the basis of being one of the few universal gene segments that is conserved across all species. Constructing a universal tree is predicated on there being orthologous genes, shared genes related through speciation events, that can provide a common foundation for comparative study. Finally, it solidified the tree paradigm as an organizing principle for relating extant species. Even though reticulate processes had been known since the nineteenth century, the idea that evolutionary relationships should be described by a bifurcating tree had been paramount since Darwin. Reticulate processes were either ignored completely, or expected to occur at such low frequencies that they need not be considered.

## 1.2 Reticulate Processes and the Universal Tree

Despite the significant impact of Woese’s observation, there remains a subtle difficulty, which Woese himself would come to contemplate in later work [37, 23]. Woese’s phylogeny was based on only 1,500 nucleotides in the ribosomal RNA, less than 1% of the length of a typical bacterial genome (see [11]). Even more striking, this accounts for less than 0.00005% of the human genome. While recent work has developed approaches for constructing reference trees from larger gene sets [9], the fact remains that the vast majority of genomic information is *not* incorporated into the tree.

The reason for this situation is twofold. First, not all genes are shared universally across all species. In constructing a phylogenetic tree using sequence data, only genes that are present across all species are informative. Second, even among universal genes, the presence of reticulate evolutionary processes will confound systematic analysis. The model of a bifurcating tree will be consistent only if all loci share the same pattern of bifurcation. When organisms exchange genetic material by means other than direct reproduction, the ancestral relationships between species will depend on which genomic regions are used. If one were to use two different genomic regions, two different tree topologies may be generated, with conflicting phylogenetic relationships. It remains an open question how to best construct a consistent evolutionary history from conflicting phylogenetic signals<sup>5</sup>

Historically, reticulate processes were believed to occur at such a low frequency that they could be safely ignored when considering evolutionary relationships. However, new genomic data has shown that, particularly in microorganisms such as bacteria and archaea, reticulate processes are much more prevalent than originally expected [32]. Incompatibilities in the tree paradigm now appear as the rule, not the exception, which has led to calls for new representations of evolutionary relationships [14, 15]. Many have argued that, in light of new genomic evidence, the very notion of a universal tree of life must be discarded [27, 28].

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<sup>5</sup>There exists a cottage industry of methods for aggregating conflicting *gene trees* into a consensus *species tree*, see [30].



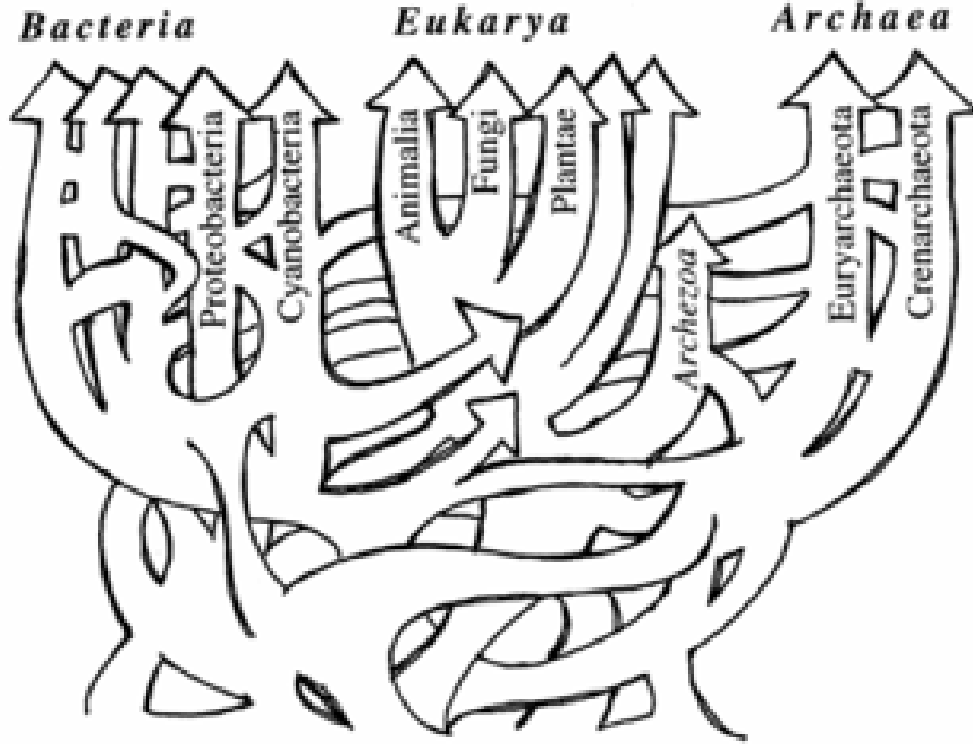


Figure 1.3: W Ford Doolittle’s representation of the universal tree of life with reticulate evolution. While the three domains of life are still recognizable, patterns of divergence no longer follow a strictly treelike model. (From *Science*, vol. 284, issue 5423, page 2127. Reprinted with permission from AAAS.)

Finally, reticulate evolutionary processes are of more than just theoretical concern. In HIV, frequent homologous recombination confounds our understanding of the epidemic’s early and present history [6]. In influenza, segmental gene reassortments lead to antigenic novelty and the emergence of epidemics [31]. In several pathogenic bacteria, including *E. coli* and *S. aureus*, horizontal gene transfer has been responsible for the spread of antibiotic resistance genes [1, 13].

### 1.3 Evolution as a Topological Space

We propose the use of new computational techniques, borrowed from the field of applied topology, to capture and represent complex patterns of reticulate evolution.

Topology as a mathematical field is concerned with properties of spaces that are invariant

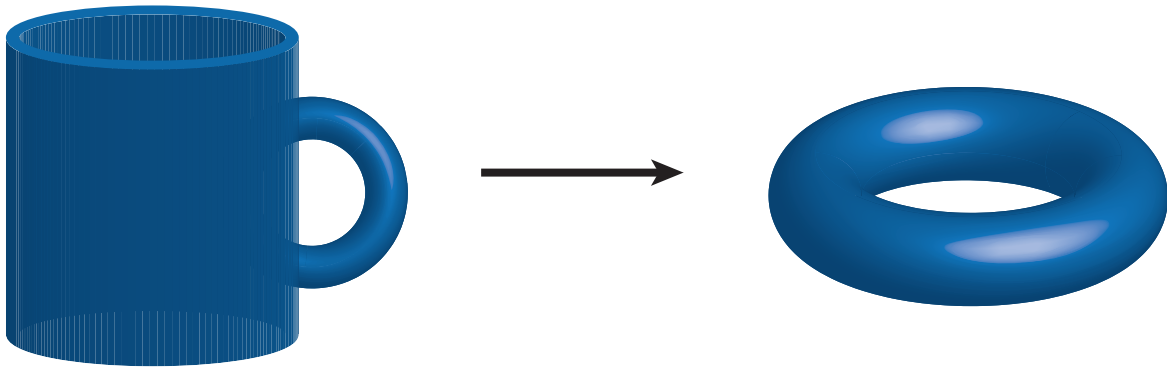


Figure 1.4: The paradigmatic example of topological equivalence. The coffee mug can be continuously deformed into the donut and are therefore topologically equivalent. Both exhibit the topology of a solid torus ( $D^2 \times S^1$ ).

under continuous deformation. Such properties can include, for example, connectedness and the presence of holes. Two objects are considered topologically equivalent if they can be deformed into one another without introducing any cuts or tears. As a paradigmatic example, consider the coffee mug and the donut (Figure 1.4). While seemingly different, it is not difficult to see that both objects consist of a single connected component that is wrapped around a single hole. Were the objects smoothly pliable they could be freely deformed into one another. Topologically, the two objects are equivalent.<sup>6</sup>

Algebraic topology quantifies our intuitive notions of shape by associating algebraic structures to different invariants. For our purposes, the most relevant invariants will be the *Betti numbers*. We give a more complete characterization of Betti numbers in Chapter 2, but the intuition is as follows. The Betti numbers are a collection of integers indexed by integer  $n$  describing the connectivity of a space at different dimensions. First, we can think of  $b_0$  as representing the number of connected components, or clusters, in our space. Next, we can

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<sup>6</sup>The two objects are topologically equivalent to a solid torus, which is represented as  $D^2 \times S^1$ , a solid two-dimensional disk wrapping around a circle.

think of  $b_1$  as representing the number of one-dimensional loops in our space. Equivalently, this is the number of cuts needed to transform the space into something simply connected. Higher Betti numbers,  $b_n$  for  $n > 1$  will correspond to higher dimensional holes. In our coffee mug example, because both objects have the same Betti numbers ( $b_0 = 1$ ,  $b_1 = 1$ , and  $b_n = 0$  for  $n > 1$ ), they can be considered topologically equivalent. Our goal in this work will be to adopt a similar perspective as this example and characterize evolutionary spaces as topological spaces using their Betti numbers.

To give the very simplest example, consider Figure 1.5. The example presents two possible scenarios describing the evolutionary relationships of three species, labeled  $a$ ,  $b$ , and  $c$ . The objects are to be read such that moving vertically corresponds to moving backwards in time. Branch lengths will correspond to some notion of evolutionary divergence. Internal vertices represent extinct ancestors of the three species, up to the root of the tree,  $r$ , which represents the most recent common ancestor. On the left, we have a simple tree topology relating the three species. Considering the shape of the tree, there is a single connected component, giving  $b_0 = 1$ . Further, we see that there are no loops formed by the branches, giving  $b_1 = 0$ . The object is trivially contractible, a property which will hold for all tree topologies. On the right, we have a reticulate topology relating the three species. We can envision species  $b$  as being the reticulate offspring of parents ancestral to species  $a$  and  $c$ . That is, species  $b$  carries unique genetic material from both species  $a$  and species  $c$ . To account for this, two branches merge into the vertex that is directly ancestral to  $b$ . Considering the shape, there is again a single connected component, giving  $b_0 = 1$ . However, because of the reticulate event mixing material from  $a$  and  $c$ , there is now a loop formed in the topology, giving  $b_1 = 1$ . The object is no longer treelike and is characterized by a nontrivial topology. The Betti numbers capture the essential difference in the two evolutionary histories.

Consider again Darwin’s branching phylogeny (Figure 1.1) and Doolittle’s modified tree accounting for reticulate evolution (Figure 1.3). The two objects can be imagined to be representations of two different topological spaces. Darwin’s branching phylogeny is a tree

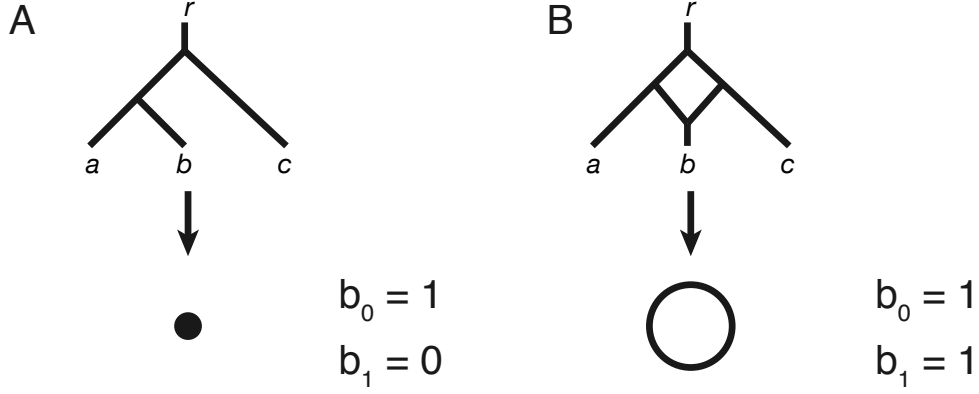


Figure 1.5: (A) A simple treelike phylogeny is contractible to a point. (B) A reticulate phylogeny that is equivalent to a circle and not contractible without a cut. The two spaces are not topologically equivalent and can be distinguished by their Betti numbers.

and hence trivially contractible ( $b_n = 0$  for  $n > 0$ ). In contrast, Doolittle's construction has a much more complex topology, with loops being formed at points where reticulate events have occurred. The object will have nonvanishing Betti numbers, which will be associated with the amount of reticulation. The remainder of this thesis focuses on expanding this idea and applying it to real data sets with the goal of measuring the prevalence and scale of reticulate evolutionary events. Our aim will be to characterize reticulate exchanges of genetic material by the parental sequences involved in the exchange, by the amount and identity of material exchanged (i.e., the genes or loci involved), and the frequency with which similar exchanges occur. Several important questions will be dealt with, such as how to construct topological spaces from finite samples, how to make comparisons among gene sets, and how to make statistical statements about reticulate events. We will address these questions, and in doing so develop new techniques to construct and extract topological and statistical information from evolutionary data. In doing so, we provide a fuller understanding of evolutionary relationships than allowed by current phylogenetic methods.

## 1.4 Thesis Organization

The remainder of this thesis is organized as follows.

In Chapter 2 we present background material on the topics discussed in this thesis. This discussion is chiefly structured into two pieces: (1) background on phylogenetics and population genetics, and (2) background on the methods we use from TDA.

In Part ??, we develop two complementary approaches for analyzing sequence data using TDA. In Chapter ??, we propose methods of constructing topological spaces that generalize standard constructions but are suited to the particular requirements of phylogenetic applications. We draw on previous work in phylogenetic networks and use homology to provide a quantitative assessment of reticulate processes. This work was published in [17]. In Chapter ??, we develop methods for performing statistical inference using summary statistics computed using methods from TDA. This is the first such use of TDA as a tool for performing parametric inference and should generalize to a wide range of application settings. This work was published in [18]

In Part ??, we apply our approach to several problems in evolution and genomics. In Chapter ?? we study bacteriophages. In Chapter ?? we study influenza. In Chapter ?? we study pathogenic bacteria and use topological techniques to represent the spread of antibiotic resistance. In Chapter ?? we study prokaryotic evolution and species tree topologies. In Chapter ?? we use population data to measure human recombination rates and identify recombination hotspots. We identify variation in recombination hotspots in different human populations. In Chapter ?? we analyze Hi-C data to explore patterns of chromatin folding in the nucleus in both prokaryotic and human datasets.

Finally, in Chapter ?? we summarize these results and present future research directions.



# Chapter 2

## Background

This thesis uses newly developed approaches from applied topology to study problems in evolutionary biology and genomics. Here we supply sufficient background to motivate our approach.

### 2.1 Biology

In this section we present a basic introduction to molecular sequence data: what the data looks like, the processes by which it is generated, and the methods by which it is analyzed. Particular attention is paid to modes of reticulate evolution. Exposition for specific applications can be found in their respective individual chapters.

#### 2.1.1 Genes and Genomes

The information required to express an organism's biological form and function is contained in the genome. Physically, the genome is manifest as a sequence of nucleotides (DNA), at least one copy of which is packaged inside each cell of an organism. Abstractly, the genome is represented as a linear sequence of characters defined over the alphabet  $\{A, C, G, T\}$ .<sup>1</sup>

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<sup>1</sup>The linear representation can be misleading, as many organisms, primarily viruses and bacteria, have circular genomes.

Contained in this sequence are subsequences representing genes, which code for the protein products that ultimately affect function. Further embedded in the genome is a complex regulatory pattern of transcription factors controlling the expression of particular genes and directing cellular differentiation and development.

Following the central dogma of biology, DNA is transcribed into RNA, RNA is translated into amino acids, and amino acids are folded into proteins [10]. Proteins comprise the functional unit of biology.

Beyond simply coding for function, the genome includes an imprint of the evolutionary history that gave rise to the organism. By comparing the genomes of multiple organisms, inferences can be drawn about the evolutionary relationships among extant organisms as well as the processes that generated observed diversity. The field concerned with exploring these relationships is *comparative genomics*.

## 2.1.2 Evolutionary Processes

Evolution describes the gradual change in phenotypes arising from random variation and subject to natural selection. The processes giving rise to diversity can be classified into two types: clonal and reticulate.

### 2.1.2.1 Clonal Evolution

Clonal evolution, or vertical evolution, is a process of self-reproduction whereby genetic material is transferred directly from parent to offspring. Population diversity is generated by stochastic mutation and maintained over multiple generations by random drift.

It is clonal evolution that Darwin had in mind when he described the idea of descent with modification, whereby a parent passes genomic information to an offspring subject to random drift. Importantly, because there is always a direct parent–offspring relationship, clonal evolution will be consistent with a phylogenetic tree model.



### 2.1.2.2 Reticulate Evolution

Reticulate evolution, or horizontal evolution, refers to exchange or acquisition of genetic material via processes that do not reflect a direct parent–offspring relationship. As we will see, these processes can make inferences about historical evolutionary relationships difficult. Different types reticulate processes occur in different types of organisms (summarized in Table 2.1.2.2).

Viruses replicate by infecting a host cell and then using the host cell machinery and resources to produce multiple copies of viral genetic material. The genetic material is then packaged into new virus particles which are shed off in order to infect new cells. Reticulation can occur when two virus particles coinfect the same host cell. During the replication process, genetic material can be exchanged in one of two ways: *reassortment* or *recombination* (the two processes are contrasted in Figure 2.1). Reassortment occurs in viruses whose genomes are segmented, such as influenza. Segments are similar to chromosomes, such that a single virus particle will contain a single copy of each segment. Reassortment occurs when coinfection results in packaging of segments taken from different virus particles. The result viral progeny will then be a genetic mixture of segments from each parental strain. Recombination, more common in nonsegmented viruses such as HIV, involves a break-rejoin mechanism during the replication process. Here, an error in the polymerase during replication can result in an incomplete copy of the genome (a break). At this point, several cellular processes involved in repair can be recruited to complete the replication process using a homologous region. If coinfection has occurred, it is possible for these processes to initiate repair using material from a different parental strain. The outcome will be novel genetic material that includes a crossover from one strain to another. Break-rejoin crossover is a type of *homologous recombination*.

In bacteria and other prokaryotes, reticulate evolution can occur when foreign DNA from a donor is acquired by a target organism and integrated into its genome. Three generic mechanisms have been identified, depending on the route by which foreign DNA is acquired

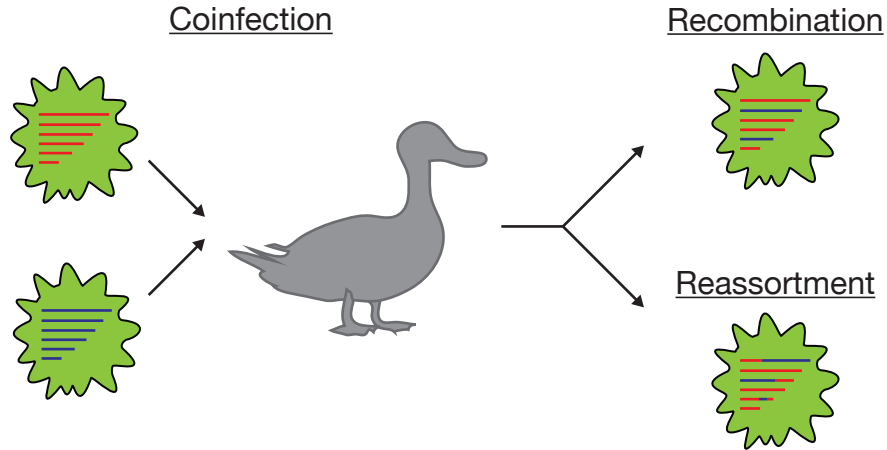


Figure 2.1: The two modes of viral reticulation. Coinfection of the same host cell can lead to either reassortment, in which whole viral segments are exchanged, or recombination, in which breakpoints can occur within segments. The former process is common in influenza, the latter in HIV. The end result, however, is a novel virus particle which shares genetic information from both parents.

[32]:

1. *Conjugation*. Direct cell-to-cell contact between donor and recipient resulting in transfer of plasmid.
2. *Transformation*. Foreign DNA acquired via uptake from freely circulating DNA in the environment.
3. *Transduction*. Virus-mediated transfer for foreign DNA from an infected donor cell.

A visualization of these three mechanisms is shown in Figure 2.2. Because these mechanisms can often lead to the acquisition of novel sequences coding for genes not in the recipient organism, reticulate evolution in prokaryotes is often called *horizontal gene transfer* or *lateral gene transfer*.

In eukaryotes, several reticulate processes have been identified. We mention two such processes: hybrid speciation and meiotic recombination. These two processes act at very different scales, but the outcome is the same: a unique offspring with genetic material drawing from both parents.

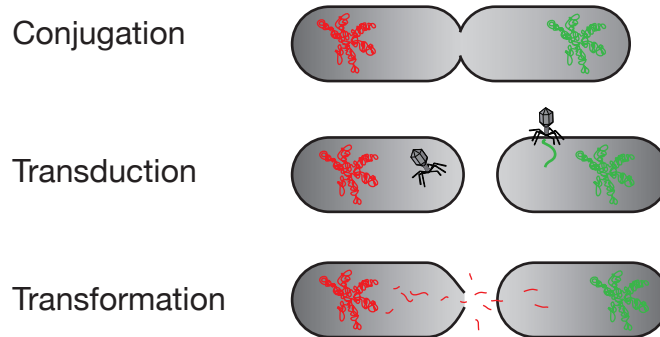


Figure 2.2: Three modes of viral reticulation. (1) Conjugation, in which direct cell-to-cell contact results in transfer of genetic material; (2) Transformation, in which foreign DNA is acquired via uptake from freely circulating DNA in the environment; and (3) Transduction, in which exchange of genetic material is mediated by a virus or phage particle.

First, hybrid speciation refers to the cross-breeding of animals or plants of different species. This mixing of genetic material can lead to the development of a third species with a phenotype distinct from both parents. Hybrid speciation was originally believed to be a rare occurrence in nature and hybrid offspring to be infertile. However, recent genomic data has demonstrated that hybrid speciation occurs quite frequently in plants [2, 3]. Indeed, Mendel’s early experiments in hybridization were themselves an artificially induced form of reticulate evolution.

Second, meiotic recombination refers to a specialized process for generating diversity that occurs in sexually-reproducing polyploid organisms, such as humans, during meiosis. Meiosis is the process by which a single cell containing  $n$  copies of each chromosome results in four distinct cells each with  $n/2$  copies of each chromosome. These special cells are called gametes. Sexual reproduction consists of the fusion of two gametes during fertilization to form a zygote, which ultimately develops into a viable offspring. Meiosis is a multistep process consisting of an initial round of DNA replication followed by two rounds of cell division. Meiotic recombination occurs after the initial round of DNA replication and prior to cell division. After DNA replication, there are two copies of each homologous chromosome that are joined at a centromere. The two sets of chromosomes then pair with each other and

exchange DNA through physical interactions known as crossovers.<sup>2</sup> This is another example of homologous recombination and results in new allelic patterns mixing genetic information from both parents.<sup>3</sup> After crossover occurs, two phases of cellular division result in gametes with  $n/2$  copies of each chromosome.

At this point, one might wonder about sexual reproduction – an offspring can be seen as a hybridization of genetic material donated from both mother and father. On the one hand, the answer could be yes, particularly because the presence of meiotic recombination involves a shuffling of genomic material such that the chromosome each parent donates is a unique combination of alleles not previously present in the donor organism. On the other hand, the answer could be no, because both mother and father donate a complete copy of the genome to the offspring. Each copy can be considered as an independent transfer of genetic information defining both a matrilineal and a patrilineal line of inheritance. Indeed, researchers in human population genetics generally distinguish between these two cases – looking at genomic regions that do not recombine they define a matrilineal common ancestor known as *Mitochondrial Eve* and a patrilineal common ancestor known as *Y-chromosomal Adam*. Mathematically, the evolutionary relationships of a population of  $N$  organisms with ploidy  $n$  are often analyzed as a haploid population of size  $nN$  with random mating.

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<sup>2</sup>These crossovers have been shown to not occur randomly, but rather at recombination hotspots regulated by binding motifs for by the PRDM9 protein. [Cite Pablo Paper and a Molly paper.](#)

<sup>3</sup>Patterns of shared alleles define the concept of *linkage*.

Organism	Process	Description
Virus	Reassortment	Exchange of discrete genomic segments
	Recombination	Intragenomic homologous crossover
Bacteria	Transformation	Acquisition of foreign DNA in environment
	Transduction	Viral-mediated exchange
	Conjugation	Cell-to-cell contact and exchange
Eukaryotes	Meiotic Recombination	Homologous crossover during meiosis
	Hybrid Speciation	Fertilization across species boundaries

The presence of reticulate processes in a set of organisms can be most clearly identified by comparing phylogenetic relationships built from different genomic segments. A general practice is to construct the set of *gene trees* which reflect ancestral branching patterns at specific loci. If a reticulate event has occurred, it implies that the branching patterns of different genes will not agree. A subfield of comparative genomics is concerned with building *species trees* from sets of gene trees.

However, in the case where there is substantial disagreement among gene trees, the very notion of a species tree may be flawed. Traditionally, evolutionary biology has concerned itself with characterizing relationships in light of vertical evolution alone. However, increasing evidence ([what evidence?](#)) has pointed to the important role played by horizontal evolution, particularly in prokaryotic evolution. [\[Further citations and exposition of Doolittle, Koonin, and Gogarten.\]](#)

### 2.1.3 Mathematical Models of Evolution

Mathematical population genetics is concerned with properties of populations as they are subject to evolutionary forces over long time scales. These forces include natural selection, genetic drift, mutation, and recombination. Historically the input data for population ge-

netics models was comparative studies of allele frequencies across populations. These studies have primarily been replaced by large-scale genomic surveys which have provided unprecedented insight into ancient population structure and historical migrations.

These models allow two things: 1) simulate genomic data under realistic processes and 2) build statistical models to estimate biological parameters from real data.

### 2.1.3.1 The Wright-Fisher Model

The Wright-Fisher model is a forward time simulation of an evolving population. In the simplest case, the model describes neutral evolution of a constant population size with no structure and constant genome length. The model proceeds in units of generations. At each generation, a member of the population is an offspring of a randomly selected ancestor from the previous generation. This offspring inherits its ancestors genomes, with mutations introduced at some base rate  $\mu$ . A member of previous generation with no offspring will be considered extinct.

### 2.1.3.2 The Coalescent Process

The coalescent process is a stochastic model that generates the genealogy of individuals sampled from an evolving population [35]. The genealogy is then used to simulate the genetic sequences of the sample. This model is essential to many methods commonly used in population genetics. Starting with a present-day sample of  $n$  individuals, each individual's lineage is traced backward in time, towards a mutual common ancestor. Two separate lineages collapse via a coalescence event, representing the sharing of an ancestor by the two lineages. The stochastic process ends when all lineages of all sampled individuals collapse into a single common ancestor. In this process, if the total (diploid) population size  $N$  is sufficiently large, then the expected time before a coalescence event, in units of  $2N$  generations, is approximately exponentially distributed:

$$P(T_k = t) \approx \binom{k}{2} e^{-\binom{k}{2}t}, \quad (2.1)$$

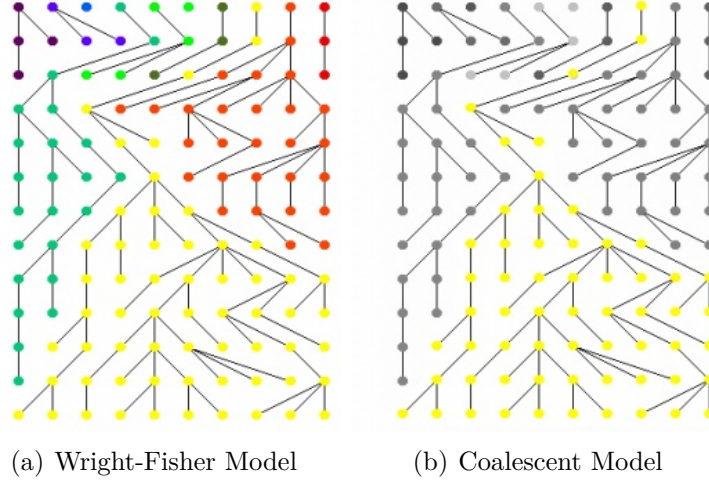


Figure 2.3: Two models for simulating evolutionary data.

where  $T_k$  is the time that it takes for  $k$  individual lineages to collapse into  $k - 1$  lineages.

After generating a genealogy, the genetic sequences of the sample can be simulated by placing mutations on the individual branches of the lineage. The number of mutations on each branch is Poisson-distributed with mean  $\theta t/2$ , where  $t$  is the branch length and  $\theta$  is the population-scaled mutation rate. In this model, the average *genetic distance* between any two sampled individuals, defined by the number of mutations separating them, is  $\theta$ .

The coalescent with recombination is an extension of this model that allows different genetic loci to have different genealogies. Looking backward in time, recombination is modeled as a splitting event, occurring at a rate determined by population-scaled recombination rate  $\rho$ , such that an individual has a different ancestor at different loci. Evolutionary histories are no longer represented by a tree, but rather by an *ancestral recombination graph*. Recombination is the component of the model generating nontrivial topology by introducing deviations from a contractible tree structure, and is the component which we would like to quantify. Coalescent simulations were performed using `ms` [25].

### 2.1.3.3 Metrics on Sequences

Evolutionary models require a notion of genetic divergence between sequences. This leads to a discussion of the types of metrics that can be put on sets of sequences.<sup>4</sup>

The simplest model, and the one most commonly adopted in this thesis, is the Hamming metric, which simply counts the proportion of sites that differ between two aligned sequences. For example, for two sequence  $s_1 = ACTTGAC$  and  $s_2 = AAGTGGC$ ,  $d_H(s_1, s_2) = 3/7$ . In general, the Hamming metric will underestimate divergences by not accounting for the possibility of back mutations.<sup>5</sup>

More biologically motivated models will introduce corrections to account for assumptions about how sequences evolve. These assumptions include the base frequency of each nucleotide as well as the substitution rates for each type of mutation. The simplest of these models is the *Jukes-Cantor model*. This model defines an equal substitution rate  $\mu$ . Inverting the probability of an alteration gives the divergence. The Jukes-Cantor metric is defined as

$$d_{JC} = -\frac{3}{4} \ln(1 - \frac{4}{3}p), \quad (2.2)$$

where  $p$  is the proportion of sites that are different.

### 2.1.4 Phylogenetic Methods

A phylogenetic tree is a binary tree in which leaves are associated with particular species or taxa, and the branching pattern of the tree reflects diverging evolutionary relationships. Branch lengths on the tree are associated with evolutionary divergence between sets of taxa. Molecular phylogenetics refers to a large collection of methods for inferring branching pat-

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<sup>4</sup>Before sequence can be compared, they must first be *aligned*. A sequence alignment arranges the characters in a set of sequences into columns such that characters sharing evolutionary identity are in the same column. Alignment is necessary because random insertions and deletions can change . The difficulty of performing an alignment will largely depend on the amount of evolutionary divergence in the set of sequences under consideration. Sequence alignment is a well studied topic but largely beyond the scope of this thesis, where we assume sufficient sequence similarity such that alignment can be performed with high confidence.

<sup>5</sup>A double mutation of the form  $A \rightarrow C \rightarrow A$ .



terns from aligned molecular sequence data.<sup>6</sup> In general, the problem of finding an optimal tree associated with sequence data is NP-complete [21], however several approximate methods have been developed. The primary types of methods include maximum parsimony, distance-matrix methods, maximum likelihood (ML), and Bayesian inference. Maximum parsimony attempts to find the phylogenetic tree that minimizes the number of evolutionary changes required to explain the observed sequences. Distance-matrix methods first compute a matrix of pairwise distances between taxa and then find the tree that best approximates these distances. ML and Bayesian methods use specific models of evolution to assign probability distributions over trees. In this work we concentrate on distance-matrix methods because of their close connection with the finite metric spaces considered in applied topology.

#### 2.1.4.1 Distance-Matrix Methods

Given a set of aligned molecular sequences, distance-matrix methods first compute the pairwise matrix of genetic distances using one of the metrics as described in Section 2.1.3.3. Then, the binary tree that best approximates those distances is iteratively fit to this data. This approach to phylogenetic inference were introduced by Cavalli-Sforza and Edwards in 1967 [8] and Fitch and Margoliash in 1967 [20]. The Fitch-Margoliash method uses a weighted least squares approach to tree-fitting, such that larger distances are weighted less, due to higher chances for random error. Distance-matrix methods are popular for their high speed and scalability as well as high accuracy in most cases.

Currently, the most widely implemented distance-matrix method is neighbor-joining.<sup>7</sup> One particular reason neighbor-joining is popular is that under certain conditions, discussed below, it has been shown to exactly recover the correct tree. The neighbor-joining algorithm is described in Algorithm 1.

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<sup>6</sup>See Felsenstein, *Inferring Phylogenies* for a readable and thorough introduction to the field [19].

<sup>7</sup>Neighbor joining was introduced by Saitou and Nei in 1987 [34].

**Data:**  $n \times n$  distance matrix  $D$   
**Result:** Phylogenetic tree on  $n$  leaves  
**while** *blah* **do**  
    | Compute  $Q$  matrix;  
**end**

**Algorithm 1:** The Neighbor Joining Algorithm. Adapted from Wikipedia entry on Neighbor-Joining.

#### 2.1.4.2 Additive Metrics and the Four Point Condition

Arbitrary distance matrices are unlikely to admit a tree representation. Those that do are called *additive metrics*, because they can be represented as an additive tree. Additivity is the property that the distance between any two nodes will be equal to the sum of the branch lengths between them. A distance matrix admits a tree representation if and only if it is additive.

There is a straight-forward condition that must be satisfied for additivity, known as the *four point condition*. For a distance matrix to admit a tree representation,

$$d_{ij} + d_{kl} \leq \max\{d_{ik} + d_{jl}, d_{il} + d_{jk}\} \quad (2.3)$$

for any four nodes  $\{i, j, k, l\}$ . The condition implies that there is a labeling on the four nodes such that

$$d_{ij} + d_{kl} \leq d_{ik} + d_{jl} = d_{il} + d_{jk}. \quad (2.4)$$

A visual interpretation of this condition is shown in Figure 2.4.

Sequence data can fail to be additive for several reasons. First, sequencing error. Errors can introduce noise into the measured genetic distances. Second, homoplasy. A homoplasy occurs when the same mutation is introduced multiple times in a set of organisms. The presence of homoplasy will underestimate genetic distance between taxa. Third, reticulate evolution. As described previously, in cases of reticulate evolution no tree will accurately describe the observed data.

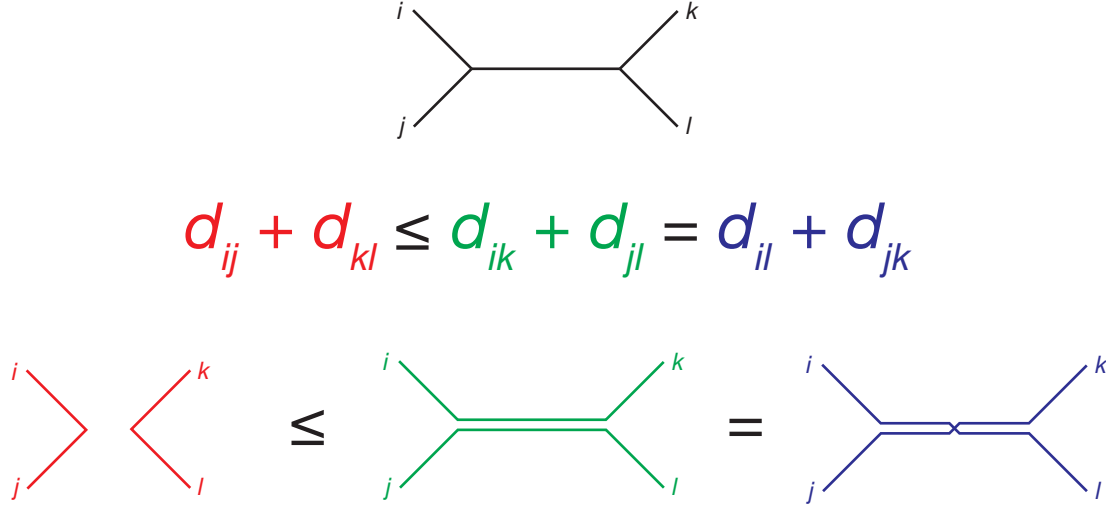


Figure 2.4: A visual interpretation of the four point condition for additivity. For any four leaves, there exists a labeling  $\{i, j, k, l\}$  such that  $d_{ij} + d_{kl} \leq d_{ik} + d_{jl} = d_{il} + d_{jk}$ . Of the three possible ways of arranging the sums of distances, two will involve traversing the internal branch, while one will involve only external branches.

### 2.1.4.3 Number of Tree Topologies

The number of unrooted bifurcating tree topologies with  $L$  leaves is  $(2L - 5)!!$ .<sup>8</sup> This can be easily shown using induction. For  $L = 3$ , we have  $\mathcal{T}(3) = 1$  and 3 branches. To pass to  $L = 4$ , we can add the fourth leaf to any of the 3 branches, resulting in 3 different topologies. For  $L = 4$ , we have  $\mathcal{T}(4) = 3$ . Every time we add a leaf, we add two branches – one external and one internal. For  $L = n$ , we have  $\mathcal{T}(n) = (2n - 5)!!$  and  $2n - 3$  branches. For  $L = n + 1$ , we can add the new external branch to any of the current  $2n - 3$  branches. A rooted tree with  $L$  leaves can be considered as an unrooted tree with  $L + 1$  leaves. Therefore, the number of rooted bifurcating tree topologies with  $L$  leaves is  $(2L - 3)!!$ . As can be seen, the number of tree topologies explodes with the number of leaves.<sup>9</sup>

<sup>8</sup>The double factorial is defined as  $n!! = n(n - 2)(n - 4) \cdots$ .

<sup>9</sup>It was observed by Walter Fitch that for more than 20 species there are more than Avogadro's number of topologies.

#### 2.1.4.4 Space of Phylogenetic Trees (Needs Expansion)

A phylogenetic tree is characterized by its number of leaves, the particular tree topology, and the length of each branch. Tree space refers to an abstract construction that represents each possible tree as a point in a geometric space. Abstract studies of tree space were initiated by Billera, Holmes, and Vogtmann (BHV) in [4].

In the BHV model, each point represents an unrooted binary tree with  $L$  leaves and strictly positive branch lengths. Number of interior edges  $r = L - 3$ , a particular additive tree can be plotted as a point in the positive open orthant  $(0, \infty)^r$ . A single orthant corresponds to a single tree topology.

The tree space construction allows one to define statistics, such as means and variance, on sets of trees. This has been utilized

#### 2.1.4.5 Phylogenetic Networks

There are several existing methods for representing reticulate evolution. Most of these methods generalize phylogenetic trees into *phylogenetic networks*, which attempt to reconcile the presence of horizontal evolution in sequence data. However, most simply present corrections to phylogenetic trees, which can fail in cases where horizontal evolution is pervasive, as in many prokaryote datasets. Additionally, the resulting networks can be complex and difficult to interpret quantitatively. This can make it difficult to distinguish between phylogenetic incompatibilities due to noisy sampling and due to true reticulations. An example of a phylogenetic network using the split network approach is shown in Figure 2.6.

[Expand. See Morrison review..]

## 2.2 Topological Data Analysis

Topology is the branch of mathematics that formalizes our intuitive notions of shape. More concretely, topology provides the methods to characterize the properties of objects and spaces

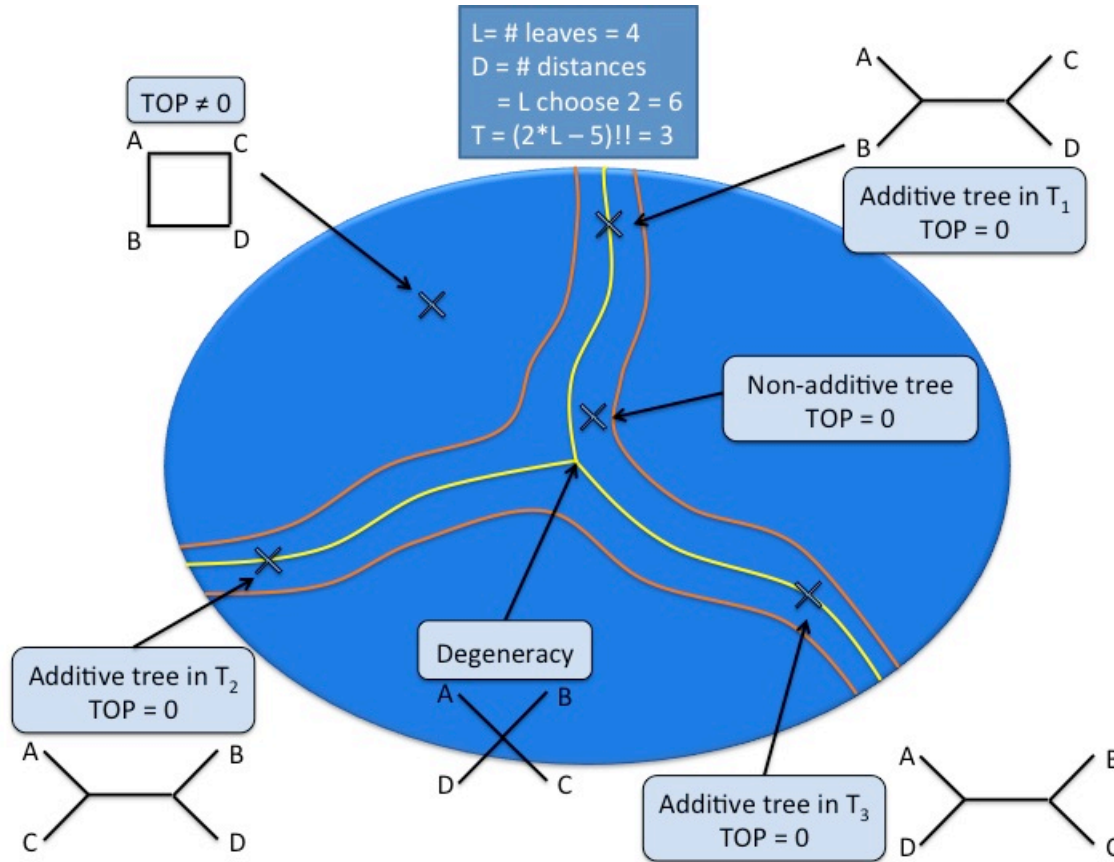


Figure 2.5: The construction of Tree Space. Each point in this space represented a particular unrooted tree topology with branch lengths.

that remain invariant under continuous deformation. For example, squeezing a circle into an ellipse by compressing along one axis does not change the fact that the object encloses a single loop. Or, as we saw in the introduction, the coffee mug can be continuously deformed into the donut. Likewise, if we take a tree and change the lengths of its branches, the tree remains a tree.<sup>10</sup> In each of these examples, while the deformation has substantially altered *local* properties of the space, on a *global* level certain essential characteristics have remained unchanged. From the perspective of topology, the spaces are considered identical. The question then is how to formalize our idea of global shape in order to systematically reason about it.

<sup>10</sup>It is important to draw a distinction between the notion of tree topology, in which the branch patterns determines the topology, and global topology, in which all trees are equivalent. While the former is more common in the phylogenetics community, here we consider the latter.

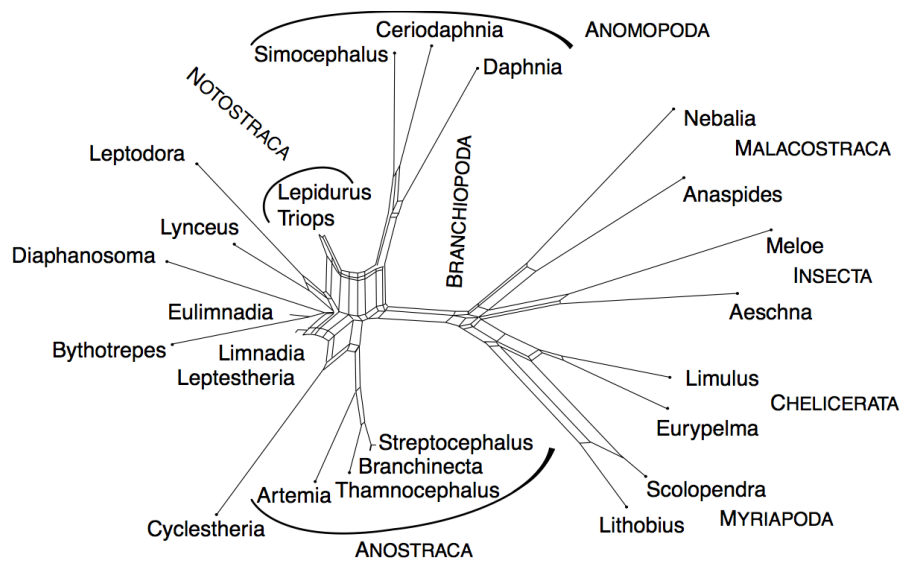


Figure 2.6: Example of a split network of genus Branchiopoda and outgroups. Computed using the Neighbor-Net algorithm. Phylogenetic incompatibilities are represented by conflicting splits. reprinted from BMC Evolutionary Biology 7:147 (2007).

Algebraic topology solves this problem by associating algebraic objects (an integer, for instance) that do not change under continuous deformation. These objects may capture properties like the number of connected components, the number of loops, and the number of holes in an object, and represent *topological invariants* of a space. Two spaces can only be deformed into one other if they share the same invariants. The circle and ellipse are identified as equivalent by the presence of one loop. Neither can be deformed into a tree without introducing a cut, which would be a type of discontinuous deformation. Using these invariants, powerful ideas from abstract algebra can then be used to manipulate and reason about shape.

While topology has traditionally developed through the study of abstract spaces, leading to very rich and beautiful constructions<sup>11</sup>, real data does not come in the form of perfect continuous spaces. Recent effort over the past 15 years has focused on developing methods to apply topology to real world problems in science and engineering. This work, collectively falling under the heading of *topological data analysis* (TDA), has focused on efficient algorithms for computing topological invariants from finite, noisy data. TDA now encompasses a range of efforts and can now be considered a branch of applied mathematics in its own right. It has emerged from substantial interdisciplinary effort between mathematicians, computer scientists, and domain experts.

In practice, a typical workflow for applying TDA to real data is as follows. Data comes in the form of a set of  $n$  observations with  $p$  attributes, where  $p$  is often very large. The data is assumed to be a finite sample from some more complex space, from which we wish to infer either some sort of global structure or underlying model. The data is represented as a finite point cloud: a set of  $n$  points in  $p$  dimensions with an associated notion of distance. The point cloud is then transformed into a topological space by associating different sets of points with each other. The associations can be constructed in different ways – for instance, one of the most common constructions associates points within a certain distance  $d$  from

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<sup>11</sup>For example, see the work of Thurstan on low-dimensional topology

one another. Computational approaches are then used to measure informative topological properties from the space.

In this thesis, we use methods from TDA to study problems in evolutionary biology and genomics. Our data is typically aligned genomic sequences from sets of related organisms. If our sequences are each of length  $l$ , then we can imagine our data as points in an  $l$ -dimensional sequence space. A sequence metric, such as the Hamming metric, measures distance.

The two main methods from TDA that we employ are *persistent homology* and *mapper*. Persistent homology provides a way to efficiently compute the topological invariants of a space across multiple scales, while mapper provides an approach for a condensed representation and visualization. In this section, we provide an overview and discussion of these two methods from the perspective of an end-user. We treat each method as a pipeline for transforming from raw data to a topological summary. While the mathematical literature on these methods is extremely deep, our goal is to explain things in sufficient detail for a wide audience to grasp the main ideas. As such, we will define technical terms where necessary, but focus primarily on developing a working understanding of the main aspects of each method as they are used in practice. The primary concept we require is *homology*, a particular way in which topological invariants can be assigned to spaces. We include a more thorough review of the requisite mathematical concepts leading to a definition of simplicial homology in Appendix XX.

The following sections draw on several excellent reviews of TDA, including [7], [16], and [22].

### 2.2.1 Persistent Homology

## 2.3 Applying TDA to Molecular Sequence Data

Aligned genomic sequences can be naturally viewed as points in a high-dimensional sequence space.



Table 2.1: Dictionary connecting algebraic topology and evolutionary biology

Algebraic Topology	Evolutionary Biology
Filtration value $\epsilon$	Genetic distance (evolutionary scale)
0-dimensional Betti number at filtration value $\epsilon$	Number of clusters at scale $\epsilon$
Generators of 0-D homology	A representative element of the cluster
Hierarchical relationship among generators of 0-D homology	Hierarchical clustering
1-D Betti number	Lower bound on number of reticulate events
Generators of 1-D Homology	Reticulate events
Generators of 2-D Homology	Complex horizontal genomic exchange
Non-zero high-dimensional homology (topological obstruction to phylogeny)	No treelike phylogenetic representation exists
Number of higher-dimensional generators over a time interval (irreducible cycle rate)	Lower bound on recombination/reassortment rate

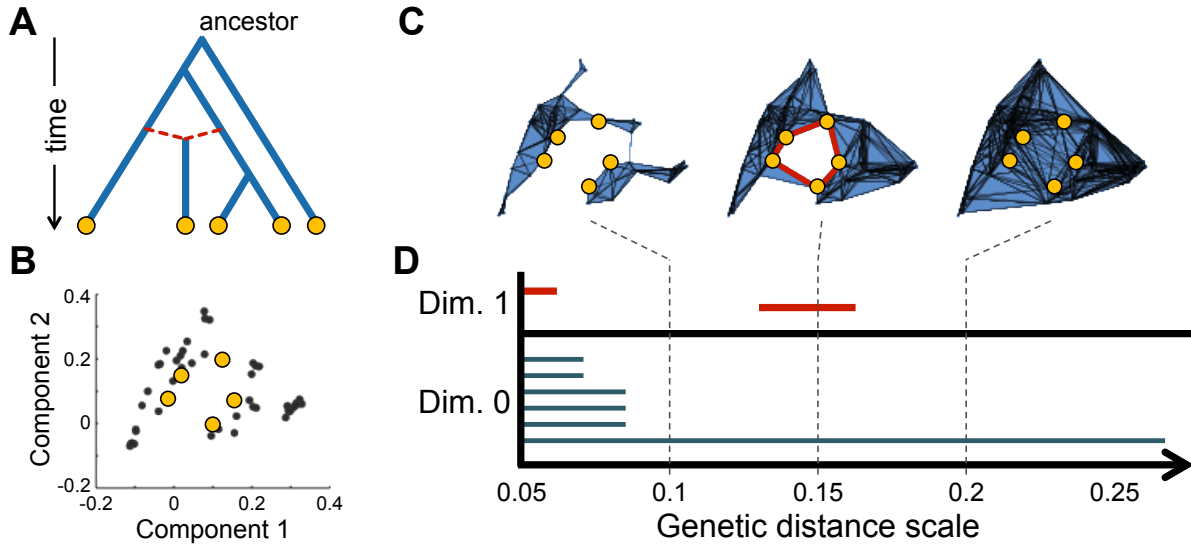


Figure 2.7: Applying persistent homology to genomic data. (A) An evolutionary genealogy including reticulation. (B) Data projected into 2-dimensions. (C) Construction of a filtered simplicial complex. (D) The resulting multiscale barcode diagram.

We summarize the connection between genomic data and TDA in Table 2.1.

### 2.3.1 The Fundamental Unit of Reticulation

Describe the four-gamete test.

### 2.3.2 The Space of Trees, Revisited

In Section XX we introduced Tree Space as an abstract construction to represent the space of trees. Tree Space was shown to be the subspace of the space of finite metrics that satisfy the condition of additivity.

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