Bridging time scales in evolutionary biology

Diego A. Hartasánchez[1,7,*], Thibault Latrille[1,8,*], Marina Brasó-Vives[2,9], and Arcadi Navarro[3,4,5,6,10]

[1]Department of Computational Biology, Université de Lausanne
[2]Department of Ecology and Evolution, Université de Lausanne
[3]Institute of Evolutionary Biology (Universitat Pompeu Fabra - CSIC)
[4]Centre for Genomic Regulation, Barcelona Institute of Science and Technology
[5]Institució Catalana de Recerca i Estudis Avançats (ICREA)
[6]Barcelonaβeta Brain Research Center, Fundació Pasqual Maragall
[7]Corresponding author: diego.hartasanchezfrenk@unil.ch
[8]thibault.latrille@ens-lyon.org
[9]marina.brasovives@unil.ch
[10]arcadi.navarro@upf.edu
[*]Equal contributions

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Abstract

Evolution is, fundamentally, a tug-of-war around biological diversity in which two main types of opposing processes are involved. Diversity is generated by means of mutation, migration and recombination; and diversity is reduced by natural selection of fitter individuals over less-fit ones among a population. An additional component, known as genetic drift, is commonly acknowledged as the main random force in evolution. The diversity resulting from this tug-of-war can be observed at two different time and biological scales: between individuals of the same species at the population-genetic scale and between different species at the phylogenetic scale. The goal of this chapter is to formally describe the bridge between these two biological scales by defining diversity within each and by relating the underlying evolutionary processes between them. To this aim, we will describe the neutral and nearly-neutral theories of evolution, setting the mathematical framework that will allow us to link the mutation rate at the population-genetic scale, which defines the amount of diversity present within a population, with the substitution rate, which defines the divergence between species at the phylogenetic scale. We here review how the neutral theory not only allows, conceptually, to establish a null model to study evolution but actually sets clear connections between two sub-disciplines, namely, population genetics and phylogenetics, which study manifestations of the same phenomena at different time scales. We discuss some consequences of bridging these time scales in current research in evolutionary biology.

Keywords: phylogenetics, population genetics, neutral theory, genetic drift, mutation rate, and substitution rate

Introduction

We have heard over and over again the phrase *the survival of the fittest* to explain how natural selection acts upon individuals. Indeed, this phrase, having been coined by Spencer [1864, p. 444] after studying Charles Darwin's work, is what Darwin somehow envisaged when thinking about organisms better adapted to their environment having higher chances to survive and to leave offspring which would inherit their better adapted characteristics [Darwin, 1859]. In the early years of the twentieth century, the ideas of Darwin were integrated into the context of the laws of inheritance first described by Mendel [1866]. Sewall Wright, Ronald A. Fisher and John B.S. Haldane, considered today as the founders of theoretical population genetics, described in mathematical terms how the frequencies of advantageous, neutral and deleterious[1] mutations varied from generation to generation under selective forces, setting the basis of what came to be known as the *Modern Synthesis* [Huxley, 1942]. Basically, they provided the probabilistic framework to describe changes in biological diversity through time, where *biological diversity* refers to either differences between individuals of the same species[2] or between individuals of different species, which is called *divergence*. At that time, biological diversity was mostly evaluated through differences in phenotype, namely observable traits such as the eye color of fruit flies [Morgan, 1910]. Today, the availability of DNA sequences from different individuals and species allows us to study diversity with great insight. We will here consider biological diversity as differences in the DNA sequence carried by germ cells of individuals.

We can think of the evolution of DNA sequences in terms of creation and loss of biological diversity from two different perspectives: within a species or a population[3]; and between species. *The survival of the fittest* concept can then be evaluated in each framework. In the first case, some individuals in the population leave offspring whereas others do not; in the second case, some species' lineages survive whereas others go extinct. In other words, as a genealogical tree represents the birth and death of individuals in a population, similarly, a species tree represents the birth and death of different species' lineages. In fact, studying diversity from these two perspectives has led to two distinct fields of evolutionary biology: population genetics, within a species or a population; and phylogenetics, between species.

The process underlying the evolution of sequences within both the population-genetic and phylogenetic frameworks is the same phenomenon occurring at two different time scales. If we focus, for example, on one gene in particular, we can observe differences in its nucleotide sequence between individuals, where each genetic variant is referred to as an *allele*. Ultimately, each allele present in part of the population (a segregating allele) will either fix in the entire population or be lost. At every moment in time, the probability for any segregating allele to attain any of these two fates is greatly determined by its current frequency in the population. Allele frequencies can thus be used to estimate diversity as well as to examine phenomena such as adaptation and population structure [Lewontin and Krakauer, 1973]. This change in frequencies of different alleles across time is at the core of population genetics' formalism. In phylogenetics, on the other hand, the focus is not on allele frequency changes, but rather, on the pattern of alleles that have reached fixation in the population. To reconstruct such a pattern, single representative sequences for each species, called *reference sequences*, are extracted from one or several individuals from each species. By comparing reference sequences from different species, a phylogenetic tree can be reconstructed, such as the example shown in Figure 1. Nevertheless, by using single representative sequences for each species, as was usually done when reference genomes were scarce, the underlying mechanisms generating the

[1] *Advantageous* mutations can be defined as mutations that increase the fitness of the individuals carrying such a mutation with respect to individuals not carrying it; *neutral* mutations do not affect fitness; and *deleterious* mutations decrease fitness.

[2] Although the definition of *species* has changed with time and is controversial, we will here use the Biological Species Concept as proposed by Mayr [1940, 1942], which defines species as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups".

[3] A *population* is a group of individuals belonging to the same species which live in the same geographical area and interbreed with each other.

observed differences between species were (and still are) often not taken into account. It is because of this particular fact that population genetics and phylogenetics have been, traditionally, two different fields of study. However, they fundamentally deal with the same information, which comes to light when we realize that differences in the molecular sequence of two different species are ultimately the product of a mutation event that originated at the population level, increased in frequency and reached fixation, in what is referred to as a *substitution* event (Figure 1).

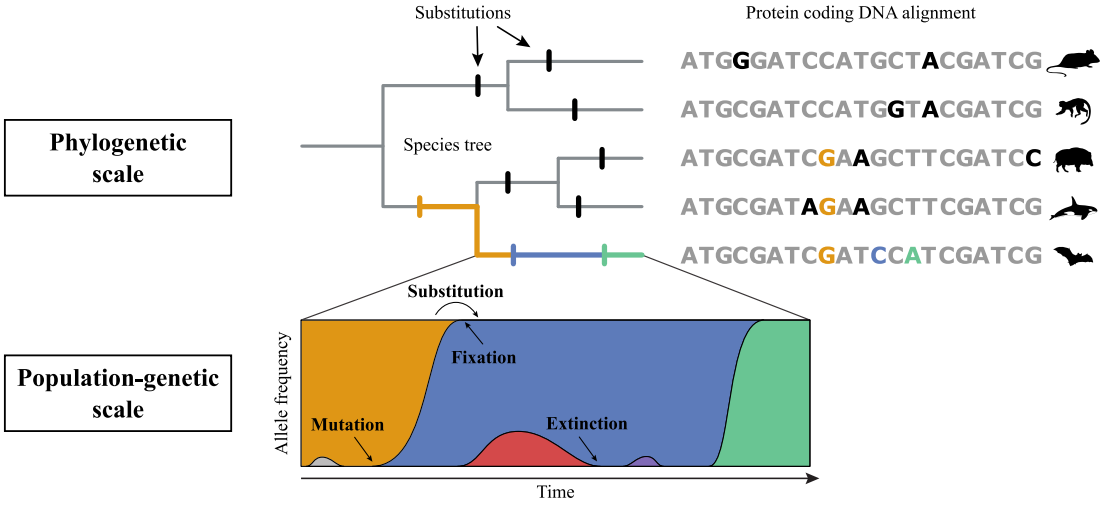


Figure 1: At the phylogenetic scale (top), the relationship between species is usually represented as a phylogenetic tree (top center) derived from a multiple-sequence alignment (top right). In a phylogenetic species tree, the tips of the tree represent extant species, and the internal nodes represent the last common ancestor between the respective merging lineages. Sequence changes along different species lineages are typically depicted by a single representative sequence per species. Nevertheless, differences between species are, ultimately, originated at the population-genetic scale (bottom). That is, they are the ultimate product of a mutation event that originated in one individual in the population. At the population scale, several mutations (alleles) might be segregating (present in the population at the same time). Some mutations might rise in frequency in the population and reach fixation replacing the previous allele in a substitution process (e.g. blue and green alleles), whereas other alleles might only segregate in the populations for a short time before becoming extinct (e.g. grey, red and purple alleles). The within-population diversity conferred by these segregating mutations is not accounted for by the phylogenetic tree, which only considers a representative sequence including the substitutions that happened during the evolution of the lineage post-speciation (e.g. orange-blue-green colored branch corresponding to the bat lineage). In this example, only mutations eventually leading to substitution events are shown in the tree as black bars, with branch lengths indicating time. The mutations that occurred in the branches leading to bats are highlighted in color in the multiple sequence alignment (e.g. the orange allele, characterized by a C to G substitution event in the common ancestor of wild boars, orcas and bats, which is not shared by mice and monkeys). Image made by authors.

We can study the evolution of sequences by thinking about the mechanisms through which diversity is generated and lost during evolution. On the one hand, the main generator of diversity is mutation, which changes the genomic sequence of one individual in the population bringing a novel, generally previously unseen variant of the sequence (a new allele) into the population (Figure 1). Recombination and migration can also produce diversity by generating new combinations of mutations. Recombination can be thought of as the process through which genetic information from ancestors is shuffled when producing new offspring, and migration is the exchange of individuals between populations which can result in the introduction of genetic information from divergent populations. Fundamentally, genetic diversity among the individuals of a population is likely to produce diversity in the observable characteristics, or traits, of the individuals. To each of these traits we

can assign a value of how beneficial or detrimental it is to carry such a trait for any individual. The sum of these values across all traits is referred to as *fitness*, which, when reduced to its ultimate consequences, represents the expected number of offspring produced by an individual. Therefore, by generating more diversity within a population, there will be more diversity in fitness.

On the other hand, the main reducer of diversity is selection, which is the process of evaluating every individuals' fitness against all the other individuals of the population. Individuals with a lower fitness will leave less offspring than individuals with high fitness. Through this process of selection, some of the biological diversity present in the population at a given moment will be inevitably lost.

Apart from mutation and selection, an additional process known as *genetic drift* also influences biological diversity. To understand the effect of genetic drift, it is first useful to think of the expected fate of a mutation in a population. From a completely deterministic point of view, any deleterious mutation should be purified away, while any advantageous mutation should invade the population. However, the fate of a mutation is not completely deterministic and does not only depend on its effect on fitness. Indeed, an individual can leave more or less offspring if at all, just by chance. Consequently, there is an inherent random component in evolution altering the frequency of alleles each generation, which is precisely the genetic drift.

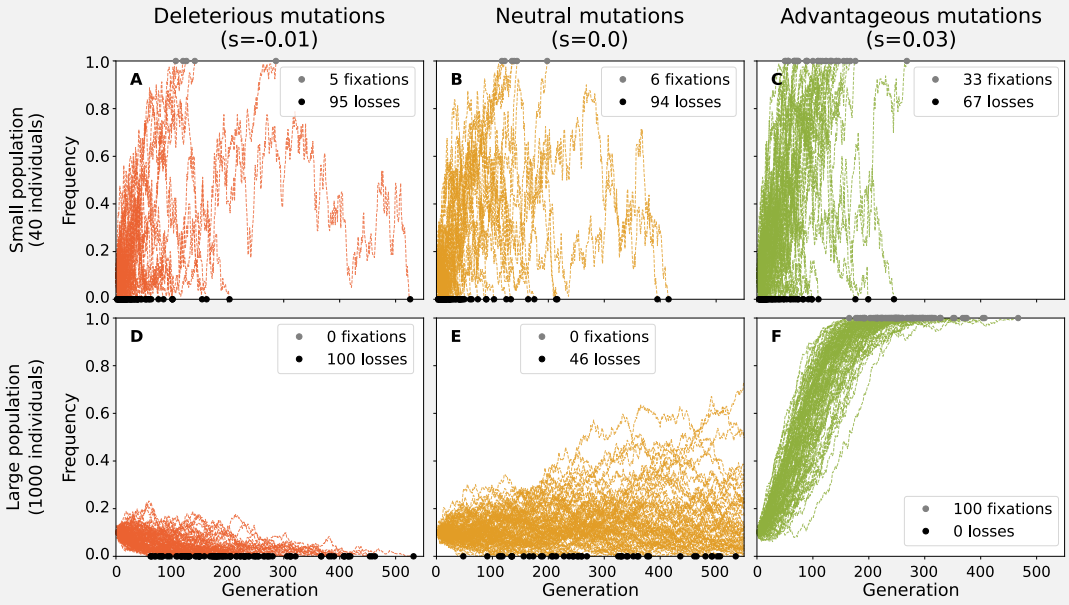
Genetic drift therefore represents the random component of the evolutionary process, which has unintuitive consequences at odds with the deterministic point of view. For example, as depicted in Box 1, a deleterious allele can ultimately reach fixation (panel A), while an advantageous allele can be lost from the population (panel C). The theory that accounted for genetic drift and formally derived the probability for a neutral mutation (panel B) to reach fixation in the population is the neutral theory of evolution [Kimura, 1962, 1968, 1983]. In the case of a mutation under selection, the theory that derived its probability of fixation in the population, while accounting for genetic drift, is the nearly-neutral theory of evolution [Ohta and Kimura, 1971; Ohta, 1973, 1992]. Altogether, these two theories describe, quantitatively, the relationship between substitution on one hand, and mutation, selection and genetic drift on the other hand. By doing so, they allow for the formalization of a bridge between the phylogenetic time scale, describing divergence between species, and the population-genetic time scale, describing the lifespan of a mutation in a population. If we take mammals, for example, the last common ancestor of all mammals is thought to have lived around 180 million years ago [Kumar et al., 2017]. Divergence between mammalian species is therefore on the scale of millions of years. However, a typical mutation in mammals, from its appearance to its fixation or extinction, only exists in the population during thousands of years. These two time scales differ by three orders of magnitude. Nevertheless, phylogenetic and population-genetics times scales can be bridged by the neutral and nearly-neutral theoretical formalism.

The neutral theory of molecular evolution

During the second half of the twentieth century, with the advent of molecular genetics, it became possible to adequately evaluate biological diversity through changes in DNA sequences. It was then observed that the number of point substitutions was approximately proportional to the time since their last common ancestor [Zuckerkandl and Pauling, 1965; Salser et al., 1976]. These observations led Zuckerkandl and Pauling [1965] to posit the molecular clock hypothesis, which states that the rate with which point substitutions accumulate is approximately constant through time. This apparently constant rate of molecular evolution is in sharp contrast with phenotypic evolution, which has a much more variable rate (observed through changes in morphology, for example) [Simpson, 1944, 1953]. Moreover, early measures of protein sequence diversity uncovered surprisingly high levels of genetic variability within populations, such that most proteins were found to be naturally variable in terms of their sequence [Harris, 1966; Hubby and Lewontin, 1966; Lewontin and Hubby, 1966]. In many cases, this sequence diversity had no visible phenotypic effects and showed no obvious correlation with any other trait. Finally, by comparing DNA sequences between related species, it was observed

that the overall (genome-wide) rate of DNA changes (substitutions) is very high, of at least one nucleotide base per genome every two years in a mammalian lineage [Lewontin, 1974].

Box 1: Genetic drift



Genetic drift is the random component affecting the frequency of mutations in the population. Each plot above shows the result of 100 simulations. In each simulation, a mutation initially present in 10% of the population was tracked within the population for 550 generations. As can be observed, the frequency of mutations as a function of time (generations) is not smooth but rather fluctuates from one generation to the next. Some key differences can be observed between plots. The top row shows the case for deleterious (A, in red), neutral (B, in yellow) and advantageous (C, in green) mutations within a small diploid population ($N_e = 40$). The bottom row shows the same three types of mutations for a larger population ($N_e = 1000$). If we compare the top row with the bottom row, we can see that the change in frequency from one generation to the next is much larger in the top row. This shows that genetic drift is much stronger in a small population size compared to a larger population size. The extent of these fluctuations of the mutation frequency has important consequences for the probability of fixation or loss of a mutation. In a large population, all deleterious mutations are ultimately lost (D), while all advantageous mutations ultimately reach fixation (F). Differently, in a small population, a few deleterious mutations actually reach fixation (A), and an important proportion (one third in this particular case) of advantageous mutations are lost (C). The size of the population and hence, the relative strength of genetic drift, also affects the average number of generations for a mutation to either reach fixation or be lost from the population. We can observe that mutations segregate in the population for a shorter period of time in the smaller population. In particular, the examples for neutral mutations show a clear difference. Whereas for the small population all neutral mutations have reached either fixation or loss by 500 generations, more than 50% of mutations are still segregating in the large population after 550 generations. The effective population size (N_e) has a direct effect on the strength of genetic drift and consequently, on the efficiency of selection: the larger the effect of genetic drift, the lower the efficiency of selection. Image made by authors.

These observations could not be easily explained in purely adaptive terms. Instead, they led Kimura [1968], and independently, King and Jukes [1969], to propose the neutral theory of molecular evolution [Kimura et al., 1986; Kimura, 1991]. The main tenet of the neutral theory is that a big part of the intra- and inter-specific molecular diversity is in fact adaptively neutral with mutations underlying this variation having no effect on fitness. According to the neutral theory, the vast majority of the nucleotide substitutions in the course of evolution have been the result of the random fixation of neutral mutants through genetic drift rather than the result of positive Darwinian selection with the

fixation of advantageous alleles precisely because of their selective advantage. Of note, the neutral theory does not state that all mutations are neutral or that adaptation does not take place. A substantial fraction of all mutations are in fact strongly deleterious, but are quickly eliminated from the population and thus, rarely observed. Contrarily, a very small fraction of mutations is advantageous, but since they are likely to increase in frequency by selection, they are generally more visible and indeed often constitute substitution events.

In an epic *tour de force*, Ohta and Kimura [1971] refined the neutral theory, by proposing that even mutations that have an effect on phenotype and fitness, should still behave neutrally and have their fate dictated solely by genetic drift, if their effect on fitness is sufficiently small. Ohta [1973] later proposed a mathematical formalization of this argument, incorporating weakly selected mutations to propose the nearly-neutral theory. Within this theory, one can compare a mutation's effect on fitness, and hence, the impact that it will have on its frequency change with time, with the corresponding impact of genetic drift. The parameter that allows for this comparison is the *effective population size*, N_e, introduced by Wright [1931, p. 110]. N_e can be thought of as the size of an *idealized* population, with characteristics such as a constant size, simultaneous birth of each new generation, random mating and equal number of offspring per parent. It is sometimes useful to think of N_e as the total number of breeding individuals of a population (although this is only true for simple scenarios). In any case, N_e is the hypothetical size that a real population would need to have for it to exhibit a particular observed quantity under the predictions of population genetics theory. Accordingly, N_e is used as a quantitative measure of the strength of genetic drift: if N_e increases, the effect of genetic drift decreases (Box 1). For example, in the case of a small N_e (Box 1, panels A-C), allele frequencies can change considerably from one generation to another just by genetic drift. In contrast, in the case of a high N_e (Box 1, panels D-F), the effect of genetic drift is smaller, and allele frequencies are expected to be less variable from one generation to another.

A bridge between two time scales

The link between the phenomena occurring at the population versus the phylogenetic scale is provided, fundamentally, by the effective population size N_e. Working out the long-term molecular evolutionary process first requires to formalize what happens in a short time period within populations. If we define the mutation rate μ as the average number of mutations that appear within a region of interest[4] per individual per generation, then the number of mutations that will appear per generation within a population will be $N_e\mu$. This result is valid for a haploid population, in which each individual has only one copy of each chromosome. In a diploid population, such as humans and almost all mammals, in which each individual contains two complete sets of chromosomes, the number of mutations that will appear per generation within a population will be $2N_e\mu$. Each of these newly appeared mutations has a probability $\mathbb{P}_{\text{fix}}$ to rise in frequency and be ultimately fixed in the population, resulting in a substitution. Altogether, we can calculate the substitution rate, as in McCandlish and Stoltzfus [2014, eq. 1-3], denoted Q, as the number of mutations per generation ($2N_e\mu$) in a diploid population, multiplied by the probability of fixation for each of these newly arisen mutations $\mathbb{P}_{\text{fix}}$:

$$Q = 2N_e\mu\mathbb{P}_{\text{fix}}. \tag{1}$$

Under a neutral scenario, at any given time, the probability for any allele to become ultimately fixed in the population is equal to its frequency at the time [Kimura, 1962, p. 717]. That is, in particular the probability of random fixation of a neutral mutation just after being originated within a diploid population will equal its initial frequency $1/2N_e$. As a consequence, the substitution rate for neutral

[4]Mutation rates can be calculated for any region of interest, including the whole genome.

mutations simplifies to:

$$Q = 2N_e\mu\mathbb{P}_{fix}, \tag{2}$$

$$= 2N_e\mu\frac{1}{2N_e}, \tag{3}$$

$$= \mu. \tag{4}$$

If we consider that all mutations that appear in the population are neutral, the substitution rate will equal the mutation rate [Kimura, 1968]. That is, the rate with which a new neutral allele is fixed in the population in a genomic region of interest equals the rate with which new mutations arise per generation for the same region of interest. It is important to note that Equation 4 is only valid when both the substitution rate and the mutation rate are measured in the same units. However, this also means that Equation 4 is valid whether the rates are measured in units of chronological time or per generation. As a convention, in this chapter, mutation rate is denoted as μ when measured per generation. Hence, this means that Q is also measured per generation.

Despite its simplicity, the consequences of this equality (Equation 4) in terms of linking population-genetics with phylogenetics are profound. On the one hand, the mutation rate μ is the defining parameter when evaluating diversity within a population since differences in sequences originate via mutation events. On the other hand, the substitution rate Q defines the speed with which two species' lineages diverge since substitutions result in differences between species. The fact that these two fundamental parameters are equal within the framework of the neutral theory allows us to make clear quantitative predictions about the rate and patterns of molecular evolution, and about the structure of genetic diversity within and between species. Ultimately what this equality implies is that the rate of change of individuals with respect to the population to which they belong (which determines biological diversity within a population) is the same as the rate of change that we expect to see between that particular species with respect to its closest relatives in a phylogenetic context (which determines biological diversity between species).

Consequences of this relation

As seen in the previous section, under the assumption of neutral mutations, the substitution rate at the phylogenetic scale is equal to the mutation rate at the individual level. This equality sets a well-defined framework against which one can test empirical sequence data from both population-genetics and phylogenetic contexts. When accounting for additional factors, such as natural selection, we will encounter deviations from this equality. We will discuss here some of the consequences of this relation and deviations from it.

Selection and the nearly-neutral theory

The neutral theory sparked a long-standing controversy between neutralists and selectionists [Smith, 1968; Nei, 2005]. Neutralists, following the argumentation of the neutral theory, considered that most mutations that are not deleterious are essentially neutral. To them, adaptive mutations are rare, relative to neutral mutations, and as a consequence, adaptive arguments do not need to be invoked in order to explain most of the variation observed at both the intra- and inter-specific levels. Some selectionists, on the other hand, maintained that most mutant alleles achieving fixation in a population must have had some selective advantage. As of today, it is widely accepted that both genetic drift and directional selection (which increases the frequency of advantageous mutations) participate in the evolution of genomes. The controversy is no longer strictly dichotomous but rather concerns the quantitative contributions of adaptive and of non-adaptive evolutionary processes, and their articulation with regards to mutation, selection, genetic drift, migration [Holderegger et al., 2006; Cortázar-Chinarro et al., 2017], recombination [Felsenstein, 1974; Roze, 2021], gene

conversion [Duret and Galtier, 2009; Hartasánchez et al., 2014; Hartasánchez et al., 2018], and other evolutionary processes [Yeaman, 2013; Pouyet and Gilbert, 2021; Latrille and Lartillot, 2021].

Quantitatively, one can evaluate the strength of selection associated with a particular mutation through its selection coefficient, s. The selection coefficient of a mutation is a measure of the differences in relative fitness of the individual carrying such a mutation compared to a reference fitness (usually set to 1 for the reference individual). A negative selection coefficient ($s < 0$) associated with a particular mutation implies that the individual carrying that mutation is less likely to leave offspring compared to other individuals without the mutation. As an example, a negative selection coefficient of $s = -0.01$ roughly means that the individual carrying the mutation has an increased 1% of dying before reproduction. Contrarily, a positive selection coefficient ($s > 0$) implies higher chances of the carrier to leave offspring. The probability of fixation was derived by Kimura [1962, eq. 11] for an allele with a small selection coefficient s as:

$$\mathbb{P}_{\text{fix}} = \frac{2s}{1 - \mathrm{e}^{-4N_{\text{e}}s}}. \tag{5}$$

As a result, the substitution rate is not equal to the mutation rate, but instead is contracted or dilated by a factor:

$$\begin{aligned} Q &= 2N_{\text{e}}\mu\mathbb{P}_{\text{fix}}, && (6)\\ &= 2N_{\text{e}}\mu\frac{2s}{1 - \mathrm{e}^{-4N_{\text{e}}s}}, && (7)\\ &= \mu\frac{S}{1 - \mathrm{e}^{-S}}, && (8) \end{aligned}$$

where $S = 4N_{\text{e}}s$ is called the *scaled selection coefficient*. Qualitatively, negative scaled selection coefficients ($S < 0$) result in a reduction of the substitution rate compared to the mutation rate, such that the rate of evolution between species is decelerated. On the opposite side, positive scaled selection coefficients ($S > 0$) result in an increase of the substitution rate compared to the mutation rate, with an accelerated rate of evolution:

$$\begin{cases} S < 0 \Rightarrow Q < \mu \\ S > 0 \Rightarrow Q > \mu. \end{cases} \tag{9}$$

The fact that selection (s) only appears as a product of effective population size (N_{e}) in Equation 8 has important consequences, namely that genetic drift and selection are intrinsically confounded factors. As an example, increasing N_{e} by a factor of 2 while reducing s by the same amount leads to the exact same equation, such that they are indistinguishable. As a result, only the scaled selection coefficients are empirically accessible from patterns of substitutions [Rodrigue et al., 2010]. Furthermore, Equation 8 also shows that increasing the population size ultimately increases the efficiency of selection, or opposingly, that reducing N_{e} reduces the efficiency of selection. In fact, the nearly-neutral theory states that mutations that have selection coefficients between $-1/2N_{\text{e}}$ and $1/2N_{\text{e}}$ have a ratio of substitution over mutation rate sufficiently close to 1, and therefore behave very similarly to neutral mutations (Figure 2). It is only for selection coefficients beyond this range that we can consider deleterious mutations to be effectively purified away ($s \leq -1/2N_{\text{e}}$) or advantageous mutations to effectively reach fixation ($s \geq 1/2N_{\text{e}}$). This threshold formally explains the choice of s and N_{e} shown in Box 1. Panel A shows the case for $s = -0.01$ and $2N_{\text{e}} = 80$. Since $-1/2N_{\text{e}} = -1/80 = -0.0125$, then the condition that $s \leq -1/2N_{\text{e}}$ is not satisfied, and therefore, this case of deleterious mutations is very similar to panel B, which is the case for neutral mutations ($s = 0$). Hence, mutations in panel A can be appropriately labeled as nearly-neutral mutations.

In this light, if one seeks to find evidence of selection for a particular genomic region, one could search for regions in which the substitution rate, Q, differs from the mutation rate, μ. Since the

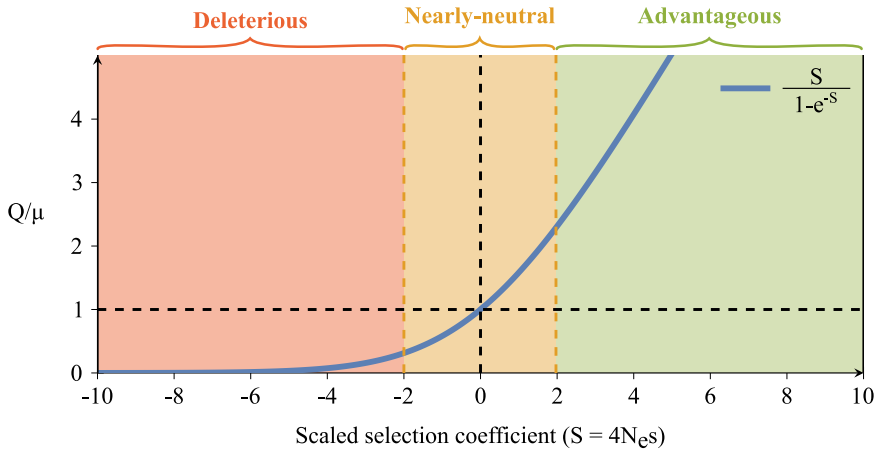


Figure 2: Ratio of substitution rate (Q) over mutation rate (μ) for a selected allele, shown in the vertical axis, as a function of the population-scaled selection coefficient $S = 4N_e s$ in the horizontal axis. For a substantially negative S ($s \leq -1/2N_e$, red-filled area), the probability of fixation is greatly reduced, with the substitution rate decaying exponentially with decreasing S values. In contrast, for a substantially positive S ($s \geq 1/2N_e$, green filled area), the increase in substitution rate is approximately linear with regard to S. In between, whenever the absolute value of s is between $-1/2N_e$ and $1/2N_e$ (orange filled area), the allele behaves approximately neutrally, with a ratio of substitution over mutation rate not substantially different from 1 according to the nearly-neutral theory [Ohta, 1973]. Image made by authors.

mutation rate is variable across regions of the genome and difficult to measure, one can instead use a proxy measurement. It so happens that in regions of the genome that code for proteins, there are regions that are, *a priori*, neutral, for which we can assume that $Q = \mu$. So, by measuring the substitution rates at these neutral sites we can establish Q_{neutral} for a particular region and hence search for regions in which Q differs from Q_{neutral}. The reason why this can be done in protein-coding regions of the genome is because, in these regions, DNA nucleotide sequences are translated into amino acid sequences that constitute proteins. During translation, a consecutive sequence of 3 nucleotides (a *codon*) encodes one particular amino acid. Since there are 4 different nucleotides in DNA, there are $4^3 = 64$ possible permutations, that is, 64 codons[5] which are more than enough to encode the 20 different amino acids. There is, hence, a redundancy in the translation code from codons to amino acids, which is known as the *genetic code*. In the genetic code, synonymous codons encode the same amino acid allowing mutations to be classified as synonymous or non-synonymous, where synonymous mutations do not modify the protein and are deemed neutral ($Q = \mu$), while non-synonymous mutations modify the protein and are considered as potential candidates to be under selection. Thus, by contrasting the substitution rates in non-synonymous positions (Q) against substitution rates in synonymous positions (Q_{neutral}), one can estimate the impact of selection in the region, effectively factoring out the variability of mutation rates across the genome [Kimura, 1977; Goldman and Yang, 1994; Muse and Gaut, 1994; Nielsen and Yang, 2003].

The idea of comparing non-synonymous to synonymous mutations was already present in the earliest landmark contributions in neutral molecular evolution [Kimura, 1968; King and Jukes, 1969], using simple statistical approaches. These studies showed that non-synonymous substitutions occur less frequently than synonymous substitutions [King and Jukes, 1969]. Similarly, radical amino acid replacements were observed to be less frequent than conservative changes to chemically similar amino acids [Kimura, 1983, ch. 7]. These results reflect purifying selection preventing most non-synonymous changes to prevail and can also be observed within populations where non-synonymous mutations

[5]There are in fact only 61 codons that encode amino acids because 3 codons, known as *stop codons*, signal the end of protein synthesis.

segregate at lower frequencies compared to synonymous mutations [Akashi, 1999; Cargill et al., 1999; Hughes, 2005]. This pervasive purifying process affecting proteins can be explained by the fact that protein sequences are relatively close to their optimum such that mutations occurring in their sequence are likely to disrupt their function. Consequently, the rate of non-synonymous mutations of protein-coding genes is primarily determined by the strength of selective pressure to maintain the gene unchanged, such that slowly evolving genes are just more constrained than fast-evolving genes [Kimura, 1983, ch. 4]. Altogether, along with the adoption of the nearly-neutral theory by evolutionary biologists, the common perception about the nature of selection shifted from selection being a driver of changes mediated by adaptive mutations to being a mainly purifying force discarding and filtering out strongly deleterious mutations [Lynch and Walsh, 2007].

Molecular clock

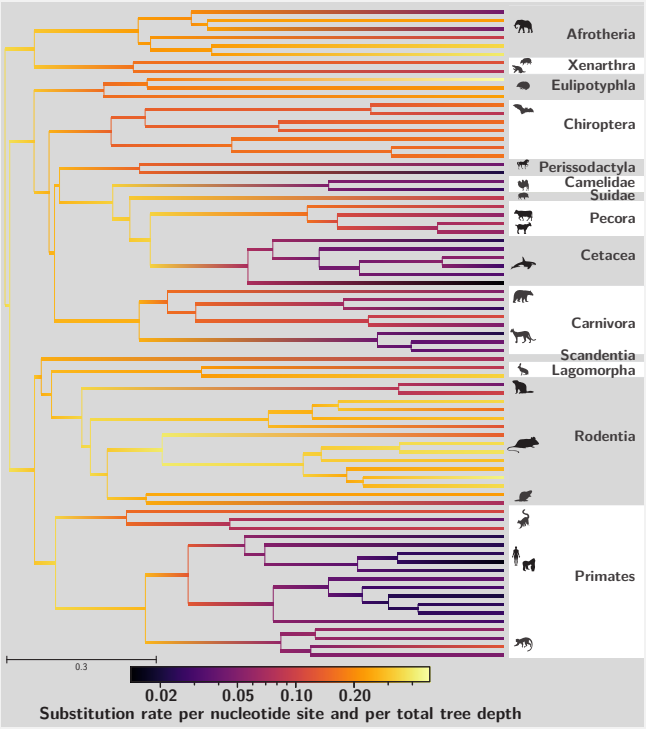
Originally, the neutral theory relied heavily on the molecular clock hypothesis of Zuckerkandl and Pauling [1965], which, as mentioned above, posits that the rate of sequence evolution is constant through time and across evolutionary lineages. Although appealing, it became clear that the rate of evolution was not constant [Wu and Li, 1985; Li et al., 1987; Bulmer et al., 1991; Gaut et al., 1992]. The rejection of a strict molecular clock motivated important methodological developments for modeling the fluctuations of the substitution rate along a phylogeny [Sanderson, 1997; Thorne et al., 1998; Kishino et al., 2001; Aris-Brosou and Yang, 2002; Drummond et al., 2006; Lepage et al., 2007]. The primary motivation for these relaxed clock models was to achieve more accurate molecular dating of the last common ancestor between species. However, these developments also fostered comparative analyses, trying to explain the causes of the variation of substitution rate between lineages [Lanfear et al., 2010; Lartillot and Poujol, 2011], which are still debated. Empirically, generation time, but also metabolic rate, are potential explanations for the variation in substitution rate [Lartillot and Delsuc, 2012], as highlighted in Box 2. Ultimately, substitution rate variation is mostly reflecting mutation rate variation, which in turn is determined by differences in life history traits [Hodgkinson and Eyre-Walker, 2011; Amster and Sella, 2016] and molecular mechanisms of mutation [Moorjani et al., 2016] and cell division [Gao et al., 2016].

Adaptation

The neutralist view of selection as mostly purifying raises an important question: where, and to what extent, does adaptation leave traces in molecular sequences? The fact that the neutral theory has been relatively silent on this question has largely contributed to its rejection by many biologists, and in many respects the question is still open [Jensen et al., 2019]. At first, methods for detecting adaptation were developed, integrating either the neutral or the nearly-neutral regime as a null model. Departures from one of these null models are then typically interpreted as traces of adaptation. This idea to detect traces of adaptation has been explored in a phylogenetic context, whenever the null model is neutral [Goldman and Yang, 1994; Muse and Gaut, 1994; Yang and Swanson, 2002; Zhang and Nielsen, 2005] or nearly-neutral [Rodrigue and Lartillot, 2016; Bloom, 2017]. Similarly, in a population-genetics context, adaptation is detected as a deviation from the null model, considered originally neutral [McDonald and Kreitman, 1991; Charlesworth, 1994; Smith and Eyre-Walker, 2002], and subsequently improved to account for slightly deleterious mutations in a nearly-neutral regime [Eyre-Walker and Keightley, 2009; Galtier, 2016]. These methods have clearly revealed important traces of adaptation [Bustamante et al., 2005; Halligan et al., 2010; Enard et al., 2014], in particular, in genes implicated in host-pathogen interactions [Enard et al., 2016; Grandaubert et al., 2019]. However, this might represent only the most extreme adaptive events. Much of adaptation might still have been missed at the molecular level. There might actually be important mechanistic and physical constraints or developments, such as cell polarity (as proposed by Søren Toxværd in a Chapter in this book) that affect the limits of adaptation.

Kimura [1983, ch. 6] proposed a more radical insight about the link between phenotypic adaptation and neutral molecular evolution by showing an example of a phenotypic trait under stabilizing selection and controlled by numerous loci with small effects. There can therefore be traits which have been efficiently optimized by selection, but for which the underlying molecular evolutionary processes at loci associated with said traits are indistinguishable from a neutral process. More recent work, using the empirical knowledge acquired by large-scale population-genomic projects, draws similar conclusions in the case of height and body mass index in humans [Simons et al., 2018]. Namely, many traits turn out to be highly polygenic [Pritchard and Cox, 2002], and the frequency changes contributing to their adaptive fine-tuning can be highly stochastic [Sella and Barton, 2019].

Box 2: Substitution rate reconstruction



Substitution rates can be calculated within a phylogenetic context as is shown here for placental mammals, based on an analysis of a concatenation of 18 genes [Latrille et al., 2021]. The substitution rate scale is per nucleotide site and per total tree depth (i.e., the total time from the most recent common ancestor to the present), and ranges from ≈ 0.01 to ≈ 1. If we assume the root to be 105 million years ago [Kumar et al., 2017], the re-scaled substitution rate per site per year is thus between 1.1×10^{-10} and 7.8×10^{-9} across this tree. Some clades, such as *Cetacea*, *Primates* and *Camelidae*, exhibit particularly low substitution rates, whereas other clades, such as *Rodentia* and *Chiroptera*, exhibit higher substitution rates. In general, one can observe a trend, in which the substitution rates are higher for species with shorter generation times. Substitution rates across mammals can be compared against genome-wide mutation rate estimates. Kumar and Subramanian [2002] calculated the per year per site mutation rate in mammals as 2.2×10^{-9}, which is within the same order of magnitude as the substitution rate range shown here. Image adapted from Latrille et al. [2021].

Conclusions

The neutral and nearly-neutral theories of evolution have existed for just about half a century. Consequences of these theories, such as substitution rates at the phylogenetic scale being equal to mutation rates at the population-genetics scale for neutral mutations, are well known. However, testing deviations from this prediction have remained inaccessible due to the lack of appropriate experimental data until very recently, with methods and studies becoming available. So, despite population-genetics and phylogenetics having been historically regarded as two different fields conceptually and methodologically, in contemporary evolutionary biology, they are coming closer together with studies working with intra-population and inter-species data simultaneously [Wilson et al., 2011; Brevet and Lartillot, 2021]. For example, modeling substitutions as mutation events followed by a gradual fixation along the phylogeny makes it possible to estimate mutation, selection and drift from genetic variation within and between species [De Maio et al., 2013; Bergman and Eyre-Walker, 2019; Schrempf et al.,

2019]. Moreover, integrating genetic variation within and between populations allows to resolve conflicts such as those in which the history of a particular gene (the gene tree) might not be the history of a species (the species tree) [Rannala and Yang, 2003; Degnan and Rosenberg, 2009]. Ultimately, phylogenetic and population-genetic approaches could be unified in the context of a single modeling framework [Thorne et al., 2012]. Establishing a link between these two sub-disciplines [Harmon et al., 2021] has been possible because of the framework provided by the neutral and nearly-neutral theories, which has allowed for a bridge to be constructed between two fundamentally different time scales.

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