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A maximum principle for the mutation-selection equilibrium of nucleotide sequences

Tini Garske and Uwe Grimm

Applied Mathematics Department, Faculty of Mathematics and Computing,
The Open University, Walton Hall, Milton Keynes MK7 6AA, UK

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Abstract

We study the equilibrium behaviour of a deterministic four-state mutation-selection model as a model for the evolution of a population of nucleotide sequences in sequence space. The mutation model is the Kimura 3ST mutation scheme, and the selection scheme is assumed to be invariant under permutation of sites. Considering the evolution process both forward and backward in time, we use the ancestral distribution as the stationary state of the backward process to derive an expression for the mutational loss (as the difference between ancestral and population mean fitness), and we prove a maximum principle that determines the population mean fitness in mutation-selection balance.

1 Introduction

The mathematical modelling of populations subjected to the competing evolutionary forces of mutation and selection has a long and rich history, see, e.g., Ewens (1979). The various approaches that have been employed to describe DNA evolution at the molecular level can be classified into two main categories, comprising stochastic approaches on the one hand and deterministic on the other.

The stochastic models deal with finite populations using Wright-Fisher sampling (Wright (1931); Ewens (1979), Ch. 3). The stochastic formulation lies at the heart of the neutral theory and had a strong influence on the methods used today to analyse population sequence data, see, e.g., Hartl and Clark (1997); Li and Graur (1990) for reviews. Selection can be treated as well; however, this is limited to very simple situations with two alleles only. The analysis of more complicated settings becomes impractical.

In the deterministic formulation, more challenging selection schemes can be treated, at the cost of neglecting genetic drift. Classical mutation-selection models formulate differential equations for the evolution of gene frequencies in infinite populations (Fisher (1922); Haldane (1928); Crow and Kimura (1970), Ch. 6; for an up-to-date review see Bürger (2000)). These have been adapted to sequence space by identifying alleles with sequences, and choosing an appropriate mutation mechanism — a tradition that was started by Eigen (1971) and reviewed by Eigen *et al.* (1989). A number of results can be found, e.g., in O'Brien (1985); Leuthäusser (1986, 1987); Rumschitzky (1987); Tarazona (1992) and, in a time-continuous formulation where mutation and selection are decoupled from each other, in Baake (1995); Baake *et al.* (1997); Baake and Wagner (2001). But this body of literature almost exclusively deals with two-state models, where each site in the sequence can be occupied with one of two states (or alleles), wildtype or mutant, in the assumption that this captures the essential behaviour of DNA sequences which are written in a four-letter alphabet.

In a recent article, Hermisson *et al.* (2001) refined this approach to a four-state model within a physical framework, concentrating on linear and quadratic fitness functions. The results obtained

for the four-state model show a much richer behaviour than is observed for the two-state case. Therefore, four-state models clearly deserve further investigation. In this article, we consider a four-state model for general permutation-invariant fitness functions, formulated entirely within the biological framework. Our main result is a maximum principle that allows us to determine the population mean fitness in mutation-selection balance by maximising the difference between the fitness and a suitably defined function that describes the mutational loss.

In practice, permutation-invariant fitness means that the fitness only depends on the type and the number of mutations relative to a reference sequence, not on their position in the sequence. This is, of course, a strong restriction; however, because of the difficult accessibility and complexity of realistic fitness landscapes, it is a widely used assumption in the population genetics literature (e.g., Charlesworth (1990); Wiehe (1997)). Furthermore, a permutation-invariant fitness function describes the accumulation of many small mutational effects surprisingly well. It is actually a good approximation for the fitness function in some models for concrete experimental situations like the DNA-binding models treated by von Hippel and Berg (1986); Gerland and Hwa (2002), which are formulated as two-state models and would certainly profit from a generalisation to a four-state description.

The four-state model of Hermisson *et al.* (2001) is a generalisation of the two-state model or biallelic chain (Baake *et al.*, 1997). Mutation-selection models of this type are closely connected to certain models of statistical physics, so-called quantum spin chains. Whereas the biallelic chain is related to the quantum Ising chain, the four-state model considered in this article corresponds to the Ashkin-Teller quantum chain, see, e.g., Kohmoto *et al.* (1981) and Baxter (1982), Ch. 12. However, this correspondence does not mean that results from statistical physics can be transferred directly to biology, because some of the quantities considered in the context of statistical physics are not those that are of interest here, compare the discussion by Baake and Wagner (2001).

The outline of this paper is as follows. We start with a general introduction to mutation-selection models, defining the ancestral distribution and the observable quantities in this class of models. This is then specialised to the sequence space of the four-state model which we are interested in, choosing appropriate models for mutation and selection. Exploiting the permutation invariance of the fitness function and the symmetries of the mutation model, the sequence space can be reduced to the permutation-invariant subspace. Subsequently, we define the mutational loss function and state the maximum principle. This principle holds exactly in three special cases which we discuss in detail. Finally, this is followed by a summary and a brief outlook. The rather technical proofs of the maximum principle are given in two appendices.

2 Mutation-selection models

We consider a population of haploid individuals¹ whose genotypes are chosen from a sequence space, a set of a finite number ν of possible genotypes i . The population is described by the population distribution \mathbf{p} , a ν -dimensional vector with entries $p_i \geq 0$, indicating the relative frequency of type i in the population. Hence, \mathbf{p} has to be normalised such that $\sum_{i=1}^{\nu} p_i = 1$. For finite population size, the p_i are rational numbers. In this article, we concentrate on the deterministic limit of an infinite population size, where the relative frequencies can take real values.

Ignoring environmental effects, mutation and selection are assumed to depend only on the genotypes of individuals. In this framework, the evolutionary processes to be considered are birth and death of individuals, and mutation from one type to another.

In the time-continuous model, each individual of type i gives birth to an identical copy with a rate b_i and dies with a rate d_i , hence we have an effective reproduction rate $r_i = b_i - d_i$, also called the *Malthusian fitness* (Bürger (2000), Ch. 1). These values are collected in a diagonal *reproduction matrix* $\mathbf{R} = \text{diag}(r_1, \dots, r_\nu)$.

¹The theory applies as well to populations of diploids without dominance, where the evolution equations reduce to those of a haploid population (cf. Bürger (2000), Ch. 2.2)

Mutation from type j to i occurs with a rate M_{ij} . To preserve the normalisation of the population distribution \mathbf{p} , the diagonal entries M_{ii} of the *mutation matrix* $\mathbf{M} = (M_{ij})$ are chosen such that \mathbf{M} has a vanishing sum over the columns, $\sum_i M_{ij} = 0$, which makes \mathbf{M} a Markov generator. Unless we talk about unidirectional mutation (cf. Sec. 5.1), we will assume that the mutation matrix is irreducible, i.e., each genotype can be reached from any other by mutation, possibly in several steps. With the definition of the *time-evolution operator* $\mathbf{H} = \mathbf{R} + \mathbf{M}$, this leads to the evolution equation

$$\dot{\mathbf{p}}(t) = (\mathbf{H} - \bar{r}(t)\mathbf{1}) \mathbf{p}(t), \quad (1)$$

where \bar{r} is the *population mean fitness* $\bar{r}(t) = \sum_i r_i p_i(t)$ and $\mathbf{1}$ denotes the $\nu \times \nu$ identity matrix, cf. Crow and Kimura (1970), Ch. 6, and Bürger (2000), Ch. 3.

Irreducibility of \mathbf{M} implies that of \mathbf{H} , and the Perron-Frobenius (PF) theorem guarantees that there exists a unique stable equilibrium solution, which is given by the strictly positive eigenvector \mathbf{p} corresponding to the largest eigenvalue λ_{\max} of \mathbf{H} ,

$$\mathbf{H} \mathbf{p} = \lambda_{\max} \mathbf{p}. \quad (2)$$

In the limit as $t \rightarrow \infty$, the population distribution converges towards this equilibrium solution, $\lim_{t \rightarrow \infty} \mathbf{p}(t) =: \mathbf{p}$.

2.1 The ancestral distribution

We are particularly interested in the equilibrium solutions $\dot{\mathbf{p}} = 0$. In this case, Eq. (1) becomes an eigenvalue equation for \mathbf{H} with eigenvalue $\lambda_{\max} = \bar{r}$. The *right* PF eigenvector \mathbf{p} is the population distribution in equilibrium, whereas the entries z_i of the *left* PF eigenvector \mathbf{z} determine the relative reproductive success of type- i individuals, as shown by Hermisson *et al.* (2002). The *ancestral distribution*, also introduced by Hermisson *et al.* (2002), is a probability distribution defined as $a_i = z_i p_i$, with the normalisation of \mathbf{z} chosen such that $\sum_i a_i = 1$. Here, a_i specifies the fraction of the equilibrium population whose ancestors, an infinitely long time ago, were of type i .

In analogy to the way that the population distribution is defined as a time-dependent quantity, this can also be done for the relative reproductive success \mathbf{z} and the ancestral distribution \mathbf{a} . However, this demands some notational efforts, and as we do not need this property later on, we limit ourselves to the definition of the ancestral distribution as an equilibrium quantity.

2.2 Means

A population is macroscopically described by mean quantities. We introduced two probability distributions, hence there are two types of averages that are relevant in our model. Every mapping o that assigns a value o_i to each possible genotype i can be averaged with respect to the population distribution or the ancestral distribution.

The *population mean* of o , denoted by $\bar{o}(t)$, is given by

$$\bar{o}(t) := \sum_i o_i p_i(t). \quad (3)$$

The population mean in equilibrium, i.e., in the limit as $t \rightarrow \infty$, is denoted by \bar{o} .

The *ancestral mean* of an operator o , denoted by \hat{o} , is defined as

$$\hat{o} := \sum_i o_i a_i. \quad (4)$$

Note that the ancestral mean does not depend on time, as we defined the ancestral distribution as an equilibrium quantity only.

3 The four-state model

The genetic information is coded in the DNA as a string composed of the purines adenine and guanine (A,G) and the pyrimidines cytosine and thymine (C,T).

We consider DNA strands of fixed length N , which may, for instance, code for an enzyme, as modelled by Hermisson *et al.* (2001). The four basic states $\{A,G,C,T\}$ are mapped onto $\{0,1,2,3\}$, or, as it is done by Hermisson *et al.* (2001), onto $\{++,+,-,-\}$. Conveniently, one can exploit the freedom in the choice of this mapping by introducing a relative rather than an absolute mapping between the bases $\{A,G,C,T\}$ and the symbols $\{0,1,2,3\}$. This essentially means that one can choose independent mappings at each position along the DNA strand. At any position, the mapping can be defined such that the symbol 0, or $++$, corresponds to the corresponding base in a given preferred sequence, which is usually chosen to be the *wildtype* or *master sequence* of maximal fitness r_{\max} . Thus the wildtype sequence is mapped onto the sequence $\{0\}^N = 000\dots 0$, see Hermisson *et al.* (2001) for details. The mapping between the remaining nucleotides and the symbols 1,2,3 will be discussed below. The sequence space consists of all possible N -letter sequences in these four symbols, so it is given by $\{0,1,2,3\}^N$ and has dimension 4^N .

In what follows, we shall not really need the complete information about the sequences. It will be sufficient to characterise a sequence by its *mutational distance* with respect to the wildtype sequence $\{0\}^N$, which just counts the deviations from the wildtype sequence. Whereas in the two-state model the mutational distance is given by a single integer, which counts the number of bases along the DNA strand that differ from those in the wildtype, we now need three non-negative integers d_1, d_2 and d_3 , according to the different types of mutations that can occur. We define the mutational distance \mathbf{d} of a sequence as

$$\mathbf{d} = \begin{pmatrix} d_1 \\ d_2 \\ d_3 \end{pmatrix} := \begin{pmatrix} \#(1) \\ \#(2) \\ \#(3) \end{pmatrix}, \quad (5)$$

where $\#(1)$, $\#(2)$ and $\#(3)$ denote the number of entries 1, 2 and 3 in the sequence, respectively. The total mutational distance is defined as the sum $d := d_1 + d_2 + d_3$, which takes values $0 \leq d \leq N$.

3.1 Mutation

Mutation is taken to be a point process that acts at each site independently. Disregarding more complicated mechanisms such as deletions and insertions, we only take into account the replacement of one base by another. Taken over the whole sequence, this happens with certain rates μ_k , where k indicates the type of replacement. We allow only one mutation at a time, as modelled by a Poisson process. This leads to a single step mutation model, which was first introduced by Ohta and Kimura (1973). We work with the *Kimura 3ST mutation scheme* shown in Fig. 1 (Kimura (1981); Swofford *et al.* (1996); Ewens and Grant (2001), Ch. 13) which assumes that, of a possible 12 mutation rates that can be chosen in our setting, only three different mutation rates μ_1, μ_2 and μ_3 occur. In particular, forward and backward mutation rates are the same, and the mutation process respects a symmetry between exchanges of purines and pyrimidines.

This mutation scheme can be treated to various degrees of sophistication. Apart from the full Kimura 3ST scheme, where all three mutation rates are different, there are also two simpler models that are worth mentioning.

The simplest approach is to take all mutation rates to be equal, $\mu_1 = \mu_2 = \mu_3$. This case is known as the *Jukes-Cantor mutation scheme* (Jukes and Cantor, 1969).

Due to the similar shapes of the nucleotides, *transitions*, i.e., the replacement of one purin/pyrimidine by the other, are more frequent than *transversions*, i.e., the replacement of a purin/pyrimidine by a pyrimidine/purin. The mutation rates describing the transversions are fairly similar, so $\mu_1 \approx \mu_3$, whereas the mutation rate for the transitions μ_2 is typically larger by a factor of about 2 to 40. This is taken into account in the *Kimura 2 parameter model* (Kimura, 1980) by assuming that $\mu_1 = \mu_3 < \mu_2$.

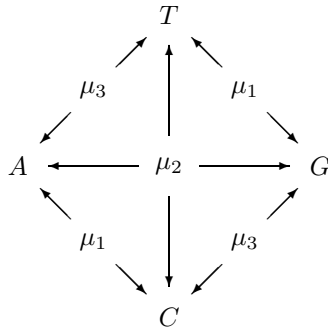


Figure 1: The Kimura 3 ST mutation scheme.

Assume there is one particular sequence s_0 with maximal fitness r_{\max} , the wildtype or master sequence, which is mapped onto $\{0\}^N$. For any other sequence, the corresponding representation in terms of the symbols $\{0, 1, 2, 3\}$ is then obtained by comparing it to the wildtype sequence, and assigning one of the labels 1, 2, 3 at each position where it differs from the wildtype sequence, according to the type of mutation as given in Fig. 1. Analogously to the mutational distance, we can define the *Hamming distance* (Hamming (1950); van Lint (1982), Ch. 3) between two sequences s_i and s_j , by comparing the sequences with each other. The restricted Hamming distances $d_k(s_i, s_j)$ are the numbers of type- k mutations between the sequences s_i and s_j , i.e., mutations that occur with rate μ_k ; the total Hamming distance is $d(s_i, s_j) = d_1(s_i, s_j) + d_2(s_i, s_j) + d_3(s_i, s_j)$.

In the Kimura 3ST setting, the entries M_{ij} of the mutation matrix are given by

$$M_{ij} = \begin{cases} \frac{\mu_k}{N} & \text{for } d(s_i, s_j) = d_k(s_i, s_j) = 1, \\ 0 & \text{for } d(s_i, s_j) > 1, \\ -\sum_{\ell \neq i} M_{\ell i} = -\sum_{k=1}^3 \mu_k & \text{for } i = j, \end{cases} \quad (6)$$

where, as mentioned above, the diagonal entries are chosen such that \mathbf{M} is a Markov generator. Here, the mutation rates are scaled as mutation rates per site, with the mutation rate over the whole DNA string being constant, see the discussion in Baake and Wagner (2001).

3.2 Selection

Whereas the process of mutation is well understood and straightforward to model, the choice of the fitness landscape on the molecular level is far from being clear. Realistic fitness landscapes would be rather rugged and strongly dependent on the function of the DNA sequence, but they are hard to access experimentally.

We shall use the severe simplification of a permutation-invariant fitness function, which is nevertheless a rather common (and usually implicitly made) assumption in theoretical investigations of mutation-selection models such as Charlesworth (1990); Wiehe (1997) as well as in the modelling of concrete experimental settings like in DNA-binding models (von Hippel and Berg, 1986; Gerland and Hwa, 2002). Using permutation-invariant fitness, one assumes that the fitness of a sequence depends only on the *number* of mutations of the various kinds, *not* on their location within the sequence. Hence, we can describe a sequence completely by its mutational distance $\mathbf{d} = (d_1, d_2, d_3)$ with respect to the wildtype sequence. As there are 4^N different sequences, but only $(N+1)(N+2)(N+3)/6$ different distances \mathbf{d} with $0 \leq d \leq N$, this reduces the effective type space enormously. In the permutation-invariant fitness model, the number of possible different genotypes, and thus the dimension of the permutation-invariant subspace, is given by $\nu = (N+1)(N+2)(N+3)/6$.

4 Reduction to the permutation-invariant subspace

In our model, three different spaces are relevant: (i) the 4^N -dimensional full sequence space, (ii) the reduced sequence space of dimension ν , and (iii) the three-dimensional space of the mutational distances.

In the *full sequence space* of dimension 4^N , each sequence corresponds to a different basis vector, and the population \mathbf{p} is then completely determined as a point on the $(4^N - 1)$ -dimensional hyperplane defined by $\sum_{i=1}^{4^N} p_i = 1$, where the projection on each axis gives the frequency p_i of the corresponding sequence.

Analogously, the ν -dimensional *reduced sequence space*, which is the permutation-invariant subspace of the full sequence space, is spanned by unit vectors, each of which corresponds to the set of all sequences which have the same number of mutations of each type, i.e., to one of the ν different mutational distances \mathbf{d} . Here, the population \mathbf{p} is also given as a point on a hyperplane (of dimension $\nu - 1$). In general, the transition from the full to the reduced sequence space is accompanied by a loss of information. As long as we consider systems with a unique equilibrium population, we know that this equilibrium will be permutation-invariant, because starting from a permutation-invariant initial population, we will reach a permutation-invariant equilibrium because the fitness function, as well as the mutation scheme, disregard the order in the sequences. As the equilibrium is unique, it will be reached from any initial condition. Therefore, sequences with the same numbers of each type of mutation, i.e., the same \mathbf{d} , must occur with the same frequency in the equilibrium population. Thus, as long as we are only interested in equilibrium properties of systems with a unique equilibrium, it suffices to restrict ourselves to the reduced sequence space.

Finally, we have the three-dimension *mutational distance space* of the mutational distance vectors \mathbf{d} with Cartesian coordinates d_1 , d_2 and d_3 . The basis of this space is formed by the Cartesian unit vectors $\mathbf{e}_1 = (1, 0, 0)^t$, $\mathbf{e}_2 = (0, 1, 0)^t$ and $\mathbf{e}_3 = (0, 0, 1)^t$, which are the basic directions of mutation. The condition $0 \leq d = d_1 + d_2 + d_3 \leq N$ restricts the possible mutational distance vectors \mathbf{d} to a simplex in the positive quadrant, as shown in Fig. 2. There is a one-to-one correspondence between the sequences in the reduced sequence space and the mutational distance vectors \mathbf{d} in this simplex, and we label the elements of the reduced sequence space by the corresponding mutational distance vectors \mathbf{d} . Whenever we speak of a sequence \mathbf{d} , we refer to the corresponding sequences in the reduced sequence space.

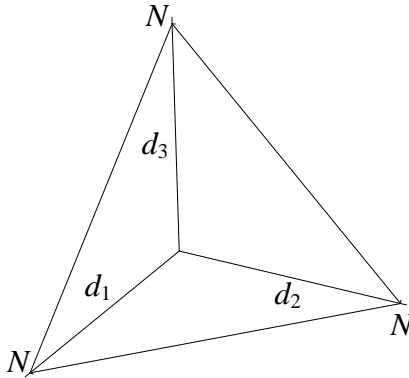


Figure 2: Mutational distance space in the case of permutation-invariant fitness.

The mutational distance space is required to define the neighbourhood of sequences. As we use a single step mutation model, each sequence, now labelled by \mathbf{d} , has at most 12 neighbours, i.e., sequences to which they can mutate within a single mutation step. The neighbours of a sequence \mathbf{d} have mutational distances $\mathbf{d} \pm \mathbf{e}_\xi$, where ξ determines the direction of mutation and the \mathbf{e}_ξ are

where the triples at the top give the mutational distances \mathbf{d} to which each column corresponds, whereas on the right the actual sequences s corresponding to each line are displayed. Only non-zero entries are shown. In this case, the remaining six columns of \mathbf{T} , shown symbolically as $(*)$, correspond to the antisymmetric subspace, which contains sequences with mutational distances $\mathbf{d} = (100)^t, (010)^t, (001)^t, (110)^t, (101)^t, (011)^t$.

The diagonal entries of \mathbf{H}_{piv} remain unchanged compared to the original \mathbf{H} ; they are $\mathbf{H}_{\text{piv},\mathbf{d}\mathbf{d}} = r_{\mathbf{d}} - \sum_k \mu_k$. The off-diagonal entries, i.e., the mutation rates u in the permutation-invariant subspace, depend on the direction of mutation. Using the normalised versions of the mutational distances $x_k := d_k/N$, they are given by

$$\begin{aligned} \mathbf{d} \rightarrow \mathbf{d} + \mathbf{e}_k : \quad & u_{\mathbf{d}}^{+k} = \mu_k x_0 & (3 \text{ eqns.}) \\ \mathbf{d} \rightarrow \mathbf{d} - \mathbf{e}_k : \quad & u_{\mathbf{d}}^{-k} = \mu_k x_k & (3 \text{ eqns.}) \\ \mathbf{d} \rightarrow \mathbf{d} + \mathbf{e}_k - \mathbf{e}_\ell : \quad & u_{\mathbf{d}}^{+k,-\ell} = \mu_m x_\ell & (6 \text{ eqns.}) \end{aligned} \quad (10)$$

where $k, l, m \in \{1, 2, 3\}$ are pairwise different, so $\{k, l, m\} = \{1, 2, 3\}$. Our notation is such that $u_{\mathbf{d}}^{+k}$ and $u_{\mathbf{d}}^{-k}$ denote the rates for mutations from distance \mathbf{d} in the positive and negative k direction, respectively, and $u_{\mathbf{d}}^{+k,-\ell}$ the corresponding rate in direction $\mathbf{e}_k - \mathbf{e}_\ell$. The mutation rates now depend on \mathbf{d} , reflecting the fraction of sites that can mutate with the specified effect. In particular, note that this implies that \mathbf{H}_{piv} is not a symmetric matrix. The above definition of mutation rates takes care of the boundary condition $u_{\mathbf{d}}^{\pm\xi} = 0$ for \mathbf{d} on the boundary of the relevant simplex in the mutational distance space with $\pm\mathbf{e}_\xi$ pointing outwards.

4.2 Symmetrisation of the time-evolution operator \mathbf{H}_{piv}

There is an alternative way to arrive at a matrix that describes the permutation-invariant subspace, which, however, does not preserve the Markov property for \mathbf{M} . As the original $4^N \times 4^N$ matrix \mathbf{H} is real symmetric, we can block-diagonalise it by an orthogonal transformation \mathbf{O} . This is an L^2 -transformation, and it preserves the symmetry of the matrix, so the corresponding $\nu \times \nu$ matrix $\widetilde{\mathbf{H}}_{\text{piv}}$ given by

$$\mathbf{O}^t \mathbf{H} \mathbf{O} = \begin{pmatrix} \widetilde{\mathbf{H}}_{\text{piv}} & 0 \\ 0 & \widetilde{\mathbf{H}}' \end{pmatrix} \quad (11)$$

is symmetric, where $\mathbf{O}^t = \mathbf{O}^{-1}$ denotes the transpose of the orthogonal matrix \mathbf{O} . In this case, the states in the permutation-invariant subspace are again superpositions of all sequences of equal distance \mathbf{d} , but now with coefficients $1/\sqrt{n_{\mathbf{d}}}$, as opposed to $1/n_{\mathbf{d}}$ for the L^1 -transformation \mathbf{T} . Knowing this connection, we can symmetrise the permutation-invariant part \mathbf{H}_{piv} of the time-evolution operator. By slight abuse of notation, we get for the permutation-invariant part

$$\widetilde{\mathbf{H}}_{\text{piv}} = (\mathbf{O}^t \mathbf{H} \mathbf{O})_{\text{piv}} = (\mathbf{O}^t \mathbf{T} \mathbf{T}^{-1} \mathbf{H} \mathbf{T} \mathbf{T}^{-1} \mathbf{O})_{\text{piv}} = \mathbf{D}^{-1} \mathbf{H}_{\text{piv}} \mathbf{D}, \quad (12)$$

where $\mathbf{D} := (\mathbf{T}^{-1} \mathbf{O})_{\text{piv}}$, and the subscripts mean that we restrict to the permutation-invariant part. As the relevant columns of the matrices \mathbf{T} and \mathbf{O} differ only by factors $\sqrt{n_{\mathbf{d}}}$, the transformation \mathbf{D} that symmetrises the time-evolution operator \mathbf{H}_{piv} is in fact *diagonal*, with entries $D_{\mathbf{d}\mathbf{d}} = \sqrt{n_{\mathbf{d}}}$.

The corresponding mutation rates \widetilde{u} of the symmetrised system are given by

$$\begin{aligned} \widetilde{u}_{\mathbf{d}}^{+k} &= \sqrt{\frac{n_{\mathbf{d}}}{n_{\mathbf{d}+\mathbf{e}_k}}} u_{\mathbf{d}}^{+k} = \mu_k \sqrt{x_0 \left(x_k + \frac{1}{N}\right)} = \sqrt{u_{\mathbf{d}}^{+k} u_{\mathbf{d}+\mathbf{e}_k}^{-k}}, \\ \widetilde{u}_{\mathbf{d}}^{-k} &= \sqrt{\frac{n_{\mathbf{d}}}{n_{\mathbf{d}-\mathbf{e}_k}}} u_{\mathbf{d}}^{-k} = \mu_k \sqrt{x_k \left(x_0 + \frac{1}{N}\right)} = \sqrt{u_{\mathbf{d}}^{-k} u_{\mathbf{d}-\mathbf{e}_k}^{+k}}, \\ \widetilde{u}_{\mathbf{d}}^{+k,-\ell} &= \sqrt{\frac{n_{\mathbf{d}}}{n_{\mathbf{d}+\mathbf{e}_k-\mathbf{e}_\ell}}} u_{\mathbf{d}}^{+k,-\ell} = \mu_m \sqrt{x_\ell \left(x_k + \frac{1}{N}\right)} = \sqrt{u_{\mathbf{d}}^{+k,-\ell} u_{\mathbf{d}+\mathbf{e}_k-\mathbf{e}_\ell}^{+\ell,-k}}. \end{aligned} \quad (13)$$

The property that \mathbf{H}_{piv} is symmetrisable by means of a diagonal transformation allows us to write the eigenvalue equation (2) in ancestral formulation, which is the starting point for the proofs of the maximum principle. In fact, the proofs presented below can be formulated for *any* mutation matrix that is symmetrisable by a diagonal transformation, which is equivalent to the property that it describes a reversible process,

$$M_{ij} q_j = M_{ji} q_i, \quad (14)$$

where \mathbf{q} is the equilibrium distribution of the mutation process without selection. In this case, we can use the diagonal transformation $Q_{ij} = \delta_{ij} \sqrt{q_j}$, and from (14), we obtain the symmetrised mutation rates $\widetilde{M}_{ij} = M_{ij} \sqrt{q_j/q_i} = \sqrt{M_{ij} M_{ji}}$. Therefore, the mutational distances are not bound to be the numbers of mutations of a DNA sequence, but the model can be reinterpreted in the multilocus model context, where the genetic distances are three arbitrary traits that determine the fitness. In fact, the maximum principle discussed below can also be derived for a model with n states at each site of the sequence, which then has an interpretation as n traits contributing to the fitness, as long as we talk about a n -dimensional single step model with a mutation matrix that describes a reversible process. A more general and more systematic approach thus seems feasible, it will be described by Baake *et al.* (2003).

In what follows, we drop the subscript, and use \mathbf{H} and $\widetilde{\mathbf{H}}$ to denote the submatrices corresponding to the permutation-invariant subspace. Both time-evolution operators \mathbf{H} and $\widetilde{\mathbf{H}}$ have the same eigenvalues, but the eigenvectors differ. The left and right eigenvectors $\widetilde{\mathbf{z}}$ and $\widetilde{\mathbf{p}}$ of $\widetilde{\mathbf{H}}$, for the largest eigenvalue, are related to the corresponding eigenvectors \mathbf{z} and \mathbf{p} of \mathbf{H} by

$$\widetilde{\mathbf{z}} = \mathbf{z} \mathbf{D} \quad \text{and} \quad \widetilde{\mathbf{p}} = \mathbf{D}^{-1} \mathbf{p}. \quad (15)$$

The relation between the ancestral distribution \mathbf{a} and the symmetrised population $\widetilde{\mathbf{p}}$ is given by

$$a_i = z_i p_i = (\widetilde{\mathbf{z}} \mathbf{D}^{-1})_i (\mathbf{D} \widetilde{\mathbf{p}})_i = \widetilde{z}_i \widetilde{p}_i \sim \widetilde{p}_i^2, \quad (16)$$

as $\widetilde{\mathbf{z}} \sim \widetilde{\mathbf{p}}$ due to the symmetry of $\widetilde{\mathbf{H}}$. With the relation $\widetilde{\mathbf{p}} \sim \sqrt{\mathbf{a}}$, the eigenvalue equation of $\widetilde{\mathbf{H}}$ in ancestral formulation becomes $\widetilde{\mathbf{H}} \sqrt{\mathbf{a}} = \bar{r} \sqrt{\mathbf{a}}$, which explicitly reads

$$\left[r_{\mathbf{d}} - \sum_{\xi} \left(u_{\mathbf{d}}^{+\xi} + u_{\mathbf{d}}^{-\xi} \right) \right] \sqrt{a_{\mathbf{d}}} + \sum_{\xi} \left[\widetilde{u}_{\mathbf{d}-\mathbf{e}_{\xi}}^{+\xi} \sqrt{a_{\mathbf{d}-\mathbf{e}_{\xi}}} + \widetilde{u}_{\mathbf{d}+\mathbf{e}_{\xi}}^{-\xi} \sqrt{a_{\mathbf{d}+\mathbf{e}_{\xi}}} \right] = \bar{r} \sqrt{a_{\mathbf{d}}} \quad (17)$$

for some distance \mathbf{d} . Here, ξ determines the six possible directions of mutation, and the sign indicates the forward and backward direction.

5 Maximum principle

For the permutation-invariant system, we can derive a maximum principle for the population mean fitness that involves maximisation only over the three components of $\mathbf{x} = \mathbf{d}/N$ of the mutational distance space, as opposed to the maximisation over the ν -dimensional reduced sequence space according to Rayleigh's principle. It finds its analogue in the scalar maximum principle given in (Hermisson *et al.*, 2002, Eqs. (30) and (33)). In the four-state model, we have

$$\bar{r} = \sup_{\mathbf{x}} (r(\mathbf{x}) - g(\mathbf{x})) \quad (18)$$

with the mutational loss function $g(\mathbf{x})$ defined as

$$g(\mathbf{x}) := \sum_{\xi} \left(u^{+\xi}(\mathbf{x}) + u^{-\xi}(\mathbf{x}) - 2\sqrt{u^{+\xi}(\mathbf{x})u^{-\xi}(\mathbf{x})} \right) \quad (19)$$

with summation over all six directions of mutation ξ .

This is exact for three special cases, namely (i) for unidirectional mutation, (ii) for linear fitness and mutation functions, and, most importantly, (iii) in the limit of infinite sequence length. These cases will be explained in what follows, and the derivation of the maximum principle for each case is presented as well. For other systems, the maximum principle gives an approximation, which, under reasonable assumptions, one might expect to differ from the true result by correction terms of order $1/N$.

If the supremum in Eq. (18) is assumed at a unique value \mathbf{x} , which is the generic case, this value is the ancestral mean mutational distance $\hat{\mathbf{x}}$, and we have, in addition to Eq. (18),

$$\bar{r} = r(\hat{\mathbf{x}}) - g(\hat{\mathbf{x}}) = \hat{r} - g(\hat{\mathbf{x}}), \quad (20)$$

which again is exact for the three special cases mentioned before. This maximum principle, in the terminology of physics, is akin to the principle of minimal free energy.

5.1 Unidirectional mutation

We now consider the first of the three situations where the maximum principle is exact, the case of unidirectional mutation.

Unidirectional mutation means that only such mutations that increase d happen, the mutation rates towards types with smaller or equal d are zero. In the most important case of a monotonically decreasing fitness function, this means that all mutations are deleterious. This mutation scheme does not go in line with the mutation rates for the DNA system as given in Eq. (10), but it is equivalent with a collapse of the mutation scheme as shown in Fig. 3.

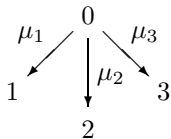


Figure 3: Simplified mutation scheme for unidirectional mutation.

For unidirectional mutation, the mutation matrix \mathbf{M} is no longer irreducible. Hence, in this case, the Perron-Frobenius theorem does not apply. The equilibrium is not unique, but depends on initial conditions. Once the wildtype is lost in the population, it can never occur again, because the mutation rates back to types with smaller d are zero. The structure of the mutational distance space is shown in Fig. 4, where the wildtype on the top corner of the mutational distance space “feeds” all mutants underneath.

Although unidirectional mutation rates do not represent the mutation model for DNA sequences as set up in this article, they still are a reasonable approximation. If in the DNA model the selection is sufficiently strong, most individuals present in the population will have a genotype with a small mutational distance from the wildtype. The mutations that leave d constant ($\mathbf{e}_\xi = \mathbf{e}_l - \mathbf{e}_k$), and those that decrease d ($\mathbf{e}_\xi = -\mathbf{e}_k$), which in the case of a decreasing fitness function are neutral and advantageous, respectively, occur with rates proportional to x_k , and therefore are small for individuals with small mutational distances $x = \sum_{k=1}^3 x_k = d/N$, which form the main part of the population, whereas the mutations that increase d ($\mathbf{e}_\xi = \mathbf{e}_k$) happen with rates proportional to $1 - x$, which are of order 1 for small \mathbf{x} . Therefore, it is reasonable to approximate the mutation rates of the DNA model by unidirectional mutation rates, which is the well known infinite sites limit (see Kimura (1969) or Ewens (1979), Ch. 8).

In the case of unidirectional mutation, the mutational distance space can be divided into three domains with respect to each sequence \mathbf{d} , namely the ancestral cone, the offspring cone and the sibling domain. Here, all sequences that can mutate to \mathbf{d} lie in the ancestral cone $AC(\mathbf{d})$, all sequences that \mathbf{d} can mutate to lie in the offspring cone $OC(\mathbf{d})$, whereas the sequences that are

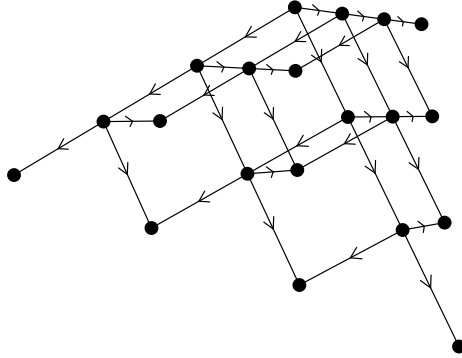


Figure 4: Structure of the mutational distance space for unidirectional mutation.

not connected to \mathbf{d} via a mutational path form the sibling domain $SD(\mathbf{d})$, which is the remainder of the mutational distance space.

The eigenvalue equation of \mathbf{H} for unidirectional mutation is given by

$$(\bar{r} - \lambda_{\mathbf{d}}) p_{\mathbf{d}} = \sum_k u_{\mathbf{d}-\mathbf{e}_k}^{+k} p_{\mathbf{d}-\mathbf{e}_k} \quad \text{with} \quad \lambda_{\mathbf{d}} = r_{\mathbf{d}} - g(\mathbf{x}_{\mathbf{d}}) = r_{\mathbf{d}} - \sum_k u_{\mathbf{d}}^{+k}, \quad (21)$$

where the diagonal entries are the eigenvalues $\lambda_{\mathbf{d}}$, because \mathbf{H} is a lower triangular matrix. In this case, the situation is particularly simple, and we can directly infer some properties of the population distribution \mathbf{p} , which allow us to show that the maximum principle holds in this case.

Suppose there is one $\hat{\mathbf{d}}$ such that $p_{\hat{\mathbf{d}}} > 0$, but $p_{\hat{\mathbf{d}}-\mathbf{e}_k} = 0$ for $k \in \{1, 2, 3\}$. This can only happen if $\bar{r} = \lambda_{\hat{\mathbf{d}}}$. An evaluation of Eq. (21) for $\hat{\mathbf{d}} - \mathbf{e}_k$ and for other sequences in the ancestral cone yields that no sequence in the ancestral cone can contribute to the population, i.e., $p_{\mathbf{d}} = 0$ for all $\mathbf{d} \in AC(\hat{\mathbf{d}})$.

If there was a sequence \mathbf{d}^+ in the offspring cone of $\hat{\mathbf{d}}$ with $\lambda_{\mathbf{d}^+} > \lambda_{\hat{\mathbf{d}}}$, we would get $p_{\mathbf{d}^+} < 0$, which is a contradiction to the condition $p_i \geq 0$. Thus, $\lambda_{\mathbf{d}} \leq \lambda_{\hat{\mathbf{d}}}$ for all $\mathbf{d} \in OC(\hat{\mathbf{d}})$, which corresponds to the maximum principle (18) as $\bar{r} = \max_{\mathbf{d} \in OC(\hat{\mathbf{d}})} [r(\mathbf{x}_{\mathbf{d}}) - g(\mathbf{x}_{\mathbf{d}})]$. If now $\lambda_{\mathbf{d}^+} = \lambda_{\hat{\mathbf{d}}} = \bar{r}$, we get $p_{\mathbf{d}} = 0$ for all $\mathbf{d} \in AC(\mathbf{d}^+)$, including $\hat{\mathbf{d}}$, so that in this case the offspring cone $OC(\mathbf{d}^+)$ spans the population rather than $OC(\hat{\mathbf{d}})$. Evaluating the eigenvalue equation for the sequences in the offspring cone, we get $p_{\mathbf{d}} > 0$ for all $\mathbf{d} \in OC(\hat{\mathbf{d}})$ or $\mathbf{d} \in OC(\mathbf{d}^+)$, respectively.

All sequences in the sibling domain $SD(\hat{\mathbf{d}})$ descend originally from the ancestors of $\hat{\mathbf{d}}$ which are not present in the population. Thus, their frequencies must vanish, unless there is a sequence $\mathbf{d}^+ \in SD(\hat{\mathbf{d}})$ with $\lambda_{\mathbf{d}^+} = \lambda_{\hat{\mathbf{d}}}$. In this case, if $p_{\mathbf{d}^+} > 0$, the sequences in both offspring cones have non-vanishing frequency $p_{\mathbf{d}} > 0$ for all $\mathbf{d} \in OC(\hat{\mathbf{d}}) \cup OC(\mathbf{d}^+)$, the frequencies of all other sequences vanish.

As the mutation matrix is not irreducible for unidirectional mutation, the population distribution in equilibrium is not unique, but depends on the initial conditions. The equilibrium with the highest mean fitness is always assumed if the wildtype initially occurs with non-zero frequency, or if we consider the limit of small, but non-vanishing back mutations. In this case, we have $\bar{r} = \lambda_{\max} = \sup_{\mathbf{d}} [r(\mathbf{x}_{\mathbf{d}}) - g(\mathbf{x}_{\mathbf{d}})]$.

If, however, the wildtype is not present in the initial population $\mathbf{p}(t=0)$ and the mutation rates for constant or decreasing d are exactly zero, we have to consider only the part of the mutational distance space that is spanned by the offspring cones $\bigcup_{\mathbf{d}_{\text{in}}} OC(\mathbf{d}_{\text{in}})$ of all initially present sequences \mathbf{d}_{in} . Now, the mean fitness assumes the highest possible value in this subspace $\bar{r} = \sup_{\bigcup OC(\mathbf{d}_{\text{in}})} [r(\mathbf{x}_{\mathbf{d}}) - g(\mathbf{x}_{\mathbf{d}})]$, which is assumed for at least one sequence \mathbf{d}^+ .

If this maximum is unique, the equilibrium population is given by the right eigenvector corresponding to this eigenvalue $\lambda_{\mathbf{d}^+}$, which has non-zero entries only for the sequences in the offspring cone $OC(\mathbf{d}^+)$. The left eigenvector corresponding to $\lambda_{\mathbf{d}^+}$ has non-zero entries only for the ancestors of \mathbf{d}^+ , so that the only \mathbf{d} with $a_{\mathbf{d}} \neq 0$ is \mathbf{d}^+ , and hence $\mathbf{d}^+ = \hat{\mathbf{d}}$ is the only ancestor. This yields Eq. (20).

An interesting case arises, however, if the maximum is not unique, but attained at two sequences $\hat{\mathbf{d}}$ and \mathbf{d}^+ in the subspace under consideration. In this degenerate case, the ancestral distribution cannot be obtained as easily as shown above, as the left and right eigenvectors have no non-zero overlap and thus it is not possible to normalise \mathbf{z} such that $\sum_i a_i = 1$.

We now have to distinguish between two cases. If the sequences lie in parent-offspring relation, i.e., $\mathbf{d}^+ \in OC(\hat{\mathbf{d}})$, $\hat{\mathbf{d}}$ is the single ancestor, whereas the population is formed by the offspring cone of \mathbf{d}^+ , i.e., $p_{\mathbf{d}} > 0$ if and only if $\mathbf{d} \in OC(\mathbf{d}^+)$. Note that Eq. (20) still holds, although in this special case the only ancestor $\hat{\mathbf{d}}$ has zero frequency in the population.

If, however, $\hat{\mathbf{d}}$ and \mathbf{d}^+ lie in sibling relation to each other, the population is formed by the unification of their offspring cones $OC(\hat{\mathbf{d}}) \cup OC(\mathbf{d}^+)$; and $\hat{\mathbf{d}}$ and \mathbf{d}^+ both may have non-vanishing ancestral frequency, which are then determined by the initial conditions.

5.2 Linear model

We now consider the second case where the maximum principle applies exactly. Here, both the fitness function and the mutation rates depend linearly on functions y_k of the genotype components x_k , and thus can be written as

$$r(\mathbf{x}) = r_0 - \sum_k \alpha_k y_k(x_k) \quad \text{and} \quad u^{\pm\xi}(\mathbf{x}) = u_0^{\pm\xi} + \sum_k \beta_k^{\pm\xi} y_k(x_k), \quad (22)$$

with parameters α_k and $\beta_k^{\pm\xi}$. This type of model has been used, e.g., in von Hippel and Berg (1986) and Gerland and Hwa (2002) in a two-state version.

In this case, the mean fitness can be obtained by maximisation over the three components of \mathbf{x} , and the maximum principle in the form of Eqs. (18) and (20) holds true for $y_k(x_k) = x_k$. This can be shown by a direct calculation starting from Eq. (17), which is carried out in Appendix A.

5.3 Infinite sequence length

Finally, we consider the limit as the sequence length becomes infinite. In the limit as $N \rightarrow \infty$, we use $\mathbf{x}_{\mathbf{d}} = \mathbf{d}/N$ to describe the mutational distance to the wildtype. The quantities $x_{d_k} = d_k/N$ and $x_{\mathbf{d}} = \sum_{k=1}^3 x_{d_k}$ fulfill the inequalities $0 \leq x_{d_k} \leq 1$ and $0 \leq x_{\mathbf{d}} \leq 1$. As long as we operate with finite N , the x_{d_k} take discrete rational values only; in the limit $N \rightarrow \infty$, however, they become dense, and it is thus reasonable to pass to a continuum formulation. Consider the fitness function $r(\mathbf{x})$ and the mutation rates $u^{\pm\xi}(\mathbf{x})$ as functions defined on the mutational distance space. The mutation rates are positive, continuous functions, obeying the boundary condition that they vanish for all \mathbf{x} at the boundary of the simplex in the mutational distance space, where they correspond to mutations out of the set of possible mutational distances. The fitness function has to be piecewise continuous, i.e., it can have discontinuities only along (finitely many) surfaces in the mutational distance space; at the discontinuities, it must be either left or right continuous. This allows for a number of biologically meaningful fitness functions, like, for example, truncation selection. For any finite N , the fitness and mutation functions are sampled at all possible $\mathbf{x}_{\mathbf{d}}$. The limit $N \rightarrow \infty$ is carried out such that the functions are kept constant, but the sampling gets finer with increasing N .

In the limit as $N \rightarrow \infty$, Eqs. (18) and (20) hold true. The proof is presented in Appendix B. Even for rather short sequence lengths, the maximum principle yields a reasonable approximation.

Figures 5 and 6 show numerical results obtained using the maximum principle in comparison with those for finite systems obtained by a direct diagonalisation of the time-evolution operator. For both figures, a quadratic symmetric fitness function $r(\mathbf{x}) = (1 - \sum x_k)^2$ has been used, but they

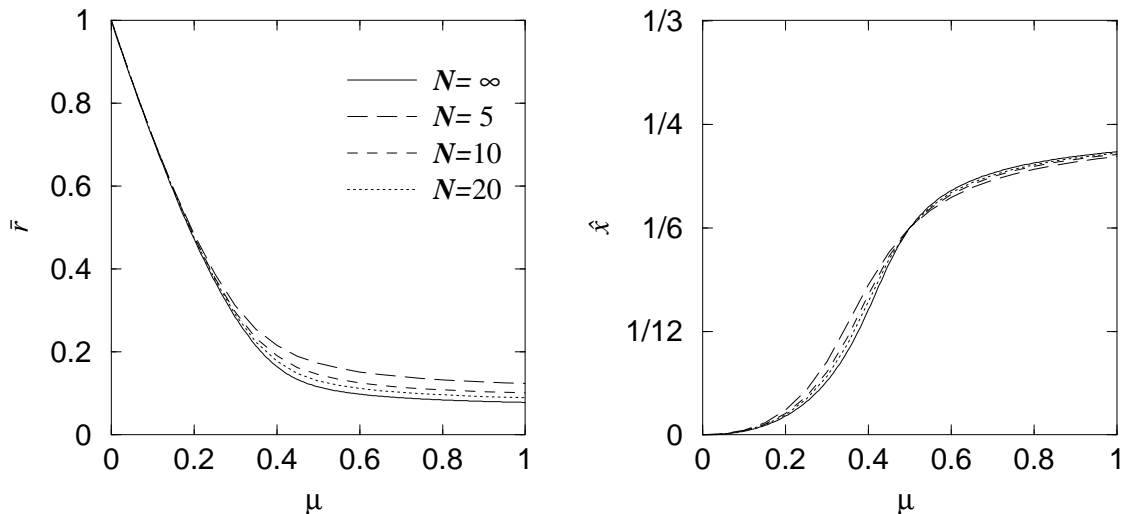


Figure 5: Population mean fitness \bar{r} and ancestral mean mutational distance \hat{x}_k for varying mutation rate μ for a model with Jukes-Cantor mutation scheme and quadratic fitness $r = (1 - \sum_{k=1}^3 x_k)^2$. The curves for finite sequence lengths $N = 5, 10, 20$ are obtained by direct diagonalisation of the time-evolution operator, the curve for $N = \infty$ is the result of the maximum principle.

differ in the mutation scheme. The mutation scheme used in Fig. 5 is the Jukes-Cantor mutation scheme, whereas in Fig. 6, we used a Kimura 2 parameter (K2P) model with $\mu_1 = \mu_3 := \mu = \frac{1}{10}\mu_2$. For the totally symmetric Jukes-Cantor model, the ancestral means of the mutational distances all coincide, whereas they differ in the K2P model, according to $\hat{x}_1 = \hat{x}_3 < \hat{x}_2$.

6 Summary

In this article, we investigated the mutation-selection balance in the mutation-selection model introduced by Hermisson *et al.* (2001). There, a deterministic approach to model the DNA evolution of asexual populations was taken. We consider four-state sequences subject to the forces of mutation and selection. For simplicity, selection is taken to be permutation invariant, which leads to a three-dimensionally structured mutational distance space, and the mutation model is a single step model on this structure.

Using the concept of the ancestral distribution, as introduced by Hermisson *et al.* (2002), we derived a maximum principle for the population mean fitness \bar{r} and the ancestral mean genotype \hat{x} in equilibrium, which involves a maximisation over the three dimensions of the mutational distance space.

This maximum principle gives the exact mean fitness in the three limiting cases of (i) unidirectional mutation, (ii) linear fitness and mutation functions, and (iii) the limit of infinite sequence length. For finite sequence lengths, it is an approximation which we expect to be correct up to terms of the order $1/N$. Numerically, we found that already rather small sequence lengths were well reproduced.

The maximum principle generalises the results of Hermisson *et al.* (2002), where the case of a one-dimensional mutational distance space was treated, which can be interpreted on the level of DNA sequences as a two-state model, with states representing wildtype and mutant. In that case, \bar{r} , \hat{x} and $\bar{x} = r^{-1}(\bar{r})$ could be obtained by a maximisation over one dimension. In our model, we have to maximise over the three dimensions of the mutational distance space to obtain \bar{r} and \hat{x} , whereas the population mean genotype \bar{x} cannot be derived as easily, because the fitness function

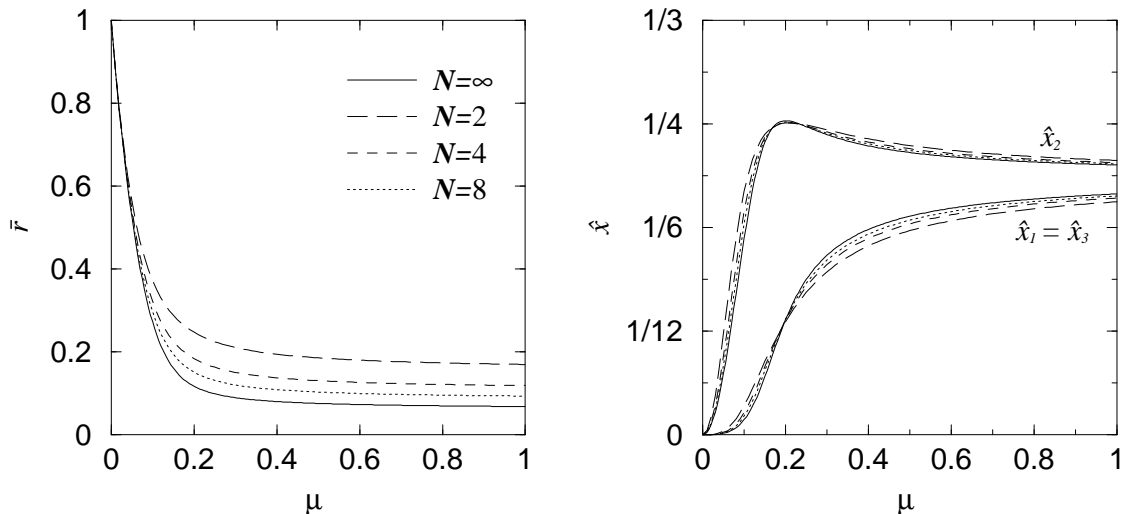


Figure 6: The quantities \bar{r} and \hat{x}_k for varying mutation rate $\mu := \mu_1 = \mu_3$ for a model with K2P mutation scheme, with $\mu_2 = 10\mu$, and quadratic fitness $r = (1 - \sum_{k=1}^3 x_k)^2$. The curves for finite sequence lengths $N = 2, 4, 8$ are obtained by direct diagonalisation of the time-evolution operator, the curve for $N = \infty$ is the result of the maximum principle.

is not uniquely invertible in three dimensions.

Other quantities of interest are the corresponding variances. The expressions for the variance of the fitness given by Hermisson *et al.* (2002) can only be generalised to our model in the linear case, not for the case of infinite sequence length. Neither can the variance of mutational distance be obtained in a simply way, because this involves inversion of the fitness function, which does not have a unique solution in more than one dimension.

Although our setup is motivated by a model for DNA evolution, it is valid for a system where the fitness depends on three arbitrary traits d_1 , d_2 and d_3 with a single-step mutation model, as long as the mutation matrix describes a reversible process, i.e., $M_{ij}q_j = M_{ji}q_i$ with \mathbf{q} being the equilibrium distribution of the mutation process without selection. In fact, the maximum principle can be generalised to an n -state model, where we have $n - 1$ different traits determining the fitness. In this case, we have to maximise over these $n - 1$ quantities.

Similarly, the restriction to permutation-invariant fitness functions could be dropped. In this case, however, we would have to maximise over the N sites of the sequence, so that it loses its use, which lies primarily in the simplicity.

A Proof of the maximum principle for the linear model

Starting with the eigenvalue equation of the symmetrised time-evolution operator $\tilde{\mathbf{H}}$ (17)

$$\begin{aligned} \bar{r}\sqrt{a_{\mathbf{d}}} &= \left(r_{\mathbf{d}} - \sum_{\xi} (u_{\mathbf{d}}^{+\xi} + u_{\mathbf{d}}^{-\xi})\right)\sqrt{a_{\mathbf{d}}} + \sum_k \left(\sqrt{u_{\mathbf{d}-e_k}^{+k} u_{\mathbf{d}}^{-k}} \sqrt{a_{\mathbf{d}-e_k}} + \sqrt{u_{\mathbf{d}+e_k}^{-k} u_{\mathbf{d}}^{+k}} \sqrt{a_{\mathbf{d}+e_k}}\right) \\ &+ \sum_{\substack{k,l \\ k \neq l}} \sqrt{u_{\mathbf{d}-e_k+e_l}^{+k-l} u_{\mathbf{d}}^{-k+l}} \sqrt{a_{\mathbf{d}-e_k+e_l}}, \end{aligned} \quad (23)$$

we make an ansatz for the $a_{\mathbf{d}}$ such that

$$\frac{a_{\mathbf{d}-e_k}}{a_{\mathbf{d}}} = C_k \frac{u_{\mathbf{d}}^{-k}}{u_{\mathbf{d}-e_k}^{+k}}. \quad (24)$$

This is equivalent to

$$\frac{a_{\mathbf{d}+e_k}}{a_{\mathbf{d}}} = \frac{1}{C_k} \frac{u_{\mathbf{d}}^{+k}}{u_{\mathbf{d}+e_k}^{-k}} \quad \text{and} \quad \frac{a_{\mathbf{d}-e_k+e_l}}{a_{\mathbf{d}}} = \frac{C_k}{C_l} \frac{u_{\mathbf{d}}^{-k+l}}{u_{\mathbf{d}-e_k+e_l}^{+k-l}}. \quad (25)$$

The latter can be seen using the condition for reversibility (14). To determine the constants C_k , we multiply Eq. (24) by its denominators and sum over all \mathbf{d} , which yields the ancestral means of the mutation rates

$$\sum_{\mathbf{d}} u_{\mathbf{d}-e_k}^{+k} a_{\mathbf{d}-e_k} = C_k \sum_{\mathbf{d}} u_{\mathbf{d}}^{-k} a_{\mathbf{d}} \iff C_k = \frac{\widehat{u}^{+k}}{\widehat{u}^{-k}}. \quad (26)$$

Now, we divide Eq. (23) by $\sqrt{a_{\mathbf{d}}}$, and insert the ansatz (24) and (25),

$$\bar{r} = r_{\mathbf{d}} - \sum_{\xi} (u_{\mathbf{d}}^{+\xi} + u_{\mathbf{d}}^{-\xi}) + \sum_k \left(\sqrt{C_k} u_{\mathbf{d}}^{-k} + \sqrt{\frac{1}{C_k}} u_{\mathbf{d}}^{+k} \right) + \sum_{\substack{k,l \\ k \neq l}} \sqrt{\frac{C_k}{C_l}} u_{\mathbf{d}}^{-k+l}. \quad (27)$$

Multiplication by $a_{\mathbf{d}}$ and summation over all \mathbf{d} yields, using the explicit form (26) of the C_k ,

$$\begin{aligned} \bar{r} &= \hat{r} - \sum_{\xi} (\widehat{u}^{+\xi} + \widehat{u}^{-\xi}) + 2 \sum_k \sqrt{\widehat{u}^{+k} \widehat{u}^{-k}} + \sum_{\substack{k,l \\ k \neq l}} \sqrt{\widehat{u}^{+k-l} \widehat{u}^{-k+l}} \\ &= \hat{r} - \sum_{\xi} \left(\widehat{u}^{+\xi} + \widehat{u}^{-\xi} - 2\sqrt{\widehat{u}^{+\xi} \widehat{u}^{-\xi}} \right). \end{aligned} \quad (28)$$

So far, we did not use linearity. If r and u depend linearly on some functions $y_k(x_k)$, we have $\hat{r} = r(\hat{\mathbf{y}})$ and $\widehat{u}^{\pm\xi} = u^{\pm\xi}(\hat{\mathbf{y}})$. With the definition of the mutational loss function (19) and in the case of $y_k(x_k) = x_k$, this is Eq. (20).

In order to obtain the supremum condition (18), we consider Eq. (27) for two different sequences \mathbf{d} and \mathbf{d}' and take the difference, using the explicit representation of fitness and mutation functions given in Eq. (22),

$$\begin{aligned} 0 &= \sum_m \left(-\alpha_m - \sum_{\xi} (\beta_m^{+\xi} + \beta_m^{-\xi}) + \sum_k \left(\sqrt{\frac{\widehat{u}^{+k}}{\widehat{u}^{-k}}} \beta_m^{-k} + \sqrt{\frac{\widehat{u}^{-k}}{\widehat{u}^{+k}}} \beta_m^{+k} \right) \right. \\ &\quad \left. + \sum_{\substack{k,l \\ k \neq l}} \sqrt{\frac{\widehat{u}^{+k-l}}{\widehat{u}^{-k+l}}} \beta_m^{-k+l} \right) (y_m(x_m) - y_m(x'_m)). \end{aligned} \quad (29)$$

This is just the condition

$$0 = \sum_m \frac{\partial}{\partial y_m} [r(\mathbf{y}) - g(\mathbf{y})]_{\mathbf{y}=\hat{\mathbf{y}}} (y_m(x_m) - y_m(x'_m)), \quad (30)$$

which has to be fulfilled for arbitrary \mathbf{x} and \mathbf{x}' . Hence, we have

$$0 = \frac{\partial}{\partial y_m} [r(\mathbf{y}) - g(\mathbf{y})]_{\mathbf{y}=\hat{\mathbf{y}}} \quad \text{for } m \in \{1, 2, 3\}. \quad (31)$$

This is a necessary condition for the existence of an extremum at $\hat{\mathbf{y}}$. A sufficient condition for the existence of a maximum of the function $r - g$ in $\hat{\mathbf{y}}$ is that the Hessian

$$\mathcal{H}_{mn}(\hat{\mathbf{y}}) := \left[\frac{\partial^2 (r(\mathbf{y}) - g(\mathbf{y}))}{\partial y_m \partial y_n} \right]_{\mathbf{y}=\hat{\mathbf{y}}} \quad (32)$$

of the second derivatives in the point $\hat{\mathbf{y}}$ is a negative definite matrix. We have

$$\mathcal{H}(\mathbf{x}) = -\sum_z c_z(\mathbf{x}) \mathbf{U}_z(\mathbf{x}) \mathbf{U}_z^t(\mathbf{x}), \quad (33)$$

with $c_z(\mathbf{x}) = \frac{1}{2} (u^{+z}(\mathbf{x}) u^{-z}(\mathbf{x}))^{-3/2}$, $\mathbf{U}_z^t(\mathbf{x}) = (U_{z,1}(\mathbf{x}), U_{z,2}(\mathbf{x}), U_{z,3}(\mathbf{x}))$, and $U_{z,m}(\mathbf{x}) = \beta_m^{+z} u^{-z}(\mathbf{x}) - \beta_m^{-z} u^{+z}(\mathbf{x})$. To test the Hessian for negative definiteness, we evaluate the quadratic form for an arbitrary vector \mathbf{w} . We have

$$\mathbf{w}^t \mathcal{H} \mathbf{w} = -\sum_z c_z(\mathbf{x}) (w_1 U_{z,1}(\mathbf{x}) + w_2 U_{z,2}(\mathbf{x}) + w_3 U_{z,3}(\mathbf{x}))^2, \quad (34)$$

which is ≤ 0 for all \mathbf{w} , and generically negative unless all terms in the sum vanish.

Hence, there is a maximum at $\hat{\mathbf{y}}$, and, together with Eq. (28), we have

$$\bar{r} = \sup_{\mathbf{x}} [r(\mathbf{y}(\mathbf{x})) - g(\mathbf{y}(\mathbf{x}))] = r(\hat{\mathbf{y}}) - g(\hat{\mathbf{y}}). \quad (35)$$

In the case $y_k(x_k) = x_k$, this is the maximum principle as stated in Eq. (18).

B Proof of the maximum principle for infinite sequence length

The proof of the maximum principle in the case $N \rightarrow \infty$ closely follows the corresponding proof in the two-state model, compare Hermisson *et al.* (2002). The idea is to establish upper and lower bounds for a system with finite N , which can be shown to converge towards each other in the limit as $N \rightarrow \infty$.

In order to obtain a lower bound, we look at the system locally. To be specific, we consider a volume V_{s,\mathbf{d}_0} in the mutational distance space around \mathbf{d}_0 , containing the sequences that can be reached from sequence \mathbf{d}_0 in at most s mutational steps. If this volume intersects a region where r has a jump, we take as V_{s,\mathbf{d}_0} only that part containing \mathbf{d}_0 where r is continuous. The number of sequences contained in V_{s,\mathbf{d}_0} is denoted by $n(V_{s,\mathbf{d}_0})$.

The part of the time-evolution operator associated with this volume consists of those matrix elements where both the row and the column index correspond to sequences in V_{s,\mathbf{d}_0} . This $n(V_{s,\mathbf{d}_0}) \times n(V_{s,\mathbf{d}_0})$ -dimensional submatrix describes a system with an effective mutational outflow, and hence the local growth rate is lower than the global growth rate. Thus, the largest eigenvalue \bar{r}_{s,\mathbf{d}_0} of this submatrix yields a lower bound for the largest eigenvalue \bar{r}_N of the whole (but still finite) system.

In order to obtain estimates for \bar{r}_{s,\mathbf{d}_0} , we use the symmetrised system described by $\widetilde{\mathbf{H}}$. This can be done because the eigenvalues of the corresponding submatrices are the same.

We evaluate Rayleigh's principle for the quadratic form for the vector $\mathbf{y}^t = (1, 1, \dots, 1)$ and get as lower bound for the largest eigenvalue of the whole system

$$\bar{r}_N \geq \bar{r}_{s,\mathbf{d}_0} = \sup_{\mathbf{y}} \frac{\mathbf{y}^t \mathbf{H} \mathbf{y}}{\mathbf{y}^t \mathbf{y}} \geq \frac{\sum_{ij} (\widetilde{\mathbf{H}}_{s,\mathbf{d}_0})_{ij}}{n(V_{s,\mathbf{d}_0})}. \quad (36)$$

To write this more explicitly, but in a compact way, we introduce the function

$$g_{N,\mathbf{d}} = \sum_{\xi} \left(u_{\mathbf{d}}^{+\xi} + u_{\mathbf{d}}^{-\xi} - \sqrt{u_{\mathbf{d}-e_{\xi}}^{+\xi} u_{\mathbf{d}}^{-\xi}} - \sqrt{u_{\mathbf{d}+e_{\xi}}^{-\xi} u_{\mathbf{d}}^{+\xi}} \right), \quad (37)$$

which sums over all mutational terms in the row labelled by \mathbf{d} of the full matrix $\widetilde{\mathbf{H}}$. Using this, we get as a lower bound

$$\bar{r}_N \geq \bar{r}_{s,\mathbf{d}_0} \geq \frac{1}{n(V_{s,\mathbf{d}_0})} \left(\sum_{\mathbf{d} \in V_{s,\mathbf{d}_0}} (r_{\mathbf{d}} - g_{N,\mathbf{d}}) + \sum (\text{boundary terms}) \right). \quad (38)$$

The boundary terms are the terms describing the mutational flow through the surface S_{s, \mathbf{d}_0} of the volume V_{s, \mathbf{d}_0} into V_{s, \mathbf{d}_0} from the outside, which are contained in the full $\widetilde{\mathbf{H}}$, but not in $\widetilde{\mathbf{H}}_{s, \mathbf{d}_0}$. They are of the form $\sqrt{u_{\mathbf{d}-\mathbf{e}_\xi}^{+\xi} u_{\mathbf{d}}^{-\xi}}$ with $\mathbf{d} \in V_{s, \mathbf{d}_0}$ and $\mathbf{d} + \mathbf{e}_\xi \notin V_{s, \mathbf{d}_0}$.

To perform the limit $N \rightarrow \infty$ as described in section 5.3, let $r_{\mathbf{d}} = r(\mathbf{x}_{\mathbf{d}})$ and $u_{\mathbf{d}}^{\pm\xi} = u^{\pm\xi}(\mathbf{x}_{\mathbf{d}})$ be given as continuous functions, and analogously, $g_{N, \mathbf{d}} = g_N(\mathbf{x}_{\mathbf{d}})$. The size of V_{s, \mathbf{d}_0} shall be scaled such that $s_N \sim \sqrt{N}$. With increasing N , the mutational distances \mathbf{x} of neighbouring sequences approach each other, and so do the values of the functions r , $u^{\pm\xi}$ and g_N as they are continuous functions. More precisely, the total Hamming distance $\sum_{k=1}^3 |d_k - d'_k|$ between any two sequences $\mathbf{d}, \mathbf{d}' \in V_{s, \mathbf{d}_0}$ is at most $2s$. For the mutational distances we then have $\mathbf{x}_{\mathbf{d}} - \mathbf{x}_{\mathbf{d}'} = \frac{\mathbf{d} - \mathbf{d}'}{N} \rightarrow 0$ with increasing N because $\sum_k \frac{|d_k - d'_k|}{N} \leq \frac{2s}{N} \sim N^{-1/2} \rightarrow 0$. For every \mathbf{x} in the mutational distance space, we can choose a suitable sequence $(\mathbf{d}_N) = (\mathbf{d}_N(x))$ such that $\mathbf{x}_{\mathbf{d}_N} \rightarrow \mathbf{x}$. For any distance \mathbf{d}' , such that $\mathbf{d}_N - \mathbf{d}'$ lies in V_{s_N, \mathbf{d}_N} , we then have $\lim_{N \rightarrow \infty} [r(\mathbf{x}_{\mathbf{d}_N + \mathbf{d}'}) - g_N(\mathbf{x}_{\mathbf{d}_N + \mathbf{d}'})] = r(\mathbf{x}) - g(\mathbf{x})$.

On the other hand, the number of sequences in the volume V_{s_N, \mathbf{d}_0} increases with $N^{3/2}$, whereas the number of sequences in the surface S_{s_N, \mathbf{d}_0} of the volume, and likewise the number of surface terms in Eq. (39), only increases with N . Therefore, we get $\lim_{N \rightarrow \infty} \bar{r}_{s_N, \mathbf{d}_N} \geq r(\mathbf{x}) - g(\mathbf{x})$ for arbitrary \mathbf{x} .

For an upper bound, we consider a global maximum of the ancestral distribution, i.e., a \mathbf{d}^+ such that $a_{\mathbf{d}^+} \geq a_{\mathbf{d}}$ for all \mathbf{d} . An evaluation of Eq. (23) for \mathbf{d}^+ and $\sqrt{a_{\mathbf{d}'}} \leq \sqrt{a_{\mathbf{d}^+}}$ yields

$$\bar{r}_N \leq r_{\mathbf{d}^+} - g_{N, \mathbf{d}^+} \leq \sup_{\mathbf{d}} (r_{\mathbf{d}} - g_{N, \mathbf{d}}). \quad (39)$$

Performing the limit in the same way as above, we get $r_{\mathbf{d}^+} - g_{N, \mathbf{d}^+} \rightarrow r(\mathbf{x}^+) - g(\mathbf{x}^+)$, and combining this with the lower bound, we have

$$\sup_{\mathbf{x}} [r(\mathbf{x}) - g(\mathbf{x})] \leq \bar{r}_\infty \leq r(\mathbf{x}^+) - g(\mathbf{x}^+) \leq \sup_{\mathbf{x}} [r(\mathbf{x}) - g(\mathbf{x})], \quad (40)$$

which proves Eq. (18).

Now, it remains to be shown that the ancestral distribution is peaked around \mathbf{x}^+ , and that indeed $\mathbf{x}^+ = \hat{\mathbf{x}}$ as well as $\hat{r} = r(\hat{\mathbf{x}})$. For this, we start again with the eigenvalue equation in ancestral form (23), multiply by $\sqrt{a_{\mathbf{d}}}$ and sum over the mutational distance space

$$\begin{aligned} \bar{r}_N = & \sum_{\mathbf{d}} \left[\left(r_{\mathbf{d}} - \sum_{\xi} (u_{\mathbf{d}}^{+\xi} + u_{\mathbf{d}}^{-\xi}) \right) a_{\mathbf{d}} \right. \\ & \left. + \sum_{\xi} \left(\sqrt{u_{\mathbf{d}-\mathbf{e}_\xi}^{+\xi} u_{\mathbf{d}}^{-\xi}} \sqrt{a_{\mathbf{d}-\mathbf{e}_\xi} a_{\mathbf{d}}} + \sqrt{u_{\mathbf{d}+\mathbf{e}_\xi}^{-\xi} u_{\mathbf{d}}^{+\xi}} \sqrt{a_{\mathbf{d}+\mathbf{e}_\xi} a_{\mathbf{d}}} \right) \right]. \end{aligned} \quad (41)$$

Using $\sqrt{a_{\mathbf{d} \pm \mathbf{e}_\xi} a_{\mathbf{d}}} \leq \frac{1}{2} (a_{\mathbf{d} \pm \mathbf{e}_\xi} + a_{\mathbf{d}})$, we get

$$\bar{r}_N \leq \sum_{\mathbf{d}} (r_{\mathbf{d}} - g_{N, \mathbf{d}}) a_{\mathbf{d}}. \quad (42)$$

As $\bar{r}_N \rightarrow \bar{r}_\infty$ and $g_{N, \mathbf{d}} \rightarrow g(x_{\mathbf{d}})$ uniformly, for every $\epsilon > 0$ we can find an N_ϵ such that for every $N > N_\epsilon$

$$\bar{r}_\infty - \epsilon^2 < \sum_{\mathbf{d}} (r(x_{\mathbf{d}}) - g(x_{\mathbf{d}})) a_{\mathbf{d}}. \quad (43)$$

Due to Eq. (40), we have $r(x_{\mathbf{d}}) - g(x_{\mathbf{d}}) \leq \bar{r}_\infty$. Splitting the sum into two parts, $\sum_{\mathbf{d}_>} + \sum_{\mathbf{d}_\leq}$ with $r(x_{\mathbf{d}_>}) - g(x_{\mathbf{d}_>}) > \bar{r}_\infty - \epsilon$ and $r(x_{\mathbf{d}_\leq}) - g(x_{\mathbf{d}_\leq}) \leq \bar{r}_\infty - \epsilon$, yields

$$\bar{r}_\infty - \epsilon^2 < \bar{r}_\infty \sum_{\mathbf{d}_>} a_{\mathbf{d}_>} + (\bar{r}_\infty - \epsilon) \sum_{\mathbf{d}_\leq} a_{\mathbf{d}_\leq} = \bar{r}_\infty - \epsilon \sum_{\mathbf{d}_\leq} a_{\mathbf{d}_\leq}. \quad (44)$$

Thus, we have $\sum_{\mathbf{d}_{\leq}} a_{\mathbf{d}_{\leq}} < \epsilon$, which means that for N sufficiently large, only sequences with $r(\mathbf{x}) - g(\mathbf{x})$ arbitrarily close to its maximum \mathbf{x}^+ contribute to ancestral means. Thus, in the generic case that the maximum is unique, the ancestral distribution is peaked around $\mathbf{x}^+ = \hat{\mathbf{x}}$, and thus $\hat{r} = r(\hat{\mathbf{x}})$, which implies Eq. (20).

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