Adaptive evolution on neutral networks

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Abstract
We study the evolution of large but finite asexual populations evolving in fitness landscapes in which all mutations are either neutral or strongly deleterious. We demonstrate that despite the absence of higher fitness genotypes, adaptation takes place as regions with more advantageous distributions of neutral genotypes are discovered. Since these discoveries are typically rare events, the population dynamics can be subdivided into separate epochs, with rapid transitions between them. Within one epoch, the average fitness in the population is approximately constant. The transitions between epochs, however, are generally accompanied by a significant increase in the average fitness. We verify our theoretical considerations with two analytically tractable bitstring models.

1. Introduction
Sudden bursts of adaptive activity which punctuate long periods of stagnancy seem to be a common observation in evolving systems. Such epochal evolution [van Nimwegen et al., 1997], has been found in the fossil record [Eldredge and Gould, 1972, Gould and Eldredge, 1977], in evolving bacteria [Lenski and Travisano, 1994, Elena et al., 1996], the evolution of tRNA structures [Huynen et al., 1996, Fontana and Schuster, 1998] or artificial systems such as digital organisms [Adami, 1995] and evolutionary optimization [Vose and Liepins, 1991, van Nimwegen et al., 1997]. The most complete theoretical analysis of epochal evolution has probably been presented in a series of papers by van Nimwegen and coworkers [van Nimwegen et al., 1997, 1999b, van Nimwegen and Crutchfield, 2000a,b]. The general picture is as follows. A population can easily climb to the nearest local optimum, but escape from there only with difficulty. Once a local optimum has been reached, the population is trapped and experiences a metastable equilibrium. With a relatively low probability, the population can discover a portal genotype leading to the next local optimum, i.e., a genotype
with a higher fitness than what is currently present in the population. Once a portal is discovered, the population moves quickly away from its current peak, and towards the new peak. There, it settles down again in equilibrium, until the next portal is discovered.

The above description focuses on the dynamics between local optima, but not on the dynamics at one local optimum. However, in the presence of neutrality, i.e., when a number of genotypes share the same identical replication rate, the dynamics around one local optimum can be quite intriguing. A population does not drift over a set of neutral genotypes, like a set of random walkers, but has a quite different, even to some extent deterministic dynamics. In a completely flat fitness landscape, for example, a population does not assume a Gaussian distribution with a variance that increases over time, as would be expected from a simple diffusion process. Rather, the population stays clustered together and the cluster moves about as a whole [Derrida and Peliti, 1991]. If neutral and deleterious genotypes are mixed, i.e., if each genotype has as direct neighbors in genotype space both neutral and deleterious genotypes, then the population moves to the regions of the most connected neutral genotypes, as long as the mutation rate is small [van Nimwegen et al., 1999a].

In this paper, we study the dynamics of a finite but large population of asexually replicating genetic sequences on a fitness landscape that contains both neutral and strongly deleterious genotypes. We show that the neutral genotypes naturally decompose into disjunct sets, and that an evolving population can be trapped within such sets. Moreover, different sets yield different reproductive success for the populations residing on them. When a population discovers a set of genotypes with higher reproductive success, the population moves over to that set. For the average fitness of the population, such transitions are reflected in a stepwise increase, exactly as it is observed in standard scenarios of epochal evolution. However, the increase observed here is not due to the discovery of faster replicating genotypes, but solely to the discovery of genotypes with increased robustness against mutations. Our results are valid for arbitrary mutation rates, and they generalize the previous findings of van Nimwegen et al. [1999a].

2. Theory

We assume that there exist two classes of genotypes, those with a relatively high replication rate $\sigma$, and the ones with a much lower replication rate. For reasons of simplicity, we assume the latter to have replication rate 0. This assumption is quite common in the literature [Gavrilets, 1997, 1999, van Nimwegen et al., 1999a]. Our analysis is similar to the standard treatment of the quasispecies model (see for example Schuster and Swetina [1988], Eigen et al. [1988, 1989], Wilke et al. [2001]) and to the work of van Nimwegen et al. [1999b,a]. Let the vector $a$ define the set of neutral genotypes, i.e., the genotypes with replication
rate $\sigma$:

$$a_i = \begin{cases} 
1 & \text{if } i \text{ has replication rate } \sigma, \\
0 & \text{else},
\end{cases}$$  \hspace{1cm} (1)

where $i$ runs over all possible genotypes. We assume a discrete time model, and write the average population fitness as $\langle f \rangle$. In equilibrium, we have [van Nimwegen et al., 1999b]

$$x = \frac{\sigma}{\langle f \rangle} Q A x ,$$  \hspace{1cm} (2)

where $x$ is the vector of concentrations, the diagonal matrix $A = \text{diag}(a_0, a_1, \ldots)$ contains the set of neutral genotypes, and the matrix $Q$ defines the mutation probabilities between different genotypes, i.e., genotype $j$ mutates into genotype $i$ with probability $Q_{ij}$.

In the following, we assume that the genotypes can be represented as sequences of length $l$ over an alphabet of $A$ different symbols. Moreover, we assume a uniform copy fidelity $q$ per symbol. That means, the $l$ symbols in a sequence mutate independently from each other, and the substitution probability in one generation is $1 - q$ for each symbol. With this assumption, the mutation matrix can be written as [Swetina and Schuster, 1982]

$$Q_{ij} = q^l \left( \frac{1 - q}{q(A - 1)} \right)^{d(i,j)} ,$$  \hspace{1cm} (3)

where $d(i,j)$ is the Hamming distance between sequences $i$ and $j$. It is useful to introduce the reduced mutation rate $\tilde{\mu}$,

$$\tilde{\mu} = \frac{1 - q}{q(A - 1)} ,$$  \hspace{1cm} (4)

which allows us to write $Q$ as a sum of matrices,

$$Q = q^l \sum_{k=0}^{l} \tilde{\mu}^k D^{(k)} .$$  \hspace{1cm} (5)

The matrices $D^{(k)}$ define the connection graphs at Hamming distance $k$ in sequence space, i.e.,

$$D^{(k)}_{ij} = \begin{cases} 
1 & \text{if } d(i,j) = k, \\
0 & \text{else.}
\end{cases}$$  \hspace{1cm} (6)

We insert (5) into (2), and obtain

$$x = \frac{\sigma q^l}{\langle f \rangle} \sum_{k=0}^{l} \tilde{\mu}^k D^{(k)} A x .$$  \hspace{1cm} (7)
It is useful to introduce the matrices

\[ G^{(k)} = AD^{(k)} A. \]  

(8)

These matrices define the connection graphs at Hamming distance \( k \) for the neutral genotypes. We now disregard all non-neutral sequences, and introduce the concentration vector \( p \), which holds the concentrations of all neutral sequences. The total number of neutral sequences is then \( P = \sum_i p_i \). Moreover, we assume all columns and rows corresponding to non-neutral sequences to be deleted from the matrices \( G^{(k)} \). From equation (7), we obtain the eigenvalue equation

\[
\left( \frac{\langle f \rangle}{\sigma q^l} - 1 \right) p = \left( \sum_{k=1}^l \tilde{\mu}^k G^{(k)} \right) p. 
\]  

(9)

Consequently, the equilibrium state of the population is fully determined by the matrix

\[
G = \sum_{k=1}^l \tilde{\mu}^{k-1} G^{(k)}
\]

\[
= G^{(1)} + \tilde{\mu} G^{(2)} + \tilde{\mu}^2 G^{(3)} \ldots
\]  

(10)

In the following, we will call matrices such as \( G \) generalized connection matrices. The difference to the normal connection matrices \( G^{(k)} \) as defined in equation (8) is that generalized connection matrices may contain powers of \( \tilde{\mu} \), i.e., they define a connection graph with weighted edges, whereas for the graphs defined by \( G^{(k)} \), all edges have the same weight.

We can obtain further insight from relating the average population fitness \( \langle f \rangle \) to the average fraction of neutral offspring, \( \langle \nu \rangle \). Under the assumption that the non-neutral sequences have a vanishing replication rate (this assumption is equivalent to neglecting back mutations), we have [van Nimwegen et al., 1999a]

\[
\langle \nu \rangle = \frac{\langle f \rangle}{\sigma}.
\]  

(11)

The probability \( \nu_i \) for a single sequence \( i \) to have a neutral genotype as offspring is given by

\[
\nu_i = \sum_j a_j Q_{ji} 
\]

\[
= q^l \sum_{k=0}^l \tilde{\mu}^k \sum_j a_j D^{(k)}_{ji}
\]

\[
= q^l \left( 1 + \sum_{k=1}^l \tilde{\mu}^k d^{(k)}_i \right).
\]  

(12)
where $d_i^{(k)}$ gives the number of neutral neighbors at Hamming distance $k$ of sequence $i$. When we take the average over all viable sequences in the population on both sides of equation (12), we arrive at

$$
\langle \nu \rangle = q^l \left( 1 + \sum_{k=1}^{l} \tilde{\mu}^k \langle d^{(k)} \rangle \right).
$$

(13)

The quantities $\langle d^{(k)} \rangle$ give the average number of neutral neighbors at Hamming distance $k$ in the population. They can be expressed as

$$
\langle d^{(k)} \rangle = \frac{1}{P} \sum_i p_i \sum_j G^{(k)}_{ij}.
$$

(14)

With equation (11), we can rewrite equation (13) as

$$
\sum_{k=1}^{l} \tilde{\mu}^k \langle d^{(k)} \rangle = \frac{\langle f \rangle}{\sigma q^l} - 1.
$$

(15)

We notice that the right-hand-side of (15) is identical to the factor in front of $p$ on the left-hand-side of (9). Therefore, we arrive at

$$
\left( \sum_{k=1}^{l} \tilde{\mu}^{k-1} \langle d^{(k)} \rangle \right) p = \left( \sum_{k=1}^{l} \tilde{\mu}^{k-1} G^{(k)} \right) p.
$$

(16)

In the limit of a small mutation rate, (16) becomes

$$
\langle d^{(1)} \rangle p = G^{(1)} p.
$$

(17)

In that case, the equilibrium distribution $p$ depends solely on the one-mutant connections of the neutral sequences, and the population neutrality $\langle d^{(1)} \rangle$ is given by the spectral radius of the first-order connection matrix. Equation (17) corresponds to the result of van Nimwegen et al. [1999a]. Thus, we find that equation (16) is the generalization of that result to arbitrary mutation rates. For larger mutation rates, the equilibrium distribution is influenced by the higher order connection matrices, and the population neutrality may deviate from the spectral radius of $G^{(1)}$.

### 2.1. Separate neutral networks

Following the conventional nomenclature in the literature [Forst et al., 1995], we call a set of neutral sequences that can be transversed by one-point mutations a neutral network. The full set of neutral sequences in genotype space will in general decompose into several disjunct such neutral networks. Assume there
are \( n \) disjunct neutral networks. By reordering sequences, we can arrange \( G \) in block-matrix form

\[
G = \begin{pmatrix}
G_1 & \mathcal{O}(\tilde{\mu}) \\
\mathcal{O}(\tilde{\mu}) & G_3 & \ddots \\
& & & \ddots & G_n
\end{pmatrix},
\]

where the matrices \( G_i \) are the generalized connection matrices for the different neutral networks, and all off-diagonal terms are at least of the order of \( \tilde{\mu} \). Let us further assume that the matrices \( G_i \) are ordered with descending spectral radius, i.e., the spectral radius of \( G_i \) is larger than the one of \( G_{i+1} \) for all \( i \).

For a finite population initially residing on network \( n \), we can then expect the following dynamics. If the mutation rate is small, the population will equilibrate within network \( n \). The discovery of a sequence that is part of a neutral network \( i < n \) is very unlikely, due to the small off-diagonal terms. However, eventually such a sequence will be discovered. Since a progeny of that sequence has a smaller probability to fall off its neutral network (\( G_i \) has a larger spectral radius than \( G_n \)), the sequences on network \( i \) will have a higher reproductive success than the sequences on network \( n \), and the population will move over to the newly discovered network. There, the population will equilibrate, until the next higher-connected network is discovered. If the mutation rate is large, on the other hand, the off-diagonal terms cannot be considered small anymore. In that case, the discovery of higher connected regions is much more likely, and a population will move straight away into the more densely connected regions of the genotype space. In Sec. 4, we present examples for both of these behaviors.

The above considerations show that the decomposition of the full set of neutral sequences into neutral networks according to the definition given at the beginning of this subsection is somewhat arbitrary. Depending on the mutation rate, it might be justified, for example, to disregard contributions of order \( \mathcal{O}(\tilde{\mu}^2) \) to the matrix \( G \), but not the ones of order \( \mathcal{O}(\tilde{\mu}) \). In that case, it would be more natural to group the sequences into sets that can be transversed by a combination of one- or two-point mutations. In general, the neutral sequences should therefore be subdivided into sets such that two arbitrary sequences of two disjunct sets are at least a Hamming distance \( k \) apart, where \( k \) represents the smallest number of simultaneous mutations that can be considered rare at the respective mutation rate. For the remainder of this paper, we will understand the term neutral network in this more general sense.

### 3. A simple exactly solvable landscape

In this section, we study a simple example landscape for which the matrix \( G \) can be diagonalized exactly, to all orders in \( \tilde{\mu} \). We consider binary sequences
of length \( l = 2n \), and break them down into \( n \) pairs of bits. For each pair, we assume that there are three states (00, 01, 10) which are neutral, and one state (11) which is lethal. Therefore, a sequence which contains at least one pair for which both bits are set to 1 has a fitness of 0, and all other sequences have a fitness of \( \sigma \). For simplicity, we set \( \sigma = 1 \). We will refer to this landscape as the **Neutral Bitpairs** landscape.

For a single pair, the matrix \( G_1 \) reads (in this section, the subscript \( i \) in \( G_i \) indicates the number of pairs we are considering)

\[
G_1 = \begin{pmatrix}
0 & 1 & 1 \\
1 & 0 & \tilde{\mu} \\
1 & \tilde{\mu} & 0
\end{pmatrix},
\]

and its largest eigenvalue is \( \lambda_1 = (\tilde{\mu} + \sqrt{8 + \tilde{\mu}^2})/2 \). For a sequence with 2 pairs, the corresponding matrix \( G_2 \) can be written as a tensor product (see Rumschitzki [1987], Dress and Rumschitzki [1988], Wilke [1999]),

\[
G_2 = \tilde{\mu}^{-1} [(1 + \tilde{\mu}G_1) \otimes (1 + \tilde{\mu}G_1) - 1].
\]

The symbol 1 stands for the identity matrix in the appropriate matrix space. In general, for a sequence consisting of \( n \) pairs, we can define the matrix \( G_n \) recursively,

\[
G_n = \tilde{\mu}^{-1} [(1 + \tilde{\mu}G_1) \otimes (1 + \tilde{\mu}G_{n-1}) - 1].
\]

As a consequence, the largest eigenvalue of \( G_n \) reads

\[
\lambda_n = \tilde{\mu}^{-1}[(1 + \tilde{\mu}\lambda_1)^n - 1],
\]

\[
= \tilde{\mu}^{-1}[(1 + \frac{\tilde{\mu}^2}{2} + \frac{\tilde{\mu}}{2}\sqrt{8 + \tilde{\mu}^2})^n - 1].
\]

The average population fitness in this landscape follows from equation (9). We obtain

\[
\langle f \rangle = q^{2n}(1 + \frac{\tilde{\mu}^2}{2} + \frac{\tilde{\mu}}{2}\sqrt{8 + \tilde{\mu}^2})^n.
\]

For small mutation rates, we can write the average fitness as

\[
\langle f \rangle = q^{2n}(1 + \sqrt{2n}\tilde{\mu}),
\]

and the average population neutrality becomes

\[
\langle d^{(1)} \rangle = \sqrt{2n}.
\]

Let us now compare the full solution for the average population fitness to the approximation given by van Nimwegen et al. [1999a],

\[
\langle f \rangle = 1 - \mu \left(1 - \frac{\langle d^{(1)} \rangle}{l(A - 1)}\right),
\]

where

\[
l = 2n, \quad A = \frac{(1 + \sqrt{8 + \tilde{\mu}^2})}{2}, \quad \tilde{\mu} = 2n.\]
Figure 1: a: Average fitness as a function of the mutation rate in a Neutral Bitpairs landscape with $n = 10$. The solid line represents equation (23), the dotted line is the approximation equation (26) with $\langle d^{(1)} \rangle$ given by equation (25), and the points stem from simulations with a population of size $N = 10000$. b: Average population neutrality $\langle d^{(1)} \rangle$ as a function of the mutation rate for the simulations from graph a. The solid line indicates the spectral radius of the first-order connection matrix $G^{(1)}$, and the dotted line gives the network neutrality $\nu = \frac{2}{3} l$.

where $\mu$ is the genomic mutation rate,

$$\mu = l (1 - q).$$

Equation (26) follows from equation (24) if we disregard all terms of the order $O((1-q)^2)$ or higher. Figure 1a shows the exact solution for the average fitness $\langle f \rangle$ equation (23) and the approximation equation (26) as a function of the genomic mutation rate $\mu$. For comparison, we have also displayed results from numerical simulations with a genetic algorithm. As was to be expected, the approximation works well for $\mu \ll 1$, but breaks down for higher mutation rates. For $\mu \gtrsim 1$, the approximation significantly underestimates the average population fitness. Interestingly, this goes along with a decrease in the population neutrality (Fig. 1b). What happens is the following. In general, a population moves to that region of the genotype space where the probability of neutral offspring is maximized for the given mutation rate. Clearly, when the mutation rate is low, only the immediate neighbors influence that probability. If, however, the mutation rate is high, such that offspring with two or even more mutations become common, the immediate neighbors lose their importance. In the extreme case of a per symbol copy fidelity of $q = 0.5$, the probability of giving birth to a viable sequence becomes identical for all sequences. Hence, in that extreme we can expect the population neutrality to coincide with the network neutrality $\nu$ (the network neutrality is the average number of neutral neighbors of a viable sequence), which amounts to $\nu = \frac{2}{3} l$ in our case. At the same time, most of the average population fitness does not stem from the one-mutants anymore, but from mutants further away, which explains why the approximation equation (26) underestimates the true average fitness.
pairs

$n$ pairs

$\overbrace{00\ldots000} \ldots \overbrace{0\ldots001} \ldots \overbrace{00101} \ldots \overbrace{1100\ldots1001}$

block $b$

block 2

block 1

Figure 2: A valid string in the Neutral Staircase landscape. None of the two active blocks (block 1 and 2) contains a pair 11, all $k$ bits between blocks 1 and 2 are set to 1, and all bits to the left of block 2 are set to 0.

4. The Neutral Staircase landscape

The fitness landscape that we have studied in the previous section contains only a single large neutral network, and hence epochal dynamics as predicted in Sec. 2.1 cannot be observed in that landscape. However, with a small modification, we can create a landscape that possesses the required properties. We subdivide a binary sequence into $b$ blocks of length $2n$, with a set of $k$ bits in between each block (Fig. 2). The total length of the sequence is thus $l = 2bn + k(b - 1)$. The blocks can be active or inactive. An active block has properties similar to the sequences of the previous section. The block is subdivided into $n$ pairs. If any of those $n$ pairs are in the configuration 11, the fitness of the whole sequence is zero. Otherwise, the fitness is not affected by that block. The rightmost block is always active. A block further to the left becomes active if the block immediately to its right is active, and all $k$ bits between the two blocks are set to 1. Finally, any bit to the left of the leftmost active block that is set to 1 results in fitness zero for the whole sequence. We call this landscape the Neutral Staircase landscape, in analogy to the Royal Staircase introduced by van Nimwegen and Crutchfield [2000b]. Note that the Neutral Staircase differs from the Royal Staircase in an important aspect: all sequences have either fitness 0 or fitness 1. No higher fitness genotypes can be discovered, and the population’s dynamics is determined only by the topology of the neutral sequences.

The analytical treatment of the Neutral Staircase landscape is straightforward for $k > 1$. The neutral sequences decompose into $b$ neutral networks, one for each possible number of blocks that can be active. Within one neutral network, the average population fitness is readily available from (23). Namely, if $i$ counts the number of active blocks, we have

$$\langle f_i \rangle = q^i\left(1 + \frac{\hat{\mu}^2}{2} + \frac{\hat{\mu}}{2}\sqrt{8 + \hat{\mu}^2}\right)^n i \cdot$$

Likewise, it is simple to derive the probability with which a new block is discovered. First, we note that the probability with which an offspring sequence remains on the neutral network $i$ is given by $\langle \nu_i \rangle = \langle f_i \rangle$, according to (11). Hence, a single offspring sequence ends up on the next neutral network with probability $\langle f_i \rangle \hat{\mu}^k q^{-2n}(1 - q^2\hat{\mu}^2)^n$ (we need $k$ extra mutations to set the $k$ bits left of the leftmost active block to one, but we are allowed some miscopies in the newly activated block). In a finite population of $N$ sequences, there are on
average $N\langle f_i \rangle$ sequences that can give rise to offspring. A sequence that belongs to the network $i+1$ is therefore created in one generation with probability

$$P_{\text{crea},i} = 1 - \left[ 1 - \langle f_i \rangle \tilde{\mu}^k q^{-2n} (1 - q^2 \tilde{\mu}^2)^n \right]^{N(J_i)}. \quad (29)$$

Next, we are interested in the probability of fixation of that newly discovered sequence, $\pi_i$. For large populations, $\pi_i$ can be approximated by $2s$ [Haldane, 1927, Kimura, 1964], where $s$ is the selective advantage of the newly discovered network. In our case, we obtain thus

$$\pi_i = 2\langle f_{i+1} \rangle / \langle f_i \rangle - 1. \quad (30)$$

The probability of a transition from network $i$ to network $i+1$, $P(i \to i+1)$, is given by $P(i \to i+1) = P_{\text{crea},i} \pi_i$, and hence the average epoch length $\tau_i$ follows as

$$\tau_i = \frac{1}{P(i \to i+1)} = \frac{1}{P_{\text{crea},i} \pi_i}. \quad (31)$$

The time to convergence $\tau_{\text{conv}}$, i.e., the time until the optimum sequence distribution has been found, is given by

$$\tau_{\text{conv}} = \sum_{k=1}^{b-1} \tau_k. \quad (32)$$

Equations (28), (31), and (32) capture most of the immediately observable quantities in an evolving population in the Neutral Staircase landscape. We have tested their applicability with numerical simulations of a genetic algorithm, and
Figure 4: Convergence time $\tau_{\text{conv}}$ in the Neutral Staircase landscape. The solid line is the analytical expression (32), and the points are derived from 10 independent simulations each. The standard error of the measured convergence times is of the size of the symbols. We used $n = 3$, $b = 5$, $k = 3$, and a population size of $N = 10000$.

have found good agreement. In Fig. 3, we compare the average fitness in the different epochs, $\langle f_i \rangle$, to the simulation results. Figure 3a shows that the population fitness fluctuates around the predicted value during the metastable equilibrium, and that transitions between two equilibria happen fast. In Fig. 3b, we have made the entropic barrier between two networks smaller (we have reduced $k$). Then, all neutral sequences actually form only a single large neutral network, and the population transitions immediately to the highest possible level of the average fitness.

In Fig. 4, we compare the time to convergence $\tau_{\text{conv}}$ with simulation results. We find that for $\mu \lesssim 3$, our analytical prediction agrees well with the simulations, whereas for larger $\mu$, the prediction fails to capture the observed behavior. The origin of this failure is the following. We have derived equation (32) under the assumption that it is hard to discover the next higher network. Now, when $\mu$ becomes of the order of $k$ (we have $k = 3$ in Fig. 4), mutations that flip all $k$ bits at once to activate the next block become likely, and hence this assumption fails. As in the case of Fig. 3b, the neutral sequences then form a single gigantic network, and an expression derived under the assumption of disjunct sub-networks must break down.

5. Discussion

The analysis presented in the preceding sections has shown that the topology of the neutral sequences in genotype space has an important influence on the dynamics of a population. It is therefore not justified to regard the evolution of a population of neutral genotypes as a simple diffusion process, unless either the population size or the mutation rate are very small (when the product of the population size, $N$ and the genomic mutation rate, $\mu$, is much smaller than one,
the population as a whole performs essentially a random walk on the neutral network [van Nimwegen et al., 1999a]. We have demonstrated that evolution on neutral networks can lead to the same kind of epochal dynamics that previously was thought to be caused solely by the discovery of genotypes of higher fitness. Of course, our results do not imply that sudden transitions in a population are never caused by such discoveries. Normally, a transition that we observe in a population evolving in an unknown fitness landscape will be due to the discovery of a faster replicating genotype. However, sometimes we may observe a transition to a higher average fitness without an increase (or even with a decrease, see below) in the fitness of the dominant or fastest replicating genotype. Such a transition is then due to the effects described in this paper.

In most cases, selection acts first and foremost on replication rates. However, if all viable genotypes are identical in terms of their replication rate, as is the case in the fitness landscapes we have studied in this paper, the next important quantity selection acts upon is the probability with which genotypes have viable offspring. This probability is more a property of a set of genotypes than of a single genotype, because a genotype that itself has high reproductive success, but produces mainly offspring with poor reproductive success, will ultimately have only a small number of progeny. Therefore, the selective pressure we have described here acts solely on clouds of mutants, in an extreme form of quasispecies-like selection [Eigen and Schuster, 1979, Nowak, 1992].

Throughout this paper, we have assumed that all sequences have the same replication rate $\sigma$. It is natural to ask what happens when sequences with different replication rates are present. The simplest such situation occurs when all sequences within a single neutral network $i$ have the same replication rate $\sigma_i$. In that case, we can take the analysis given in Sec. 2.1 one step further. The equilibrium fitness $\langle f_i \rangle$ within a network $i$ is then given by

$$\langle f_i \rangle = \sigma_i q^i (1 + \tilde{\mu} \rho_i),$$

(33)

where $\rho_i$ is the spectral radius of the connection matrix of the network, $G_i$. A population will of course try to move to the particular network that gives the maximum equilibrium fitness. For a small mutation rate $\tilde{\mu}$, it is clear that the network with the largest $\sigma_i$ yields the maximum equilibrium fitness. If, however, the mutation rate is large, such that $\tilde{\mu} \rho_i$ becomes of the order of unity or even exceeds that value, then depending on the distribution of the $\rho_i$’s and the $\sigma_i$’s over the different networks, a population may actually transition from a network $j$ with a higher replication rate $\sigma_j$ to a network $i$ with a smaller replication rate $\sigma_i$, while at the same time increasing its average fitness. A similar effect was already predicted by Schuster and Swetina [1988], although they investigated peaks with different support from slightly deleterious mutations, rather than neutral networks with different connection densities. In recent work, Ofria et al. [2000] have observed this selection for neutrality empirically in digital organisms. In a large number of evolution experiments with different mutation rates, they
observed that the number of neutral neighbors of the dominant genotype (a crude but practical measure for the spectral radius of the connection matrix) would increase at higher mutation rates, at the expense of the digital organisms’ replication rate. Similarly, Wilke et al. [2000] showed that digital organisms with a vastly inferior replication rate could outcompete seemingly superior digital organisms at high mutation rates if the slower replicating organisms had a higher robustness against mutations.

It is interesting to relate our results for the average population fitness to the mutational load $L$, which is defined as [Haldane, 1937, Muller, 1950, Crow, 1970]

\[ L = 1 - \frac{\langle f \rangle}{\sigma}. \]  

(34)

From equation (9), we obtain

\[ L = 1 - q^{l}(1 + \tilde{\mu}\rho), \]  

(35)

where $\rho$ is the spectral radius of the connection matrix $G$. As was already noted by van Nimwegen et al. [1999a], the load can deviate significantly from Haldane’s result $L = \mu$ [Haldane, 1937] if neutrality is present [note that equation (35) becomes identical to Haldane’s result in the absence of neutrality ($\rho = 0$) and in the limit of a small mutation rate]. More importantly, as a generalization of Haldane’s result, it is often cited that the genetic load in an asexual population is independent of the fitness landscape, and therefore also of epistasis, and that it is equal to

\[ L = 1 - e^{-\mu}. \]  

(36)

(see e.g. Kondrashov [1988], Charlesworth [1990], derivation given by Kimura and Maruyama [1966], Crow [1970]). This result, however, holds only in the absence of neutral mutants. If neutral mutants are present, then the topology of the neutral genotypes in the genotype space is coupled to the mutational load, by virtue of $\rho$ in equation (35). Since the topology is also coupled to the type of epistasis that we observe [Wilke and Adami, 2000], epistasis must have an influence on the mutational load, even in asexual populations, as long as neutral mutations can occur. However, equation (36) is the basis of the deterministic mutational hypothesis of the evolution of sex [Kondrashov, 1988]: if the mutational load is independent of epistasis for asexual populations, but depends strongly on the sign of epistasis for sexual populations, then for certain types of epistasis the mutational load of a sexual population may be much smaller than that of an asexual population. Because of the differences between equations (35) and (36), the deterministic mutational hypothesis could break down if neutrality were taken into account. As a consequence, this hypothesis should be reconsidered for the case of landscapes with neutrality.
6. Conclusions

We have shown that adaptive evolution can take place in the complete absence of what is ordinarily understood as advantageous genotypes. Even if the fitnesses of all viable genotypes are completely identical does selection favor particular regions of genotype space over others. What makes the difference is the density of neutral sequences. In a region of genotype space with a higher density of neutral sequences, chances are higher that a mutated offspring is neutral rather than deleterious. Therefore, neutral sequences in such a region have a higher robustness against mutation, and hence a higher reproductive success. This gives them sufficient selective advantage to outcompete sequences from a less densely connected region. The transitions between different such regions will often occur in sudden jumps, followed by relatively long periods of stasis. Evolution on neutral networks alone can thus lead to epochal dynamics observed in so many natural and artificial evolving systems.

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