Evolution of clonal populations approaching a fitness peak.

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Populations facing novel environments are expected to evolve through the accumulation of adaptive substitutions. The dynamics of adaptation depends on the fitness landscape and possibly on the genetic background on which new mutations arise. Here we model the dynamics of adaptive evolution at the phenotypic and genotypic levels, focusing on a Fisherian landscape characterized by a single peak. We find that Fisher's geometrical model of adaptation, extended to allow for small random environmental variations, is able to explain main observations of experimentally evolved populations. Consistent with data of populations evolving under controlled conditions, the model predicts that: mean population fitness increases rapidly when populations face novel environments and then achieves a dynamic plateau; the rate of molecular evolution is remarkably constant over long periods of evolution; mutators are expected to invade and patterns of epistasis vary along the adaptive walk. Negative epistasis is expected in the initial steps of adaptation but not at later steps, a prediction that remains to be tested. Furthermore, populations are expected to exhibit high levels of phenotypic diversity at all instances of their evolution. This implies that populations are possibly able to adapt rapidly to novel abiotic environments.

Keywords: experimental evolution, clonal interference, molecular clock, epistasis, fitness peak

1. Introduction

In asexual populations the process of adaptation can be complex due to interference between selected mutations. This is called the Hill-Roberson effect, which leads to increased rates of fixation of deleterious mutations (Muller's ratchet)

and decreased rates of fixation of beneficial mutations (clonal interference (CI) [1]). Theoretical models, which assume a rate and distribution of mutation effects that is time homogeneous, predict that both the rate of fitness increase and the rate of molecular evolution will be constant (reviewed in [2]). These models are valid when the rate of environmental change is large, since the supply of beneficial mutations will never be depleted. But when the environment changes very slowly the rate of adaptation declines as the population adapts [3]. Experimental evolution in microbes and other organisms has provided indications that this can be the case. Indeed the rate of increase of the competitive ability of populations of *Escherichia coli* declines considerably after a few thousands of generations [4]. Recently, a genomic analysis of the mutations associated with those phenotypic changes suggested an interesting and paradoxical pattern of variation: while the rate of phenotypic (fitness) change is highly non-linear, the rate of molecular evolution is approximately constant over the period studied (20,000 generations) [5]. Since most mutations fixed have been shown to be advantageous, it is rather surprising that their rate of accumulation is constant.

Two other interesting patterns are observed in these and other experimentally evolved populations: the emergence of mutator alleles [6,7,8] and the occurrence of negative epistasis between beneficial mutations [9,10]. Mutators of intermediate strength emerge when the rate of fitness **increase** is already very small [6].

Here we explore **whether** these patterns can be expected under theoretical models of adaptation. A geometrical model of adaptation towards a single fitness peak, was provided by Fisher (FGM). It has been extended [11,12,13] and successful in interpreting experimental data [14,15,16,17,18]. We consider FGM, in conditions of intense CI, to explore under what conditions it can predict those patterns. We consider different values of the parameter space, but focus on genomic mutation rates that are biologically relevant for bacteria [19].

2. Methods

In FGM an organism is a point in a *n*-dimensional Euclidean trait space and a Gaussian fitness landscape is assumed. In individual based simulations each individual is represented by its phenotypic trait values (vector $\mathbf{x}=(x_1,x_2,\ldots,x_n)$), which correspond to a given fitness $W=\mathrm{Exp}(-\Sigma_i\, x_i^2)$ and by its number of mutations. Initially no mutations are present. Since all individuals are assumed to be derived from a

single clone, all have the same trait values and fitness W_0. Mutations are Poisson distributed with rate *U* and are modeled as random vectors (from a normal distribution with mean 0 and variance σ^2 that move individuals in trait space. If an individual is located at the origin, then all mutations are deleterious with $E(s_d)=-n\sigma^2$. Individuals are selected to the next generation according to their fitness. Given that we are targeting microbial systems, population size (*N*) is typically large. Since genomic mutation rates of most DNA based microorganisms, *U*, is around 0.001 per generation, we explore a regime where $NU>>1$ with strong Hill Robertson effects. This is contrary to the weak mutation strong selection assumption in previous studies, which allows to obtain many analytical results [20].

We extended FGM by modifying a single assumption: that the optimum is unique and constant. Assuming that the organism is part of the environment and when it changes the environment changes [21], we extended FGM in the following way. During adaptation of large populations many mutations arise per generation (in a typical microbial evolution experiment it can easily reach 10^4). Many will be deleterious and soon eliminated. This means that a small fraction of the population is composed by different genotypes at any given time. These mutations are random, mostly deleterious and occasionally beneficial. If organisms are adapting to the environment, which includes themselves, then random fluctuations of the environment are always occurring. We model this by assuming that the optimum in Fisher's geometrical model changes location constantly in a stochastic way, i.e. the classical location at the center of the space performs a random walk with no preferential direction. We call this model the shaking optimum model.

To simulate FGM with this extension we assume that the optimum at time t=0 is set at position $\mathbf{x_{opt}}(0)=(0,\ldots,0)$, and at subsequent generations its position changes recursively as $\mathbf{x_{opt}}(t)=\mathbf{x_{opt}}(t-1)-D\mathbf{x}$, where $D\mathbf{x}=(Dx_1,Dx_2,...,Dx_n)$ is the displacement vector. Each of the Dx_i's is a random variable from a normal distribution with mean zero and variance v, typically varying from 10^{-7} to 10^{-5}. Note that every generation the optimum value of each trait varies randomly, by a tiny amount which is mimicking the assumption that as populations evolve novel phenotypes they can change the environment, which then leads to novel trait values as the optimal. At least 10 simulations are run for each set of parameter values.

To study the probability of fixation of mutators during the adaptive walk, we

introduce a single clone in the population, which has a higher genomic mutation rate (*smut**U)- **modifier mutation rate**. The mutator is introduced after the population has evolved for T generations and its fate is followed during this adaptive walk. Hundreds of thousands of independent introductions are performed to estimate the probability of fixation for each time T. The mutator is considered to be advantageous if its probability of fixation is above 1/*N* (that of a neutral allele).

3. Results

Dynamics of adaptation under FGM with CI

We study the evolution of clonal populations where the mutation input is large and both beneficial and deleterious mutations occur, so we study FGM under intense CI. FGM with a static optimum generally predicts that the mean population fitness increases very rapidly and then achieves a plateau. The rate of molecular evolution parallels the fitness increase and the accumulation of mutations stops after the fitness plateau is achieved (Figure 1). Simulations with different sets of parameters predict that organisms with bigger phenotypic complexity (larger *n*) adapt more slowly [3] and that in small populations mean fitness increases at a slower pace, as expected (see Supplement). In FGM the mean effect of deleterious mutations changes along the walk towards the peak. In supplement we present simulations with different selection coefficients. As expected, only when the majority of mutations are effectively neutral a nearly constant rate of mutation accumulation is observed. But this is also accompanied by a very slow and nearly constant rate of fitness increase. From other simulations similar qualitative behaviors were observed leading to the conclusion that FGM, in its classical form, generally does not predict an abrupt increase in fitness accompanied by a constant rate of molecular change.

We now consider that the optimum is not constant, but moves with no preferential direction. If organisms are adapting to the environment, which includes themselves [21], then random fluctuations (of variance v) of the environment are always occurring. Figure 1 shows that when the fluctuations are very small (low values of v), we observe the same patterns as in Fisher's classical model: an initial high rate of both fitness increase and divergence at the molecular level, followed by a fitness plateau and a drastic reduction of the rate of molecular evolution. Strikingly, as the optimum diffuses around its initial location, we observe a similar fitness dynamics

but a quite different pattern at the molecular level. Whereas at the fitness level the populations reach a plateau, at the molecular level evolution is non-stopping, and divergence increases at a constant rate. Importantly, the fitness plateau is dynamic, characterized by oscillations around a mean. In the supplement we show the results for smaller *N*. Figure 1, as well as simulations with other parameters sets (Supplement), show three important features: a) an initial rapid increase of fitness, which means that populations become well adapted to the environment in the earlier stages; b) a plateau that is only plateau as a time average, in fact mean fitness fluctuates in time (interestingly, strong fluctuations in fitness are also observed in Lenski's experimental lines); c) a constant rate of molecular evolution underlying a phenotypic plateau. These features are similar to a Red Queen ("it takes all the running you can have to stay in the same place") for a single species [22]: at the molecular level evolution is not stopping, whereas the fitness level remains unaltered.

Invasion of mutator alleles, epistasis and trait variation

We studied the advantage of mutator alleles along the adaptive walk. Figure 2A shows the relative probability of fixation of a mutator during the adaptive walk. In static fitness landscapes (v->0)**,** the probability of fixation of mutators decreases as the population adapts. But for a shaking optimum the opposite can occur. There the probability of fixation of the mutator is lower in the beginning of the adaptive walk, and then becomes constant as populations wander around the fitness plateau. The probability of fixation also increases with the variance v of the shaking peak. Consistent with the hypothesis that mutators hitchhike with beneficial alleles, we find that the advantage of the mutator augments with an increased population size (Supplement), because beneficial mutations are more abundant in larger populations.

The pattern of epistasis between beneficial alleles can be studied by measuring the mean effect of the mutations that get fixed at each step of climbing the landscape in FGM. Figure 2B shows that during the first step of the adaptive walk very strong effect mutations fix. The mean effect of fixed mutations decreases as more steps take place. However, in latter stages of adaptation, the static and the shaking optimum models are distinct: whereas in the static negative epistasis is the rule, in the shaking no epistasis is detected. In other words, after an initial rapid fitness increase, which is characterized by negative epistasis in both models, multiplicative fitness is expected under the shaking optimum. This prediction can be tested in experimental lines by

choosing those mutations that have fixed latter in the adaptive process. In addition, the levels of variation at the trait level are also distinct. Figure 2C shows that under the shaking peak the variance in trait values is kept large along the adaptive walk, in particular after a dynamic fitness plateau has been achieved (grey bar at generation 10000). This contrasts with the expectation under the static fitness landscape where trait variation is expected to decrease as the populations adapt. Therefore in the landscape with a shaking peak, populations exhibit high levels of trait diversity, implying that they can more readily respond to novel selection pressures from changes in the abiotic environment.

4. Discussion

We have explored theoretically the conditions under which major observed patterns in experimentally evolving populations are likely to emerge. We studied FGM under CI and extended it to include the possibility that the optimal value of traits is not static. Such extension predicts the emergence of the paradoxical pattern of phenotypic and molecular change observed in the experiments [23]. The model predicts a dynamic fitness plateau and also a high probability of invasion of mutator alleles in populations that have reached such a plateau. It also implies that some amount of non-transitive fitness interactions may be detectable when competing different clones with the ancestral population, as well as a change in the pattern of epistasis along the adaptive walk. While some evidence for non-transitive fitness interactions has been observed in experimentally evolved populations [24], the pattern of epistasis between mutations along the walk has not yet been measured.

In a recently theoretical framework of dynamic fitness landscapes, seascapes, conditions were found where a rapid rate of fitness increase could be accompanied by a much slower rate of change at the molecular level [25]. In such model, as in FGM, the fraction of beneficial and deleterious mutations changes along the adaptive walk. It would be interesting to explore if such a genotypic model can also capture the main observations made in experimental evolution with asexual microbes.

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FIGURE LEGENDS:

Figure 1 A) Dynamics of fitness increase and B) divergence (number of mutations accumulated) under FGM with a shaking peak. W_0=0.5, N=10^7, U=0.001, σ^2=0.001 and n=20. The optimum shakes every generation with a variance v. In the limit v-> 0 we recover the classical FGM, where the mean effect of mutations at the optimum is -2%.

Figure 2 A) Relative probability of fixation of a mutator, with strength *smut*=100, introduced at generation T. N=10^4 σ^2=0.001, n=20, U=0.001, W_0=0.8. B) Mean fitness effect of fixed mutations along the adaptive walk. The back bars represent a static landscape (v=0), and the grey bars a randomly shaking optimum. N=10^6 σ^2=0.001, n=20, U=0.001, W_0=0.5. C) The mean within population variance in trait values observed along the adaptive walk (at generation 10000 the fitness plateau was already achieved). Black bars static landscape (v=0), and grey bars a randomly shaking optimum (v=10^{-5}). N=10^5 σ^2=0.001, n=20, U=0.001, W_0=0.5.

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