Understanding the Evolutionary Fate of Finite Populations: The Dynamics of Mutational Effects

Olin K. Silander1,2*, Olivier Tenaillon1,3,4*, Lin Chao1

1 Division of Biology, University of California San Diego, La Jolla, California, United States of America, 2 Institute of Integrative Biology, Eidgenössische Technische Hochschule (ETH) Zurich, Zurich, Switzerland, 3 INSERM U722, Faculté de Médecine Xavier Bichat, Paris, France, 4 Université Paris 7, Paris, France

The most consistent result in more than two decades of experimental evolution is that the fitness of populations adapting to a constant environment does not increase indefinitely, but reaches a plateau. Using experimental evolution with bacteriophage, we show here that the converse is also true. In populations small enough such that drift overwhelms selection and causes fitness to decrease, fitness declines down to a plateau. We demonstrate theoretically that both of these phenomena must be due either to changes in the ratio of beneficial to deleterious mutations, the size of mutational effects, or both. We use mutation accumulation experiments and molecular data from experimental evolution to show that the most significant change in mutational effects is a drastic increase in the rate of beneficial mutation as fitness decreases. In contrast, the size of mutational effects changes little even as organisal fitness changes over several orders of magnitude. These findings have significant implications for the dynamics of adaptation.

Introduction

The process of adaptation in finite populations is determined by the distribution of mutational effects on fitness and the size of the population. In large populations, beneficial mutations are frequently fixed, whereas deleterious mutations will only be fixed if they are of vanishingly small effect size (approximately the inverse of the effective population size [1]). In small populations, beneficial mutations may be frequently lost due to drift, whereas the same process may lead to the fixation of moderately sized deleterious mutations. Whether fitness increases or decreases in a population of a given size thus depends on the relative rates of beneficial and deleterious mutations, and the effect sizes of such mutations. An accurate description of the distribution of mutational effects is thus fundamental to understanding how adaptive changes occur in biological populations. Experimental manipulation of organisms in the laboratory (i.e., mutation accumulation [MA]) [2–15] and genomic and molecular analyses [16–23] have indicated that deleterious mutations are more common than beneficial mutations. However, experimental evolution of large populations in the laboratory has clearly demonstrated the existence of beneficial mutations [24], and several studies have shed some light on their rate and the distribution of their effects [25–27]. Recently, comparative studies have allowed considerable insight into the rate of fixation of both beneficial and deleterious mutations [28–31]. These comparative studies have frequently come to different conclusions, and it has not yet been determined whether this is a result of different methods employed, biases in the datasets used, differences in the demography of the populations (e.g., population bottlenecks or inbreeding), differences in the evolutionary pressures on the populations (e.g., changes in organisal ecology), or some other factor. Resolving both the rate and the effect size of beneficial and deleterious mutations thus requires experiments expressly designed for this purpose.

Importantly, the rate and effect size of beneficial and deleterious mutations are not constant. These quantities change if the fitness of the evolving organism changes. One manifestation of this effect is the fitness plateaus reached by populations adapting to constant environments [24,32,33]. The appearance of fitness plateaus in adapting populations requires that either beneficial mutations become more rare relative to deleterious mutations, or that mutations become smaller in effect size (which has a 3-fold effect, increasing the fixation probability of deleterious mutations, decreasing the fixation probability of beneficial mutations, and decreasing adaptive step sizes when substitutions do occur). It has previously been observed that populations starting from the same genotype often reach plateaus at similar levels of fitness [34–36], despite fundamental differences in the genetic paths taken [37]. This suggests that changes in the rate and distribution of mutational effects have less to do with the specific genetic background of an organism than with the fitness that an organism has attained. In other words, history plays less of a role than adaptation in reaching these fitness plateaus [34].

There is an analogous process that occurs in populations that experience decreases in fitness. This process is equally important for evolutionary dynamics, but has received less attention. When populations are so small that drift over-whelms selection, deleterious mutations frequently fix [16,22,23], causing fitness to decline. If these populations...
Author Summary

In any population, two factors determine whether the average fitness of individuals will increase (adaptation) or decrease: the size of the population and the distribution of mutational effects (i.e., the relative rates and effect sizes of beneficial and deleterious mutations). Although it is relatively simple to get quantitative information on population size, it is much harder to gain insight into the distribution of mutational effects. Very little information exists on the relative rates of beneficial versus deleterious effects, on the shapes of mutational distributions, or on whether the distributions change over time. Thus, it remains difficult to even speculate whether a given population will adapt over time. Here, we use laboratory evolution of a bacterial virus to quantify the distribution of mutational effects. Our results reveal that the average impact of a mutation is approximately constant with respect to fitness, that most mutations have small effects, and that the rate of beneficial mutation depends on the fitness of the organism. Our study demonstrates the simple, but perhaps underappreciated fact that mutational effects are dynamic. It also proposes and tests an explicit model of adaptation in which organismal fitness specifies both the rate and distribution of deleterious and beneficial mutations, and it presents specific and testable predictions of the circumstances under which populations will adapt.

are not all destined to become extinct, then as deleterious mutations are fixed, a fundamental change must occur in the distribution of mutational effects. Two possibilities have been proposed: that the accumulation of deleterious mutations is accompanied by an increase in the mean effect size of deleterious mutations (negative epistasis) or by an increase in the rate of beneficial mutations (compensatory epistasis) [16,22,23] (Figure 1). Both of these ideas are termed epistatic because the effect of a mutation, and thus the distribution of mutational effects, changes according the genetic background of the organism in which it appears.

We used experimental evolution of a bacteriophage to investigate how the distribution of beneficial and deleterious mutations changes (i.e., epistasis) when fitness changes in the course of evolution, and how this impacts adaptation. We corroborate the finding that in large populations, fitness increases to a plateau. We also report the complementary effect that in small populations, fitness does not decrease indefinitely, but reaches a lower plateau. The equilibrium level of fitness is thus largely determined by the effective population size, and we show that this is a consequence of simple changes in mutational effects that cause qualitative changes in the processes of selection and drift.

Results

Experimental Evolution of Fitness Plateaus

To study the impact of population size on adaptation, we used experimental evolution in the bacteriophage φX174, an organism in which population sizes can be controlled by adjusting the bottleneck size at transfer. We used phage populations to investigate two related questions: First, do populations evolve to a fitness equilibrium? Second, do populations that differ in effective population size reach different fitness equilibria? As a measure for fitness, we used competition with a reference strain [38]. Because there are few interactions between viral plaques during evolution, nontransitive fitness interactions are not expected to affect the outcome; thus competition assays with a common ancestor give an accurate assessment of fitness.

To identify fitness equilibria, we initiated experimental populations with either an ancestor of high fitness or an ancestor of low fitness. We then investigated whether the populations converged toward the same fitness value, irrespective of their starting condition. If populations that were initialized with high- or low-fitness ancestors converged in fitness during evolution, the evolved fitness levels of these populations were concluded to bracket a fitness equilibrium. The high-fitness ancestor was a clone derived from a phage line that had been passaged in our laboratory for 100 transfers at a large population size (~10^8). Two lower fitness ancestors were derived by serially bottlenecking this clone and selecting small plaques. This procedure resulted in two strains that had low fitness in competition with the ancestral strain.

We initiated three populations from the high-fitness ancestor at each of four population sizes: three, ten, 30, and 100. We also initiated three populations from one low-fitness ancestor (two at a population size of ten and one at a population size of three, as well as one population from a very low-fitness ancestor, at a population size of three (Figure 2).
During experimental evolution, all phage populations were subject to mutagenesis (see Materials and Methods).

After 90 transfers, the fitness of populations bottlenecked at sizes of three, ten, 30, and 100 were measured to test for convergence (Figure 2). We focus first on the ten populations bottlenecked at effective population sizes of three and ten, which exhibited evidence of convergence in fitness (Figure 2). Of these ten populations, all six populations that were initiated from a high-fitness ancestor declined significantly in fitness. Of the populations initiated from lower fitness ancestors, one increased in fitness \( N_e = 3 \), two maintained almost constant fitness \( N_e = 10 \), and one declined only slightly in fitness \( N_e = 3 \) (Figure 1). In the populations bottlenecked at larger effective sizes \( N_e = 30 \) and \( N_e = 100 \), five of the six evolved to a mean fitness above that of the high-fitness ancestor. It was thus difficult to ascertain whether these populations were evolving towards an equilibrium fitness. To establish whether these larger populations were evolving towards an equilibrium, we took the fitness ancestor. It was thus difficult to ascertain whether these populations were evolving towards an equilibrium fitness due to errors. Some populations are slightly displaced on the x-axis for clarity.

The population bottleneck sizes were three, ten, 30, and 250. Five lineages were propagated at bottleneck sizes three and ten, and three lineages were propagated for all other bottleneck sizes. The color and shape of each point indicates the ancestral clone from which that population was derived. The fitness of the high-fitness ancestor was set equal to zero on a log10 scale, and all other fitness values are relative. doi:10.1371/journal.pbio.0050094.g002

Figure 2. Changes in Mean Population Fitness
Dotted lines indicate the fitness of ancestral clones: the high-fitness clone (black circles), lower fitness clones (bright red triangles and dark red diamonds), and the evolved highest fitness ancestor (green squares). This ancestor was derived from the population having the highest fitness after 90 transfers (population 100c). Each point indicates the average fitness of an evolved population, with error bars indicating one standard error. Some populations are slightly displaced on the x-axis for clarity. The population bottleneck sizes were three, ten, 30, and 250. Fifty lineages were propagated at bottleneck sizes three and ten, and three lineages were propagated for all other bottleneck sizes. The color and shape of each point indicates the ancestral clone from which that population was derived. The fitness of the high-fitness ancestor was set equal to zero on a log10 scale, and all other fitness values are relative. doi:10.1371/journal.pbio.0050094.g002

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Although care was taken to minimize the opportunity for cross-contamination between populations, it was important to directly rule out that contamination was responsible for the fitness convergence. To ascertain this, we sequenced the complete genome of one clone from each evolved population. Convergence of substitutions at a large fraction of sites would imply contamination. However, the number of sites at which convergence occurred was not significantly greater than expected by chance (see Materials and Methods), so cross-contamination was ruled out as a cause of fitness convergence.

The sequence data revealed that the fitness convergence was achieved despite little indication of genetic convergence. Hence our system reproduced one previously observed phenomenon in experimental evolution: fitness in a large, adapting population does reach a plateau with different starting genotypes. Interestingly, our results show that this effect can be extended to small population sizes: small populations converge to low-fitness equilibria without genetic convergence.

Models Underlying Population Size–Dependent Fitness Plateaus
The presence of fitness equilibria has specific implications for how epistasis influences the rate and shape of the mutational distribution. In previous studies that have focused on the accumulation of deleterious mutations in small populations, two general hypotheses have been put forth that can prevent continuous fitness decline [16,17,22,23]. One hypothesis, commonly referred to as negative epistasis (synergistic epistasis in the case of deleterious mutations), postulates that the effect size of novel deleterious mutations increases as fitness decreases (i.e., as more deleterious mutations accumulate), and that this makes selection more efficient in removing these deleterious mutations [39] (Figure 1B). The second hypothesis, compensatory epistasis, suggests that some fraction of (formerly) deleterious mutations become beneficial (i.e., compensatory) when organismal fitness decreases (Figure 1C) and that this effect slows the fitness decline in small populations.

We focus on testing these two models here, by using an explicit model to describe how epistatic changes affect fitness convergence and equilibria. In this model, the deleterious and beneficial substitution rates \( k_d \) and \( k_b \) respectively change as fitness changes. For simplicity, we illustrate the case in which the effect on fitness of all beneficial mutations is identical, as is the effect on fitness of all deleterious mutations (in all later analyses, we use simulations in which mutational effects are modeled as a distribution). The substitution rates of deleterious and beneficial mutations are thus:

\[
k_d = N_e u_d p_d(N_e, s_d) \\
k_b = N_e u_b p_b(N_e, s_b)
\]

in which \( N_e \) is the population size, \( u_d \) is the deleterious mutation rate, \( u_b \) is the beneficial mutation rate, \( p_d(N_e, s_d) = (1 - e^{s_d})/(1 - e^{-2N_e}) \) [1] is the probability of fixation of a deleterious mutation of effect size \( s_d \), and \( p_b(N_e, s_b) = (1 - e^{-s_b})/(1 - e^{-2N_e}) \) is the probability of fixation of a beneficial mutation of beneficial...
We can thus dichotomize the two epistatic hypotheses: The first hypothesis, negative epistasis, proposes that a decrease in the left side of Expression 4 occurs through an increase in the average selective coefficients, $s_d$ and $s_b$. The second hypothesis, compensatory epistasis, states that decreases in fitness cause an increase in the ratio between the beneficial mutation rate and the deleterious mutation rate, such that the right side of Expression 4 increases. We focus here on testing these two forms of epistasis, because they are the most commonly modeled forms. If there is either no epistasis or positive epistasis, then the fitness decline will continue unchecked, and no fitness equilibrium occurs. Similar logic applies to the case of large populations adapting to a constant environment. In the absence of epistatic effects, or if positive epistasis operates, fitness would increase continuously without reaching an equilibrium.

### Mutation Accumulation

In order to understand which mechanism was responsible for the fitness equilibria we observed in our experimental populations, we needed to discriminate between the two main epistatic mechanisms (negative or compensatory epistasis). This required determining the ratio of beneficial and deleterious mutations, and the distribution of the effects of these mutations. To estimate these quantities, we used MA. In an MA experiment, mutations are allowed to accumulate by randomly collecting the progeny of a parental phage with no bias against low-fitness progeny. By measuring the fitness of a number of parents and a number of randomly chosen progeny, it is possible to estimate the fitness effects of mutations that occur in a single generation. We determined the fitness of 50 parents and 50 progeny for three high-fitness populations and three low-fitness populations (Figure 3; Materials and Methods). If negative epistasis is the primary mechanism promoting fitness equilibria, then we expect that mutations occurring in low-fitness populations will have larger selective effects than those occurring in high-fitness populations. If compensatory epistasis is the main mechanism operating, a higher proportion of the mutations occurring in low-fitness populations should be beneficial.

We used maximum-likelihood (ML) analysis [5] to estimate the distribution of mutational effects, $s$, and the proportion of beneficial mutations, which we refer to as $B$, and define as $u_b/(u_b + u_d)$. Importantly, this analysis assumes that the distribution of mutational effects can be modeled as a reflected gamma distribution. The gamma distribution is used because it has great flexibility in terms of the shapes it can assume, with shapes ranging from highly leptokurtic to exponential to log-normal to Gaussian (Figure S2). However, the assumption of a reflected gamma distribution constrains beneficial and deleterious mutational to follow the same distribution. Additionally, the model is limited in its power to detect asymmetric epistasis between beneficial mutations (i.e., if only beneficial mutations increase in effect size as fitness decreases). However, such asymmetric epistasis does not seem very plausible. If the distribution of deleterious mutations does not depend on fitness, but the distribution of beneficial mutations does depend on fitness, then the fitness of an individual carrying one beneficial and one deleterious mutation would depend on the order in which the mutations occur, which is nonsensical. We discuss this idea in more detail in Text S1.

Estimating $s$ and $B$ requires an estimate of the mutation rate ($U$). We derived this estimate from sequence data rather than the ML analysis to avoid conflating estimates of $s$ and $U$ [5]. We used the sequence data to determine the rate of synonymous substitutions, from which we inferred the mutation rate imposed under experimental evolution. There was a slight, but not significant, decrease in the rate of mutation of effect size $s_b$. If a population is reduced in size such that fitness begins to decline, by definition,

$$k_d s_d > k_b s_b$$

or

$$\frac{p_d(N_c, s_d)}{p_b(N_c, s_b)} > u_b / u_d$$

For the fitness decline to stop, Expression 4 must become an equality.

We can thus dichotomize the two epistatic hypotheses: The first hypothesis, negative epistasis, proposes that a decrease in the left side of Expression 4 occurs through an increase in the average selective coefficients, $s_d$ and $s_b$. The second hypothesis, compensatory epistasis, states that decreases in fitness cause an increase in the ratio between the beneficial mutation rate and the deleterious mutation rate, such that the right side of Expression 4 increases. We focus here on testing these two forms of epistasis, because they are the most commonly modeled forms. If there is either no epistasis or positive epistasis, then the fitness decline will continue unchecked, and no fitness equilibrium occurs. Similar logic applies to the case of large populations adapting to a constant environment. In the absence of epistatic effects, or if positive epistasis operates, fitness would increase continuously without reaching an equilibrium.

### Figure 3. Mutation Accumulation

(A) Fitness measurements of the high-fitness MA lines. The fitness values of the parents are shown in gray, and the offspring in black. Note that all fitness values for each MA experiment were scaled such that the mean of the parental log10 fitness values was zero; this was done so that the error in fitness measurements was approximately normal, and so all three separate experiments in the low- and high-fitness lines could be combined on the same axis.

(B) Fitness measurements of the low-fitness MA lines. Shading and scaling are the same as in (A). In both (A) and (B), a considerable shift to the left (lower fitness) can be observed after a single round of mutagenesis. In the low-fitness lines, several offspring clones with fitness greater than the parental clones can be observed. In the high-fitness lines, no such offspring clones are observed.

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The Dynamics of Mutational Effects

(A) Shape of the joint ML gamma distribution of mutational effects for all MA datasets. The distribution is extremely leptokurtic (L-shaped), with the majority of mutations having very small selection coefficients, although a significant proportion have large selection coefficients. (B) Relationship between fitness and rate of compensatory mutation. Filled circles indicate the inferred proportion of compensatory mutations, but not in the effect size of mutations. This provides our first support for compensatory epistasis.

We tested the reliability of our results by using a different method to estimate $B$ for all the evolved populations. For a certain $N_e$ and a specified distribution of $s$, it is possible to use numerical simulations to find the value of $B$ at which mean population fitness no longer increases or decreases. Using the above estimate for the shape of the distribution of mutational effects, we could thus obtain an estimate of $B$ at which each of the evolved populations should not increase or decrease in fitness (see Materials and Methods). Using this method to estimate $B$ and a constant distribution of $s$ (as was found using the maximum likelihood analysis of the MA data), suggested that $B$ must increase to approximately 18% to maintain fitness equilibrium in the $N_e = 3$ populations. We used additional simulations to derive the value of $B$ required for all other population sizes to maintain fitness equilibria. We then used the median values of the experimentally determined equilibrium fitness values at each $N_e$ (Figure 2) to plot $B$ as a function of fitness (Figure 4B). $B$ increased in a nonlinear manner with fitness. For comparison, the two ML estimates of $B$ from the MA data are also plotted in Figure 4B. The close match between the two methods suggests that the estimates of $B$ are robust.

**Figure 4. Mutational Parameters**

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**Testing for the Effect of Lethal Mutations**

The results of the MA experiment and the above analysis suggest that an increase in the ratio between the rates of beneficial and deleterious mutations ($B$) allows low-fitness populations to reach a fitness equilibrium. There are two different ways in which this ratio can increase, either through an increase in $u_{lel}$, the rate of beneficial mutations, or through a decrease in $u_{dele}$. The two alternatives have different biological meanings. An increase in $u_{lel}$ with decreasing fitness would correspond to the hypothesis of compensatory epistasis. A decrease in $u_{dele}$, the synonymous substitution in larger populations, suggesting that synonymous substitutions may have been slightly deleterious (unpublished data). We therefore used the rate of synonymous substitution observed in the populations bottlenecked at $N_e = 3$ and $N_e = 10$ to estimate the neutral mutation rate. (We expect that in these populations synonymous substitutions behaved neutrally, i.e., the selective coefficient is less than the inverse of the population size.) The average rate of synonymous substitution in these lineages was 0.18 per transfer. We extrapolated this rate to a genomic rate by multiplying by the ratio of total sites to synonymous sites in the ancestral genome while accounting for a proportion of lethal mutations (see Materials and Methods). The estimated genomic mutation rate was 0.56 per transfer.

The ML estimate of the mean mutational effect over all populations had an expected value of 0.35 (0.082 per generation) (Figure 4A). The estimate of the mean effect was not different within the groups of low- or high-fitness populations (likelihood ratio test [LRT], $\chi^2$ 4 df, $p = 0.21$, $\chi^2$ 4 df, $p = 0.22$). Although the estimate was slightly lower in the high-fitness populations (0.29 as compared to 0.40), this difference was not significantly different between the two groups ($\chi^2$ 2 df, $p = 0.49$; Figure S1). The ML estimate of beta, which describes the shape of the distribution, was 1.0, and again, did not differ significantly between the two groups (Figure S1). This distribution of mutational effects agrees well with values reported for other viruses [40,41]. The ML estimate of $B$, the proportion of beneficial mutations, was zero for all high-fitness populations and greater than zero in all low-fitness populations. For two of the low-fitness populations, the model in which $B$ was greater than zero gave a significantly better fit than the model in which $B$ was constrained to zero (LRT, $\chi^2$ 1 df, $p = 0.39$, 0.010, and 0.0044 for these three ML analyses). The joint ML estimate of $B$ for all low-fitness populations was 16%, and was not significantly different between these populations (LRT, $\chi^2$ 2 df, $p = 0.63$, Figure 4B). These results suggest that populations with high and low fitness may differ in the proportion of beneficial mutations, but not in the effect size of mutations. This provides our first support for compensatory epistasis.

We tested the reliability of our results by using a different method to estimate $B$ for all the evolved populations. For a certain $N_e$ and a specified distribution of $s$, it is possible to use numerical simulations to find the value of $B$ at which mean population fitness no longer increases or decreases. Using the above estimate for the shape of the distribution of mutational effects, we could thus obtain an estimate of $B$ at which each of the evolved populations should not increase or decrease in fitness (see Materials and Methods). Using this method to estimate $B$ and a constant distribution of $s$ (as was found using the maximum likelihood analysis of the MA data), suggested that $B$ must increase to approximately 18% to maintain fitness equilibrium in the $N_e = 3$ populations. We used additional simulations to derive the value of $B$ required for all other population sizes to maintain fitness equilibria. We then used the median values of the experimentally determined equilibrium fitness values at each $N_e$ (Figure 2) to plot $B$ as a function of fitness (Figure 4B). $B$ increased in a nonlinear manner with fitness. For comparison, the two ML estimates of $B$ from the MA data are also plotted in Figure 4B. The close match between the two methods suggests that the estimates of $B$ are robust.
rate of deleterious mutations, could result if a large fraction
of deleterious mutations become lethal in less fit genotypes.
In our experimental regimes, the number of lethal mutations
was not directly measured, and it was thus important to test
for such an increase directly.

A change in the number of lethal mutations with changing
fitness would manifest as a change in the genomic mutation
rate, $U$, which is the sum of $\nu_u$ and $\nu_d$. We derived an
independent measure of $U$ based on the total number of
nucleotide substitutions that occurred in all lines; we used a
ML analysis to determine the $U$ that best fit the observed
number of substitutions in each population (see Materials
and Methods). A model in which $U$ varied across populations
did not significantly improve the likelihood over a model with
a single $U$ (LRT, $\chi^2$ 15 df, $p = 0.18$). There is thus no evidence
that low-fitness populations have lower $U$, as would be
expected if the proportion of lethal mutations had increased
in these populations. The new joint ML estimate of $U$ using
the nucleotide substitution data was 0.64. This closely
matched our first estimate of 0.56, which relied only upon
the number of synonymous substitutions observed in the $N_e = 3$ and $N_e = 10$ lines. Again, this suggests that our results are
robust and internally consistent, and that increases in $\nu_u$, and
not a change in the genomic mutation rate, accounted for the
observed changes in $B$ (Figure 4B).

Discussion

In the present paper, using experimental evolution of
viruses, we have shown that populations tend to converge to a
fitness equilibrium that depends on their population size.
Large population-size lines converged to high fitness, and
small population-size lines to low fitness (Figure 2). The
observations of such fitness equilibria in all the experimental
populations presented here, together with changes in the rate
of adaptation observed in other experimental populations
[32,42,43], demonstrate a simple, but perhaps underappreciated fact about the nature of mutational effects: they are
dynamic. This means that mutational distributions are
epistatic: the effect size, the sign (beneficial or deleterious),
or both, depend on the genetic background of the organism
in which they appear. Despite the evidence that the rate and
distribution of mutation effects change as organismal fitness
changes, a considerable amount of effort has been put toward
obtaining precise estimates of these parameters [6,14,25–
27,44–48], resulting in significant disagreement over what the
relevant values may be [48,49]. We propose that a more
careful examination of how these parameters are affected by
organismal fitness may give greater insight into how
adaptation occurs, and provide some resolution to the
qualitatively different conclusions concerning the rate and
shape of mutational distributions.

This distribution of mutational effects has significant
implications for adaptation, because the direction and rate
of fitness change in any population is governed by four
quantities intrinsic to the organism: the rate and mutational
distribution of beneficial mutations and the rate and muta-
tional distribution of deleterious mutations. As shown above,
that populations at a specific effective population size
converge toward a single level of fitness necessarily constrains
the values and dynamics of these quantities. In a population
in which mean individual fitness is increasing, the rate of
fixation of beneficial mutations multiplied by the mean effect
size must outweigh this analogous quantity for deleterious
mutations. In order for the rate of adaptation to decrease as
fitness increases, one or more of the following must occur: the
mean effect size of beneficial mutations must decrease; the
rate of beneficial mutations must decrease (and consequently
the rate of deleterious mutations must increase); or the effect
size of deleterious mutations must decrease (although the
consequence of this does depend on population size. When
$1/N_e$ is less than the average mutational effect, a decrease in
effect size will make deleterious mutations less visible to the
purifying action of selection, and this effect will outweigh the
fact that each deleterious fixation event has a lesser effect on
fitness). Conversely, if a population is decreasing in fitness, to
stop this decrease, one or more of the following must occur:
The mean effect size of beneficial mutations must increase; the
rate of beneficial mutations must increase (and consequently
the rate of deleterious mutations must decrease); or the effect
size of deleterious mutations must increase.

To untangle the different possibilities, we performed MA
experiments on lines differing by approximately 300-fold in
fitness to characterize the distribution of deleterious muta-
tional effects on fitness (Figure 4). This distribution of
mutational effects is leptokurtic, with most mutations being
of small effect, and a few having large effects (Figure 4A).
Across a very wide range of fitness values, this distribution of
effects appears to remain approximately constant (Figure S1).
Additionally, the shape of the distribution is strongly
supported by the genomic data, which show that even when
populations are maintained at large population sizes and
fitness does not change, substitutions continue to accrue;
these must be of very small effect. However, if all mutations
were of small effect, they should be immune to selection in
small populations. This was not observed; both deleterious
and beneficial mutations were subject to selective forces, even
in the smallest of the populations ($N_e = 3$). Although the
distribution differs slightly from others that have been
recently proposed, it concurs well with others [41]. We also
note that the distribution we have estimated includes both
synonymous and non-synonymous mutations, and that this
also contributes to the leptokurtic shape.

The deleterious mutation distributions found do not
provide significant support for the hypothesis that the effect
size of deleterious mutations changes with fitness (i.e.,
positive or negative epistasis between deleterious mutations)
(Figure 1), despite the fact that clones differing by a 300-fold
factor in fitness were tested. Although there is some
suggestion that mutational effects increase slightly in low-
fitness populations (negative epistasis), this effect did not
approach significance. Additionally, the size of the effect was
not large enough to account for the evolution of fitness
equilibria that were observed. This conclusion of constant
mean mutational effects is further supported by the
molecular data, which show that populations maintained at
or near fitness equilibrium have the same, and possibly
greater, rates of substitution when compared to populations
experiencing declines in fitness. This outcome is precisely the
opposite of that expected if equilibria were maintained
through more efficient selection (i.e., negative epistasis, or a
larger effect size of deleterious mutations). This type of
epistasis would act to purge more, not fewer, substitutions
from the population.
The absence of any change in the effect size of deleterious mutations suggests that a significant change must occur in either the rate or effect size of beneficial mutations. We provided strong support for compensatory epistasis using a ML analysis of MA data. We also showed that the increase in B, the proportion in beneficial mutations, is not driven by a decrease in the deleterious mutation rate (i.e., an increase in the nonviable mutation rate). This is shown through an analysis of the molecular data and is also suggested by the magnitude of change: it is likely that the beneficial mutation rate changes by much greater than 10-fold between the high- and low-fitness lines. A comparable change in the overall mutation rate would be easily detected; indeed, it is very unlikely that the rate of lethal mutation in the low-fitness lines increased more than 2-fold (see Materials and Methods).

The epistasis we have observed in our data is a result of a change in the probability that a given mutation is beneficial or deleterious and is dependent on the fitness of the organism in which it appears. The shape of the distribution of beneficial and deleterious effects remains approximately the same. This type of epistasis has been studied less often than other forms, but is consistent with several experimental estimations of epistasis that have focused only on deleterious mutations. The positive epistasis found to be operating between deleterious mutations in several recent studies could be due to the presence of compensatory mutation. Moreover, several recent comparative studies have emphasized the importance of compensatory epistasis in evolution. Together these results encourage reconsidering classic models to take compensatory epistasis into account. We must also emphasize that the model presented here is based on statistical properties of the distribution of mutational effects, and only makes predictions about the expected values of mutations. Thus we do not expect that all individual mutational interactions will conform to the model; there is already good empirical evidence for other types of epistatic interactions between mutations (e.g., antagonistic epistasis).

Additionally, it is important to note that the model of compensatory epistasis suggested here is not subsumed by any of the simpler models of epistasis (Figure 1) (although similar dynamics can be observed in some more complex models of adaptation). All of the simpler models of epistasis are defined according to deviations from expected fitness when there are combinations of either deleterious or beneficial mutations, and therefore involve a change in the mean of the distribution of deleterious or beneficial effects (Figure 1). The epistasis we have observed here relies simply on the change from beneficial to deleterious (and vice versa) of a given mutation according to the genetic background and fitness of the organism in which it appears; the mean of the distribution of beneficial and deleterious effects remains the same (Figure S1). Compensatory epistasis also suggests that few mutations are strictly deleterious or beneficial; they are conditionally deleterious or beneficial.

The nonlinear relationship that we observed between B (the proportion of beneficial mutations) and log-fitness is noteworthy. Because the shape of the mutational distribution does not change with fitness, log-fitness should, on average, scale linearly with the number of deleterious mutations that an individual has. Under the simplest mutational model, deleterious mutations interact multiplicatively (with i deleterious mutations, \(W = (1 - s)^i\)), and each deleterious mutation would result in a single beneficial mutation becoming available—the back mutation. Because the expected value of log-fitness and the number of deleterious mutations scales linearly (\(\log(W) = i\log(1 - s)\)), and the number of deleterious mutations scales linearly with the number of beneficial mutations (in a 1:1 manner), then log-fitness should also scale linearly with the beneficial mutation rate. This is true under any model in which each deleterious mutation results in a constant average number of newly available beneficial mutations. The nonlinearity of the relationship between B and fitness thus implies that the number of beneficial (compensatory) mutations that become available in the context of each additional deleterious mutation increases as fitness decreases. This suggests that there may be changes in the number of loci involved in compensatory mechanisms as fitness declines. One interpretation of this is that pleiotropic interactions between genetic loci (functions) increase as fitness decreases. Although a negative relationship between the rate of compensatory mutation and fitness has been predicted by modeling, little has been predicted concerning pleiotropy. We hope that our results will spur new interest in this topic.

This study is the first to propose and test an explicit model of adaptation in which organismal fitness specifies both the rate and distribution of deleterious and beneficial mutations. Although the model necessarily relies upon an idealized form of the genotypic landscape, it presents specific and testable predictions of the circumstances under which populations will increase or decrease in fitness.

Additionally, the model offers a simple mechanistic explanation for the diminishing rate of fitness increase observed within large populations adapting in a constant environment, as well as for the prevention of continued fitness degradation in small populations that have accumulated deleterious mutations. Finally, these data suggest that attempts to measure the rate (or distribution) of mutational effects must take into account that these rates may change by orders of magnitude as the fitness of an organism increases or decreases.

Materials and Methods

Strains. The original 4X174 bacteriophage used in this study was derived from a clone kindly provided by B. Fane. This clone was passaged for 100 transfers at 32°C to allow general adaptation to laboratory conditions. The deleterious mutants were obtained through serial bottlenecks with mutagenesis (see below), during which small plaques were purposefully chosen. The host was Escherichia coli (mutT), obtained from D. Bregon at INSERM.

Passaging. During passaging, phage were mutagenized in 250 mM hydroxyamine (HA) [58], 1 mM EDTA at 37°C for 140 min. Mutagenesis limited back mutation, prevented the mutation rate from evolving [59], and allowed simplifications to be made in modeling mutagenesis. Mutagenic treatment was stopped by 100- to 1,000-fold dilution into Luria-Bertani media with 0.5X NaCl (LC media). The phage were then plated on LC agar plates with 3 ml of soft agar and 0.3 ml of E. coli (mutT), and then grown overnight at 32°C. From these plates, a number of plaques equal to the bottleneck size were randomly selected and diluted into culture tubes containing 3 ml of 1 mM EDTA. These tubes were vortexed and centrifuged, after which 0.5 ml was removed to a fresh Eppendorf tube. Chloroform was added, the tubes were vortexed and centrifuged, and 0.5 ml was removed. This was used for further mutagenesis, and the remainder of the stock was stored in 40% glycerol at −20°C.

Competition experiments. Competition experiments were designed and performed as described previously in Burch and Chao.
Briefly, each strain was mixed in an approximately 1:1 ratio with a marked ancestral strain. This mixture was then plated twice to obtain a precise measure of the starting ratio of the two strains. At least three competition plates were plated for each strain (except when assessing the fitness of the mutation accumulation lines, for which only one plate was used); each of these were grown for 18 h (the time it takes for a doubling change to occur), and the fraction of the phage from each plate were harvested and re-plated once to find an ending ratio for the two strains.

**Mutation accumulation.** In an MA design, mutations are allowed to accumulate by randomly collecting the progeny of a parental phage with no bias against low-fitness progeny. The high-fitness parents were derived from two of the \( N_s = 100 \) populations (Figure 3A), and the low-fitness parents from two of the \( N_s = 3 \) populations (Figure 3B), after fitness convergence. To ensure that changes in mutational parameters were not due to the particularities of one clone, populations were used, one from each of two independent clones, and the fitness of 50 parental clones.

Fifty progeny clones were chosen randomly without respect to fitness. The MA experiment was performed simultaneously. For the analysis, log-transformed values of fitness were used because without this transformation, error was not normally distributed. Additionally, the means of the ancestral and descendant clones were performed simultaneously. The likelihood analysis assumes a normally distributed measurement error; the estimate of the distribution of fitness was derived from two of the \( N_s = 100 \) populations, as well as the fitness of 50 parental clones. Fifty progeny clones were chosen randomly without respect to fitness.

The likelihood surfaces were generated by calculating the likelihood values over a grid of parameter values while maintaining the other parameters at their ML-estimated values (for \( B = 0 \) in the high-fitness lines and 0.16 in the low-fitness lines).

**Estimation of the mutation rate.** The simplest method of estimating the mutation rate is to estimate the rate of mutation in a theoretically neutral class of sites, and then to extrapolate this rate to the entire genome. However, two problems arise in using such an analysis. Firstly, the mutagenesis generated in a significant bias toward cytosine \( \rightarrow \) thymine transitions; of all observed substitutions, 89% were of this nature. Additionally, a significant number of mutations are either lethal or highly deleterious, and such mutations will never occur in the population during evolution. It is very likely that the majority of such mutations are missense or nonsense substitutions. Because the ML model of the mutational distribution can only account for a unimodal distribution of mutations, with a maximum value of 50% for the two parameters were extrapolated (power law fit, \( r^2 > 0.98 \)), and the following equation was numerically solved for each population size:

\[
B \left( 0.0, \infty \right) \left( s \right) \left( \frac{0.01}{N} \right) \left( d \right) = \left( B + 1 \right) \left( e^{-\left( 0.001 \right) d s} \left( N \right) \right) \tag{5}
\]

in which \( B \) is the fraction of beneficial mutations, \( s \) is the ML gamma distribution of mutational effects, and \( p_\text{mut}(N,d,s) \) is the probability of fixation, for all simulations, thymine substitutions were simulated using plaques (not phages) as the individuals.

The relationship between log fitness and \( B \) was calculated using the mean equilibrium fitness of each population size and assuming a hyperbolic relationship, which implies the existence of a maximum fitness and a maximum value of 50% for \( B \). For each value of \( U \), the simulation took into account population size, initial fitness, and number of transfers. At least 1,000 simulations were run for each set of parameters, and the total number of mutations in each individual in the population was recorded. Likelihoods were calculated as the
probability of picking an individual with the same number of fixed mutations as the number observed.

### Supporting Information

**Figure S1.** Confidence Limits for the Parameters of the Mutational Distributions in Low- and High-Fitness Clones When $B$ Is Held Constant

(A) Confidence limits for the ML parameter values of the gamma distribution in the low-fitness clones. The proportion of beneficial mutations, $B$, was left at its ML value of 0.16. The joint likelihood surface for the three MA analyses in the low-fitness lines is shown.

(B) Confidence limits for the ML parameter values of the gamma distribution in the high-fitness lines. The proportion of beneficial mutations, $B$, was left at its ML value of zero.

(C) Confidence limits for the ML parameter values of the gamma distribution in all MA lines. The proportion of beneficial mutations, $B$, was left at its ML value (0.16 or 0). The joint log-likelihood value is indicated to the right of each panel; the ellipses indicate the 50%, 80%, 90%, and 95% confidence limits. The y-axis shows the estimated mean of the distribution, while the x-axis is the coefficient of variation ($\sqrt{\beta}$). The estimated mean of the distribution is relatively constant for the two groups, providing little support for any hypothesis under which the mean value of the selection coefficient changes with fitness. However, there is less confidence in the shape of the distribution (beta). Found at doi:10.1371/journal.pbio.0050094.sg001 (146 KB PDF).

**Figure S2.** Confidence Limits for the Parameters of the Mutational Distributions in Low- and High-Fitness Clones When Beta Is Held Constant

(A) Confidence limits for the ML parameter values of the gamma distribution in the low-fitness clones. The value of beta was kept constant, at one.

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### Text S1. Asymmetric Epistasis

Found at doi:10.1371/journal.pbio.0050094.ssth001 (26 KB DOC).

### Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) accession numbers for the genome sequences of the bacteriophage phiX174 lineages discussed in this paper are EF380009—EF380032.

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