Dependence of Epistasis on Environment and Mutation Severity as Revealed by 
in Silico Mutagenesis of Phage T7

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Manuscript received October 4, 2001
Accepted for publication January 14, 2002

Abstract

Understanding how interactions among deleterious mutations affect fitness may shed light on a variety of fundamental biological phenomena, including the evolution of sex, the buffering of genetic variations, and the topography of fitness landscapes. It remains an open question under what conditions and to what extent such interactions may be synergistic or antagonistic. To address this question, we employed a computer model for the intracellular growth of bacteriophage T7. We created in silico 90,000 mutants of phage T7, each carrying from 1 to 30 mutations, and evaluated the fitness of each by simulating its growth cycle. The simulations sought to account for the severity of single deleterious mutations on T7 growth, as well as the effect of the resource environment on our fitness measures. We found that mildly deleterious mutations interacted synergistically in poor-resource environments but antagonistically in rich-resource environments. However, severely deleterious mutations always interacted antagonistically, irrespective of environment. These results suggest that synergistic epistasis may be difficult to experimentally distinguish from nonepistasis because its effects appear to be most pronounced when the effects of mutations on fitness are most challenging to measure. Our approach demonstrates how computer simulations of developmental processes can be used to quantitatively study genetic interactions at the population level.

The interaction among mutations in their effects on fitness, known as epistasis, plays a major role in evolutionary processes (Wolf et al. 2000). It affects the mutation load of a population (Kimura and Maruyama 1966; Crow 1970), the drift and fixation of deleterious mutations (Phillips et al. 2000), and the topography of fitness landscapes (Whitlock et al. 1995; Phillips et al. 2000). Epistasis is also an essential component of models of the evolution of sex (Kondrashov 1993; Peters and Lively 2000). In small populations Muller’s ratchet (Muller 1964) offers sexual organisms an advantage over their asexual counterparts even in the absence of interactions among deleterious mutations (Haigh 1978). However, the mutational deterministic hypothesis, which addresses the advantages to sex for any population size, requires synergistic epistasis (Kondrashov 1993); in this case the collective effect of two or more deleterious mutations is more severe than their combined effect, had they acted independently. Following Lenski et al. (1999), one may distinguish among different forms of epistasis using a power model,

\[ \log(w) = -\alpha n^p, \]  

where \( w \) is the fitness of the organism relative to wild type, \( n \) is the number of deleterious mutations the organism carries, and \( \alpha \) and \( \beta \) are parameters. For deleterious mutations, \( \alpha > 0 \) and \( \alpha \) is larger for more severe mutations. The type and strength of the epistasis among the mutations are determined by \( \beta \), where \( \beta = 1 \) for independent interactions (nonepistasis), \( \beta > 1 \) for synergistic epistasis, and \( 0 < \beta < 1 \) for antagonistic epistasis (Figure 1). The degree of epistasis increases as \( \log(\beta) \) increases. The power model has an advantage over the more commonly used log-quadratic model (Charlesworth 1990), \( \log(w) = - (\alpha' n + \beta' n^2) \), which can erroneously predict an increase in fitness for large \( n \) in the case of antagonistic epistasis (\( \beta' < 0 \)).

Numerous experimental studies have been conducted to determine the dependence of fitness on the number of deleterious mutations, in particular, whether synergistic epistasis is ubiquitous in nature. Several of them have examined directly the variation in epistasis and found that both synergistic and antagonistic interactions are prevalent among individual sets of mutations (de Visser et al. 1997b; Elena and Lenski 1997; Whitlock and Bourguet 2000). However, the nature of epistasis across populations is all but clear. Some studies find synergistic epistasis (Spaskey et al. 1965; Mukai 1969), while others find multiplicative interactions (de Visser et al. 1997b; Elena and Lenski 1997). Further complications follow as different forms of epistasis may occur for different fitness components, such as the longevity or the productivity of an organism (de Visser et al. 1997a; de Visser and Hoekstra 1998; Peters and Keightley 2000; Whitlock and Bourguet 2000).

The confusion results from several factors. First, it is difficult to accurately estimate the fitness because of the
Figure 1.—Three forms of epistasis as described by the power model (Equation 1). Multiplicative, $\alpha = 0.002$, $\beta = 1$; synergistic, $\alpha = 0.0001$, $\beta = 2$; antagonistic, $\alpha = 0.009$, $\beta = 0.5$.

complex life cycles of the model organisms (de Visser et al. 1997b; de Visser and Hoesstra 1998; Peters and Keightley 2000; Whitlock and Bourguet 2000). Second, although the Darwinian fitness is the appropriate measure for $w$ in Equation 1 (West et al. 1998), the Malthusian parameter, which is the natural logarithm of the Darwinian fitness, has been used instead in some studies (de Visser et al. 1997a). This is problematic because synergism in the Malthusian scale can correspond to multiplicative or antagonistic interactions in the Darwinian scale. Third, theories on the advantages of sex have suggested that the most relevant interactions are those among mildly deleterious mutations, because deleterious mutations with large effects can easily be eliminated, even in small populations (Keightley et al. 1998). The effect of deleterious mutations, however, has yet to be adequately quantified or controlled. Fourth, mutants with many deleterious mutations are difficult to construct systematically in experiments, even for a relatively simple organism like the bacterium Escherichia coli; the resulting data are thus insufficient to distinguish at the population level among different types of epistasis (Elena and Lenski 1997). Efforts to accumulate many mutations, for instance, by applying mutagens (de Visser et al. 1996; Peters and Keightley 2000) or by growing the organism under minimum pressure of natural selection (Mukai 1969; Wloch et al. 2001a), replace this difficulty with that of accurately estimating the number of deleterious mutations. Finally, two related methods that detect epistasis by comparing the mean log-fitness of the parents with that of the offspring after a cross (de Visser et al. 1996) or by testing the skewness of the log-fitness distribution of these offspring (de Visser et al. 1997a; Wloch et al. 2001a) suffer several methodological limitations that may obscure their conclusions (West et al. 1998).

Given current limitations in generating, characterizing, and quantifying the effects of mutations on the fitness of organisms in the laboratory or in the field, we chose to study how simulated mutations affect the development of bacteriophage T7 in a computer model of its life cycle. The construction of this model is detailed elsewhere (Endy et al. 1997, 2000; You and Yin 2000; You et al. 2002). Briefly, it incorporates biochemical, genetic, and mechanistic data accumulated on phage T7 over the last four decades, and it uses ordinary differential equations and algebraic equations to describe the major molecular processes of T7 development: entry of T7 DNA into the host, transcription and translation of T7 genes, protein-protein interactions that regulate transcription, procapsid assembly, DNA replication, and progeny formation. It predicts, as a function of time postinfection, the intracellular levels of mRNAs, proteins, DNA, and, eventually, phage progeny. In essence, the model bridges the genotype of a phage with its growth phenotype, a feature we exploit here to predict how simulated phage mutations affect fitness.

Our use of the phage T7 model here to study epistasis shares advantages with approaches based on artificial-life programs (Lenski et al. 1999; Wilke and Adami 2001). Both approaches allow for the efficient creation and appraisal of thousands of in silico mutants. However, the T7 model is based on established biochemical mechanisms while the artificial-life programs are not. Simulated T7 mutations correspond to changes in molecular functions, such as binding constants between interacting proteins, promoter strengths, or terminator efficiencies, just as mutations alter molecular functions in laboratory mutants of T7. Mutations in any function can quantitatively span a broad parameter range and produce a correspondingly broad range of effects on phage growth, a unique feature of the T7 model that enables us to probe, at the level of individuals or populations, the severity of mutation effects on growth.

By incorporating the results of extensive experimental studies on T7, we have sought with our simulation to create a faithful quantitative representation of its intracellular infection dynamics. Nevertheless, gaps in our knowledge remain. Functions and mechanisms for many T7 genes are lacking (Endy et al. 2000; Molineux 2001; You et al. 2002) and only sparse data exist for the effects of host-cell physiology on phage growth (You et al. 2002). Our imperfect knowledge was most evident when we employed the simulation to predict the behavior of T7 mutants carrying permuted genomes (Endy et al. 2000); while the simulation captured qualitatively the detrimental effects on growth of repositioning an important early gene to locations downstream of its wild-type position, it was unable to account quantitatively for the resulting highly perturbed protein expression profiles. In contrast, under conditions that are closer to wild type, the simulation has performed well. Specifically, it has enabled us to better understand how a mutant carrying a single-gene deletion grows faster than wild type (Endy et al. 1997), and more recently, the simulation has, together with laboratory experiments,
provided a means for us to identify likely host-resource limitations to T7 growth (You et al. 2002). Our current work here employs no permuted genomes or other large excursions from wild type. Instead, we study how slightly deleterious mutations interact against a backdrop of essentially wild-type T7 behavior. As a result, we expect that deficiencies or imperfections in our detailed knowledge and simulated implementation of T7 biology will have little if any effect on the outcomes of this study.

**MATERIALS AND METHODS**

**Phage T7 model:** By accounting for and incorporating existing experimental data and mechanisms, we developed a genetically structured model to simulate the infection of a single E. coli BL21 cell by a single wild-type T7 particle (Endy et al. 1997). The present T7 model recasts the previous versions using an object-oriented approach. It treats the genome as an array of 145 functional genetic elements, including 59 genes, 7 E. coli RNA polymerase (EcRNAP) promoters, 17 T7 RNA polymerase (T7RNAP) promoters, two terminators, 10 RNase III splicing sites, and 50 spacer DNAs. These genetic elements constitute 74 genetic blocks, where each “block” consists of one genetic element or multiple overlapping genetic elements. This model accounts for the mechanisms of the entry of T7 DNA into the host cell; the logical definition of mRNA species based on the order of promoters, terminators, and RNase III splicing sites; synthesis of T7 mRNAs and proteins; regulation of T7 gene expression; degradation of host DNA and replication of T7 DNA; assembly of procapsids; and finally, formation of T7 progeny. It extends the earlier versions by accounting for the stoichiometric relation of the T7 helicase/primase (gp4A) and the DNA polymerase (gp5) in forming replication complexes, or replisomes, as well as the stoichiometric balance between the number of replication complexes and the maximum number of replication forks that can form on the newly synthesized T7 genomes. In addition, it allocates EcRNAPs and T7RNAPs to the synthesis of different mRNAs on the basis of the relative strengths of the promoters. Further, it incorporates a module that facilitates the sensitivity analysis of the model with respect to single parameters or two parameters simultaneously. All simulations in this study were conducted with a host growth rate of 1.5 doublings per hour.

Although in previous simulations we assumed that host resources were unbounded (Endy et al. 1997, 2000; You and Yin 2000), this model incorporates an empirically based host-cell model that accounts for experimentally observed correlations between the E. coli growth rate and resources such as the numbers of EcRNAPs and ribosomes, the pool sizes of NTPs and amino acids, and the cell volume (You et al. 2002). In setting up the host-cell model, we treated the host cell as a spatially homogeneous resource reservoir, where the levels of the resources were defined at the initiation of the T7 infection. We also assumed that (1) cell volume was constant over the course of infection, (2) there was negligible exchange of metabolites between the infected cell and its extracellular environment during infection, (3) the model host cell represented an average of a cell population that was growing at the exponential phase immediately prior to phage infection, (4) the initial NTP pool size was equivalent to the total RNA content of the cell, (5) NTPs were not consumed as the energy source for the reactions, and (6) the initial amino acid pool size was equivalent to the total protein content of the cell (You et al. 2002).

All versions of the T7 model have been compared with available experimental data. Simulated one-step growth curves agree well with experiments for wild-type T7 and a gene 1-deletion mutant complemented by constitutive expression of gene 1 in a recombinant host (Endy 1997). Although previous simulations that assumed unlimited host resources predicted poorly the growth of two T7 mutants carrying permuted genomes (Endy et al. 2000), better agreement was found when levels of host resources were adjusted to empirically observed levels (You and Yin 2001; L. You, unpublished data). Further, we recently demonstrated that the present model predicted well the dependence of T7 growth on the host cell growth rate (You et al. 2002).

**Definition of fitness:** The infection of an E. coli cell by phage T7 is often characterized by a one-step growth curve, from which we can extract parameters to define its fitness (Figure 2). Fitness is essentially determined by the interplay between the genotype of an organism and the environment in which it grows; so one genotype can exhibit different fitnesses in different environments. Here we consider two extreme scenarios. First, if a phage grows in an environment that permits only one cycle of infection, the fecundity of the phage, characterized by its burst size (Y), or the number of progeny produced per infected host bacterium, will be the most crucial parameter in determining its fitness. Phage that maximize their burst size in such poor-resource environments maximize their chances of survival. Therefore, an appropriate fitness measure (Wpoor) in this poor-resource environment is the burst size, or $W_{poor} = Y = \max(N(t))$, where $t$ is the time after infection initiation and $N(t)$ is the number of phage particles at $t$ (Figure 2a).

![Figure 2.—Definition of fitness. (a) The fitness measure for a poor-resource environment ($W_{poor}$) is defined from the simulated one-step growth curve as the maximum value of $N(t)$, where $t$ is the time (minutes) after infection initiation, and $N(t)$ is the number of phage progeny at $t$. (b) The fitness measure for a rich-resource environment ($W_{rich}$) is defined as the maximum value of $N(t)^{1/2}$.](image)
2a). In the second scenario, if the phage grows in a rich environment that allows an infinite number of infection cycles, then both the burst size \( (Y) \) and the burst time \( (t) \), the time when the burst occurs, will contribute to its fitness. For example, let us start with a single phage at time zero and ask how many phage there will be at time \( t \), assuming infinite host resources. For large \( t \), the number of phage will be \( \sim Y^{t/\tau} = Y^{n/\tau} \), where \( \tau = t \) corresponds approximately to the number of phage generations elapsed at time \( t \). This expression suggests a different measure of fitness: \( Y^{t/\tau} \). Since the phage with the highest fitness will burst at a time that maximizes \( Y^{t/\tau} \), we define \( W_{\text{rich}} = \max \{ Y(t)^{1/\tau} \} \) (Figure 2b).

We further denote the relative fitness values of a mutant phage for the cases above with \( W_{\text{poor}} = W_{\text{rich}} \) and \( W_{\text{rich}} = W_{\text{rich}}/W_{\text{poor}} \), respectively, where the subscript \( \text{w} \) indicates the fitness values for the wild-type phage. Note that both \( W_{\text{poor}} \) and \( W_{\text{rich}} \) are fitness measures in the Darwinian scale.

**Constructing T7 mutants in silico** Although we cannot yet predict how mutations at the DNA, RNA, or protein level influence molecular function, we do know that such changes can quantitatively alter function. By altering function they change molecular properties that are typically described by parameters such as enzymatic rates, equilibrium binding constants that characterize interactions between molecular components, promoter strengths, or extents of regulatory inhibition or enhancement of other molecular functions. Taking this perspective, we simulated the effects of mutations on specific T7 functions by altering T7 parameters from their wild-type default values. We then used one or more altered parameters in our simulation to calculate how one or more mutations could affect the intracellular development of the phage. Further, we defined a deleterious mutation as a single-parameter change that would reduce the T7 relative fitness, \( W_{\text{rich}} \), to a value below one. Different fitness metrics can be used to characterize phage growth, so the same mutation may be deleterious for one metric but not for another. However, for all cases examined, single mutations that were deleterious in \( W_{\text{rich}} \) were also deleterious or at least neutral in \( W_{\text{poor}} \).

Thirty T7 parameters were specified as potential targets for mutations; for each parameter, a range was specified from which random selected values corresponded to random deleterious mutations. The parameters, their default values, and their ranges normalized to the default values are listed in Table 1. These parameters were identified and their ranges were determined on the basis of a single-parameter sensitivity analysis on all T7 parameters. In the sensitivity analysis, each parameter varied from 0 to 100-fold in its default value and the resulting change in \( W_{\text{rich}} \) was examined for deleterious effect. For instance, if we reduce the T7RNAS elongation rate from its default value (200 bp/sec) to 0, the simulated \( W_{\text{rich}} \) will decrease from 1 to 0; thus the deleterious range for this parameter is (0, 1). To control the magnitude of deleterious mutations, we further partitioned each parameter range into five equal-width subranges. Each subrange was labeled by an index from 1 to 5, on the basis of its deviation from the default parameter value, 1 for the least deviant and 5 for the most deviant. Again consider the elongation rate of the T7 RNA polymerase as an example. Its complete range \((0, 1)\) was partitioned into the five subranges: subrange 1, \((0.8, 1.0)\); subrange 2, \((0.6, 0.8)\); subrange 3, \((0.4, 0.6)\); subrange 4, \((0.2, 0.4)\); and subrange 5, \((0, 0.2)\). We call a mutation in subrange \( k \) a class \( k \) mutation. For a given parameter, the deleterious effect of a mutation increases with its class index. A special subrange—\((0.9, 1.0)\)—was created to represent mutations with very mild effects, which we called class 0.5 mutations.

A T7 mutant with \( n \) random class \( k \) deleterious mutations was constructed by randomly selecting \( n \) parameters and then setting each selected parameter to a value randomly sampled from the class \( k \) subrange following a uniform distribution.

**Simulation and statistical analysis** For each \( n \), where \( 1 \leq n \leq 30 \), 500 T7 mutants carrying the same class of random deleterious mutations were constructed, and a simulation was performed for each mutant to compute its fitness using two measures, \( W_{\text{poor}} \) and \( W_{\text{rich}} \). The means and the standard deviations of \( \log(W_{\text{poor}}) \) and \( \log(W_{\text{rich}}) \) were calculated for each \( n \); the means were then fitted by least squares against \( n \) using Equation 1 to obtain \( \alpha \) and \( \beta \) values. For each fitted curve, \( R^2 \) was calculated as the ratio of the difference between the corrected total sum of squares and the residual sum of squares to the corrected total sum of squares. The magnitudes of \( \beta \) values obtained for \( W_{\text{poor}} \) and \( W_{\text{rich}} \) may be compared; however, because the \( \alpha \) values from \( W_{\text{rich}} \) curves depend arbitrarily on the dimensions of time \( t \), comparisons between \( \alpha \) values obtained for \( W_{\text{poor}} \) and \( W_{\text{rich}} \) will not be meaningful. A sample size of 500 appeared to be sufficient; sampling 1000 did not yield significantly different results. Further, all the simulations were conducted assuming a host growth rate of 1.5 doublings per hour. The same conclusion as presented here was reached when assuming other host growth rate values. The statistical analysis was conducted using Matlab and Mathematica.

**RESULTS AND DISCUSSION**

**Effects of environment and mutation severity on epistasis** The power model matched well the fitness loss for phage strains carrying up to 30 class 1 mutations in poor- and rich-resource environments (Figure 3, a and b). Moreover, these strains exhibited either synergistic or antagonistic epistasis, when tested in a poor- or rich-resource environment, respectively. When the analysis was extended to other mutation classes, the power model also served well to capture data trends (Figure 4). By extracting the \( \alpha \) and \( \beta \) values from each curve fit and then plotting \( \beta \) vs. \( \log(\alpha) \) we were able to probe how the form of epistasis, measured by \( \beta \), depended on the mutation severity, measured by \( \alpha \), in different environments (Figure 5). In a poor environment \( \beta \) was >1 for mildly deleterious mutations (classes 0.5, 1, and 2), indicating synergism, but it rapidly decreased with increasing \( \alpha \); it was nearly 1 for class 3 mutations and then became <1 for the most severe mutations (classes 4 and 5), reflecting antagonism (Figure 5a). These results show that the form of epistasis can depend on the severity of the mutations. By contrast, in a rich environment the epistasis was always antagonistic, but the antagonism decreased with decreasing severity of mutations (Figure 5b). Note the almost linear dependence of \( \beta \) on \( \log(\alpha) \) in either case. This relationship was stronger in the poor environment, where a least-squares linear fit between \( \beta \) and \( \log(\alpha) \) yielded an \( R^2 \) of 0.9971 compared with an \( R^2 \) of 0.9655 in the rich environment. Further, the rich environment deviation of the data from linearity suggests that \( \beta \) may asymptotically approach unity instead of crossing it (Figure 5b), a trend that is further discussed below.

From these results we draw two main conclusions, summarized in Table 2. First, mildly deleterious mutations tend to interact synergistically in a poor-resource environment, where fecundity is the primary determi-
TABLE 1
The list of parameters investigated in this study

<table>
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<th>Parameter</th>
<th>Default value</th>
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*a In the given range of values, each single parameter change alone is deleterious for T7 growth.
*b When the relative activity of promoter 13 is within the range of (0.3, 1), T7 growth is enhanced.
^c Relative activities of *E. coli* RNA polymerase promoters.
^d Relative activities of T7 RNA polymerase promoters.

nant of fitness. This result is consistent with the notion that synergistic epistasis can emerge from competition for food or limited resources (Peck and Waxman 2000). Second, deleterious mutations tend to interact antagonistically in a rich-resource environment, and the degree of antagonism increases as the mutation severity increases. This conclusion may provide an intuitive explanation to the controversy over the nature of epistasis: Perhaps previous studies reached different conclusions because they focused on different environments or were based on mutations of differing severity.

**Correlation between epistasis and mutation severity:** Inverse correlations between $\beta$ and $\alpha$ (Figure 5) have also recently been observed by Wilke and Adami (2001) in self-replicating computer programs and models for RNA folding. To probe the relationship between $\beta$ and $\alpha$ they considered sequences composed of a finite fixed number of monomers, where the fitness of mutants was defined as either neutral or lethal. Assuming that the fitness always decreased with the number of mutations, following a power model (Equation 1), they suggested that $\beta$ must be inversely correlated with $\alpha$ due to a conservation law that the total number of neutral mutants in the genetic space is constant (Wilke and Adami 2001).

However, this argument cannot be directly mapped to our study because of the infinite diversity of mutational effects on fitness in our system. Furthermore, the apparent linear dependence of $\beta$ on log($\alpha$) in Figure 5 suggests an alternative mechanism. If $\beta$ and log($\alpha$) followed an exact linear relation, then the different log($\omega$)-vs.-$n$ curves, each specified by a different pair of $\alpha$ and $\beta$ values, would share two points of intersection, one at $n = 0$ (wild type) and the other at some large number of mutations ($n \gg 30$, for our examples). The reasoning is as follows. At the large $n$ intersection point, we have
log(w_rich) = -\alpha N^\beta, where N is the number of mutations and \( w_rich \) is its corresponding fitness. This equation defines a linear relation between \( \beta \) and \( \log(\alpha) \), since \( \log(-\log(w_rich)) = \log(\alpha) + \beta \log N \). We assume that the power model is valid if and only if \( n > N \); it would predict that mutations carrying severe mutations would have a higher average fitness than mutants carrying the same number of mild mutations. Within this framework mutations that yield a fitness \( w_{rich} \) are effectively lethal since a fitness equal to zero is not defined. Further, the dependence of fitness on the accumulation of mutations will, for differing degrees of mutation severity, all originate from wild type but follow different paths that ultimately converge to the same fitness \( w_{rich} \) of the effectively lethal mutants. Hence, to reach this fitness with \( N \) mutations, mild mutations will tend to reinforce each other, leading to synergistic epistasis, whereas severe mutations will tend to buffer each other, resulting in antagonistic epistasis.

**Antagonism in rich environments:** In light of the above argument for a linear correlation between \( \beta \) and \( \log(\alpha) \), it appears contradictory that mildly deleterious mutations should still exhibit antagonistic epistasis in rich environments. We found that the relative fitness in a rich environment, \( w_{rich} \), is generally more sensitive to mutations than the measure in a poor environment, \( w_{poor} \); mutations that changed the latter also changed the former, but the inverse was not true. It would be thus conceivable that the same mutations could have a greater deleterious effect on \( w_{rich} \) than on \( w_{poor} \). (Note that the higher sensitivity of \( w_{rich} \) to mutations is not reflected by the \( \alpha \) values, because the absolute value of \( \alpha \) depends on the time units we use in the definition of \( w_{rich} \).) Perhaps if mutations were sufficiently mild, their interactions in a rich environment would become synergistic, following the pattern of epistasis in a poor environment. To test this possibility we examined interactions among mutations that were >100-fold milder than our 0.5 class mutations. These very mild mutations exhibited very slight antagonism in the rich environment (not shown) and behavior that was indistinguishable from wild type in the poor environment. This result further confirms the overall trend of changes in \( \beta \) in a rich environment (Figure 5b): As severity of mutations decreases, the interaction among these mutations asymptotically approaches multiplicativity (\( \beta = 1 \)).
fore, the differences in the forms of epistasis between our metrics do not merely reflect differences of degree to which these metrics are affected by mutations, but rather intrinsic differences in the nature of their responses to deleterious mutations.

**Limits to observability:** Besides its implication that epistasis and mutational effect could evolve only in a coordinated fashion (Wilke and Adami 2001), the correlation between $\beta$ and $\log(\alpha)$ further leads to a dilemma for any attempts to distinguish synergistic epistasis ($\beta > 1$) from nonepistasis ($\beta = 1$): If synergistic epistasis is present (for example, in a poor environment), it will be highest under conditions where the effects of mutations on fitness are minimal and most challenging to accurately measure (mutation severity class 0.5). This challenge may be better understood by considering a quantitative example. Weak synergistic epistasis is apparent for class 2 mutations, where 30

**TABLE 2**
The dependence of epistasis on growth environment and the magnitude of deleterious mutations

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Poor</th>
<th>Rich</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Synergism</td>
<td>Weak antagonism</td>
</tr>
<tr>
<td>Severe</td>
<td>No epistasis or weak</td>
<td>Antagonism</td>
</tr>
</tbody>
</table>

**Figure 5.**—The dependence of $\beta$ on $\alpha$ for (a) the poor environment and (b) the rich environment. Dashed lines indicate multiplicative interactions among mutations ($\beta = 1$, nonepistasis). A linear equation fits well the dependence of $\beta$ on $\log(\alpha)$ in each case, with $R^2 = 0.9971$ for the poor environment and $R^2 = 0.9655$ for the rich environment. All $\beta$ values are significantly different from 1.0, with $P < 0.01$ for $\beta = 1$, except the $\beta$ value for $\alpha_{\text{poor}}$ and class 3 mutations, where $P = 0.087$ for $\beta = 1$. 
mutations decrease the fitness by about one-half (Figure 4a). The average deleterious effect of each mutation should thus be no greater than $\sim 1 - (0.5)^{1/30} = 0.023$. That is, each mutation on average should decrease the fitness by $< 3\%$.

Although fitness effects of $\sim 2\%$ might be experimentally established in competition experiments using microbes, the resulting synergistic epistasis would be mild ($\beta$ is only slightly $< 1.0$). To measure such synergistic epistasis, one would need to quantify the fitness of a large number of mutants, ranging from those with single mutations to those with a large number of mutations. This would be a daunting experimental task. If the mutations overall are mild enough to demonstrate a high degree of synergistic epistasis, the effects of individual mutations may be too small and fall within the experimental variability of most fitness measures. For example, most experimental studies to date have measured only mutations with average selection coefficients $> 0.01$ (Spaskey et al. 1965; Mukai et al. 1972; Elena and Lenski 1997; Fry et al. 1999; Wloch et al. 2001b), and it has been suggested that the majority of deleterious mutations have effects that are immeasurably small under laboratory conditions (Davies et al. 1999).

**Extensions of in silico mutagenesis:** We have focused here on probing the effects of mutational severity and resource environment on the form and extent of epistasis in the simulated intracellular growth of a well-studied bacteriophage. This work may serve as a foundation to test the consequences of additional mechanisms or assumptions. For example, to study the effects of mutation severity on fitness we have assumed the mutations carried by each strain are uniformly distributed across each class of mutation severity. More natural distributions could be implemented by making mild mutations more frequent than severe mutations. From this perspective, our simulations of low severity (class 0.5 and class 1) mutations more likely reflect the effects of natural distributions than those involving severe mutations. Our current study has also neglected pleiotropic effects, where a mutation in one gene may affect more than one phenotypic trait. For example, a mutation that altered the processivity of the T7 RNA polymerase could at the same time influence the strength of its association with the T7 lysozyme, which downregulates the polymerase activity. To account for such effects, one would need to obtain data that quantitatively described the nature of each pleiotropic effect or assume and implement a mathematical model for its form.

We thank J. F. Crow, S. F. Elena, R. Kishony, and R. E. Lenski for helpful comments and suggestions and H. Wang for assistance with the statistical analysis. Support was provided by the National Science Foundation.

**LITERATURE CITED**


Communicating editor: P. D. Keightley