Evolution, 51(4), 1997, pp. 1058–1067

LONG-TERM EXPERIMENTAL EVOLUTION IN *ESCHERICHIA COLI*. VII. MECHANISMS MAINTAINING GENETIC VARIABILITY WITHIN POPULATIONS

SANTIAGO F. ELENA AND RICHARD E. LENSKI
Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824

Abstract.—Six replicate populations of the bacterium *Escherichia coli* were propagated for more than 10,000 generations in a defined environment. We sought to quantify the variation among clones within these populations with respect to their relative fitness, and to evaluate the roles of three distinct population genetic processes in maintaining this variation. On average, a pair of clones from the same population differed from one another in their relative fitness by approximately 4%. This within-population variation was small compared with the average fitness gain relative to the common ancestor, but it was statistically significant. According to one hypothesis, the variation in fitness is transient and reflects the ongoing substitution of beneficial alleles. We used Fisher's fundamental theorem to compare the observed rate of each population's change in mean fitness with the extent of variation for fitness within that population, but we failed to discern any correspondence between these quantities. A second hypothesis supposes that the variation in fitness is maintained by recurrent deleterious mutations that give rise to a mutation-selection balance. To test this hypothesis, we made use of the fact that two of the six replicate populations had evolved mutator phenotypes, which gave them a genomic mutation rate approximately 100-fold higher than that of the other populations. There was a marginally significant correlation between a population's mutation rate and the extent of its within-population variance for fitness, but this correlation was driven by only one population (whereas two of the populations had elevated mutation rates). Under a third hypothesis, this variation is maintained by frequency-dependent selection, whereby genotypes have an advantage when they are rare relative to when they are common. In all six populations, clones were more fit, on average, when they were rare than when they were common, although the magnitude of the advantage when rare was usually small (~1% in five populations and ~5% in the other). These three hypotheses are not mutually exclusive, but frequency-dependent selection appears to be the primary force maintaining the fitness variation within these experimental populations.

Key words.—Experimental evolution, Fisher's fundamental theorem, frequency-dependent selection, mutation-selection balance, relative fitness, within-population variation.

Received November 12, 1996. Accepted March 6, 1997.

Bacteria such as *Escherichia coli* provide a powerful experimental system for addressing a wide range of evolutionary questions (see reviews by Dykhuizen and Hartl 1983; Levin and Lenski 1983; Dykhuizen and Dean 1990; Lenski 1992; Sniegowski and Lenski 1995). Their short generation times make it feasible to observe population dynamics over tens, hundreds, and even thousands of generations. Their large population sizes ensure a substantial input of new mutations every generation, so that one can study the origin as well as the fate of genetic variation. Bacteria can be stored in suspended animation at low temperatures, which makes it possible to measure the relative fitness of derived and ancestral genotypes by placing them in direct competition, using genetic markers to tell them apart. Finally, they are easy to handle and propagate, which permits intensive replication of experiments so that even subtle effects can be measured. More than 1000 competition experiments were performed for this paper alone.

Since its beginnings early in this century, the field of population genetics has focused on quantifying the extent of variation *within* populations and identifying the forces responsible for maintaining that variation (Fisher 1930; Haldane 1932; Dobzhansky 1937; Wright 1969; Lewontin 1974; Kimura 1983; Gillespie 1991). For several years, our group has carried out a long-term evolution experiment with *E. coli* (Lenski et al. 1991; Lenski and Travisano 1994; Vasi et al. 1994; Travisano et al. 1995a,b; Elena et al. 1996; Mongold and Lenski 1996; Travisano and Lenski 1996; Souza et al., in press; Sniegowski et al. 1997; Travisano 1997). In contrast to the central goals of population genetics, we have focused most of our attention on quantifying the extent of divergence *between* replicate populations and elucidating the forces that have promoted parallel changes in some traits and divergence in others.

However, several considerations lead us now to believe that it would be very worthwhile to quantify the extent of variation within these experimental populations and determine the roles of several distinct population genetic processes in maintaining this variation. First, the rate of adaptation by the bacteria to their experimental environment has slowed considerably from its initially rapid pace. Between 5000 and 10,000 generations, the mean fitness of these populations (relative to their common ancestor) increased at a rate that was less than one-thirtieth of their rate of improvement during the first 1000 generations (Lenski et al. 1991; Lenski and Travisano 1994). Thus, the bacterial populations seem to have reached a state of quasi-equilibrium, which is presumably more similar to the state of most natural populations (except those that have recently encountered novel environmental conditions).

Second, substantial genetic diversity has been revealed by a study of the dynamics of molecular evolution in our evolving populations of *E. coli*. Michel Blot and collaborators (pers. comm.) have used IS elements to obtain DNA "fingerprints" for clones isolated from these populations. After 10,000 generations, they observed nine distinct genotypes among 11 clones sampled from one population and 11 distinct genotypes among 11 clones sampled from another population. These populations clearly must harbor considerable genetic diversity. Contamination can be ruled out as a source

of this diversity because all of the derived genotypes' fingerprints are still very similar to one another and to the ancestral genotype's fingerprint. Moreover, all of the derived genotypes have certain genetic markers that are characteristic of the ancestral strain (Lenski et al. 1991).

Third, Souza et al. (in press) performed an experiment using these same bacterial populations, as well as some additional genotypes, to examine the effects of plasmid-mediated recombination on evolutionary dynamics. During their study, they observed polymorphisms involving recombinant genotypes under the same environmental conditions used during the long-term experimental evolution of the asexual populations of *E. coli*. At least one polymorphism was stably maintained by frequency-dependent selection via a cross-feeding interaction (Turner et al. 1996). It was unclear from these experiments whether recombinant genotypes were somehow more prone to exhibit stable polymorphisms, or whether the genetic markers that allowed recombination events to be readily scored simply marked stable polymorphisms that could also arise with asexual genotypes. Therefore, they proposed a systematic examination of the extent of frequency-dependent selection in the asexual populations.

Fourth, Sniegowski (1997) have recently shown that some of the asexual *E. coli* populations have evolved genomic mutation rates that are approximately two orders of magnitude higher than the ancestor and the other derived populations. In a population of haploid organisms, the equilibrium frequency of a deleterious allele that is maintained by mutation-selection balance is proportional to the mutation rate (Nagylaki 1977). Hence, if recurrent deleterious mutations are a major source of variation in fitness, then we would expect to see much higher levels of variation in the populations with elevated mutation rates. Thus, we have a "natural experiment" that allows us to determine whether or not deleterious mutations contribute substantially to variation in fitness.

Finally, the field of population genetics has historically paid far more attention to organisms that are diploid and sexual than it has to those that are haploid and asexual. Nonetheless, it has become clear in recent years that bacterial species harbor tremendous genetic diversity, even one such as *E. coli* that is nearly asexual in nature (Milkman 1973; Selander and Levin 1980; Caugant et al. 1981; Istock et al. 1992; Souza et al. 1992; Maynard Smith et al. 1993). However, the forces that affect genetic variation may be quite different depending on the organism's genetic system. The most obvious difference is that dominance cannot play any role in maintaining genetic variability in haploid populations. A more subtle difference—but one that is probably more important—arises from the effect of beneficial mutations sweeping through a population by natural selection. In an asexual organism, all loci are effectively linked and so a selective sweep purges variation throughout the genome (Atwood et al. 1951; Levin 1981); by contrast, the selective sweep of a beneficial allele has much less of an effect on genetic variation in a sexual organism (Kaplan et al. 1989; Begun and Aquadro 1991). Therefore, it is especially interesting to examine the population genetic mechanisms that maintain variation in our experimental populations of *E. coli* precisely because they are asexual.

Our focus in this paper is on heritable variation in fitness itself. We will first quantify the amount of variation for fitness among clones within populations. We will then examine three population genetic hypotheses that postulate different mechanisms to maintain this variation. Hypothesis 1: The variation in fitness is transient and reflects the ongoing substitution of beneficial alleles by natural selection. To test this hypothesis, we use Fisher's fundamental theorem to compare for each population the rate of change in its mean fitness with the amount of within-population variation for fitness (Fisher 1930; Nagylaki 1977; Lenski et al. 1991). Hypothesis 2: The variation in fitness is maintained by recurrent deleterious mutations that give rise to a mutation-selection balance (Haldane 1937; Muller 1950; Crow and Kimura 1970). To test this hypothesis, we make use of the fact that some, but not all, of the replicate populations have evolved greatly elevated genomic mutation rates, which should lead to much higher levels of variation for fitness. Hypothesis 3: The variation in fitness is maintained by frequency-dependent selection, whereby genotypes are more fit when they are rare relative to when they are common (Haldane 1932; Wright and Dobzhansky 1946; Clarke and O'Donald 1964; Ayala and Campbell 1974). To test this hypothesis, we perform competition experiments to determine whether genotypes within the evolving populations tend to have a selective advantage when rare. (Note that we are *not* concerned with random genetic drift per se; drift may be important in maintaining molecular variation, but it cannot explain much variation in fitness in the large populations that we are studying.)

Materials and Methods

Bacteria and Culture Conditions

The bacteria in this study are part of a long-term evolution experiment with an asexual strain of *E. coli* B (Lenski et al. 1991; Lenski and Travisano 1994). Twelve populations were founded from two ancestral genotypes that were identical except for their ability to grow on L-arabinose. Each population was started from a single cell, so that initially there was no variation either within or among the populations. Therefore, all of the variation in the evolving populations has appeared by spontaneous mutation. The populations were serially propagated at 37°C for 1575 d in Davis minimal (DM) medium supplemented with glucose at 25 μg/mL (Carlton and Brown 1981; Lenski et al. 1991), which supports $\sim 5 \times 10^7$ cells/mL. Each day, 0.1 mL of the prior day's culture was inoculated into 9.9 ml of fresh medium. This dilution and subsequent regrowth allowed $\log_2 100 \cong 6.64$ generations of binary fission per day; thus, the 1575 d of serial transfer correspond to approximately 10,500 generations.

The present study used only those six populations that were founded from the Ara$^-$ ancestral genotype. Specifically, we used genetically heterogeneous samples of each Ara$^-$ population that were stored at generations 10,000 and 10,500. These samples were obtained by freezing several mL of each population after 0.1 mL had been transferred into fresh medium; hence, they include essentially all of the genotypes in the same proportions as they were present in the respective populations. We also used 25 clones that were sampled at random from each population at generation 10,000. From each of these 150 clones (6 populations × 25 clones per

population), we obtained a spontaneous Ara$^+$ mutant by plating millions of cells onto minimal agar that contained arabinose as a sole carbon source. Ara$^-$ and Ara$^+$ cells can be readily distinguished from one another by their production of red and white colonies, respectively, on tetrazolium arabinose (TA) indicator agar (Levin et al. 1977; Lenski 1988). These Ara$^+$ mutants therefore enabled us to measure the relative fitness of genotypes by placing them in direct competition with one another. Numerous experiments in our laboratory have shown that the Ara marker is selectively neutral in minimal glucose medium (Lenski 1988; Lenski et al. 1991; Bennett et al. 1992).

All of the experiments reported in this study were carried out using the same DM medium and other culture conditions employed during the long-term evolution experiment itself.

Estimation of Fitness

Competition experiments were performed to estimate either the mean fitness of a derived population relative to its ancestor or the fitness of a particular genotype relative to the population from which it was sampled. In either case, the two competitors (genotypes or populations) must have opposite Ara-marker states, so that their colonies can be distinguished on TA agar. Prior to every competition experiment, both competitors were separately acclimated for an entire growth cycle (~6.64 generations) in the same DM medium in which they would subsequently be mixed. The two competitors were usually mixed at a 1:1 volumetric ratio and diluted 100-fold into fresh DM medium to start the competition experiment. In some experiments that were performed to assess the extent of frequency-dependent selection, we also employed a 1:10 initial ratio while keeping the same overall dilution factor. Samples were taken from the competition flasks at time zero and again after 24 h (one growth cycle) and spread onto TA agar to produce colonies.

For each competitor, we computed its Malthusian parameter as $m = \ln[N(1)/N(0)]/(1\ \mathrm{d})$, where $N(0)$ and $N(1)$ are population densities at time zero and after one day. The fitness of one competitor relative to the other, W, was then calculated as the ratio of their realized Malthusian parameters (Lenski et al. 1991).

Estimation of Mutation Rates

The mutation rates used in this study were estimated previously by Sniegowski (1997). Briefly, rates of mutation were estimated at three different loci that could be readily scored: reversion from Ara$^-$ to Ara$^+$, resistance to nalidixic acid, and resistance to phage T5. Estimates were obtained using fluctuation tests (Luria and Delbrück 1943), in which many replicate cultures were grown from a few founder cells and the numbers of cells expressing the mutant phenotype in each culture were counted. Mutation rates were then calculated from the distribution of mutants across the replicate cultures using a maximum-likelihood method (Ma et al. 1992; Stewart 1994). Mutation rates were estimated for single clones that had been chosen at random from each population after 10,000 generations. Two lines of evidence indicate that the mutation rates measured for these clones reflect the average mutation rates for the populations from which they were sampled. First, a number of additional clones were sampled from the two populations that exhibited elevated mutation rates at various time points in their evolutionary history. In each case, these clones had mutation rates that were consistently elevated beginning at some time point well before 10000 generations (Sniegowski et al. 1997). Second, in the course of obtaining spontaneous Ara$^+$ mutants for 25 clones from each of the six populations in this study, we observed that characteristically few or many mutants were obtained for most, if not all, of the clones sampled from a particular population. Thus, these very large differences in mutation rate evidently distinguished populations (rather than merely clones within populations).

Experimental Designs

Within-Population Variance for Fitness.—Each of the 25 Ara$^+$ clones was placed in competition with the heterogeneous population from which the clone's Ara$^-$ progenitor was sampled. Three replicate competitions were performed for each Ara$^+$ clone and its Ara$^-$ source population, with both types at an initial volumetric ratio of 1:1. Every replicate competition yielded an estimate of relative fitness, W, as defined above. Thus, we had a total of 450 fitness estimates (6 populations $\times$ 25 clones $\times$ 3 replicates) on which to base our estimates of the within-population variance for fitness, Var W.

Change in Mean Fitness.—The heterogeneous samples from each Ara$^-$ population were placed in competition with the Ara$^+$ variant of the common ancestor. Ten replicate competitions were performed using the 10,000-generation samples and another 10 were performed using the 10,500-generation samples. Thus, we had a total of 120 estimates of fitness (6 populations $\times$ 2 time points $\times$ 10 replicates) on which to base our estimates of the change in mean fitness, $\Delta\bar{W}$.

Advantage When Rare.—Three more competitions were performed using each of the 25 Ara$^+$ clones and its Ara$^-$ source population. These competitions were started with a ratio of 1:10 for the clone and its source population, respectively, whereas the competitions used to estimate the within-population variance for fitness began with a 1:1 ratio. The competitions at these two initial ratios were performed together, in a pairwise fashion, so that we could compute for each clone three independent estimates of the difference in relative fitness when the clone was rare versus when it was common. Thus, we had a total of 450 estimates of the fitness difference (6 populations $\times$ 25 clones $\times$ 3 replicate pairs) on which to base our estimates of the advantage when rare, AWR.

Statistical Analyses

When testing the magnitude of some hypothesized effect within a particular bacterial population, the number of degrees of freedom used to determine statistical significance was equal to one less than the number of independent observations for that population (usually the number of clones that were sampled). When testing the applicability of a hypothesis to the populations in general, the number of degrees

TABLE 1. Nested analysis of variance to estimate the genetic variance for relative fitness among clones within populations.

Source	SS	df	MS	F	P
Among populations	0.3090	5	0.0617	6.008	< 0.0001
Among clones (within populations)	1.4802	144	0.0103	1.787	< 0.0001
Error	1.7261	300	0.0058		
Total	3.5154	449			

of freedom used to determine statistical significance was set by the number of replicate populations.

Variance components and their confidence intervals were calculated from nested analyses of variance as described by Sokal and Rohlf (1995). In some of the analyses that we performed, the assumption of homogeneity of variances was violated. In the case of estimates of mutation rates, which spanned orders of magnitude, this violation was severe and so we log-transformed these data to make the variances homogeneous. We also repeated all other analyses of variance using log-transformed data; when we did so, the variances were no longer significantly heterogeneous. Using the transformed data, however, we obtained statistical conclusions that were in all essential respects identical to those obtained using the untransformed data. Therefore, except for mutation rates, we have chosen to report the analyses that were performed using the untransformed data because they are easier to interpret in terms of the three population genetic models that we sought to test.

We report one-tailed significance levels for all of the statistical tests in this paper. The three population genetic hypotheses that we have tested make *directional* predictions about effects and the correlation of those effects with levels of within-population variability. For example, if frequency-dependent selection is going to promote variation within a population, then genotypes must have a systematic advantage when rare (i.e., $AWR > 0$). Similarly, this hypothesis predicts a positive correlation across the replicate populations between the average advantage when rare to genotypes in a population and the amount of variability in that population.

RESULTS

Within-Population Variance for Fitness

The analysis of variance shown in Table 1 indicates that the variability in fitness among clones within populations was highly significant. (The analysis also shows significant variation among populations, but this effect does not concern us in the present study, which is focused on the variation within populations.) The resulting estimate of the within-population variance component, Var W, is 0.0015. Taking the square-root of this variance component yields a corresponding standard deviation for fitness of 0.0387. In other words, two clones chosen randomly from the same population differed from one another in their relative fitness by ~3.9%, on average. By contrast, the mean fitness of the derived populations increased by more than 50% relative to the common ancestor (Lenski and Travisano 1994). Thus, multiple clones sampled from the same population differed in their fitnesses, but these differences were much smaller than the cumulative fitness gains that occurred during 10,000 generations of experimental evolution.

TABLE 2. Estimates for six experimental populations of the within-population variance for relative fitness, Var W. Three replicate competitions were performed for each of 25 clones from every population. Negative values for Var W reflect statistical noise and are not biologically meaningful.

Population	MS clones ($\times 10^3$)	MS error ($\times 10^3$)	P	Var W ($\times 10^4$)	Lower and upper 95% confidence limits ($\times 10^4$)	
Ara$^-$ 1	7.20	4.45	0.0754	9.18	−3.33	29.22
Ara$^-$ 2	22.36	6.84	0.0002	51.74	20.25	112.58
Ara$^-$ 3	15.20	6.97	0.0101	27.43	4.00	69.18
Ara$^-$ 4	7.99	6.42	0.2524	5.23	−11.06	27.95
Ara$^-$ 5	4.75	5.15	0.5756	−1.36	−13.93	12.70
Ara$^-$ 6	4.18	4.69	0.6109	−1.70	−13.15	10.76

Table 2 summarizes the among-clone variation for fitness estimated separately for each of the six populations. The six populations were variable in the extent of their within-population variance for fitness, as indicated by the 95% confidence intervals for their respective variance components. Thus, we have a strong signal that may be used to disentangle the role of different population genetic mechanisms in promoting that variance. In particular, population Ara$^-$2 had the highest within-population variance for fitness, and as we will show later it was unusual in other respects as well. Therefore, it is of interest to ask whether there is significant variance in fitness among clones (within populations) if population Ara$^-$2 is removed from the analysis. The resulting analysis of variance indicated that there remained significant clonal variation in fitness ($F_{120,250} = 1.421$, $P = 0.0109$) even after population Ara$^-$2 was excluded. However, the resulting within-population variance component was reduced to 0.0008, so that a pair of clones sampled randomly from the same population would have differed in fitness by only ~2.8%, on average.

Test of Fisher's Fundamental Theorem

According to Fisher's fundamental theorem, the instantaneous rate of change in the mean fitness of a population (living in a constant environment) is equal to the genetic variance for fitness within that population (Fisher 1930; Nagylaki 1977; Lenski et al. 1991). This theorem is specifically concerned with the selective sweep of beneficial alleles through a population, which simultaneously generates transient heterogeneity and a shift in the population's mean fitness. We sought to determine whether the observed variation in fitness within populations was simply a manifestation of the ongoing substitution of beneficial alleles carried by different clones.

Table 3 shows the mean fitness values estimated at 10,000 and 10,500 generations for each of the six populations. None of the populations showed any significant increase in mean fitness over this time span. This negative result cannot be attributed to a lack of sufficient replication, as the same data reveal a very strong positive correlation between mean fitnesses estimated at the two time points ($r = 0.9564$, 4 df, one-tailed $P = 0.0014$). That is, certain populations were substantially more fit (relative to the common ancestor) at

TABLE 3. Estimates for six experimental populations of the change in mean fitness, $\Delta\bar{W}$, between generations 10,000 and 10,500. Ten estimates of mean fitness were obtained at each time point for every population. *P*-values are based on one-tailed *t*-tests (with 19 df) of the hypothesis that $\Delta\bar{W} > 0$.

Population	$\bar{W}$ at 10,000 generations (± SD)	$\bar{W}$ at 10,500 generations (± SD)	$\Delta\bar{W}$	*P*
Ara⁻ 1	1.618 (± 0.129)	1.609 (± 0.090)	−0.009	0.5737
Ara⁻ 2	1.849 (± 0.210)	1.746 (± 0.103)	−0.103	0.9091
Ara⁻ 3	1.602 (± 0.151)	1.555 (± 0.172)	−0.047	0.6926
Ara⁻ 4	1.666 (± 0.120)	1.667 (± 0.134)	0.001	0.4954
Ara⁻ 5	1.536 (± 0.093)	1.536 (± 0.103)	0.000	0.4985
Ara⁻ 6	1.610 (± 0.118)	1.596 (± 0.131)	−0.014	0.5969

10,000 generations than were other populations, and these differences in fitness between populations were preserved over the subsequent 500 generations. Nonetheless, the absence of change in mean fitness over this interval implies that we do not have an adequate signal for testing the prediction of Fisher's fundamental theorem. Indeed, we failed to observe the positive correlation between Var W and $\Delta\bar{W}$ that was predicted under this hypothesis ($r = -0.9663$, 4 df, one-tailed $P = 0.9992$ for $r > 0$). In fact, there appears to be a negative correlation, although this is almost certainly spurious because none of the populations exhibited any significant change in mean fitness.

In addition to examining the statistical correlation between Var W and $\Delta\bar{W}$, we asked whether the significant within-population variation for fitness was quantitatively consistent with the failure to observe any significant increase in fitness. The following equation allows us to calculate the change in fitness that would be expected over 500 generations (= 75 d), given the observed variation in fitness, according to Fisher's fundamental theorem (Lenski et al. 1991, eq. 7b):

$$\Delta\bar{W} = \text{Var } W \times \bar{m} \times 75 \text{ d}, \quad (1)$$

where $\bar{m}$ is the average Malthusian parameter (ln 100 = 4.605 per day) and measures the rate of population turnover. The estimated clonal variation within populations (averaged over the six replicate populations) was 1.5×10^{-3}, from which we would expect to have seen an increase in mean fitness of ~0.52. This value is far greater than was actually measured for any of the six populations (Table 3). However, this calculation presumes that the within-population variance for fitness, Var W, persisted over time, whereas selection will tend to exhaust the variation in fitness. Very conservatively, we would expect under Fisher's fundamental theorem to have seen an increase in mean fitness of at least $(\text{Var } W)^{1/2}$. This calculation assumes that the populations were sampled at the moment of maximum variance (i.e., two genotypes at frequencies of 0.5), as one genotype replaced another. But even this conservative analysis gives an expected increase in fitness, $\Delta\bar{W}$, of 0.039, which is much greater than was observed for any of the six replicate populations. Evidently, the within-population variance for fitness was too great to be explained as transient variation that existed only as more fit genotypes replaced those that were less fit.

TABLE 4. Estimates for six experimental populations of mutation rates relative to the common ancestor, μ_{rel}, for three loci (Sniegowski et al. 1997). The ancestral mutation rates (per cell generation) to Ara⁺, Nal^R, and T5^R were estimated as 9.25×10^{-10}, 7.10×10^{-11}, and 8.78×10^{-8}, respectively. Mutation rates for populations Ara⁻ 2 and Ara⁻4 were significantly different from those of the ancestor at all three loci; the other populations did not differ significantly from the ancestor in their mutation rates at any of the loci.

Population	μ_{rel} to Ara⁺	μ_{rel} to Nal^R	μ_{rel} to T5^R	Geometric mean μ_{rel}
Ara⁻ 1	0.217	2.155	0.708	0.692
Ara⁻ 2	22.243	429.577	320.046	147.293
Ara⁻ 3	2.508	1.845	1.055	1.696
Ara⁻ 4	29.189	174.648	25.285	50.514
Ara⁻ 5	2.086	0.665	0.723	1.001
Ara⁻ 6	1.773	3.296	0.315	1.226

Test of Mutation-Selection Balance

Sniegowski et al. (1997) discovered that two of the six Ara⁻ populations had evolved mutation rates approximately two orders of magnitude higher than those of the ancestor and the other four populations. Table 4 shows that these differences in mutation rate were present for all three loci that were screened. An analysis of variance of the log-transformed mutation rates (with loci as blocks) confirms that the six populations were highly divergent in their relative mutation rates ($F_{5,10} = 15.513$, $P = 0.0002$). The heterogeneity in mutation rates remains highly significant even when population Ara⁻2 is excluded from the analysis ($F_{4,8} = 12.908$, $P = 0.0014$). Genetic complementation tests indicated that populations Ara⁻2 and Ara⁻4 had acquired mutations that rendered their methyl-directed mismatch repair systems defective (Sniegowski et al. 1997). These two populations must therefore have experienced much higher mutation rates throughout their genomes than did the other four populations. Thus, these two populations should have harbored much greater genetic variance for fitness under the hypothesis that the variation was maintained by a balance between recurrent deleterious mutations and selection against them.

As shown in Figure 1, we observed a marginally significant positive correlation between the clonal variance for fitness and the relative mutation rates of the six populations ($r = 0.7919$, 4 df, one-tailed $P = 0.0303$). However, this correlation could be attributed entirely to the fact that population Ara⁻2 had both the highest variance for fitness and the highest mutation rate. By contrast, population Ara⁻4 was also exceptional in its mutation rate, but it was unexceptional in its variance in fitness. Excluding population Ara⁻2 eliminates the positive correlation between the within-population variance for fitness and relative mutation rate ($r = -0.1083$, 3 df, one-tailed $P = 0.5688$ for $r > 0$). Thus, the hypothesis that genetic variation in fitness was maintained in these experimental populations primarily by a balance between recurrent deleterious mutations and selection against those mutations lacks compelling support.

Test of Frequency-Dependent Selection

To evaluate the role of frequency-dependent selection in maintaining variation within populations, we compared the

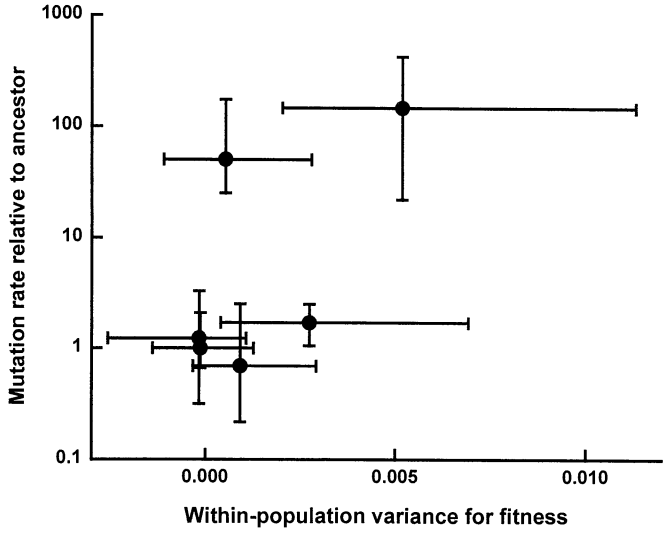

FIG. 1. Correlation between the within-population variance for fitness, Var W, and the mutation rate relative to the common ancestor, μ_{rel}, for six populations. The correlation is marginally significant ($r = 0.7919$, 4 df, one-tailed $P = 0.0303$). Error bars around estimates of Var W are 95% confidence intervals; error bars around estimates of μ_{rel} show the range of values for three loci.

fitness of clones (relative to their source population) when they were rare versus common. If frequency-dependent selection contributed to the maintenance of genetic variation within the populations, then we would expect clones to have had a systematic advantage when rare, or AWR. Table 5 shows the average value of AWR that was measured for each of the six populations. An analysis of variance indicates significant differences among the six populations in the strength of frequency-dependent selection ($F_{5,144} = 3.615$, $P = 0.0041$). Treating each population as one observation, the grand mean AWR is equal to 0.0212, which is significantly greater than zero ($t_5 = 2.1361$, 5 df, one-tailed $P = 0.0429$). Once again, population Ara$^-$2 contributed disproportionately to the overall effect. If population Ara$^-$2 is excluded, then an analysis of variance finds no significant variation among the remaining populations in terms of the effects of frequency-dependent selection ($F_{4,120} = 0.2912$, $P = 0.8832$). However, even when population Ara$^-$2 is excluded, the grand mean of AWR equals 0.0115 and remains significantly greater than zero ($t_4 = 4.0734$, 4 df, one-tailed $P = 0.0076$). Hence, we conclude that there was strong frequency-dependent selection in population Ara$^-$2 with much weaker, but still significant, selection favoring minority genotypes in the other populations.

Figure 2 shows a significant correlation between the clonal variance for fitness and the average advantage when rare across the six populations ($r = 0.8650$, 4 df, one-tailed $P = 0.0131$). As in the previous section, this correlation is highly dependent on population Ara$^-$2, which had both the highest variance for fitness and exhibited the strongest frequency-dependent selection. If population Ara$^-$2 is excluded, then the positive correlation becomes nonsignificant ($r = 0.2420$, 3 df, one-tailed $P = 0.3475$). However, there are no conspicuous discrepancies between the clonal variance for fitness and the average advantage when rare for any of the remaining five populations, whereas there was such a discrepancy in the relationship between variance for fitness and mutation rate.

TABLE 5. Estimates for six experimental populations of the average clone's fitness advantage when rare, AWR. Three paired competitions were performed for each of 25 clones from every population. P-values are based on one-tailed t-tests (with 24 df) of the hypothesis that $AWR > 0$.

Population	Average AWR	SD	t	P
Ara$^-$ 1	0.0094	0.0656	0.7188	0.2396
Ara$^-$ 2	0.0694	0.0852	4.0716	0.0002
Ara$^-$ 3	0.0165	0.0565	1.4624	0.0783
Ara$^-$ 4	0.0042	0.0747	0.2784	0.3915
Ara$^-$ 5	0.0196	0.0466	2.1080	0.0228
Ara$^-$ 6	0.0080	0.0445	0.8959	0.1896

Moreover, the quantitative agreement between the clonal variance for fitness and the average advantage when rare appears to be reasonably good. The average difference in fitness among clones from the same population was 0.039 (0.028 excluding Ara$^-$2), and the average advantage when rare was 0.021 (0.012 excluding Ara$^-$2). In other words, both analyses indicate significant fitness effects on the order of a few percent. However, we may have systematically underestimated the average advantage when rare, as shown by the following hypothetical case. If a source population contained two ecologically distinct genotypes in equal frequency, then the 1:10 initial ratio of a marked clone relative to its source population would have produced a 6:5 (i.e., 1:5:5) ratio of the two ecotypes, while a 1:1 initial ratio would have yielded a 3:1 (i.e., 1:0.5:0.5) ratio of the ecotypes. Hence, instead of spanning an order of magnitude in relative initial frequencies, the actual range may have been somewhat smaller. Even so, we conclude that frequency-dependent selection favoring genotypes when they were rare contributed significantly to the maintenance of variation within these experimental populations.

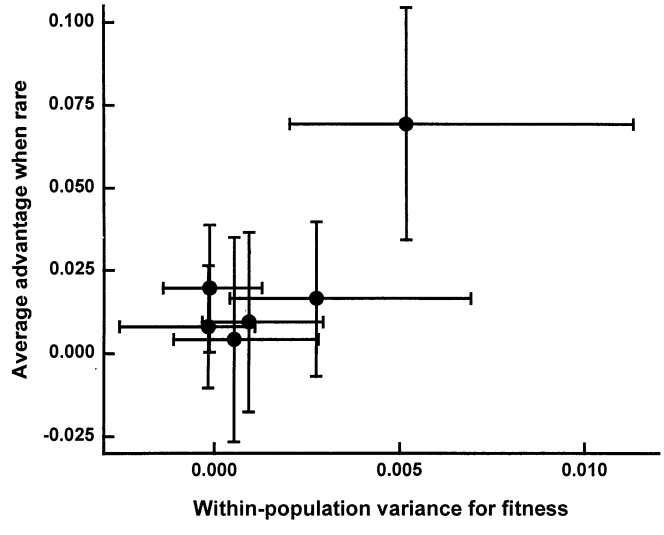

FIG. 2. Correlation between the within-population variance for fitness, Var W, and the average clone's fitness advantage when rare, AWR, for six populations. The correlation is significant ($r = 0.8650$, 4 df, one-tailed $P = 0.0131$). Error bars around estimates of both Var W and AWR are 95% confidence intervals.

Discussion

Our study has addressed a classical question in population genetics: What forces maintain genetic variability within populations? Although the question is an old one, the approach that we have taken differs in several important respects from the many other studies that have previously considered this issue. First, we have studied a haploid asexual microorganism, whereas most earlier work focused on diploid sexual animals and plants. As pointed out in the introduction, certain population genetic mechanisms may be either more or less important depending on these distinctions.

Second, we have focused on those processes that maintain variation in an organism's performance in its historical environment (i.e., its fitness). By contrast, most previous studies of the mechanisms that maintain heritable variation in populations have examined traits (including allozymes and DNA sequences) whose relationship to organismal performance is uncertain and difficult to establish. Consequently, tremendous energy was devoted to determining the relative importance of random genetic drift versus natural selection (Lewontin 1974; Kimura 1983; Gillespie 1991), while much less empirical work was done to disentangle the several distinct ways in which selection can affect heritable variation within populations—selection against deleterious mutations, selection for beneficial alleles, and frequency-dependent selection. And while many studies have examined the action of one or another of these modes of selection, our study is unique (to the best of our knowledge) in that we have measured the effects of all relevant modes of selection in the same populations. (We have emphasized modes of selection because of our interest in the processes maintaining variation in fitness; it remains to be seen whether genetic drift played a large or small role with respect to molecular variation in these same populations.)

Third, we have examined these population genetic processes in the context of a well-defined and replicated experimental system. Thus, we can have confidence about the historical environment of the organisms under study, their effective population size, and so on. Moreover, although we have been concerned with the processes that affect genetic variation within a population, we have used replicate populations as our unit of observation for the purpose of statistical inference. This approach limits the apparent statistical power of our tests because we could claim many more degrees of freedom based on intensive replication of measurements within any particular population. However, by analyzing the various effects across replicated populations, we could rigorously address their generality (within the context of the specific biological system under study).

Our major findings can be summarized as follows. Frequency-dependent selection was apparently the most important factor in maintaining heritable variation with respect to fitness properties of the bacteria within these experimental populations. Several analyses showed the importance of frequency-dependent selection in promoting genetic polymorphisms. On average, clones were at a competitive advantage when they were rare relative to when they were common ($AWR > 0$); this effect was significant whether or not population Ara$^-$2, which exhibited the most extreme effect, was included in the analysis. Moreover, there was a significant positive correlation between the average AWR for a population and the variation in fitness within that population, Var W (although this latter result depended on the inclusion of population Ara$^-$2).

We did not seek to identify the ecological interactions that promote frequency-dependent selection in these populations. However, several distinct mechanisms that can maintain genetic polymorphisms in bacteria have been mathematically modeled, experimentally demonstrated, or both (Levin 1972; Stewart and Levin 1973; Chao et al. 1977; Lenski and Hattingh 1986; Helling et al. 1987; Levin 1988; Rosenzweig et al. 1994; Turner et al. 1996). Some of these mechanisms involve viral predators or antibiotics, which were absent from our experimental system. The two ecological mechanisms that are most likely to have contributed to frequency-dependent selection in our experiments are cross-feeding interactions and fluctuations in resource concentration. In a cross-feeding interaction, one strain is superior in competition for a primary resource while another strain is better able to grow on a metabolic by-product released into the medium (Helling et al. 1987; Rosenzweig et al. 1994; Turner et al. 1996). In a serial transfer regime, temporal fluctuations in resource concentration may allow two strains to coexist stably if their relative growth rates are reversed at high and low concentrations (Levin 1972; Stewart and Levin 1973; Turner et al. 1996).

By contrast, neither the selective fixation of novel beneficial alleles nor the selective elimination of recurring deleterious mutations appears to have contributed very much to the genetic variance for fitness within these populations. If the observed variance for fitness within populations had been due to the selective sweep of beneficial alleles, thereby giving rise to transient polymorphisms, then we should have been able to see an increase in mean fitness (in accordance with Fisher's fundamental theorem). However, there was absolutely no evidence for an increase in mean fitness, $\Delta\bar{W}$, of any population during the period immediately following the measurement of the within-population variance for fitness, Var W. And if the observed variance for fitness within populations was caused by recurrent deleterious mutations, then we should have seen much more variation in the two populations that had genomic mutation rates that were orders of magnitude higher than those of the other four populations. Although one of the hypermutable populations exhibited an unusually high level of variation for fitness, the other hypermutable population did not. Moreover, the hypermutable population that harbored the greatest variance for fitness also exhibited the strongest frequency-dependent selection, which in itself provides a sufficient explanation for the high variance.

We have shown that frequency-dependent selection was more important than either the selective fixation of novel beneficial alleles or the selective elimination of recurring deleterious mutations, but we must also emphasize that these three mechanisms are not mutually exclusive. Indeed, one would expect from first principles that both of the other mechanisms must contribute something to variation in fitness within populations. Thus, one may ask why the effects of novel beneficial alleles and recurring deleterious mutations on fit-

ness variation were so small in these experimental populations.

One can use Fisher's fundamental theorem to predict the within-population variance for fitness due to the substitution of beneficial alleles, given some rate of change in mean fitness. In particular, the cumulative variance in fitness over some interval is proportional to the total change in mean fitness over that interval (Crow 1968; Felsenstein 1971), and so one can estimate the expected variance in fitness as follows:

$$\mathrm{E(Var}\ W) = (1/\bar{m})d\bar{W}/dt, \quad (2)$$

where $\bar{m}$ is the average Malthusian parameter (here, ln 100 = 4.605 per day) and $d\bar{W}/dt$ is the rate of change in mean fitness (Lenski et al. 1991). In the results section, we used this relationship in reverse to show that the observed Var W at 10,000 generations implied a subsequent change in mean fitness that was much greater than was measured between 10,000 and 10,500 generations. Now, we point out that $d\bar{W}/dt$ was independently estimated as 5.3×10^{-5} per day (= 0.008 per 1000 generations $\times$ 1000 generations per 150 d) between 5000 and 10,000 generations (Lenski and Travisano 1994), which yields an expected variance in fitness of only 1.2×10^{-5}. In fact, the observed variance in fitness, 1.5×10^{-3}, was more than 100 times greater than this expected value. Hence, we conclude that the ongoing substitution of beneficial alleles must have contributed only very slightly (perhaps 1%) to the within-population variance for fitness and, moreover, that this small effect was expected from the slow rate of increase in the mean fitness of these populations after they had evolved for many thousands of generations in the experimental environment. By contrast, during the first 2000 generations of this same experiment, the expected variance in fitness was 2.7×10^{-4}, a value that is statistically indistinguishable from the empirical estimate of 2.5×10^{-4} (Lenski et al. 1991). In other words, as the evolving populations went from a state of selective disequilibrum in a novel environment to a state of quasi-equilibrium in a familiar environment, the proportion of the within-population variance that can be attributed to selective sweeps has dropped from nearly 100% to perhaps 1%.

We can suggest two interrelated reasons why the large differences in genomic mutation rate between replicate populations had little effect on the within-population variance for fitness. First, the average base-pair mutation rate for nonmutator strains of *E. coli* has been estimated to be $\sim 5 \times 10^{-10}$ per generation (Drake 1991); with a genome size of $\sim 5 \times 10^6$ base-pairs, this figure yields a genomic mutation rate of only 2.5×10^{-3} per generation. Even for the two evolving populations that have become defective in mismatch repair, and which have a mutation rate that was increased ~100-fold, the genomic mutation rate would have been well below one. Second, a substantial fraction of all mutations may be selectively neutral and hence would not contribute to the within-population variance in fitness. In fact, using an *E. coli* clone from the same long-term evolution experiment, and under similar culture conditions, Kibota and Lynch (1996) estimated from mutation-accumulation experiments that the genomic rate of *deleterious* mutations, μ_D, was only 1.7×10^{-4} per generation, less than one-tenth the overall genomic mutation rate. Kibota and Lynch also estimated that the average effect of the deleterious mutations on fitness, S_D, was 0.012. For simplicity, assume that all deleterious mutations have this average effect. Then the mean number of deleterious mutations per genome, $\bar{U}$, is governed by $d\bar{U}/dt = \mu_D - S_D\bar{U}$. At equilibrium, the mean number of deleterious mutations per genome would be $(1.7 \times 10^{-4})/0.012 \cong 0.014$. Assuming that these mutations are distributed randomly and independently (no epistasis), then the Poisson distribution can be used to calculate the proportion of cells that carry 0, 1, 2, ... mutations and that have fitness 1, $1 - S_D$, $1 - 2S_D$, ..., respectively. The resulting distribution yields an expected variance in fitness of 2.04×10^{-6}. If the deleterious mutation rate is increased 100-fold, then the expected variance for fitness becomes 4.87×10^{-4}. Averaging the expected variance in fitness due to deleterious mutations over the six replicate populations (four with low and two with high mutation rates) yields 1.64×10^{-4}, which is approximately one-tenth of the observed within-population variance for fitness of 1.5×10^{-3}. Of course, various assumptions and uncertainties enter into these calculations. Nonetheless, recurring deleterious mutations also seem not to have contributed very much (perhaps 10%) to the within-population variance for fitness.

Evidently, frequency-dependent selection was the major force that maintained variation in fitness among the clones within these evolving populations of bacteria. One may ask whether this finding invalidates the use of mean fitness relative to the common ancestor as a measure of adaptive evolution in our system (Lenski et al. 1991; Lenski and Travisano 1994). In a strict sense, we believe that it does, because it shows that the reproductive success of a genotype may depend not only on the constant abiotic environment but also on the changing biotic milieu. However, these frequency-dependent effects are quite small in comparison with the overall gains in mean fitness measured relative to the common ancestor. While the average gain in mean fitness relative to the common ancestor was more than 50% during 10,000 generations (Lenski and Travisano 1994), the average advantage when rare to clones within populations was only ~2%. Thus, measurements of the evolving populations' competitive fitness relative to the common ancestor seem to provide a good (but not perfect) metric of adaptive evolution. However, the fact that the rate of increase in mean fitness relative to the ancestor has decelerated so dramatically suggests to us that these subtle frequency-dependent effects may, over time, become an increasingly important facet of the bacteria's adaptive evolution.

ACKNOWLEDGMENTS

We thank L. Ekunwe and N. Hajela for technical support, P. Sniegowski and P. Gerrish for sharing estimates of mutation rates, M. Blot for sharing the IS fingerprint analysis, and D. Rozen and P. Turner for helpful comments and discussion. This research was supported by a postdoctoral fellowship from the Spanish Ministerio de Educación y Ciencia to SFE and by a National Science Foundation grant (DEB-9421237) to REL.

Literature Cited

Atwood, K. C., L. K. Schneider, and F. J. Ryan. 1951. Periodic selection in *Escherichia coli*. Proc. Nat. Acad. Sci. USA 37:146–155.

Ayala, F. J., and C. A. Campbell. 1974. Frequency-dependent selection. Annu. Rev. Ecol. Syst. 5:115–138.

Begun, D. J., and C. F. Aquadro. 1991. Molecular population genetics of the distal portion of the X chromosome in *Drosophila*: evidence for genetic hitchhiking of the *yellow-achaete* region. Genetics 129:1147–1158.

Bennett, A. F., R. E. Lenski, and J. E. Mittler. 1992. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. Evolution 46: 16–30.

Carlton, B. C., and B. J. Brown. 1981. Gene mutation. Pp. 222–242 *in* P. Gerhardt, ed. Manual of methods for general bacteriology. American Society for Microbiology, Washington, DC.

Caugant, D. A., B. R. Levin, and R. K. Selander. 1981. Genetic diversity and temporal variation in the *E. coli* population of a human host. Genetics 98:467–490.

Chao, L., B. R. Levin, and F. M. Stewart. 1977. A complex community in a simple habitat: an experimental study with bacteria and phage. Ecology 58:369–378.

Clarke, B., and P. O'Donald. 1964. Frequency-dependent selection. Heredity 19:201–206.

Crow, J. F. 1968. The cost of evolution and genetic loads. Pp. 165–178 *in* K. R. Dronamraju, ed. Haldane and modern biology. Johns Hopkins Univ. Press, Baltimore, MD.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row, New York.

Dobzhansky, Th. 1937. Genetics and the origin of species. Columbia Univ. Press, New York.

Drake, J. W. 1991. A constant rate of spontaneous mutation in DNA-based microbes. Proc. Nat. Acad. Sci. USA 88:7160–7164.

Dykhuizen, D. E., and A. M. Dean. 1990. Enzyme activity and fitness: evolution in solution. Trends Ecol. Evol. 5:257–262.

Dykhuizen, D. E., and D. L. Hartl. 1983. Selection in chemostats. Microbiol. Rev. 47:150–168.

Elena, S. F., V. S. Cooper, and R. E. Lenski. 1996. Punctuated evolution caused by selection of rare beneficial mutations. Science 272:1802–1804.

Felsenstein, J. 1971. On the biological significance of the cost of gene substitution. Am. Nat. 105:1–11.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

Gillespie, J. H. 1991. The causes of molecular evolution. Oxford Univ. Press, New York.

Haldane, J. B. S. 1932. The causes of evolution. Harper, New York.

———. 1937. The effect of variation on fitness. Am. Nat. 71:337–349.

Helling, R. B., C. N. Vargas, and J. Adams. 1987. Evolution of *Escherichia coli* during growth in a constant environment. Genetics 116:349–358.

Istock, C. A., K. E. Duncan, N. Ferguson, and X. Zhou. 1992. Sexuality in a natural population of bacteria—*Bacillus subtilis* challenges the clonal paradigm. Mol. Ecol. 1:95–103.

Kaplan, N. L., R. R. Hudson, and C. H. Langley. 1989. The "hitchhiking effect" revisited. Genetics 123:887–899.

Kibota, T. T., and M. Lynch. 1996. Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. Nature 381: 694–696.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge Univ. Press, Cambridge.

Lenski, R. E. 1988. Experimental studies of pleiotropy and epistasis in *Escherichia coli*. I. Variation in competitive fitness among mutants resistant to virus T4. Evolution 42:425–432.

———. 1992. Experimental evolution. Pp. 125–140 *in* J. Lederberg, ed. Encyclopedia of microbiology. Vol. 2. Academic Press, San Diego, CA.

Lenski, R. E., and S. E. Hattingh. 1986. Coexistence of two competitors on one resource and one inhibitor: a chemostat model based on bacteria and antibiotics. J. Theor. Biol. 122:83–93.

Lenski, R. E., and M. Travisano. 1994. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. Proc. Nat. Acad. Sci. USA 91:6808–6814.

Lenski, R. E., M. R. Rose, S. C. Simpson, and S. C. Tadler. 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. Am. Nat. 138: 1315–1341.

Levin, B. R. 1972. Coexistence of two asexual strains on a single resource. Science 175:1272–1274.

———. 1981. Periodic selection, infectious gene exchange and the genetic structure of *E. coli* populations. Genetics 99:1–23.

———. 1988. Frequency-dependent selection in bacterial populations. Phil. Trans. R. Soc. Lond. B Biol. Sci. 319:459–472.

Levin, B. R., and R. E. Lenski. 1983. Coevolution in bacteria and their viruses and plasmids. Pp. 99–127 *in* D. J. Futuyma and M. Slatkin, eds. Coevolution. Sinauer, Sunderland, MA.

Levin, B. R., F. M. Stewart, and L. Chao. 1977. Resource-limited growth, competition, and predation: a model and experimental studies with bacteria and bacteriophage. Am. Nat. 111:3–24.

Lewontin, R. C. 1974. The genetic basis of evolutionary change. Columbia Univ. Press, New York.

Luria, S. E., and M. Delbrück. 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28:491–511.

Ma, W. T., G. H. Sandri, and S. Sarkar. 1992. Analysis of the Luria-Delbrück distribution using discrete convolution powers. J. Appl. Prob. 29:255–267.

Maynard Smith, J., N. H. Smith, M. O'Rourke, and B. G. Spratt. 1993. How clonal are bacteria? Proc. Nat. Acad. Sci. USA 90: 4384–4388.

Milkman, R. 1973. Electrophoretic variation in *E. coli* from natural sources. Science 182:1024–1026.

Mongold, J. A., and R. E. Lenski. 1996. Experimental rejection of a nonadaptive explanation for increased cell size in *Escherichia coli*. J. Bacteriol. 178:5333–5334.

Muller, H. J. 1950. Our load of mutations. Am. J. Human Genet. 2:111–176.

Nagylaki, T. 1977. Selection in one- and two-locus systems. Springer, New York.

Rosenzweig, R. F., R. R. Sharp, D. S. Treves, and J. Adams. 1994. Microbial evolution in a simple unstructured environment: genetic differentiation in *Escherichia coli*. Genetics 137:903–917.

Selander, R. K., and B. R. Levin. 1980. Genetic diversity and structure in *Escherichia coli* populations. Science 210:545–547.

Sniegowski, P. D., and R. E. Lenski. 1995. Mutation and adaptation: the directed mutation controversy in evolutionary perspective. Annu. Rev. Ecol. Syst. 26:553–578.

Sniegowski, P. D., P. J. Gerrish, and R. E. Lenski. 1997. Evolution of high mutation rates in experimental populations of *Escherichia coli*. Nature 387:703–705.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3d ed. Freeman, New York.

Souza, V., T. T. Nguyen, R. R. Hudson, D. Piñero, and R. E. Lenski. 1992. Hierarchical analysis of linkage disequilibrium in *Rhizobium* populations: evidence for sex? Proc. Nat. Acad. Sci. USA 89:8389–8393.

Souza, V., P. E. Turner, and R. E. Lenski. In press. Long-term experimental evolution in *Escherichia coli*. V. Effects of recombination with immigrant genotypes on the rate of bacterial evolution. J. Evol. Biol.

Stewart, F. M. 1994. Fluctuation tests: how reliable are the estimates of mutation rates? Genetics 137:1139–1146.

Stewart, F. M., and B. R. Levin. 1973. Partitioning of resources and the outcome of interspecific competition: a model and some general considerations. Am. Nat. 107:171–198.

Travisano, M. 1997. Long-term experimental evolution in *Escherichia coli*. VI. Environmental constraints on adaptation and divergence. Genetics 146:471–479.

Travisano, M., and R. E. Lenski. 1996. Long-term experimental evolution in *Escherichia coli*. IV. Targets of selection and the specificity of adaptation. Genetics 143:15–26.

TRAVISANO, M., J. A. MONGOLD, A. F. BENNETT, AND R. E. LENSKI. 1995a. Experimental tests of the roles of adaptation, chance, and history in evolution. Science 267:87–90.

TRAVISANO, M., F. VASI, AND R. E. LENSKI. 1995b. Long-term experimental evolution in *Escherichia coli*. III. Variation among replicate populations in correlated responses to novel environments. Evolution 49:189–200.

TURNER, P. E., V. SOUZA, AND R. E. LENSKI. 1996. Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. Ecology 77:2119–2129.

VASI, F., M. TRAVISANO, AND R. E. LENSKI. 1994. Long-term experimental evolution in *Escherichia coli*. II. Changes in life-history traits during adaptation to a seasonal environment. Am. Nat. 144:432–456.

WRIGHT, S. 1969. Evolution and the genetics of populations. Vol. 2. The theory of gene frequencies. Univ. of Chicago Press, Chicago.

WRIGHT, S., AND TH. DOBZHANSKY. 1946. Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. Genetics 31:125–156.

Corresponding Editor: D. Piñero