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Source: *Evolution*, Vol. 37, No. 1 (Jan., 1983), pp. 125-134

Published by: Society for the Study of Evolution

Stable URL: <http://www.jstor.org/stable/2408181>

Accessed: 04-07-2017 15:15 UTC

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COMPETITION BETWEEN HIGH AND LOW MUTATING STRAINS OF *ESCHERICHIA COLI*

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Received July 6, 1981. Revised April 8, 1982

The dynamics of bacterial populations are often characterized by several distinctive features: under optimal growth conditions they double every few hours; they usually contain in excess of 10^6 individuals; higher fitness mutants have a good chance of arising in a population since average mutation rates are approximately 10^{-6} to 10^{-7} per gene replication; new favorable mutations, in the absence of genetic recombination, always increase to fixation in linkage with the genome of the parent clone in which they originally occurred; and higher fitness mutants often exhibit 10% to 20% higher growth rates than their parental clones.

Consequently, when populations of such organisms are exposed to a new environment, a series of replacement cycles rapidly ensues, each cycle corresponding to the fixation of a higher fitness mutation in linkage with the genome of its parent clone. The linkage between the new mutation and the genome of the parent clone, and the rapidity of these clonal replacements, are two features that distinguish such asexual populations from ones that reproduce sexually.

The existence of such cycles in asexual populations was first studied systematically by Atwood et al. (1951) in a study with laboratory populations of *Escherichia coli* in long-term cultures. By comparing the relative fitness of a series of bacterial clones isolated from these cultures, Atwood et al. were able to show that populations underwent a succession of clonal changes, each clonal replacement

resulting from the fixation of a clone competitively superior to the preceding one. These workers also found a frequency dependent effect in their study; all else being equal, the more numerous population was favored because it had a greater probability of acquiring the higher fitness mutations. Whenever two genetically marked populations were cultured in competition, the rarer one was displaced after a lag period during which the two coexisted in relatively constant numbers. The lag period corresponded to the time required for the clone containing the first higher fitness mutation to go to fixation in the more numerous population. The higher fitness clone then replaced the rarer population, since, in the absence of recombination, the latter could not acquire the new mutation.

The frequency dependent result reported by Atwood et al. is of significance in that it offers a simple mechanism for selection for increased mutation rates in asexual populations. All else being equal, including population numbers, a strain with a higher mutation rate could be at an advantage in the same manner as a population that is more numerous. Clearly, the final net advantage of possessing a mutator gene would still be dependent on the starting frequency of the mutator population and as well as on whether or not the associated benefits outweigh the costs resulting from the increased mutational load.

Populations harboring mutator genes that increase mutation rates in *E. coli* are known to be at a strong selective advantage in long term competition in chemostats (Gibson et al., 1970; Nestmann and Hill, 1973; Cox and Gibson, 1974). When the relative fitness of a mutator (*mutT*) was compared with an otherwise isogenic wild

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type strain (Gibson et al., 1970) the frequency of wild type population decreased in all cases, sometimes by several orders of magnitude, and new mutants with more fit phenotypes were shown to increase with the *mutT* allele (Cox and Gibson, 1974). Cox and Gibson, however, did not find the expected correlation between the mutator allele's initial frequency and the outcome of the competition, thus leaving open the possibility that the mutator gene simply conferred an intrinsic advantage, e.g., a higher growth rate. Nestmann and Hill (1973) working with a different mutator, *mutH*, found in preliminary experiments that the advantage of the mutator population appeared to be frequency dependent: at low starting frequencies the mutator population did not displace the wild-type population. Nonetheless, Nestmann and Hill's results were inconclusive, since the frequency dependent outcome was observed in only two out of four cases and the expected lag period was not detected.

We report here the results of a study extending the work initiated by Gibson et al. (1970). We have repeated their competition experiments using the same *mutT* and wild-type strains, but have monitored the populations more closely by sampling more frequently. We have been able to demonstrate that there is a lag before the mutator population displaces the wild-type population and that the advantage of the mutator strain is frequency dependent. Our results clearly show that *mutT* confers an advantage by generating beneficial mutations at a rate higher than *mut*⁺.

MATERIALS AND METHODS

Bacterial Strains.—Four of the *E. coli* strains originally constructed by Cox and Gibson (1974), stored as lyophils since 1969, were used in this study (Table 1). In a few experiments Lac⁻ strains were made resistant to bacteriophage T5 (T5^R) for use as a selectable marker. T5^R behaves as a neutral marker in glucose limited chemostats (Novick and Szilard, 1950; Hartl and Dykhuizen, 1980). With the exception of the mutator, the *lac* and the T5^R loci, all

TABLE 1. *E. coli* strains.^a

Strain	Description ^b
P113	<i>mutT1</i> Lac ⁺ (Cox and Gibson, 1974).
P153	<i>mut</i> ⁺ Lac ⁻ (Cox and Gibson, 1974).
LC153	T5 ^R derivative of P153, this paper.
P127	<i>mut</i> ⁺ Lac ⁺ (Cox and Gibson, 1974).
LC151	T5 ^R derivative of P151, this paper.
P151	<i>mutT1</i> Lac ⁻ (Cox and Gibson, 1974).

^a Derived from W3110, an *E. coli* K12 F⁻ strain.

^b Description of genotypes and phenotypes follows the recommendations of Demerec et al. (1966).

these strains are coisogenic (see Table 1). They were stored in broth containing 40% (V/V) glycerol at -20 C for periods of up to one year.

Media.—The media used are described in Cox and Gibson (1974) except for the tetrazolium lactose (TZL) plates. Our TZL plates contained per liter 10 g "Difco" Casamino Acids, 6 g lactose, 15 g agar and 1 ml 5% filter sterilized 2,3,4 triphenyl tetrazolium chloride. TZL-T5 plates, TZL plates spread with 0.1 ml of a T5 lysate containing approximately 10¹⁰ phage/ml were used to determine T5 resistance.

Chemostats.—The chemostats used were similar to those described in Chao et al. (1977) except that a sampling port was added by attaching the top half of a 15 × 120 mm screw cap tube to the chemostat vessels. Population samples were withdrawn through the port with a sterile Pasteur pipet. We used a culture volume of 40 ml, a dilution rate of 12 ml/h, and VB minimal medium with glucose, the limiting resource, at a concentration of 500 μg/ml for all of our chemostats. These values are within the range used by Cox and Gibson (1974).

Generation time was defined as $\{(\ln 2) \cdot (\text{culture volume})/(\text{dilution rate})\}$ (see Kubitschek, 1970). The average generation time for the 11 chemostats used in this study was 2.40 ± 0.045 h.

Experimental Procedures.—Populations grown to stationary phase in batch cultures were used to inoculate our chemostats. These batch cultures were always grown in a total volume of 50 ml of the same media used in the chemostats and kept at stationary phase for at least 5 h

TABLE 2. Starting ratios, selective coefficients, and lag periods.^a

Chemostat	Bacterial strains and relevant genotype		Starting ratio $mutT/mut^+$ ^b	Lag ^b generations	S_1 ^c generations ⁻¹	S_2 ^d generations ⁻¹
1 ^e	P113 <i>mutT1</i> Lac ⁺	P153 <i>mut</i> ⁺ Lac ⁻	4.0×10^{-4}	60	1.0×10^{-2}	0.256
2 ^e	P113 <i>mutT1</i> Lac ⁺	P153 <i>mut</i> ⁺ Lac ⁻	4.5×10^{-3}	63	6.0×10^{-3}	0.139
3	P113 <i>mutT1</i> Lac ⁺	LC153 <i>mut</i> ⁺ Lac ⁻ T5 ^R	1.2×10^{-6}	90	4.6×10^{-3}	-0.154
4 ^e	P113 <i>mutT1</i> Lac ⁺	LC153 <i>mut</i> ⁺ Lac ⁻ T5 ^R	1.1×10^0	37	1.6×10^{-3}	0.232
5	P151 <i>mutT1</i> Lac ⁻	P127 <i>mut</i> ⁺ Lac ⁺	8.7×10^{-1}	53	-3.1×10^{-3}	0.104
6	P151 <i>mutT1</i> Lac ⁻	P127 <i>mut</i> ⁺ Lac ⁺	9.4×10^{-1}	65	-7.8×10^{-3}	0.156
7	LC151 <i>mutT1</i> Lac ⁻ T5 ^R	P127 <i>mut</i> ⁺ Lac ⁺	1.0×10^{-3}	67	-1.0×10^{-2}	0.144
8	LC151 <i>mutT1</i> Lac ⁻ T5 ^R	P127 <i>mut</i> ⁺ Lac ⁺	9.4×10^{-6}	79	-1.1×10^{-2}	-0.164
9	LC151 <i>mutT1</i> Lac ⁻ T5 ^R	P127 <i>mut</i> ⁺ Lac ⁺	1.1×10^{-6}	88	-1.5×10^{-2}	-0.202
10	LC151 <i>mutT1</i> Lac ⁻ T5 ^R	P127 <i>mut</i> ⁺ Lac ⁺	1.1×10^{-4}	54	-2.0×10^{-2}	0.105
11	LC151 <i>mutT1</i> Lac ⁻ T5 ^R	P127 <i>mut</i> ⁺ Lac ⁺	6.3×10^{-5}	81	-2.0×10^{-2}	-0.125

^a Data for chemostat 6 is not presented in this paper. Remaining data taken from Figures 1 and 2.

^b Calculated from the intersection of the S_1 and S_2 regression lines.

^{c,d} Determined by linear regression.

^e The cultures used to inoculate these chemostats were started with a small but unknown number of bacteria. The inocula for the cultures used to start the remaining chemostats (3, 5–11) contained less than 10^3 bacteria.

but less than 24 h before inoculation. For the first part of this study these batch cultures were started with an undetermined amount of bacteria picked from a single colony. In later experiments, however, we always started them with less than 10^3 bacteria, either from a single colony or from a liquid culture started from a single colony (see Table 2). The competing populations were mixed at the desired starting ratios, but always maintained at a total density equal to the population density at stationary phase in the batch cultures, and then introduced into an empty chemostat as an inoculum of 40 ml (the holding volume of the chemostats). The stationary phase density of our strains in batch cultures is indistinguishable by our sampling methods from their equilibrium density in chemostats with identical growth media, roughly 3×10^8 cells/ml. All batch cultures and chemostats were kept at 37 C.

We monitored the *mutT* and the *mut*⁺ populations by appropriately diluting the chemostat samples and plating them on either TZL, minimal lactose, or TZL-T5 plates. We counted on the average about 300 *mutT* and *mut*⁺ colonies per sample.

Statistical comparisons between sample groups were performed by a two sample *t*-test. Throughout this paper averaged

data are presented as the mean \pm SE of the mean.

RESULTS

We present in Figure 1 the results of a series of chemostats started with varying ratios of *mutT* Lac⁻ (or *mutT* Lac⁻ T5^R) and *mut*⁺ Lac⁺ populations. It is evident from these results that the advantage of the mutator population is frequency dependent and that the trajectory of the populations within each chemostat follows a distinct pattern.

At *mutT/mut*⁺ ratios above 7×10^{-5} , there was initially a short lag, during which the two population densities remained relatively constant. After between 50 and 70 generations the mutator population begins to increase. We believe this short lag corresponds to the time required for the first higher fitness mutation to increase to fixation within the mutator population. The *mutT/mut*⁺ slope during this lag should then provide an estimate of the fitness difference between the 'original' *mutT* and *mut*⁺ strains used to start the chemostats. By inspection it can be seen that this slope is slightly negative, suggesting either that the *mutT* allele confers a small disadvantage or that the Lac or the T5^R markers are not neutral. Whichever explanation is

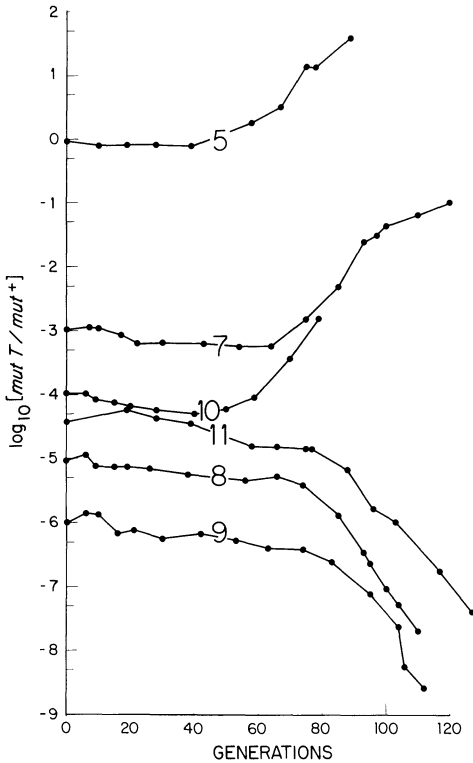


FIG. 1. Changes in the ratio of $mutT/mut^+$ over time at varying starting ratios with strains P151, LC151, and P127. For actual combinations of strains used see Table 2. Numbers in this figure (5, 7–11) correspond to the chemostat numbers used in Table 2.

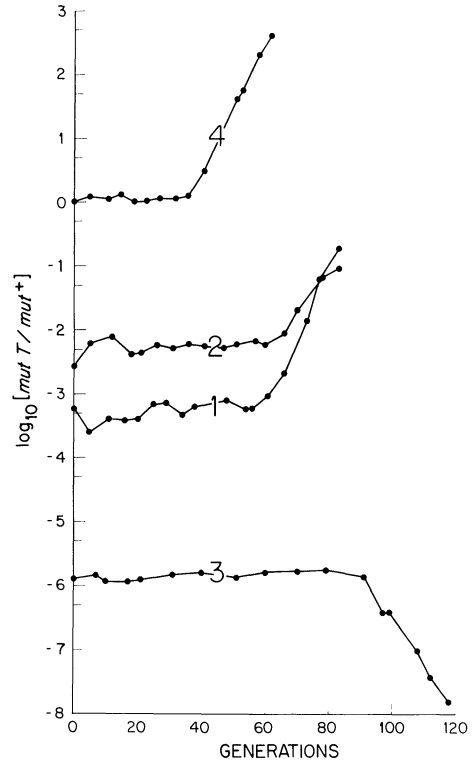


FIG. 2. Changes in the ratio of $mutT/mut^+$ over time at varying starting ratios in control chemostats with reversed markers (strains P113, P153, and LC153). For actual combination of strains used see Table 2. Numbers (1–4) in this figure correspond to chemostat numbers used in Table 2.

correct, the decline during the lag is small relative to the rise following it, and consequently the slope during the rise can be used as a measure of the fitness difference between the $mutT$ higher fitness mutant and the original mut^+ population (see below).

If the advantage of the mutator population is dependent on the fixation of a new higher fitness clone, then it should be possible to eliminate this advantage by reducing the mutator population to a size where no mutants would be present. When we reduced the $mutT/mut^+$ starting ratio below 7×10^{-5} , the $mutT$ population was displaced in every case (Fig. 1, 2). In addition, a longer lag of about 80 generations

is observed. The decline of the $mutT$ population after a lag would suggest that we are now observing the fixation of a higher fitness mutant in the wild type population. As before, the slope during the decline can be used as a measure of the fitness difference between the mut^+ higher fitness mutant and the original $mutT$ population.

mutT and mut⁺ Populations with Reversed Markers.—In order to control for possible marker effects we also examined the behavior of $mutT$ and mut^+ populations with reversed markers, that is $mutT$ Lac⁺ and mut^+ Lac⁻ (or mut^+ Lac⁻ T5^R). We initiated four chemostats using these strains, and the results, shown in Figure 2, were consistent with those obtained in

our first set of experiments. The only noticeable difference is that in these experiments the *mutT* population actually increased in frequency during the initial lag, suggesting that certain of the markers are not neutral.

Monitoring the Markers used in the Chemostats.—At periodic intervals, single colonies were isolated from the chemostats and checked for lactose fermentation, T5^R and mutator activity. The latter was accomplished by determining the mutation rate of these clones to streptomycin resistance (Cox and Gibson, 1974). In all cases, their markers matched those of the strains used to start their respective chemostats.

Estimates of the Lag and Relative Fitness.—Using the data from Figures 1 and 2 and one additional chemostat (data not presented) we estimated the lag and the relative fitness of the higher fitness mutants and the original *mutT* and *mut*⁺ clones. The results are summarized in Table 2. S_1 is the fitness difference between the original *mutT* and *mut*⁺ strains and it was estimated by a least square fit of the data points during the lag, using the relationship $\Delta \ln(\text{mutT}/\text{mut}^+) = S_1 \cdot \Delta G$, where G is the number of generations (Cox and Gibson, 1974). The *mutT*/*mut*⁺ intercepts calculated from these regressions for S_1 were used as the values for the starting ratios. S_2 corresponds to an approximate estimate of the fitness difference between the higher fitness mutants and the original *mutT* and *mut*⁺ clones and it was determined using the same relationship given above from the data points following the lag. In this purely competitive situation both S_1 and S_2 can be taken to represent differences in growth rates. The lag was estimated from the intercept of the S_1 and S_2 regression lines.

The results summarized in Table 2 rank the chemostats according to the values of S_1 and an inspection of the resulting order of the genotypes of the strains reveals that the Lac⁻ and T5^R markers are not neutral. In all cases, the strains with the Lac⁻ and T5^R markers were at a slight competitive disadvantage (compare chemostats 1 and 2 with 3 and 4; and 5 and 6 with 7–11).

Nonetheless, given the small sample size it is not entirely feasible to factor out the individual contributions of the markers and the *mutT* allele, and thus we have resorted to calculating some limiting values for the fitness difference between *mutT* and *mut*⁺. By taking the average S_1 values for chemostats 1 and 2 (8.0×10^{-3} /generation) and 5 and 6 (-5.4×10^{-3} /generation), and by assuming that the Lac and Mut contributions to fitness are additive, the following relationship can be constructed:

$$T + L = 8.0 \times 10^{-3} \text{ generation}^{-1}$$

$$T - L = -5.4 \times 10^{-3} \text{ generation}^{-1},$$

where T and L are the respective contributions of the *mutT* and the *lac*⁺ alleles. From this, $T = 1.3 \times 10^{-3}$ generation⁻¹. Similarly, by using chemostats 3 and 4 and 7 through 11, an estimate of $T = -6.1 \times 10^{-3}$ was derived. The average of these two estimates, $T = -2.4 \times 10^{-3}$, would indicate that *mutT* imposes a small cost (see Discussion).

The value of the starting ratio clearly correlated with the sign of S_2 and the length of the lag period. In every case, a starting ratio above 7×10^{-5} resulted in a positive S_2 and a shorter lag (average values of 0.162 ± 0.0225 generation⁻¹ and 60 ± 3.6 generations, respectively); at starting ratios below, a negative S_2 and a longer lag (average values of -0.161 ± 0.0159 and 84.5 ± 2.66).

The two lag period estimates were based only on chemostats started with less than 10^3 bacteria (chemostats 5, 6, 7 and 10; and chemostats 3, 8, 9 and 11, respectively; for justification see Discussion). They were significantly different ($P < .01$).

The standard error of our estimates of S_2 was about equal to the largest absolute value of our S_1 estimates, suggesting that the Lac⁻ and T5^R markers and the *mutT* allele could not have had any significant effect on our S_2 estimates. In fact the positive S_2 's estimated with the *mutT* Lac⁺ strain were not significantly different ($.1 > P > .05$) from the positive S_2 's estimated with either *mutT* Lac⁻ or *mutT* Lac⁻ T5^R.

A comparable result was obtained ($P > .2$) when we compared the *mutT* Lac⁻ T5^R strain's estimates with the remaining ones, again using only positive S_2 's.

The absolute values of the means for the positive and negative S_2 's were very close and a paired comparison between the absolute values of the two groups detected no significant differences ($P > .9$). Neither were any significant differences detected when we took all the absolute values and singled out the estimates made with the *mutT* Lac⁻ T5^R ($P > .4$) and with either *mutT* Lac⁻ or *mutT* Lac⁻ T5^R ($.1 > P > .05$).

DISCUSSION

We have investigated the competitive interaction between coisogenic *mutT* and *mut*⁺ populations of *E. coli* in chemostat cultures. Our results are consistent with previous reports that bacterial strains with higher mutation rates have a relative advantage over wild-type strains in pairwise competition (Gibson et al., 1970; Nestmann and Hill, 1973; Cox and Gibson, 1974). We have also demonstrated that the advantage of the mutator strain results from the fact that it generates more beneficial mutants and not from an intrinsic advantage, e.g., a faster growth rate. We have presented two lines of evidence for these conclusions.

First, in pairwise competition at about equal numbers a mutator population will only displace a wild-type strain after a lag of about 60 ± 3.6 generations. This lag corresponds to the time necessary for the first higher fitness mutants generated by the mutator population to go to fixation within that population.

Second, we demonstrated that the advantage of the mutator strain was frequency dependent. When the *mutT*/*mut*⁺ starting ratio was reduced to less than 7×10^{-5} , the mutator strain was displaced by the wild type strain after a lag of about 84.5 ± 2.66 generations. This result shows that if the *mutT* population is made sufficiently small it will not contain any higher fitness mutants and loses its advantage. In addition, it demonstrates that the wild-type

population can also generate similar higher fitness mutants.

Estimation of Population Parameters

We used the rate of change in the population ratio during the lag as an estimate of the difference in fitness between the "original" coisogenic mutator and wild-type before any adaptation to the chemostat has occurred, and obtained a value of 2.4×10^{-3} generation⁻¹ against the mutator. This estimate, however, was based on the average of a negative and a positive value, 1.3×10^{-3} and -6.1×10^{-3} , suggesting that the true value must be very close to zero, or of about the same magnitude as the error of our sampling methods and the stochastic factors affecting these populations. It is in fact smaller than the limit of resolution for these procedures (Hartl and Dykhuizen, 1981) and the standard error we obtained for our other estimates of mean fitness differences (see below).

The slope of the population trajectories during the rise phase following the lag, S_2 , was used as an approximate estimate of the difference in fitness between the higher fitness mutants and the original *mutT* and *mut*⁺ strains. The average slope value for chemostats starting at ratios above 7×10^{-5} was 0.162 ± 0.0225 generation⁻¹, and for those started at lower ratios the average slope was -0.161 ± 0.0159 .

Since the transition from the lag to the rise is smooth, it is very likely that both of our S_2 values are underestimates. Nonetheless, it is still noteworthy that the absolute values of those S_2 estimates, within the range of our experimental errors, were not significantly different ($P > .9$), suggesting that the higher fitness mutants generated by both the mutator and the wild-type populations are phenotypically very similar. We take this result, along with our earlier observation of the difference in lag periods, as an indication that the major difference between *mutT* and *mut*⁺ populations is not the type of mutants they produce but the number of them, *mutT* simply having a higher proportion. It is possible to calculate the ac-

tual number within each population at time zero by correcting for the shift in resource concentration that occurs when a mutant becomes fixed (for a more detailed discussion of the arguments used in the calculations to follow, see Novick, 1960).

When a chemostat like the ones in this study is initiated, the concentration of the limiting resource, glucose, is determined by the original *mutT* and *mut*⁺ clones, the most abundant types of bacteria in the system. The concentration achieved is that which allows these clones to grow at a rate just equal to a dilution rate of $\ln 2$ generation⁻¹. At this concentration, however, the higher fitness mutants grow at a rate, to be denoted a_1 , which is higher than the dilution rate and hence they will increase relative to the original clones at a rate equal to $[a_1 - \ln 2]$. As they increase they will consume more glucose and begin to lower its concentration to a level where they in turn will grow at a rate just equal to the dilution rate. The effect of the new glucose level is to reduce the growth rates of the original clones to a lower level level a_2 , where,

$$\ln 2 - a_2 = S_2. \quad (1)$$

At these reduced levels of glucose, the growth rate of these bacteria is a linear function of the glucose concentration, or

$$\frac{a_1}{\ln 2} = \frac{\ln 2}{a_2}. \quad (2)$$

Using $S_2 = 0.161$ (Table 2) and equations (1) and (2), $a_1 = 0.90$ generation⁻¹. Thus, starting at time zero the higher fitness mutants must be increasing relative to the original clones at a rate of $[0.90 - \ln(2)]$ or about 0.21 generation⁻¹.

Since it took about 60 generations for the *mutT* higher fitness mutants to go to fixation within the *mutT* population, it follows that those mutants must have increased by a factor of $e^{(0.21)(60)}$ during the lag. Alternatively stated, at time zero there must have been on the average one mutant for every 3×10^5 mutator and, by the same argument, one for every 5×10^7 wild-type bacteria. This estimate of the

number of higher fitness mutants in the mutator population is in good agreement with the frequency dependent results of our chemostats. If those mutants are already present at time zero, then it should be possible to eliminate the mutator's advantage by reducing its population to a size smaller than 3×10^5 bacteria or, with a total population size of 1.2×10^{10} bacteria per chemostat, a starting ratio lower than 3×10^{-5} . In our chemostats, the mutator population lost its advantage when the starting ratio was reduced below a cut-off point of 7×10^{-5} .

We can also use the above estimates to determine the mutation rates from the original *mutT* and *mut*⁺ strains to the higher fitness mutants and, hence, the number of bases that have to be mutated to produce a higher fitness mutant. To determine the mutation rate, we used the formulation originally developed by Luria and Delbruck (1943),

$$r = \mu N_t \ln(\mu N_t), \quad (3)$$

where "r" is the number of mutants within a chemostat, "μ" the mutation rate, and " N_t " the total number of bacteria per chemostat. Two assumptions were made in the derivation of this equation, (i) that the inocula used to start the batch cultures used to initiate the chemostats did not contain any mutants and (ii) that the growth rates of the original and higher fitness mutants were the same. We satisfy the first condition by restricting our estimates of the lag periods to chemostats started with less than 10^3 bacteria. We meet the second condition by observing that although our estimate of S_2 indicates that the growth rates of the original clones and the mutants are different, that difference was estimated in saturated chemostats with low levels of glucose. Strains selected for increased growth rates at low resource levels often lose that advantage at high concentrations (Novick, 1956; Wu et al., 1968). The more fit mutants whose trajectories we have studied here arose during growth of the batch cultures used to inoculate the chemostats. Thus they

evolved under conditions of high resource level.

For the mutator population $r = 1.2 \times 10^{10}/3 \times 10^5 = 4 \times 10^4$ and by a series of numerical approximations of equation (3) with $N_t = 1.2 \times 10^{10}$ we obtain a value of $\mu = 4 \times 10^{-7}$ mutations generation⁻¹. For the wild-type populations, with $r = 2 \times 10^2$, we find that $\mu = 5 \times 10^{-9}$ mutations generation⁻¹.

Our estimates for the mutator and wild-type mutation rates are close to the estimated base pair mutation rates for *mutT* and *mut+*, respectively, 2×10^{-6} errors per AT replication and 5×10^{-9} to 5×10^{-10} errors per base pair replication (Cox, 1970, 1976). It should be emphasized, however, that both of our present estimates are subject to large errors because they are calculated through the exponentiation of two separate parameters, S_2 and the lag; and as indicated earlier S_2 is likely to be an underestimate. Nonetheless, if we do take our mutation rate estimates as order of magnitude approximations, their match with their respective base pair mutation rates does indicate that the target for the generation of a higher fitness mutation by either *mutT* or *mut+* could be the same size, possibly the same specific base pairs. It is unlikely that the target will be two specific base pairs, but it could be as small as a single specific base pair.

Long Term Evolution

The dynamics of competition between mutator and wild-type bacteria in chemostat cultures have been analyzed by Painter (1973). Painter makes the assumptions that 1) mutator strains have a slight growth rate disadvantage, 2) the mutator population produces a larger proportion of higher fitness mutations, and 3) the higher fitness mutations are superior to their parent clones by large fitness increments. Our results are consistent with Painter's model. His model predicts the existence of the lag and the rise following it. It also predicts that the rise phase should eventually plateau because higher fitness mutations are produced by both the mutator and the

wild-type populations. The displacement of the wild-type population during the rise-phase should stop when that population in turn becomes fixed for its own higher fitness mutants, the result being a plateau with the same slope as the lag. For example, in a chemostat at a starting ratio of 10^{-3} , there should be by our estimates $(10^{-3})(1.2 \times 10^{10})/(3 \times 10^5) \cong 4 \times 10^4$ higher fitness mutants in the mutator population and $(1.2 \times 10^{10})/(5 \times 10^7) \cong 2 \times 10^2$ in the wild-type. If the fitness difference between these two new clones is slight, their abundance relative to each other should remain thereafter at a ratio of 2×10^{-1} . In such a chemostat, the populations should start with a lag at a ratio of 10^{-3} and then shift to a plateau at a ratio of 2×10^{-1} . Although the mutator population is still a minority at the plateau, it has increased by a factor of 200 since time zero and along with this increase, its chances of survival; it is now further away from the frequency dependent cut-off point (a ratio of 7×10^{-5} in our case). Being away from the cut-off point reduces the chances that the mutator population might not generate the next higher fitness mutation. If a second higher fitness mutation then appears and is produced by both the mutator and wild-type populations, the population ratio will again shift upwards by another factor of 200, to a new plateau, a ratio of 40 for this example.

In some of our chemostats started at 1:1 ratios, we observed a leveling comparable to a plateau at a ratio of about 10^2 (data not shown). Although the existence of the plateau is consistent with Painter's model, we are at this point reluctant to use it as supporting evidence because a similar outcome can result from wall growth. The walls of the chemostat could have been colonized during the lag when the ratio was 1:1, and once a wall population is established it should change more slowly than the liquid population. As a result, when the liquid population changes during the rise phase, the wall population will remain closer to a 1:1 ratio, and mi-

gration off the wall will become increasingly important. Migration will never allow the wild-type population to be completely eliminated, and this effect also results in a plateau.

Given the clear advantage mutator strains have over wild-type strains in chemostat culture, it is surprising that mutator genes are rarely found in nature. Among 110 isolates studied by Jyssum (1960) only four were mutators, and in a more recent study by Gross and Siegel (1981) only one out of 408 was a mutator.

It is possible that the low mutation rates in naturally occurring populations have been selected by a mechanism similar to that postulated by Leigh (1973) in his model for optimizing mutation rates in asexual populations. This process can be interpreted in terms of Painter's model, following the transition from the lag to the rise phase to the plateau. Consider various populations of bacteria with differing mutation rates in a habitat that fluctuates every fixed number of generations. With every fluctuation the populations will respond with an adaptation and rise to a new plateau. The strains with the higher mutation rates will increase the most in frequency, as did our *mutT* populations. Those same strains, however, will also generate more deleterious mutations and will, in turn, decrease in numbers at a faster rate at the new plateau, until the onset of the next fluctuation. The end result, as demonstrated by Leigh, is that the mutation rate that maximizes the difference between the gain during rise phase and the loss during the plateau will increase in frequency.

SUMMARY

Previous studies (Gibson et al., 1970; Nestmann and Hill, 1973; Cox and Gibson, 1974) have shown that strains of *E. coli* with high mutation rates have a marked advantage in competition with wild-type strains. Those studies, however, failed to discriminate between the hypotheses that the mutator bacteria acquired an advantage by evolving faster, on the one hand, or that they had an intrinsic competitive advantage, on the other. Our

present investigation supports the first hypothesis. We also present estimates for the mutational load in *E. coli mutT* strains and the mutation rate to higher fitness mutants in these experiments.

ACKNOWLEDGMENTS

We thank D. Culver, D. Dykhuizen, M. Newlon, P. Painter, S. Zabell and an anonymous reviewer for helpful comments and discussion.

This study was supported by grant number GM28923 from the National Institutes of Health. L. Chao was a postdoctoral trainee of the Public Health Service, grant number GM07625.

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