# SELECTION FOR HIGH MUTATION RATES IN CHEMOSTATS[1]

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ABSTRACT

Competition experiments in chemostats show that mutator populations of *Escherichia coli* are more fit than wild type. The increased fitness can be explained by the appearance of new mutants better adapted to the chemostat environment. Fitness values vary between chemostats and are strongly correlated ($P < 0.001$) with fluctuations in population density.

THE spontaneous mutation rate in eukaryotes and prokaryotes is under genetic control. Mutant genes that increase or decrease mutant frequency in populations have been isolated and studied in a variety of organisms (reviewed in Genetics **73**: 1–205, 1973). These mutant genes are called mutator if the mutation rate increases, and antimutator if the rate decreases, relative to wild type. Recent and very elegant studies with bacteriophage T4 have shown that its mutation rate is controlled by the structure of T4 DNA polymerase. The polymerase isolated from cells infected with mutator T4 causes more errors during DNA replication than does wild type, while antimutator polymerase causes fewer (MUZYCZKA, POLAND and BESSMAN 1972; HERSHFIELD and NOSSAL 1973). In other organisms the biochemical details are not as certain, but it is known that several *Escherichia coli* mutator gene products are directly involved in DNA replication (COX 1970, 1973; HALL and BRAMMAR 1973), and a mutator gene of *Drosophila melanogaster* is thought to cause errors during recombination (GREEN 1970).

If the fidelity of DNA replication is under genetic control, as the evidence suggests, will natural selection optimize mutation rates so that some balance is struck between beneficial and harmful mutations? The answer to this question is not clear, but recent papers by LEIGH suggest that natural selection probably can optimize the mutation rate in asexual populations (LEIGH 1970, 1973). This result follows because the mutator gene and the mutations it causes must always remain linked; and consequently, a mutator-induced mutation that confers high fitness on the organism will spread throughout the population, thereby fixing the linked mutator gene. LEIGH also treats sexual populations, as have LEVINS and KIMURA. Here the results for simple additive models are that selection favors the lowest mutation rate acceptable in terms of biochemical cost (KIMURA 1967; LEIGH 1970, 1973); for a class of nonadditive models, however, LEVINS has shown that selection may favor a higher mutation rate (LEVINS 1967). In models where

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mutation rates may be optimized the strength of selection is related to the stability of the environment, the general result being that selection for higher mutability is stronger in unpredictable than it is in predictable environments.

We report here experiments that study the strength of selection for or against increased mutability in a haploid asexual population. We have asked if selection can favor increased mutation rates in populations of *Escherichia coli*. In preliminary experiments, our results showed that competing mutator populations were more fit than wild type (GIBSON, SCHEPPE and COX 1970). These experiments did not, however, distinguish between increased fitness due to mutation and fitness due to unknown but intrinsic properties of the mutator gene product.

In this paper we present evidence that indeed the increased fitness of the mutator population in these kinds of experiments has a mutational origin and that mutator population fitness is strongly correlated with the stability of the chemostat population. These results show that higher mutation rates can be selected for in this simple system, and that selection is strongest when the variance in population size over many generations is largest.

## MATERIALS AND METHODS

*Bacterial strains:* Table 1 lists the origin of each strain and the crosses performed to construct it. All strains were checked for P1 lysogeny; P1 lysogens found were discarded. Lac$^-$ and Ara$^-$ derivatives of these strains arose spontaneously and were detected and purified on TZL and TZA plates, respectively.

All strains competed in the chemostat were coisogenic. Because the genetic backgrounds of mutator and nonmutator stocks begin to diverge after transduction, we lyophilized twelve to twenty samples of each transductant as soon as its purity and genotype had been verified. Sibling stocks therefore differ from each other by no more than fifty generations at the beginning of each experiment.

*Media:* Tetrazolium lactose (TZL) plates were used to identify Lac$^+$ and Lac$^-$ colonies; TZA plates are the same, supplemented with arabinose rather than lactose. VB minimal medium is a phosphate-buffered minimal medium containing citrate; M63 is similar to VB medium, but lacks citrate. Tryptone broth and tryptone plates were used for routine colony counts. Tryptone plates were supplemented with $2 \times 10^{-3}$ M sodium azide or 200 $\mu$g/ml dihydrostreptomycin for the assay of azide resistance, or for mutator activity, respectively. Minimal plates contained VB medium solidified with 1.5% agar and supplemented with the appropriate carbon source. Complete references to the composition and preparation of the above may be found in COX, DEGNEN and SCHEPPE (1972).

*Isotopes:* $^{14}$C-1,6-citric acid, 7.35 mC/mM, was obtained from the New England Nuclear Corporation. The isotopic purity of this preparation was checked by thin layer chromatography on cellulose plates developed with a diethyl ether-formic acid-water solvent (STAHL 1965).

*Experimental procedure:* Coisogenic mutator and wild-type cultures were grown from single colony isolates in minimal glucose medium to mid log phase. Then they were mixed in the appropriate ratios and densities and inoculated into chemostats containing the appropriate media. Samples were removed from the chemostat at frequent intervals and assayed for total cell number and for the mutator/wild-type ratio (Mut$^-$/Mut$^+$), either directly, or by following a Lac$^-$ derivative of the *mutT1* population (see below).

*Chemostat design:* The chemostat used in these experiments is similar in design to the chemostat of NOVICK and SZILARD (1950) and incorporates features of the "Bactogen" of MONOD (1950). It has proven to be reliable, can be built at a nominal cost from screw-cap test tubes, laboratory flasks and tubing, and is simple to maintain over several thousand generations. We have found it feasible to operate five or six at a time, with very few contamination problems.

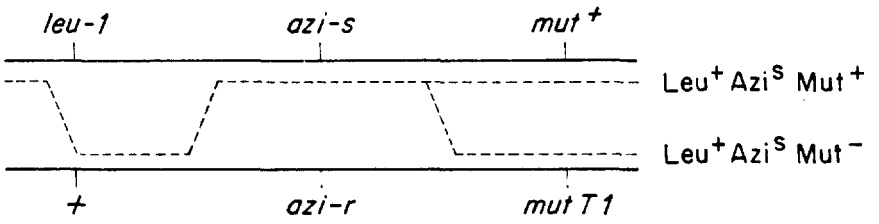


FIGURE 1.—Coisogenic *mut*+ and *mutT1* stocks. In the cross used to obtain *mut*+ and *mutT1* recombinants the following Plkc co-transduction frequencies were observed: *Leu, azi*, 26%; *Azi-mut*+, *mutT1*, 96%. The order is based on these data, as well as more extensive data involving the *ace* locus and a suppressor of *mutT1* (RAY and COX 1972). *Leu-1* maps at 1 min, and the *leu—mutT* distance is approximately 0.5% of the chromosome, or $2.1 \times 10^4$ base pairs (*ca.* 20 genes).

Copies of this chemostat design and instructions for its use may be obtained by writing either of the authors.

*The assay system:* In a previous paper we showed that the mutator population could be followed by assaying for a spontaneous Lac⁻ mutation in the *mutT1* background (GIBSON, SCHEPPE and COX 1970). We have continued to use this method in these experiments. In addition, chemostat populations were checked by P1 transduction at frequent but irregular intervals to insure that Lac⁻ clones carried the mutator gene, and that Lac+ isolates were wild type (COX and YANOFSKY 1969).

## RESULTS

*Stock construction:* Figure 1 shows the *mutT* region of the *E. coli* genome. The mapping data are based on the data of COX and YANOFSKY (1969) and the results obtained during the construction of stocks for these experiments. Azi$^s$ Mut+ and Azi$^s$ Mut⁻ stocks used in these experiments were obtained as Leu+ co-transductants, as described in Table 1. It is important to note that they are the products of a backcross between P101, the *mutT1* strain, S100, a Leu⁻ derivative of W3110, and a double recombination event between *leu, azi* and *mutT1*. Consequently, the paired strains used in the experiments reported here are co-isogenic by quite rigorous standards. Although not relevant to the purposes of this paper, the order *leu azi mutT1* deduced from the extensive series of crosses used to establish these stocks does not agree with the results earlier published (COX and YANOFSKY 1969). They do agree, however, with data obtained in *E. coli* B (DONCH *et al.* 1971).

*Competition experiments between* mut+ *and* mutT1 *populations: MutT1* populations consistently outgrow *mut*+ populations in glucose-limited chemostats (GIBSON, SCHEPPE and COX 1970). We have extended these preliminary results by examining different culture media, other independently isolated *mutT1* and *mut*+ cell lineages, and a large range of initial population ratios and cell densities. The detailed data obtained in two typical chemostat experiments are shown in Figure 2. These data illustrate features common to all but one of our experiments. First, and most striking, the mutator population out-grows wild type at a relatively constant rate, with at most a 50-generation lag; second, the cell density in each chemostat fluctuates about a mean density throughout each experiment; and third, the rate at which the *mutT1* population eliminates wild type varies

TABLE 1

*Bacterial strains used*

| Strain* | Phenotype† | | | | | | Origin |
|---|---|---|---|---|---|---|---|
| | Ara | Leu | Azi | Mut | Lac | P1 | |
| 58–278 | + | + | R | — | — | S | TREFFERS, SPINELLI and BELSER (1954) |
| W3110 | + | + | S | + | + | S | *Escherichia coli* K12; COX and YANOFSKY (1969) |
| S100 | + | — | S | + | + | S | UV induced Leu⁻ auxotroph of W3110; COX and YANOFSKY (1969) |
| P101 | + | + | R | — | + | S | P1kc(58–278)×S100 Leu⁻ → Leu⁺ $Azi^R$ Mut⁻ |
| P105 | + | + | S | — | + | S | P1kc(P101)×S100 → Leu⁺ $Azi^S$ Mut⁻ |
| P108 | + | + | S | — | + | S | As above, independent co-transductant |
| P113 | + | + | S | — | + | S | As above, independent co-transductant |
| P123 | + | + | S | + | + | S | P1kc(P101)×S100 → Leu⁺ $Azi^S$ Mut⁺ |
| P124 | + | + | S | + | + | S | As above, independent co-transductant |
| P127 | + | + | S | + | + | S | As above, independent co-transductant |
| P151 | + | + | S | — | — | S | Spontaneous Lac⁻ derivative of P105 |
| P152 | + | + | S | — | — | S | Spontaneous Lac⁻ derivative of P113 |
| P153 | + | + | S | + | — | S | Spontaneous Lac⁻ derivative of P124 |
| P510 | — | + | S | — | — | S | Spontaneous Ara⁻ derivative of P151 recovered from Chemostat 10 |
| P511 | + | + | S | + | — | S | P1kc(W3110)×P510 → Ara⁺ Mut⁺ |
| P515 | — | + | S | — | — | S | Spontaneous Ara⁻ derivative of P151 recovered from Chemostat 15 |
| P516 | + | + | S | + | — | S | P1kc(W3110)×P515 → Ara⁺ Mut⁺ |
| P512 | + | + | S | + | + | S | Mut⁺ descendant of P124 isolated from Chemostat 10 |
| P517 | + | + | S | + | + | S | Mut⁺ descendant of P124 isolated from Chemostat 15 |
| P154 | + | + | S | — | — | S | Mut⁻ descendant of P151 isolated from Chemostat 10 |
| P155 | + | + | S | — | — | S | Mut⁻ descendant of P151 isolated from Chemostat 15 |

* Strains P105 to P127 are derived from a single P1kc transduction, plated immediately after the P1kc lysate was adsorbed to the recipient cells. Thus, each strain is independent of all others.

† Ara⁺ is the ability to ferment L-arabinose, Ara⁻ the inability; Leu⁺ strains can grow without leucine, Leu⁻ require leucine; $Azi^R$ strains are resistant to $2.5\times10^{-3}$ M sodium azide in tryptone plates, $Azi^S$ strains are sensitive; Mut⁻ strains are mutator active and carry the *mutT1* allele, Mut⁺ strains are wild-type; Lac⁺ strains can ferment lactose, Lac⁻ cannot; $P1^S$ strains are sensitive to P1kc, $P1^R$ strains are resistant. Genotypes and phenotypes here and in the text follow the recommendations of DEMEREC *et al.* 1966.

from experiment to experiment, a point made more clearly in Table 2. We have not studied the generality of these phenomena with a wide range of growth media, temperature, and other variables, but have established that they hold for at least one other minimal medium, also glucose-limited (Figure 3).

Table 2 summarizes the results of eleven different competition experiments between mutator and wild type populations, and includes a measure of the relative fitness of the *mutT1* population, $\bar{w}_1$. These experiments were designed to test the consistency of our data for several independently derived mutator and wild-type populations, the influence of cell density and generation time on $\bar{w}_1$, and the effects of various initial population ratios on the outcome of each experiment. The results clearly show that the competition patterns hold for at least three independently isolated *mutT1* strains. Moreover, qualitatively similar results are

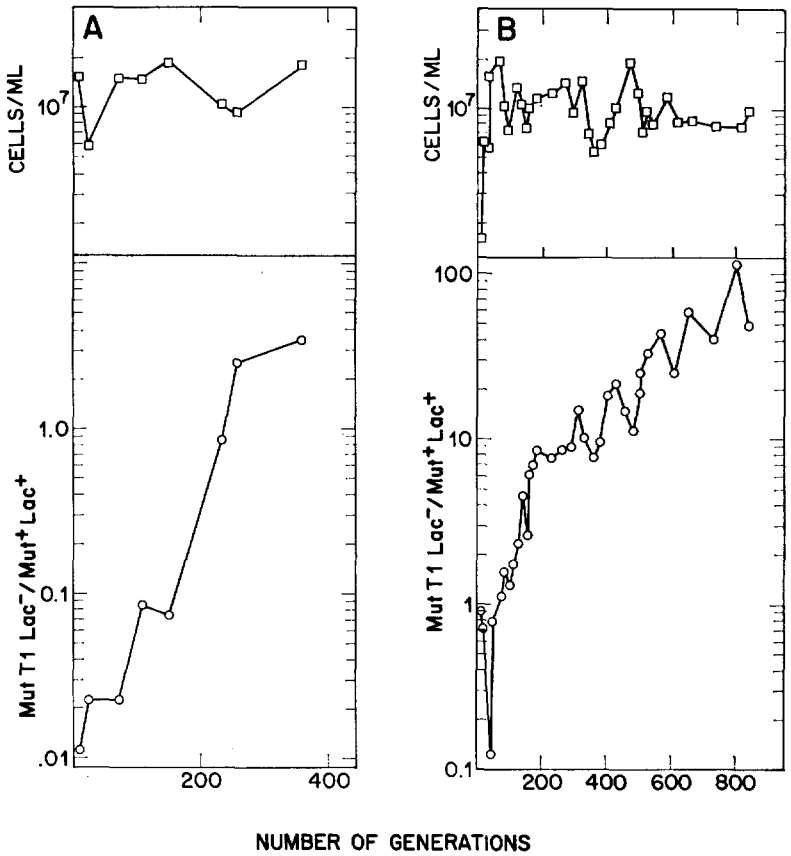


FIGURE 2.—Two competition experiments. In this figure the cell density and the *mutT1/mut*+ ratio are plotted as a function of generation time. Cells at the end of the two experiments were lyophilized and used to obtain the data presented in Table 3 and Figures 4, 5, and 6. Part of Figure 2 has been published before (GIBSON, SCHEPPE and COX 1970). A. is chemostat 15, B. is chemostat 10.

obtained in each experiment, but note that $\bar{w}_1$ obviously varies from experiment to experiment.

In each experiment described in Table 2 $\bar{w}_1$ was determined from data obtained by a least-squares fit of the logarithm of the Mut⁻/Mut⁺ ratio *versus* the number of generations. When the data are fitted in this way the values are all highly significant, with the likely exception of chemostats $29_2$ and 52, discussed below.

*Mutation and increased fitness:* The results from the above experiments could be explained on one of two hypotheses: either mutator populations are intrinsically more fit, or their variance in fitness because of mutation is much higher than wild type (GIBSON, SCHEPPE and COX 1970). In the former case, one might postulate that the *mutT1* product, or lack of product, is responsible for a slight increase in growth rate as well as increased mutability; in the latter, selection would operate on those mutants better adapted to the chemostat environment, but

TABLE 2

*Chemostat parameters*

| Chemostat | Competitors *mutT1* | Competitors *mut*+ | $\rho \times 10^7$ | CVRHO | $(r_1 - r_2) \times 10^3$ | $\bar{w}_1$ | $g$ | $n$ |
|---|---|---|---|---|---|---|---|---|
| 5 | P151 | P124 | 120 | 67 | 44 | 1.0635 | 100 | 16 |
| 8 | P151 | P124 | 16 | 94 | 28 | 1.0404 | 165 | 13 |
| $10_1$ | P151 | P124 | 1.10 | 33 | 15 | 1.0216 | 229 | 14 |
| $10_2$ | P151 | P124 | 0.95 | 35 | 3.9 | 1.0056 | 518 | 21 |
| 14 | P151 | P124 | 1.40 | 48 | 3.9 | 1.0056 | 715 | 13 |
| 15 | P151 | P124 | 1.20 | 29 | 8.5 | 1.0123 | 574 | 13 |
| 19 | P105 | P124 | 1.30 | 54 | 8.7 | 1.0125 | 368 | 8 |
| 25 | P151 | P124 | 0.57 | 39 | 2.3 | 1.0033 | 837 | 48 |
| $28_1$ | P105 | P153 | 0.73 | 62 | 24 | 1.0346 | 378 | 7 |
| $28_2$ | P105 | P153 | 0.72 | 16 | 1.7 | 1.0024 | 780 | 7 |
| $29_1$ | P105 | P153 | 0.44 | 77 | 37 | 1.0534 | 314 | 7 |
| $29_2$ | P105 | P153 | 0.64 | 9 | −2.8 | 0.9960 | 437 | 3 |
| 32 | P108 | P123 | 0.79 | 47 | 4.8 | 1.0069 | 636 | 16 |
| 52 | P151 | P124 | 16.6 | 86 | 46 | 1.0664 | 145 | 5 |

Each chemostat contained glucose-VB minimal medium, with the following exceptions: chemostat 5 was initially VB-glucose medium, and acid hydrolyzed casein was added linearly during the experiment to a final concentration of 0.1% (w/v) at 100 generations; and chemostat 25 contained M63-glucose medium. Detailed data for chemostats 10, 15, and 25 are shown in Figure 2 and Figure 3, respectively. $\rho$ is the mean cell density averaged over the chemostat lifetime. CVRHO is the coefficient of variation of $\rho$ [(standard deviation/mean) $\times$ 100]. $(r_1 - r_2)$ is the slope of the competition curve, determined from the relationship $\Delta \ln(mutT1/mut+) = (r_1 - r_2)\Delta g$. $\bar{w}_1 = r_1/r_2$, and is the relative finess of the *mutT1* population ($\bar{w}_2$, the fitness of the *mut*+ population, is 1.0). For the calculations here $r_2 = \ln 2$ by definition, since, in the absence of competition, a *mut*+ population must double each generation. $g$ is the number of generations. $n$ is the number of observations on which all other parameters are based. With the exception of chemostats $29_2$ and 52, $(r_1 - r_2)$ values are all highly significant ($P \leq 0.001$) as determined by a $t$ test of the regression line on which $(r_1 - r_2)$ is based. For $29_2$ and 52, $P = 0.05$.

doubling times and saturation densities of uncompeted populations would be identical.

With these possibilities in mind we first measured the intrinsic rates of increase ($r_o$) and the carrying capacity of the growth medium ($K_o$) for uncompeted *mut*+ and *mutT1* populations both by optical density measurements and viable cell counts. No differences were found (±3% error in optical density measurements, ±25% in five sample replicate viable counts). Whether or not the $r_o$'s and $K_o$'s are the same for uncompeted *mut*+ and *mutT1* populations probably could not be settled by measurements of this kind, however, since a glance at the $\bar{w}_1$'s of Table 2 will show that any differences in growth rates or saturation densities must be exceedingly small.

We then asked if successful *mutT1* competitors were more fit in definable ways.

*Selection for more fit mutants:* We have examined three obvious ways in which *mutT1* populations might have become more fit. We first tested the ability of clones isolated from the chemostat to colonize glass surfaces, since it is well known that bacterial cultures rapidly coat the walls of glass chemostats (CONTOIS 1959).

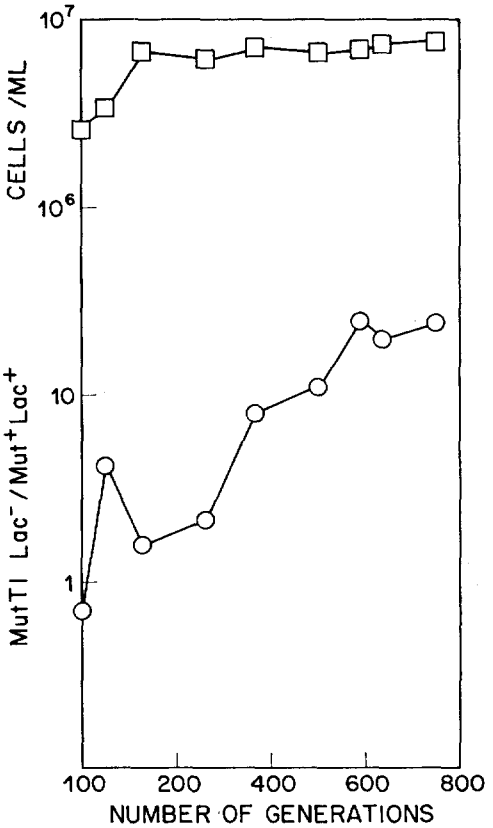


FIGURE 3.—Competition in M63 medium. Chemostat 25 is similar to 10 and 15. The conditions are described further in the text.

These experiments were conducted by mixing cells, medium, and sterile glass powder in sterile tubes under chemostat conditions. The glass powder was washed several times with sterile medium, and the last wash was allowed to grow overnight with the glass powder. Finally, the $Mut^-/Mut^+$ ratio was measured after growth had ceased. An increase in this ratio would show that mutator cells could bind more tightly than wild type to a glass surface. The results of these experiments are shown in Table 3 and Figure 4. Uncompeted populations, both mutator and wild type, clearly possess an equal ability to bind to powdered glass, whereas competed mutator populations are much more effective in this regard than either uncompeted mutator or wild type. Furthermore, the increased binding of these cells to glass varies from one experiment to another (Chemostat 10 compared to 15, Table 3 and Figure 4), suggesting that various kinds of sticky mutants have been selected during growth in different chemostats. This observation, as well as the previous one that uncompeted mutator and wild-type cells bind with equal affinity, suggests that this aspect of chemostat fitness is mutational in origin. More convincing results, however, are obtained with mutator lineages in which the

TABLE 3

*Competition for glass surfaces*

| Experiment | Stocks* | Description† | Increase in ratio‡ |
|---|---|---|---|
| Controls | P151/P124 | *mutT1* Lac⁻ / *mut*⁺ Lac⁺ | 0.85 (0.15) |
| | P113/P153 | *mutT1* Lac⁺ / *mut*⁺ Lac⁻ | 1.00 |
| | P153/P124 | *mut*⁺ Lac⁻ / *mut*⁺ Lac⁺ | 0.85 |
| Chemostat 10 | P154/P512 | *mutT1* Lac⁻ / *mut*⁺ Lac⁺ | 12.70 (8.3) |
| | P154/P124 | *mutT1* Lac⁻ / *mut*⁺ Lac⁺ | 14.67 (3.2) |
| | P511/P517 | [*mutT1*]⁺ Lac⁻ / *mut*⁺ Lac⁺ | 6.83 (1.2) |
| | P511/P124 | [*mutT1*]⁺ Lac⁻ / *mut*⁺ Lac⁺ | 3.73 (0.4) |
| Chemostat 15 | P155/P517 | *mutT1* Lac⁻ / *mut*⁺ Lac⁺ | 1.61 (0.54) |
| | P155/P124 | *mutT1* Lac⁻ / *mut*⁺ Lac⁺ | 2.40 (0.60) |
| | P153/P517 | *mut*⁺ Lac⁻ / *mut*⁺ Lac⁺ | 0.86 (0.10) |
| | P516/P517 | [*mutT1*]⁺ Lac⁻ / *mut*⁺ Lac⁺ | 4.13 (0.32) |
| | P516/P124 | [*mutT1*]⁺ Lac⁻ / *mut*⁺ *Lac*⁺ | 2.40 (0.10) |

* As described in Table 1.

† The relevant mutator genotype and lactose phenotype is described in this column. The designation [*mutT1*]⁺ indicates that the *mutT1* allele of a competed strain has been crossed out by P1 transduction, as described in MATERIALS AND METHODS, and in Table 1.

‡ The mean increase in the relevant population ratios listed under Stocks, followed by the standard deviatison of the measurement in brackets. Figure 4 is based on these data.

*mutT1* allele from competed clones has been crossed out by transduction, also included in Table 3 and Figure 4. In all cases examined, *mut*⁺ derivatives of competed *mutT1* clones retained their ability to bind more tightly to glass powder in the presence of both competed wild type and uncompeted mutator. This result shows clearly that successful mutator clones have mutated to become more efficient colonizers of the chemostat environment.

In a second experimental series designed to distinguish between intrinsic fitness and fitness due to mutation and selection, we tested the ability of various mutator and wild-type populations to utilize citric acid as a carbon source, since our minimal medium contains citrate as a $Mg^{++}$ chelating agent and a potentially exploitable resource. Figure 5 summaries these results and compares mutator, wild-type and competed mutator clones whose mutator allele had been crossed out. It is clear from these data that at least one *mutT1* clone from Chemostat 10 could utilize citrate more readily than could a *mut*⁺ co-competitor, and that the reason for this was mutational, since when the *mutT1* allele was crossed out, the *mut*⁺ recombinant (designated [MutT]⁺Lac⁻ in Figure 5) retained its ability to use citrate. Compare this result with clones isolated from Chemostat 15 at the termination of the experiment. In this case, mutator and non-mutator colonies utilized citrate at equal rates, as did [MutT]⁺ cells. These observations show that for at least two clones from two chemostats, the ability to utilize citrate is different. Increased competence to colonize glass surfaces is not, therefore, the sole reason for increased mutator fitness.

In a third series of experiments we tested the ability of various competed and uncompeted populations to withstand long periods of starvation, conditions that reflect to some degree the famine of the chemostat environment. A clone tested

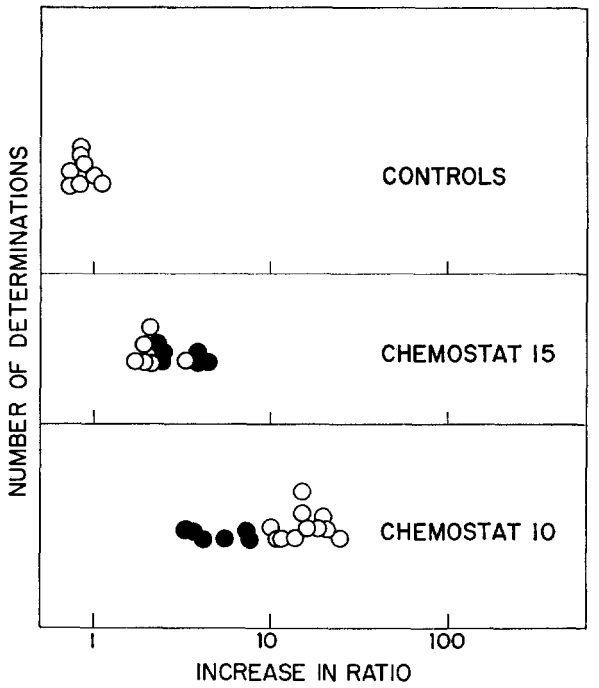


FIGURE 4.—Competition for glass surfaces. These results, taken from Table 3, show that successful *mutT1* competitors bind more effectively to powdered glass than do competed wild type. The *open circles* come from repeated trials of a single clone isolated at the end of a chemostat experiment. The *closed circles* represent results obtained with the same clone after the *mutT1* allele had been crossed out. The data labeled *Controls* were obtained with various stocks that had not been grown in the chemostat. The headings refer to Table 3. Other details may be found in the text and Table 1.

from Chemostat 15 was more resistant to glucose starvation that was a wild-type clone from the same chemostat, while a colonizer from Chemostat 10 was at most slightly more tolerant to starvation than wild type (Figure 6). Moreover, it is clear from Figure 6 that resistance to glucose starvation of a Chemostat 15 mutator clone was mutational in origin, since when the *mutT1* allele was crossed out, the *mut*$^+$ recombinant ([mutT]$^+$) was resistant still, albeit at a reduced level. A Chemostat 10 mutator, on the other hand, showed only a small increase in tolerance to glucose starvation, and when the *mutT1* allele was crossed out, it was at a decided disadvantage, as though a linked "vitality" gene had been lost concomitantly with the *mutT1* allele.

In summary, then, the above results show that successful *mutT1* competitors have mutated in several ways, such that they enjoy increased fitness for the chemostat environment. Competed clones from two independent chemostats are not identical, however: it thus seems likely that other mutational advantages remain to be discovered.

*The relationship between mutator density and increased fitness:* If mutation were the only factors increasing the fitness of *mutT1* populations, then we should

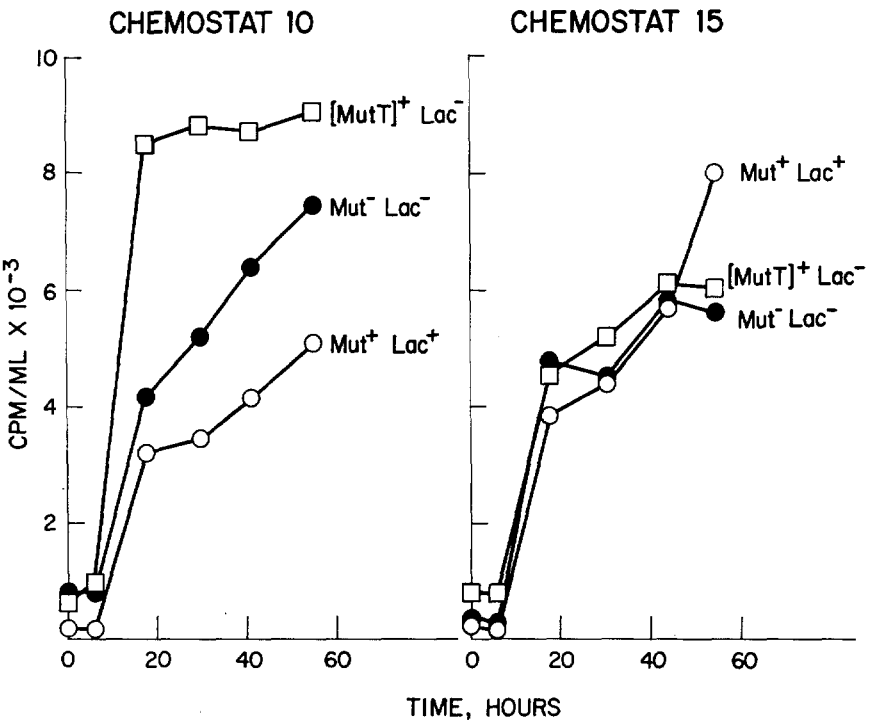


FIGURE 5.—Citrate utilization. Competed mutator and wild-type cells derived from a single colony were grown to about $10^8$ cells/ml in VB minimal glucose medium at 37°. Each culture was then diluted fivefold under the same conditions into the same medium containing 5 μC/mM $^{14}$C-citric acid. Samples were harvested periodically thereafter, precipitated with one volume of 10% trichloroacetic acid and collected on glass fiber filters, washed well with ethanol, dried, and counted in a scintillation counter using a toluene phosphor. Twenty hours is approximately when the cultures become glucose-limited. Beyond this time, cell numbers do not increase. The strains used are listed in Table 1.

find a relationship between the mutator cell density and not only the magnitude of $\bar{w}_1$ but the lag between the start of the experiment and the time at which the mutator cells begin to outgrow wild type, since the total number of new mutations appearing per generation is proportional to the product of the mutation rate and the number of cells in the chemostat. To test this expectation, we operated a series of chemostats at progressively lower initial *mutT1*/*mut*$^+$ ratios, holding the total population density at $10^7$ cells/ml. At the lowest *mutT1* population size that we have been able to study ($5.6 \times 10^6$ cells in a 40-ml chemostat, Chemostat 14) the mutator population outgrew wild type, with a relatively small value for $\bar{w}_1$ (Table 2). However, $\bar{w}_1$ and the initial *mutT1*/*mut*$^+$ ratio are not correlated ($P > 0.3$) (data not shown). These results are the opposite of those obtained by NESTMANN and HILL (1973) with a different mutator gene, *mutH*. As we discuss below, however, we do not believe that our results are inconsistent with the hypothesis that mutation is the primary explanation for the observed increased fitness of *mutT1* populations.

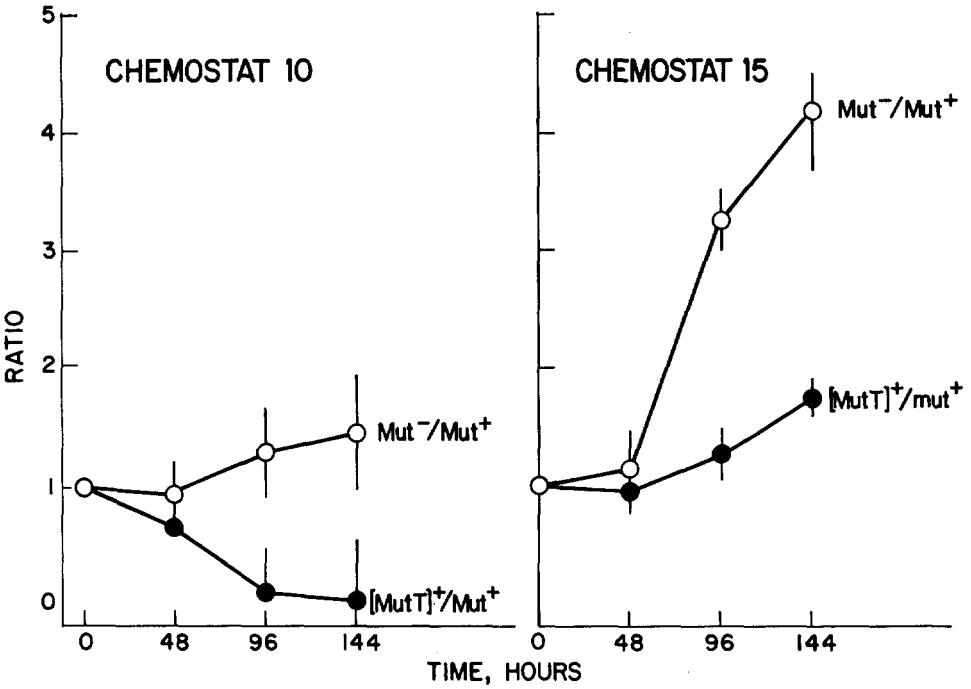


FIGURE 6.—Starvation in glucose-exhausted cultures. Mutator and nonmutator clones from lyophilized samples taken at the end of chemostat experiments 10 and 15 were mixed in equal proportion during exponential growth. A sample was taken immediately and plated for total cells and for the *Mut⁻/Mut*+ ratio, in the case of mutator and wild type, or for the *Lac⁻/Lac*+ ratio in [*MutT*]+ sub-lines. Duplicate samples were taken again at 48-, 96-, and 144-hour intervals and normalized to the 0 time sample. Each point is the average of the two samples and the error flags show the range. The genealogy of the strains used in these experiments is outlined in Table 1.

*Population stability and the magnitude of* $\bar{w}_1$: We can now ask how $\bar{w}_1$ relates to generation time, cell density, initial *mutT1/mut*+ ratio, and other variables. The only significant correlation, $P < 0.01$ ($t = 3.66$; $t_{.01[9]} = 3.25$) is between $\bar{w}_1$ and the coefficient of variation in the cell density (CVRHO) during the chemostat run. CVRHO is, of course, a measure of the stability of the chemostat population. This relationship is shown graphically in Figure 7, where we have plotted $\bar{w}_1$ *vs.* CVRHO. The correlation is even stronger ($P < 0.001$) if we compute separate values for $\bar{w}_1$ in those chemostats that show obvious changes in the slope of the competition curve during the experiment, such as Chemostat 10, shown in Figure 2, where $\bar{w}_1$ changes from approximately 1.0216 to 1.0056 at 200 generations. Another example comes from Chemostat 29. This competition experiment was unique—the only one in which the mutator population declined. The decline began at 450 generations, and continued for the ensuing 300. When these two regions of the competition curve from Chemostat 29 are used to calculate $\bar{w}_1$, we find a high positive value for the first 450 generations, and a low negative value for the next 300 (plotted in Figure 7 as $29_1$ and $29_2$, respectively). In both

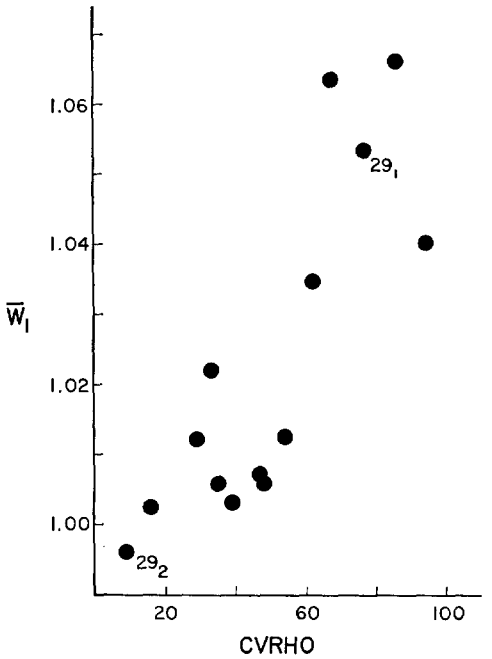


FIGURE 7.—The correlation between population fitness and population stability. The data in this figure are taken from Table 2. $\bar{w}_1$ is the relative fitness of the *mutT1* population. CVRHO is the coefficient of variation of the cell density during the entire experiment. A correlation analysis of the data according to SOKAL and ROHLF (1968) shows that the correlation between $\bar{w}_1$ and CVRHO is significant at the 0.1% level ($t = 5.09$; $t_{.001[12]} = 4.32$).

instances, even though $\bar{w}_1$ for $29_2$ is not based on enough data to be very significant, our measure of population fitness correlates well with the population stability. We will return to this result in the DISCUSSION.

## DISCUSSION

*Fitness and mutation:* The ability of the *mutT1* stocks studied here to outgrow wild type in glucose-limited chemostats is primarily a consequence of mutation. Four kinds of experiments support this conclusion: first, the growth rate constants and saturation levels are, as far as we can tell, the same for uncompeted *mut*$^+$ and *mutT1* populations; second, citrate utilization has a mutational origin, since in at least one experiment, Chemostat 10, a competed mutator isolate retained this ability even though the *mutT1* region of the chromosome had been crossed out (Figure 5); third, we showed that when adapted *mutT1* and *mut*$^+$ cultures were allowed to exhaust the glucose in the medium, a *mutT1* isolate from Chemostat 15 preferentially survived (Figure 6); and fourth, *mutT1* clones isolated from Chemostats 10 and 15 competed more effectively for glass surfaces than did either competed wild type or non-competed mutator (Table 3 and Figure 4). In this last case, Chemostat 10 colonizers were clearly more effective than those from Chemostat 15; both, moreover, retained their stickiness when the *mutT1* allele had been crossed out.

Two objections can be raised to the above interpretation of our data. In the first instance, the reader will have noticed that Mut$^+$ clones derived by transduction from a competed *mutT1* isolate did not behave precisely like the immediate parents in most of the experiments conducted to assess the contribution due to mutation. As examples, notice in Figure 5 that the strain labeled [*mutT1*]$^+$ Lac$^-$ was a more effective citrate utilizer than the Mut$^-$Lac$^-$ parent; that both [*mutT1*]$^+$ progeny isolated from Chemostats 10 and 15 were less resistant to starvation than their parents (Figure 6); that some ability to compete for glass surfaces was gained in [*mutT1*]$^+$ recombinants isolated from Chemostat 15, while considerable ability was lost by [*mutT1*]$^+$ clones from Chemostat 10 (Table 3 and Figure 4). Some of these observations might be interpreted as intrinsic *mutT1* fitness. However, the *mutT* region of the chromosome is known to contain genes involved in the tricarboxylic acid cycle, in electron transport, and in cell wall and membrane structure, each linked to *mutT1* by P1 transduction (TAYLOR and TROTTER 1972). It therefore seems likely that other loci either more (Figure 5) or less (Figures 4 and 6; Table 3) suited to the chemostat environment are gained from uncompeted wild type during transduction. Although this interpretation can be checked by examining several independent P1 transductants, with the expectation that not all will behave identically, such experiments have not been done. Until they have, this explanation must remain tentative.

As a second objection to our interpretation of these results, it might be argued that citrate utilization, resistance to starvation, and increased stickiness are all attributable to the same mutation. This argument would seem to us unlikely for biochemical reasons; but, more importantly, our data show that these three phenotypes are not correlated in a one-to-one fashion. Thus, a Chemostat 10 isolate is a better citrate utilizer and is also stickier than one from Chemostat 15; but this isolate is not tolerant to glucose starvation. On the other hand, a Chemostat 15 isolate is highly resistant to starvation, utilizes citrate no better than Mut$^+$ cocompetitors from the same chemostat, and is on the average stickier than wild type, but not markedly so. This apparent lack of correlation between the phenotypes that we have studied suggests that they are probably caused by several independent mutations, rather than one, a conclusion also supported by the behavior of these strains when the mutator allele is crossed out. This conclusion also suggests that the number of ways in which *E. coli* can adapt to this new environment is quite large, since it is unlikely that by guessing at three possibilities we have found them all.

In sum, we feel that we are observing the effects of mutation alone on the outcome of these experiments. This would seem to be true for the reasons mentioned above. In addition, it is worth pointing out that the initial 50 to 100 or so generations of each experiment are essentially unpredictable—in some the *mut*$^+$ population outgrows *mutT1* for a time (Figure 2B, as an example), while in others the converse is true (Figures 2A and 3). This observation is not expected on the hypothesis that intrinsic growth rate differences explain our results. Finally, the measured abilities of successful competitors to utilize citrate, to colonize glass surfaces, and to withstand starvation, each attributable to mutation,

seem qualitatively and quantitatively sufficient fully to explain the outcome of each experiment. In the only other case that we are aware of, more fit mutants were also shown to accumulate during periodic selection in *E. coli* 15T$^-$ (McDONALD 1955).

*Fitness and genetic load:* The *mutT1* allele of the *mutT* gene increases A:T → C:G transversions (YANOFSKY, Cox and HORN 1966). This mutational event is, as far as we know, the only one that increases in these strains, and the rate, measured accurately with bacteriophage λ (Cox 1970), is at least $2 \times 10^{-6}$ per A:T base-pair replication. The *E. coli* genome contains $4 \times 10^6$ base pairs, of which $2 \times 10^6$ are A:T piars. Thus, the *mutT1* genome accumulates about 4 base pair changes per generation, one consequence of which is an increase in the G:C content of the genome after several thousand generations (Cox and YANOFSKY 1967). Our results show that even with a very high mutation rate, *E. coli* can continue to show considerable evolutionary flexibility, without any obvious decrease in ability to grow successfully in this environment.

Is the number of targets on the *E. coli* genome that can mutate to higher fitness in the chemostat environment large or small? The results with citrate utilization, glucose starvation, and competition for glass surfaces suggest three or more such targets: but what is the upper bound consistent with our experiments? This question may be approached as follows. In the majority of our chemostats the *mutT1* population begins to outgrow wild type before 50 generations have elapsed. Using the smallest *mutT1* population size that we have studied, $5 \times 10^6$ cells, and a mutation rate of $2 \times 10^{-6}$ per A:T replication, the number of mutations in the population is $2 \times 10^{-6} \times 5 \times 10^6$ cells $\times$ $2 \times 10^6$ A:T pairs/genome or $2 \times 10^7$ new mutations each generation. Thus, surprisingly, we cannot deduce the number of A:T pairs which by mutation to G:C pairs lead to a more fit cell, because, for the population sizes studied here, all possible different A:T pairs in the chemostat are sampled by mutation at least once every generation. This seems to be the most likely explanation for our inability to find a dependence of $\bar{w}_1$ on cell density in these experiments; nor should we until we study *mutT1* populations of approximately $5 \times 10^5$ cells or less. These results are in contrast to the results of NESTMANN and HILL (1973), where the outcome of the competition experiment was thought to be dependent on the initial Mut$^-$/Mut$^+$ ratio. However, the mutation rate of the mutator used in their study, *mutH*, is substantially lower than the one studied here (Cox *et al.* 1972), so that for the population sizes studied by NESTMANN and HILL it is likely that not all genes were mutated somewhere in the population at each generation.

*Fitness and population stability:* When we correlated the various fitness values for *mutT1* populations with other parameters, such as generation time, initial ratio of *mutT1*/*mut*$^+$ cells, total cell density, and the variance of each of these with time, we found that the only significant correlation was between $\bar{w}_1$ and the coefficient of variation of the cell density (CVRHO). The correlation is positive and significant at the 1% level ($t = 3.66$; $t_{.01[9]} = 3.250$). In fact, the correlation is stronger if the data from three experiments are degraded and each treated as two separate experiments: as Figure 7 and Table 3 show, when the two obviously

different values for $\bar{w}_1$ from experiments 10, 28 and 29 are plotted as functions of CVRHO, the correlation is enhanced, and is now significant at the 0.1% level ($t = 5.09$; $t_{.001[12]} = 4.32$).

What does this relationship suggest? The fluctuation in $\rho$ might be caused by instabilities intrinsic to the experimental design—instabilities caused by changes in flow rate, aeration, temperature, and so forth—or, they might be caused by changes in the population itself—transient colonization of the chemostat walls, cross-feeding or poisoning of one population by another, and local changes in food supply. In the former, the *mutT1* population would be responding to a changed environment through mutation, with the strongest selection at work in the most unpredictable environment, while in the latter, fluctuations in $\rho$ would be caused by successful colonizers. The data presented here do not allow a choice to be made between these two explanations. The results nonetheless demonstrate that a high mutation rate confers higher fitness in these experiments, and, that whatever the cause, fitness values for the *mutT1* population correlate strongly with population instabilities.

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