Population Studies in *Escherichia coli*: Selection for Lactose Utilization.

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INTRODUCTION

The classical theory of population genetics is firmly based on the concept of "relative fitness." The process of population change may be viewed as a birth and death phenomenon, each genotype with its own rates (Fisher 1930). The selective differential between two genotypes may be described by the difference in their intrinsic growth rates, and it is natural to use this difference as a parametric measure of selection. The resulting theory of natural selection has been a very useful descriptive treatment.

As a predictive device, however, the idea of "parametric fitness" has not always proven to be reliable. Actual growth rate for genotypes are dependent upon the numbers and kinds of genotypes present; selection is therfore frequency and density dependent. As a consequence, the performance of a genotype cannot be completely predicted on the basis of pure culture performance (eg. Lewontin 1955; Harding *et al.* 1966, Ehrman 1966, Kojima and Yarbrough 1967, Allard *et al.* 1968, Huang *et al.* 1971, Kosuda 1981 a; 1981 b).

This dependence of instantaneous replacement rates of population growth upon the numbers and kinds of organisms present has long been recognized by ecologists to be the rule in interspecific dynamics (Gause 1934). The argument is usually formulated in terms of saturation phenomena, rather than as a birth and death process (Volterra 1926). Similar treatment has recently been forthcoming for intraspecific dynamics as well (MacArthur 1962; Smouse 1976; Smouse and Kosuda 1977).

It has become explicit that intrinsic growth rate and maximum population size of the various genotypes and additional parameters must be considered in predicting the outcome of competition (Pianka 1972, Ayala 1969, Ayala *et al.* 1973, Gill 1972). While it is becoming increasingly obvious that natural selection and evolution should be viewed as an ecological phenomenon, there is still no general agreement of the principles involved. The essential ecological aspects of natural selection are expected

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to be same for sexual and asexual population, whether diploid or haploid. The purely dynamic aspects of the process, are confounded in diploid sexual organisms with the necessities of reproductive interaction among genotypes. The purpose of this paper is to experimentally explore one particular ecological aspect of natural selection in asexual populations. The resource utilization efficiencies of populations having different genetic constitutions were investigated. It was also attempted to delineate the impact of limited energy resources on the genetic compositions of a population.

MATERIALS AND METHODS

Two asexual strains of *Escherichia coli* K-12 were utilized: a standard wild type strain ($F^-\lambda^-$) and a lactose negative auxotroph, derived from the wild type by UV irradiation inducing a deleteion in the lac Z cistron. A lactose negative auxotroph never reverted to the wild type. These strains will henceforth be denoted G_0 and G_1, respectively. The metabolic requirements of the organism are very simple, consisting of inorganic salts and sugar sources.

The liquid growth media employed are all based on the same minimal salt solution (KH_2PO_4 − 2 gm/l; K_2HPO_4 − 7 gm/l; $Na_3C_5H_5O_7 \cdot 2(H_2O)$ − 0.5 gm/l; $MgSo_4 \cdot 7(H_2O)$ − 0.1 gm/l; and $(NH_4)_2SO_4$ − 1 gm/l). To this minimal salts broth, growth limiting concentrations of three different carbon sources, lactose, arabinose, and glucose were added. The concentration of arabinose (0.20 gm/l) and glucose (0.05 gm/l) were held constant. Four level of lactose concentration in media were employed

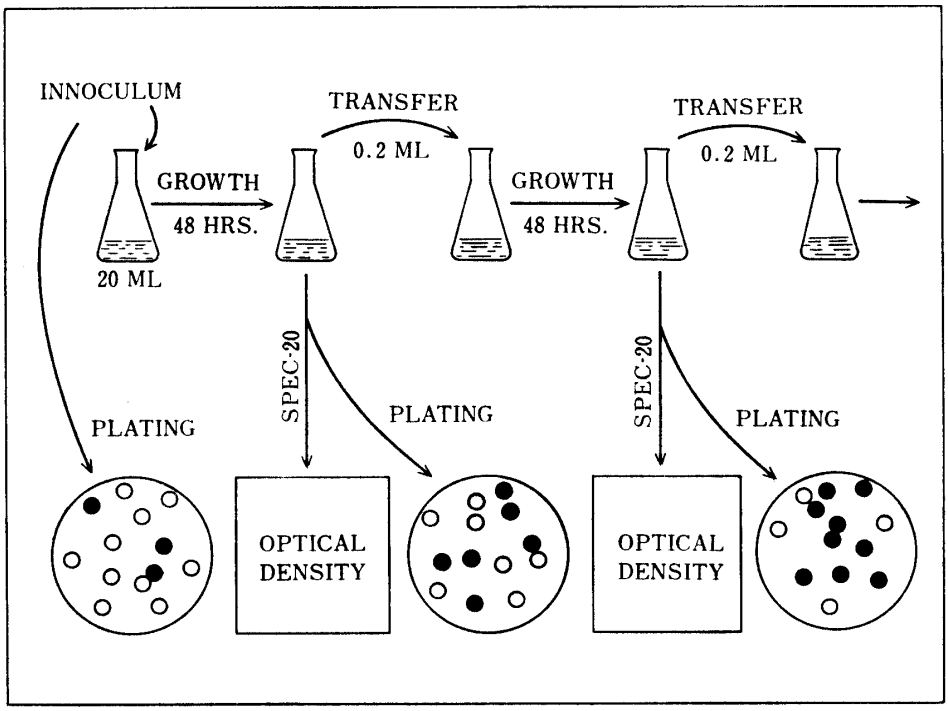

Figure 1 Growth and assay scheme for serial transfer competition experiments.

[0 gm/*l* for M(0), 0.08 gm/*l* for M(08), 0.16 gm/*l* for M(16) and 0.25 gm/*l* for M(25)]. The concentration of lactose constitutes the primary expermental variables in the present study. Three replicates for each experiment were made. Pure strain cultures of each genotype were maintained on each medium, as a pair of controls for mixed cultures. The resource base of G_1 is thus contained within that of G_0, since G_1 can not metabolize lactose.

The culture technique employed was the serial transfer procedure of Atwood *et al.* (1951 b). Each culture was grown in a 125 m*l* Erlenmeyer flask, in 20 m*l* of liquid medium, agitated at 80 cycles/min. All cultures were maintained at 37.5± 0.5 °C. At the end of 48 hours, 0.2 m*l* were transferred to fresh medium, and the cycle was repeated. At the time of transfer, population density was determined by optical scattering set at 450 nm with SPEC-20. The genetic composition of a culture was also determined at the time of transfer, by means of dilution plating. For this purpose EMB (Eosin Methylene Blue) agar was utilized, because it stains colonies of G_0 a metallic green, while leaving those of G_1 a pale pink or white (see Figure 1).

RESULTS

THE GROWTH EXPERIMENTS

The determination of population density at short intervals is most easily accomplished by the destructive sampling. Eighteen replicates were started simultaneously. Pairs of cultures were sampled at predetermined intervals. Since any given culture was sampled only once, each observation is independent of others. Repeat-

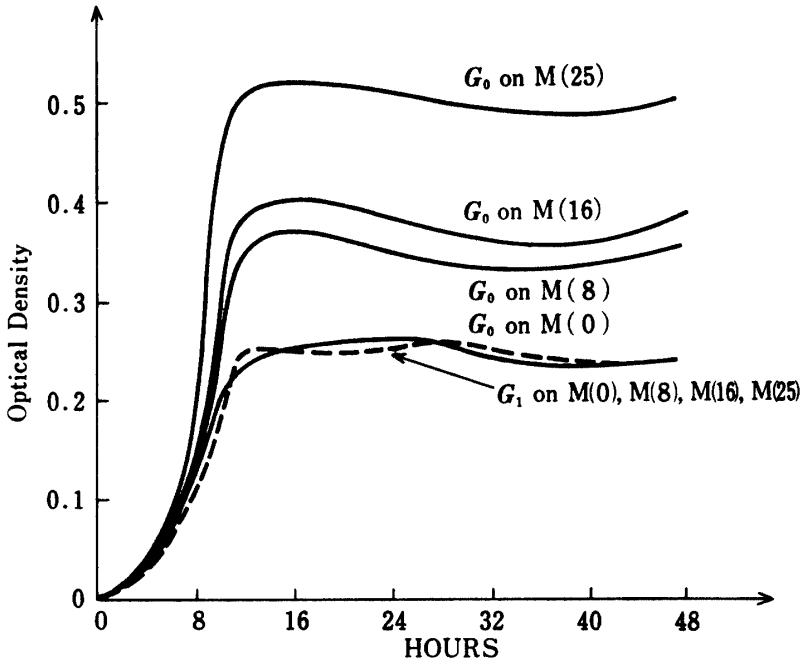

Figure 2 Pure culture growth curves of lactose positive (G_0) and lactose negative (G_1) strains of *E. coli*. K-12. Media are described in the text.

ability was very high, and the resulting trajectories are accurately depicted in Figure 2, where optical density is plotted against time.

Calibration experiments have revealed that the logarithm of living cell number is an increasing linear function of O. D. over the range of total sugar concentrations used in the experiments. The senescence characteristic of batch cultures was found not to bigin until 48 hours, so that we are dealing with live cells throughout the period between transfers. Optical scattering, measured by O. D. at 48 hours is thus a very convenient measure of population size, and it can be used as a reliable indicator of the efficiency of resource utilization.

As expected, the final population size at 48 hours of G_0 increased with lactose concentration, while that of G_1 was not responsive to this variable. It is evident that the exponential growth rate of G_0 also increases with lactose concentration, but that final size is a more sensitive indicator of population performance.

Since G_0 is more efficient in the presence of lactose, one would reasonably predict that mixed cultures grown on M(8), M(16), and M(25) would proceed to fixation for G_0. Although it is not evident from Figure 2, G_0 is, on the average, slightly more efficient than G_1 on M(0). The average values are .245 and .240, respectively, and while the difference is subtle, it appears to be real. One would thus expect that even on M(0), mixed cultures would proceed to fixation for G_0. It seems reasonable to predict that the selective differential between the two strains should increase with lactose concentration and that the rate of allelic substitution should increase accordingly.

COMPETITION IN MIXED CULTURES

We shall first describe the results of competition on M(0). Since the selective differential between the two strains was expected to be very small, we expected to encounter difficulty in detecting genetic change. Consequently, mixtures were initiated

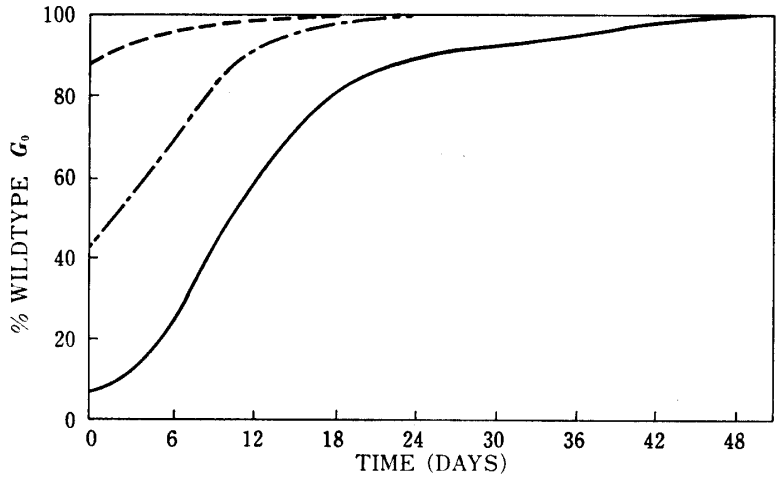

Figure 3 Trajectories of the frequency of G_0 for mixed cultures, initiated at three different frequencies. The medium was M(0), as described in the text.

at three different frequencies of G_0 (.071, .432, and .884). Each mix was represented by three replicate cultures, and three replicates each of pure G_0 and pure G_1 were initiated as controls. The behavior of replicates was quite homogeneous, with the exception of one of the (.071) replicates to be discussed later, and the frequency trajectories of G_0 are shown as averages for the replicates of each mix in Figure 3. The dilution rate is 1/101 at each transfer, and the population thus undergoes 6.658 doublings every two days. The growth trajectories of Figure 2, however, show that the 6.658 doublings occur in the first 12 hours, rather than in the full 48 hours. Cell division continues very slowly for the remainder of the time. In order to establish a convenient time reference, it may be assumed that each transfer represents 10 generations, although the exact number is not crucial.

In accord with expectation, selective replacement of G_1 by G_0 was very slow (the frequency of G_0 changed from .071 to .999 in about 255 generations). The initial frequency of G_0 did not affect the ultimate outcome, and we may concentrate on the most informative trajectory, the lower one.

Having established that even subtle differences in efficiency are sufficient to result in detectable selective differentials, we proceed to examine the fate of mixed cultures on M(8). M(16), and M(25). For M(25), we have 9 replicate mixes, all initiated at low frequency (.001) for G_0. The repeatability was so high that only three replicate cultures each were used for M(8) and M(16), and these were all run together. Pure culture controls were included in all experiments. Repeatability was high for M(16), but one of the mixes on M(8) showed unusual behavior. This culture will be discussed at a later point. The average trajectories of the mixes, excluding this last culture, are shown in Figure 4. The rate of replacement of G_1 by G_0 is an increasing function of lactose concentrtion. Not only are pure culture efficiencies good predictors of eventual outcome, but the size of the difference is a monotonic

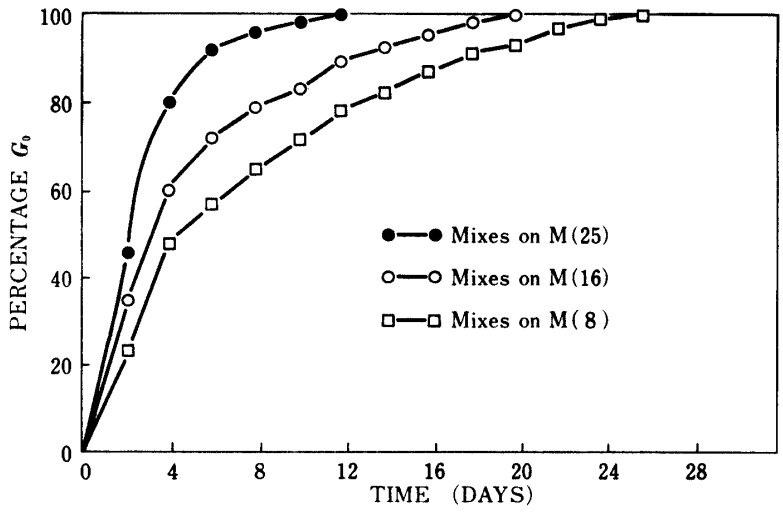

Figure 4 Trajectories of the frequency of G_0 for mixed cultures, grown on M(8), M(16), and M(25). The media are described in the text.

indicator of replacement rate.

In all cases, total population size for genotypic mixes converged very rapidly to the same level as G_0 control, even for cultures which were still in the midst of selective substitution. Although the population sizes of mixed cultures oscillate around those of their more numerous control (G_0), they never drop below the population sizes of the less numerous control (G_1). Population sizes differ between pure culture controls, even for the M(0) experiments, although the replicate to peplicate variation for all three types (Pure G_0, Pure G_1, and the mix) overlap.

If one were to view the introduction of G_0 into the cultures as a mutational event, one can see that the ensuing genetic substitution improves the poulation size relative to the initial pure G_1 state. Moreover, the greater the advantage of the rare variant, the greater the improvement. This result would seem to be in conflict with the theory of substitutional load (Haldane 1957), which would predict that while the population would ultimately benefit from the substitution, it would suffer a reduction in "fitness" in the interim. Moreover, the greater the selective differentials between two genotypes, the greater the substitutional load. This would imply that a limit to the amount of selection a population could sustain. It seems to us that this very odd theoretical claim derives from a failure to distinguish between the "relative fitness" values of individual genotypes and the "absolute fitness" value of a population. Although we hesitate to extrapolate our results to situations where selective differentials do not depend upon resource utilization, it seems clear that "substitutional load" does not pose a general barrier to rapid evolution.

CONSTANCY AND NON-CONSTANCY OF SELECTIVE DIFFERENTIALS

We now turn to a consideration of whether the selective differentials encountered are constant or whether they are frequency and/or density dependent. Smouse (1976) has shown theoretically that density regulated populations may exihibit non-constancy of selective differentials. Smouse (1976) employed a Lotka-Volterra formulation for theoretical purpose. That formulation is not entirely adequate to describe all of the features of serial transfer culture, but it does constitute a very useful conceptual aid for the present situation. We shall briefly recapitulate the salient features of that argument and since it was originally posed as a birth and death process, shall provide the translation to an ecologically more familiar analogue.

Imagine that the growth equations of the two genotyes in mixed culture may be written

$$\begin{aligned} G_0:\ \dot{N}_0 &= [r_0-\beta(N_0+N_1)]N_0 \\ &= r_0N_0(K_0-N_0-N_1)K_0^{-1} \\ G_1:\ \dot{N}_1 &= [r_1-\beta(N_0+N_1)]N_1 \\ &= r_1N_1(K_1-N_0-N_1)_1^{-1} \ , \end{aligned} \qquad (1)$$

where r_0 and r_1 are the intrinsic growth rates of the two genotypes, β is a parameter describing the intensity of density-dependent damping on population growth, and

$K_0=r_0/\beta$ and $K_1=r_1/\beta$ are the respective carrying capacities of the two genotypes. The situation could be described as one where both genotypes are equally competitive (equally damped) on all available resources, but where one genotype (G_0 for our case) has a higher intrinsic growth rate, and hence K-value. The net result is ultimate fixation of the more "fit" genotype where fitness can be measured by the K-values (or in this case the r-values). If one defines $\Lambda=\log P-\log Q$, where P and Q are the respective frequencies of G_0 and G_1, it can be shown that the trajectory of Λ is a straight line, i. e. $\Lambda=r_0-r_1=s$ (Kosuda 1981). The selective differential (s) between the two genotypes is a constant, irrespective of gene frequency (P) or population size ($N=N_0+N_1$).

Instead of employing (1) to describe the growth equations in mixed culture, we could use

$$\begin{aligned} G_0 &: \dot{N}_0=[r_0-\beta_0(N_0+N_1)]N_0 \\ &\quad =r_0N_0(K_0-N_0-N_1)K_0^{-1} \\ G_1 &: \dot{N}_1=[r_1-\beta_1(N_0+N_1)]N_1 \\ &\quad =r_1N_1(K_1-N_0-N_1)K_1^{-1} \end{aligned} \qquad (2)$$

where r_0 and r_1 are as defined for (1), β_0 and β_1 indicate different intensities of density-dependent feedback, and $K_i=r_i/\beta_i$. This situation differs from the previous one in that here the two genotypes are not equally competitive. The end result is

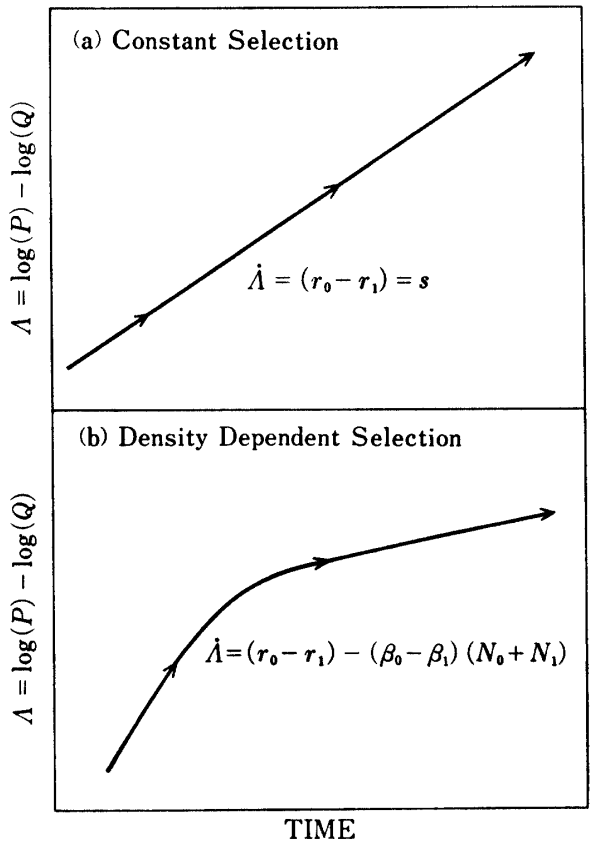

Figure 5 Theoretical trajectories of $\Lambda=\log P-\log Q$ under: (a) constant selection, (b) density dependent selection.

still fixation for the genotype (G_0) with a higher K-value, but in this case the trajectory of A is not a straight line. It can be shown that trajectory of A takes the form $A=(r_0-r_1)-(\beta_0-\beta_1)(N_0+N_1)$, which depends upon population size. As indicated above, the population size of a mixture equilibriates more rapidly than gene frequency, and this has an interesting effect upon A. Suppose initially that population contains only G_1, and maintains the size K_1. One then adds G_0 at lower frequency, and selective replacement begins. Initially, however, one may write

$$A\approx(r_0-r_1)-(\beta_0-\beta_1)K_1=\cdots\cdots\quad=\beta_0(K_0-K_1)\ , \tag{3}$$

since $(N_0+N_1)=K_1$. Population size rapidly approaches the value $(N_0+N_1)=K_0$, however, and from that point onward, one has

$$A\approx(r_0-r_1)-(\beta_0-\beta_1)K_0=\cdots\cdots\quad=\beta_1(K_0-K_1)\ , \tag{4}$$

a different slope from that of (3), but essentially a constant. The types of trajectories from (1) and (2) are shown in Figure 5.

One may adopt still another model of competitive growth, replacing (1) and (2) with

$$\begin{aligned} G_0:\ \dot{N}_0&=(r_0-\beta_{00}N_0-\beta_{01}N_1)N_0\\ &=r_0N_0(K_0-N_0-\alpha_{01}N_1)K_0^{-1}\\ G_1:\ \dot{N}_1&=(r_1-\beta_{10}N_0-\beta_{11}N_1)N_1\\ &=r_1N_1(K_1-N_1-\alpha_{10}N_0)K_1^{-1} \end{aligned} \tag{5}$$

where r_0 and r_1 are defined as before, β_{00} and β_{11} are the intensities of self damping for G_0 and G_1, respectively. β_{01} is the intensity of G_1 damping on G_0, and conversely for β_{10}. The K-values are the pure culture performances of the two genotypes, defined to be $K_0=r_0/\beta_{00}$ and $K_1=r_1/\beta_{11}$, respectively. The parameters α_{01} and α_{10} are defined as β_{01}/β_{00} and β_{10}/β_{11}, respectively. The model is particularly useful in situations where the two genotypes have different utilization capacities for different resources. The resulting "niche partition" might be very subtle; for example, one genotype might be slightly more efficient than the other in its use of one substrate, while being less efficient for an alternative substrate. In such case, a stable polymorphic mixture may sometimes arise, but regardless of the ultimate outcome, the trajectory of A takes the form

$$\begin{aligned} A&=(r_0-r_1)-(\beta_{00}-\beta_{10})N_0-(\beta_{01}-\beta_{11})N_1\\ &=s-[P(\beta_{00}-\beta_{10})+Q(\beta_{01}-\beta_{11})](N_0+N_1)\ , \end{aligned} \tag{6}$$

which is both frequency and density dependent. The trajectory will tend to curve continuously over time.

Simply by examining the trajectories of A, we should be able to cast some light on the nature of competitive interactions between the two genotypes in different ecological settings. The trajectories of A for all four experimental media are shown in Figure 6. We have artificially staggered the times of initiation for the four media so as to facilitate comparison.

The trajectories of M(25), M(16), and M(8) are all of the sort shown in Figure 5 b, while that of M(0) is the sort shown in Figure 5 a. Since we had not initiated

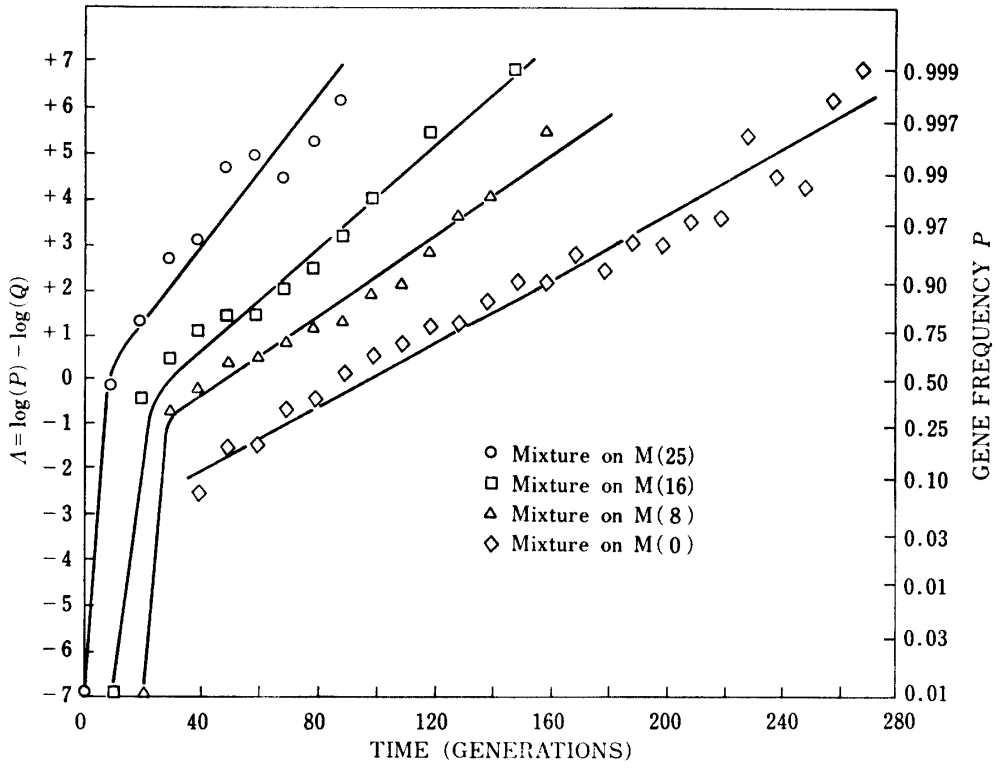

Figure 6 Trajectories of $\Lambda = \log P - \log Q$ for mixed cultures on four growth media. Media are described in the text.

the mixture on M(0) at as low a frequency as that used for the other media, one must interpret the lack of change of slope with some caution. We are lead to speculate, however that no shift should have been detected in the trajectory of Λ on M(0).

The break points for the other three trajectories correlate well with the composition of the respective media. The portion of the total ATP yield of the respective sugar mixes which are attributable to the lactose component are 52.1% for M(25), 41.1% for M(16), and 25.8% for M(8). The comparable value for M(0) is, of course, zero. The aformentioned break points occur at about $P=48\%$ for M(25), $P=39\%$ for M(16), and $P=30\%$ for M(8). One should, by extrapolation, expect no break point for M(0). It appears that G strain is entering an "ecological vacuum" at the initiation of the experiment, since G_1 is unable to metabolize lactose. By the time of the first transfer, however, this untapped resource has been largely utilized. At that point, the frequency of G_0 reflects the concentration of lactose. Subsequent change in gene frequency are largely determined by competition for the shared resources: glucose and arabinose. It appears from Figure 6 that the slope of the trajectory increases with the lactose concentration, and to the extent that this observation is valid, it indicates that competition for glucose and arabinose is not independent of the availability of lactose. The pure culture growth trajectories of Figure 2 can be interpreted, in terms of (4), to mean that r_1 and β_1 are not responsive to lactose concentration, but that r_0 and β_0 are responsive. This implies that (4)

should increase with lactose concentration, since K_0 is increasing and β_1 and K_1 are not. The changes in slope evidenced in Figure 6 are as expected. Since lactose component of M(0) is zero, we argue that the M(0) trajectory represents a base line and that no shift of slope is to be expected.

Since no general curvature was evident in the abovementioned trajectories, it does not appear that G_0 and G_1 are "partitioning" the arabinose and glucose to any detectable degree. To the extent that the models employed are appropriate (and they are admittedly approximations), these results indicate that selection is density but not frequency dependent under the conditions described.

DISCUSSION

Two mixed cultures, one grown on M(0) and one on M(8), did not exhibit the usual patterns of gene-substitution. The trajectories of logit P for both cultures are shown in Figure 7. Culture A grown on M(0), showed an initial decrease in the wild type (G_0), whereas the other replicates showed an increase. This pattern was reversed after four days, and the frequency of G_0 increased steadily for a period of sixteen days. This rate of increase exceeded the comparable rate of change for the replicate cultures (see Figure 3). This rapid increase in the frequency of G_0 was followed by a brief decrease, which was followed in turn by steady increases in the frequency of G_0. This second phase of gene substitution occurred at a slower rate than that of the replicate cultures. While a certain amount of erratic behavior in the trajectory of any mixed culture is to be expected, that for culture X was quite excessive.

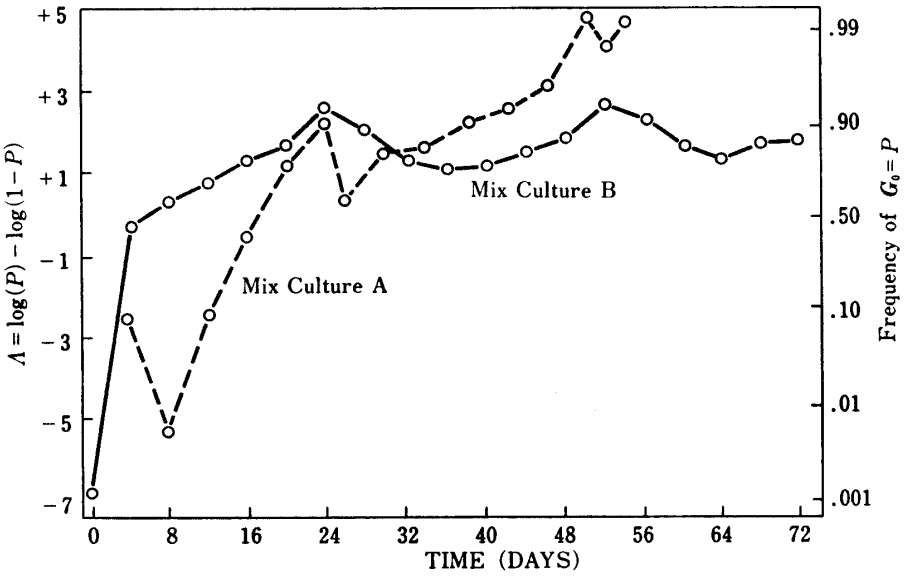

Figure 7 Trajectories of $\Lambda = \log P - \log(1-P)$ for two aberrant cultures: (a) mix culture A, grown on M(0); and (b) mix culture B, grown on M(8). Media are described in the text.

The changes may be attributed to real evolutionary (selective) phenomena, rather than to sampling errors since each point is estimated from 800–1600 colonies.

It can be speculated that the genotypes G_0 and G_1 in Culture A have mutated to new forms with different adaptive properties. It seems likly that we have the sort of "periodic selection" described by Atwood *et al.* (1951 a), which can be schematized as follows;

$$\underset{(r_0)}{G_0} \longrightarrow \underset{(r_0)}{G_0} \overset{\text{mutation}}{\longrightarrow} \underset{(r_0')}{G_0'} \longrightarrow \underset{(r_0')}{G_0'} \overset{\text{mutation}}{\longrightarrow} \underset{(r_0'')}{G_0''}$$

$$\underset{(r_1)}{G_1} \overset{\text{mutation}}{\longrightarrow} \underset{(r_1')}{G_1'} \longrightarrow \underset{(r_1')}{G_1'} \overset{\text{mutation}}{\longrightarrow} \underset{(r_1'')}{G_1''} \longrightarrow \underset{(r_1'')}{G_1''}$$

The intensity and direction of selection depends upon the difference in r-values at each stage. We postulate $r_0-r_1>0$, as in the replicates, and the frequency of G_0 increases; $r_0-r_1'<0$, and the frequency of G_0 decreases; $r_0'-r_1'>0$, $r_0'-r_1''<0$, and $r_0''-r_1''>0$, and G_0 increases and decreases accordingly.

If this speculation is correct, one should be able to isolate G_1', G_0', G_1'', and G_0'', and show that their r-values (and hence K-values) increases sequentially. In addition, the population size of Culture A should be increase. Although G_0'' and G_1'' were isolated and the progress of population size were monitored, no compelling evidence for the predicted changes was found. But such determinations are beset by the difficulty in detecting subtle difference among K-values.

In view of the likelihood that frequent "background mutation" is present in our cultures, one may legitimately query the consistent behavior of the majority of cultures described. The answer seems to be that any given mutation is vanishingly rare, and that very few mutants will possess a pronounced selective advantage. A mutant with a selective advantage of as much as .01 would take a long time to increase from an initial frequency of 10^{-6} to 10^{-3}, by which time the experiment would have been terminated. If the selective differential between G_0 and G_1 is large, it will be a rare mutant indeed that makes an appropriate impact on the outcome. Where this selective differential is small, as for the M(0) experiments, an occasional culture will experience such an event. In most cases the "signal" induced by experimental inputs outweighs the "noise" induced by background mutation,

Culture Y in Exp. B also appears to have experienced "periodic selection", but in a rather unusual fashion. G_0', G_1', G_0'', and G_1'' were isolated. Average population densities for pure culture controls and Culture Y are shown in Figure 8. As early as generation 100, the mixture manifests a consistent and clear increase in population size over the more populous of the pure culture controls. Derivative strains seems to complement each other; i. e., the mixture is more effectively utilizing the heterogeneous resource base than is either strain alone. Growth experiments with the derivative strains indicate that each is more efficient than its unselected parent strain.

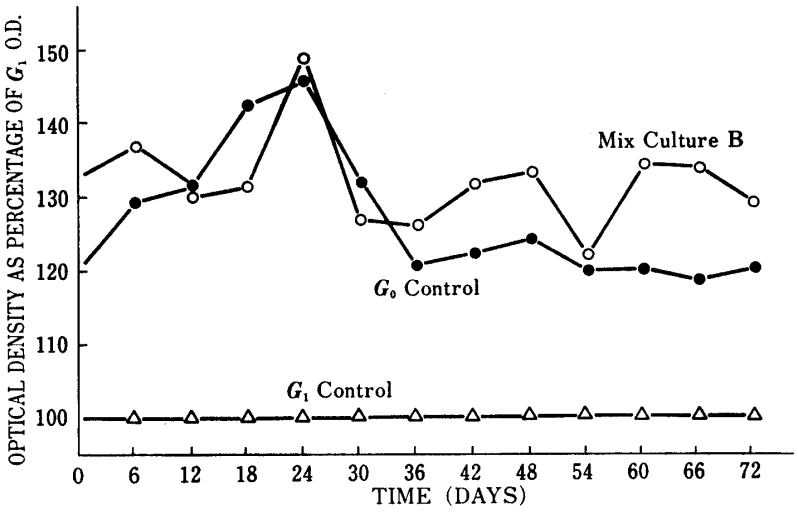

Figure 8 Trajectories of population density for mix culture B and its pure G_0 control, as percentages of the population densily of pure G_1 control.

It appears that even for this anomalous culture, the basic hypothesis on resource utilization is borne out. The genotypic array making most efficient usage of limited energy resources will prevail. In this last case, that genotypic array is a polymorphic mixture, whereas for all of the other cultures it was a pure culture.

SUMMARY

Competition experiments were conducted between twe genotypes of *E. coli* K-12 [$lac^+(G_0)$ and $lac^-(G_1)$], grown on four different culture media differing in the concentration of lactose. Trajectories of gene frequency and population size followed for the various mixed populations. In almost all cases, the lac^- genotype (G_1) were replaced by the lac^+ genotype (G_0), even when no lactose was present in the medium. The selective differential and the rate of "gene substitution" were increasing functions of lactose concentration, as expected, and the selective differentials were found to be density-dependent and frequency-independent. One culture established a persistent polymorphism, with population size greater than that of either monomorphic control. Evolution in this culture, by means of successive substitutions of more efficient G_0 and G_1 derivatives resulted in minor changes in gene frequency, and a sort of complementation of the two genotypes. These experiments suggest that the more efficient genotype will prevail, except where a mixture is more efficient than either genotype alone.

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