SEGREGATION OF THE SELECTIVE ADVANTAGE OBTAINED THROUGH ORTHOSELECTION IN ESCHERICHIA COLI[1, 2]

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THE existence of an unanticipated selective process in populations of bacteria was postulated by STOCKER in 1948. While engaged in measuring the mutation rate of antigenic variants of Salmonella, he observed that in mixtures predominantly of one phase a fluctuation occurred in the frequency of the other phase. He pointed out that the most probable explanation of this phenomenon is the appearance of a more rapidly growing mutant which displaces its predecessors in the culture.

SCHNEIDER (1950) working independently with mixtures of *Escherichia coli* mutants observed that the frequency of the minority type was constant for a time and then underwent a rapid decline. She demonstrated that a population shift had occurred because of the overgrowth of a mutant with a selective advantage. Similar results were obtained by NOVICK and SZILARD (1950) who observed the repetitive nature of the selection process. A year later ATWOOD, SCHNEIDER and RYAN (1951a) published the results of experiments which demonstrated conclusively the existence of a unique selective mechanism in strain 15 of *E. coli*. They found that in growing cultures consisting predominantly of h^- (histidine dependent) cells there was a cyclical rise and fall in the frequency of the h^+ mutants brought about by the emergence of h^- cells which enjoyed a selective advantage and therefore soon constituted the preponderant type in the culture. h^+ mutants of this new type at once began a gradual increase toward the mutational equilibrium frequency but were interrupted before this was reached by the appearance of a second fitter type among the h^- and the ensuing rapid turnover in the population. To describe this phenomenon they proposed the term "periodic selection" and suggested that this mechanism operated continuously in populations of microorganisms subject to extended periods of growth, but was detectable only when fluctuations in a suitable indicator strain could be observed. Since it is now understood that these fluctuations are only a reflection of a continuous selective process the more accurate term "orthoselection" is currently in use (RYAN 1953).

The changes brought about in the population by orthoselection seemed to be stable and heritable but a direct genetic analysis of the selective advantage was not possible because of the unfortunate lack of a known sexual phase in the strain employed. A survey of the literature immediately suggests several different genetic schemes which might be operating here. Cytoplasmic inheritance has been demonstrated in

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microorganisms (SONNEBORN 1950; EPHRUSSI 1951; MITCHELL and MITCHELL 1952) and it is at least conceivable in the present instance that the conditions imposed by continuous growth over long periods of time might be conducive to the accumulation of extranuclear components which confer a selective advantage upon the cells harboring them. On the other hand the discovery of recombination in *E. coli* K12 (LEDERBERG and TATUM 1946) has led to the conclusion that a nuclear mechanism exists in this bacterium controlling the inheritance of many different traits. Instances of single gene (LEDERBERG, LEDERBERG, ZINDER and LIVELY 1951) and polygenic inheritance (CAVALLI 1952) are known and it is suggestive in the latter instance that long periods of growth under highly selective conditions are required to produce the altered genotype. In any event, since the mechanics of inheritance in *E. coli* were sufficiently clear and a distinction between the various schemes could be made, it seemed feasible to undertake a genetic analysis of the fitter types generated through orthoselection.

EXPERIMENTAL PROCEDURES

It seemed desirable to use material which was as genetically homogeneous as possible, and for this reason a single strain, W1896, kindly supplied by DR. JOSHUA LEDERBERG, was used to produce two crossable substrains by spontaneous mutation of its markers. By the same method five additional unselected marker differences were obtained to provide a basis for a genetic analysis of a polygenic system if this proved to be the case. The genealogies of the strains are summarized in figure 1. Mutants were obtained by plating on selective media and picking a colony. All the fermentation mutants were characterized on a complete indicator agar (EMB without lactose) to which the appropriate sugar had been added. The resulting substrains R4123 and R1131 were relatively infertile under the usual crossing conditions (LEDERBERG, LEDERBERG, ZINDER and LIVELY 1951) but produced prototrophs abundantly when aerated cultures, grown in YECA for 12 to 18 hours at 37°, were mixed together 4 to 6 hours, washed twice in saline, resuspended and plated in minimal agar supplemented with 0.01 μg thiamine hydrochloride per ml and 100 μg streptomycin per ml. In control experiments of R4123 plated by itself only a few streptomycin resistant (S^r) colonies per 10^{10} bacteria were found, while R1131 under similar conditions yielded no colonies at all.

The production of fitter types involved the serial transfer system employed by ATWOOD, SCHNEIDER and RYAN (1951a). The cells were inoculated into 125 ml erlenmeyer flasks containing 50 ml of Gray and Tatum minimal medium supplemented with 200 μg DL-threonine, 60 μg DL-leucine and 0.01 μg thiamine hydrochloride per ml, and were incubated without aeration at 37°C for 12 hours, when full growth had been attained. A 0.5 ml sample was then removed to a flask of fresh medium, this was incubated for 12 hours, and a 0.5 ml sample was used to inoculate the third serial transfer (ST). This sequence was repeated until the strain had passed through sixty ST. A single colony isolated from each line at this time was designated 60A1 (from the R4123 line) and 60B1 (from the R1131 line).

To determine the selective advantage of the original types R4123 and R1131, the fitter types 60A1 and 60B1, and recombinants from any cross, two standard

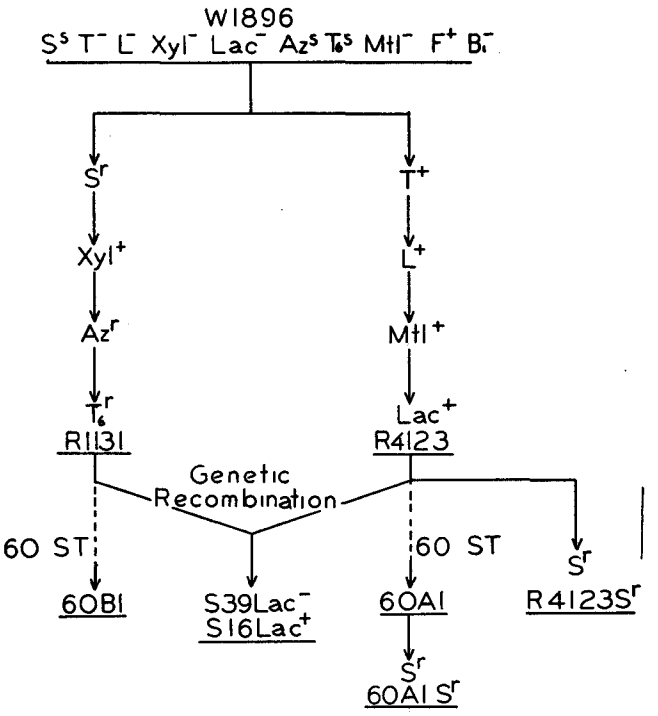

FIGURE 1.—The genealogy of the strains derived from W1896. Thiamine hydrochloride was added to all minimal media so that the B_1 marker need not be considered. Abbreviations for the markers are:

S = Streptomycin	*Xyl* = Xylose	*Az* = Sodium azide
T = Threonine	*Lac* = Lactose	T_6 = Coliphage T_6
L = Leucine	*F* = Mating type	*Mtl* = Mannitol
		B_1 = Thiamine

tester strains were obtained, S39 *Lac*$^-$ and S16 *Lac*$^+$, as prototrophs from a cross of R4123 and R1131 (fig. 1). *Lac*$^+$ strains were tested against S39 and *Lac*$^-$ against S16. Testing experiments were begun by inoculating 0.4 ml of the appropriate tester into a ST flask with 0.1 ml of the strain to be tested. This constituted the beginning of a series of eight ST carried out as described in the preceding section. A 0.5 ml sample was removed from the first ST immediately after inoculation, diluted and spread on four EMB plates. Similar samples were removed from each ST at the end of its incubation period. Thus, a set of nine samples was obtained from which any change in the ratio of the two competing strains could be calculated. Assuming that the selective advantage is independent of the ratio of the two types, the log of this ratio plotted as a function of the number of ST is a straight line, and the slope of this selection curve constitutes a comparative measure of the selective value of any strain. In these calculations the ratios were multiplied by ten for convenience in using the log tables.

The growth rate and final level of growth were obtained by the following procedure. Two ml of a stationary culture grown without aeration in ST medium were inoculated

into 400 ml of fresh medium and 50 ml were measured into each of eight 125 ml erlenmeyer flasks. These were allowed to stand undisturbed at 37°C and at appropriate intervals a flask was removed and a 0.5 ml sample was diluted and spread on four plates. The flask was then discarded. In this way the growth occurred under conditions very closely approximating those in the ST system.

RESULTS AND CONCLUSIONS

The cross of the original types

It seemed necessary to establish at the outset that the segregating markers were selectively neutral. For this reason and also to obtain the standards for further tests, the two original strains R4123 and R1131 were crossed and from among one hundred prototrophs picked and characterized, six with different arrays of markers were chosen for further analysis (table 1). A 1:1 mixture of S39 and S16 carried through eight ST underwent no change in proportion, for the slope of the line plotted in figure 2 is not essentially different from zero. Since these two strains were equal in their selective advantage and carried opposite sets of markers (with the exception of mannitol) they were chosen as standards against which all the other strains were subsequently tested. A 1:4 mixture of S125 *vs.* S16 when tested as described above underwent no significant change in the initial ratio (fig. 2). Essentially the same results were obtained with S14 *vs.* S39, S63 *vs.* S39, S25 *vs.* S39 and R1131 *vs.* S16, although here there seemed to be a small but definite rise in the proportion of R1131

TABLE 1

Recombinants from the crosses of R4123 × R1131

Strain*	*S(TL)*	*Xyl*	*Lac*	*Az*	*T*	*Mtl*	Slope
R4123	s +	−	+	s	s	+	0.288
R1131	r −	+	−	r	r	−	0.060
S39	r +	−	−	r	r	−	0.000
S16	r +	+	+	s	s	−	0.000
S63	r +	−	+	s	s	−	0.029
S25	r +	−	+	s	r	−	0.041
S125	r +	−	−	s	s	−	0.022
S14	r +	+	+	s	r	−	0.019
R4123*S*^{r}	r +	−	+	s	s	+	−0.400
2-1	r +	−	+	s	s	−	0.000
2-20	r +	+	−	r	r	−	−0.011
2-126	r +	−	+	s	r	−	0.020
2-5	r +	+	+	s	s	−	0.032
2-31	r +	+	+	r	s	−	0.019
2-206	r +	+	+	s	r	−	0.000
2-12	r +	−	+	r	r	−	0.030
2-4	r +	−	−	s	r	−	0.012
2-2	r +	+	−	s	r	−	−0.005
2-9	r +	−	−	r	r	−	0.015

* Recombinants from the first cross are labeled "S" and those from the second cross are labeled "2." Strain R4123*S*^{r} is a streptomycin resistant reversion of R4123.

(fig. 3). On the other hand, R4123 in competition with S39 was decidedly superior and rapidly replaced S39 in the ST. This was not entirely unexpected since S^s strains have been shown to have a more rapid growth rate than S^r (CAVALLI 1952). The effect of the streptomycin locus in this case was clearly demonstrated, for a S^r reversion of R4123 (R4123S^r) when pitted against S39 now proved to be inferior (table 1). These results were confirmed by a second experiment in which ten recombinants were analyzed (table 1). All were found to be without any selective advantage over the tester. It was concluded from this that the markers were selectively neutral

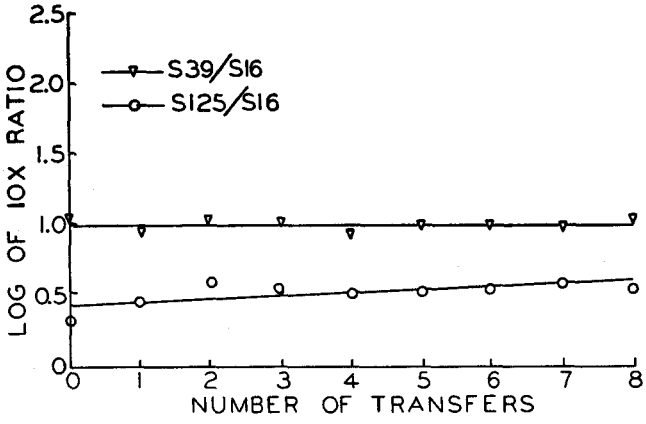

FIGURE 2.—The log of 10X the ratio plotted as a function of the number of serial transfers.

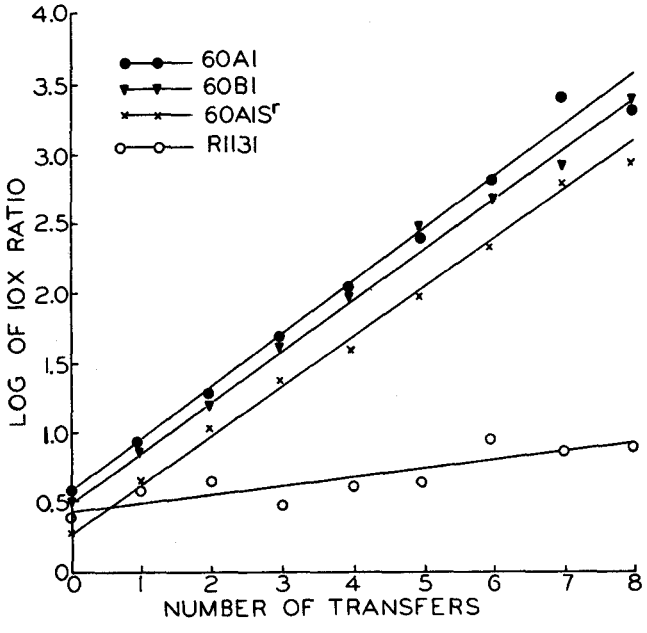

FIGURE 3.—Selection curves for 60A1, 60B1, 60A1S^r and R1131.

with the exception of the *S* locus, and since S^r recombinants apparently did not inherit the rapid growth rate of the S^s parent, the effect of this locus could be disregarded.

The selective advantage of the fitter types

When 60A1 and 60B1 were placed in competition with the standards it became at once evident that during the course of the serial transfers the original strains had acquired a pronounced selective advantage (fig. 3). A comparison of the slope of the R1131 selection curve with that of 60B1, the fitter type derived from it, reveals a six-fold difference which is definitely significant. A S^r reversion of 60A1 (60A1S^r) proved to have the same slope as 60A1 itself, thus demonstrating that the S^s locus was not contributing to the selective advantage of this fitter type. It is interesting that the slopes of 60A1 and 60B1 are nearly identical and, in fact, no significant difference between them can be demonstrated. This fact suggests that changes which occurred during the evolution of the fitter types may have been the same in the two strains despite the fact that they were evolved independently of each other. The test of this hypothesis involves the crossing of the fitter types with the original strains

TABLE 2

Recombinants from the crosses of 60A1 × R1131

Strain*	*S*(*TL*)	*Xyl*	*Lac*	*Az*	T_6	*Mtl*	Slope
60A1	s +	−	+	s	s	+	0.381
R1131	r −	−	−	r	r	−	0.060
0-37	r +	+	−	s	s	−	0.245
0-292	r +	−	−	s	s	−	0.225
0-45	r +	+	+	s	s	−	0.210
0-108	r +	−	+	r	s	−	0.299
0-99	r +	−	−	s	r	−	0.297
0-20	r +	+	+	r	s	−	0.015
0-233	r +	+	−	s	s	−	0.000
0-5	r +	+	−	s	r	−	0.017
0-13	r +	−	−	r	r	−	0.018
0-18	r +	+	+	r	r	−	0.012
60A1S^r	r +	−	+	s	s	+	0.321
1-27	r +	−	+	s	s	−	0.026
1-84	r +	+	+	s	s	+	0.238
1-284	r +	+	+	r	s	+	0.166
1-5	r +	+	−	r	r	−	0.019
1-75	r +	+	+	s	s	−	0.035
1-133	r +	−	−	s	r	+	−0.023
1-293	r +	+	+	r	s	−	0.020
1-24	r +	−	−	s	r	−	0.024
1-120	r +	+	+	s	r	−	0.200
1-13	r +	+	−	s	r	−	0.029
1-50	r +	−	−	r	r	−	0.022

* Recombinants from the first cross are labeled "0" and those from the second cross are labeled "1." Strain 60A1S^r is a streptomycin resistant reversion of 60A1.

and with themselves, and observing the segregation of the selective advantage among the recombinants.

The selective advantage of the recombinants

Ten recombinants with different markers were isolated from a cross of 60A1 with R1131 and the selective advantage of each was determined (table 2). In figure 4, the slopes of the selection curves for each, calculated by regression, are used to construct lines radiating from a common point. It is immediately apparent that the recombinants fall into two distinct classes, five without any selective advantage over the tester, and five with an intermediate advantage. There are none with a selective advantage as high as 60A1. A significant difference ($t = 11.16$, $P < 0.005$) can be shown to exist between the lowest curve in group A and the highest curve in group B. Within group B no differences among the curves can be demonstrated, while in group A probably only the curves at the extremes of the group are different. ($t = 2.93$, $P = 0.005$).

When this experiment was repeated and eleven new recombinants were tested (table 2 and fig. 4) the same general pattern prevailed. In this case there were three with an intermediate selective advantage and eight with no selective advantage. Since there is no difference between the two sets of data ($\chi^2 = 0.014$, $P = 0.90$) the results can be pooled.

On the basis of these experiments a tentative hypothesis of the genetics of the selective advantage in 60A1 could be constructed. The segregation of the selective

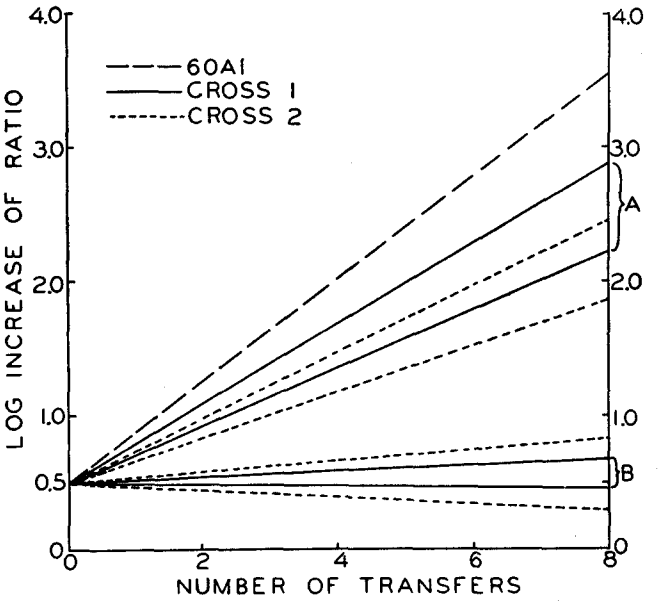

FIGURE 4.—Selection curves plotted from a common point of recombinants from the crosses of 60A1 × R1131. Only the curves at the upper and lower limits of each group are drawn. Data are given in table 2.

TABLE 3

The segregation of the selective advantage with the markers in the crosses of the filter types

Selective advantage	*Xyl*		*Lac*		*Az*		T_6		*Mtl*	
	+	−	+	−	s	r	s	r	+	−
60A1 × R1131										
Intermediate	4	4	6	2	6	2	6	2	2	6
None	8	5	5	8	7	6	5	8	1	12
60B1 × R4123										
Intermediate	3	0	2	1	3	0	2	1	2	1
High	8	9	6	11	11	6	9	8	1	16
The segregation of the markers among 221 recombinants										
Percent	56	44	30	70	80	20	28	72	0.5	99.5

advantage into distinct classes indicates the presence of discrete heritable entities whose distribution is discontinuous and controlled. It is difficult to reconcile the segregation pattern obtained with that expected where the trait is cytoplasmic, polygenic or caused by a single gene. The simplest hypothesis which embodies all the facts assumes the existence of two genes which determine the degree of selective advantage. If these two genes are not linked to each other, the unselected markers or the selective markers S and TL, then three classes of segregants would be expected: a group in which each segregant carries both genes and consequently possesses as high a selective advantage as 60A1, a group with only one of the genes and an intermediate selective advantage, and a group with neither gene and no selective advantage, similar to R1131. The proportion of the three classes would be 1:2:1, respectively. Upon comparison it is obvious that the proportion obtained is different from that expected ($\chi^2 = 17.3$, $P < 0.001$) and therefore that the genes in question are not segregating independently. The segregation pattern of the unselected markers was similar to that obtained by other investigators (LEDERBERG, LEDERBERG, ZINDER and LIVELY 1951). The *Mtl* marker seemed closely linked to the *S* locus, with *Lac* and T_6 nearby, while *Az* was closer to (*TL*). Xylose seemed independent of both *S* and (*TL*). It can be shown from the data in table 3 that the segregation of the selective advantage is not appreciably influenced by the segregation of the unselected markers. Consequently, although the recombinants were chosen with regard to their markers the sampling method was essentially random so far as selective advantage was concerned and has not significantly affected the segregation pattern. The analysis of this pattern suggests that both genes are linked to each other and are in the region of the chromosome near the streptomycin locus, for in the S^s parent 60A1, this is the region against which selection operates during the crossing procedure. The formation of a segregant without any selective advantage requires no crossing over between the postulated genes and the *S* locus. These segregants therefore are more frequent than those with intermediate selective advantage, which require at

TABLE 4

Recombinants from the crosses of 60B1 × R4123

Strain*	*S(TL)*	*Xyl*	*Lac*	*Az*	*Ts*	*Mtl*	Slope
60B1	r −	+	−	r	r	−	0.370
3-64	r +	−	+	s	s	−	0.352
3-26	r +	−	−	s	s	−	0.382
3-75	r +	+	+	s	s	−	0.243
3-1	r +	−	−	s	r	−	0.327
3-143	r +	+	−	s	s	−	0.395
3-263	r +	+	+	r	s	−	0.375
3-6	r +	−	−	r	r	−	0.357
3-4	r +	+	−	s	r	−	0.328
4-11	r +	+	+	s	s	+	0.209
4-13	r +	−	+	s	s	−	0.375
4-359	r +	−	−	s	s	−	0.401
4-53	r +	−	+	r	s	−	0.395
4-52	r +	+	+	s	s	−	0.343
4-35	r +	+	+	r	s	−	0.371
4-241	r +	+	−	s	r	+	0.221
4-2	r +	−	−	s	r	−	0.334
4-3	r +	+	−	s	r	−	0.354
4-17	r +	−	−	r	r	−	0.386
4-14	r +	+	−	r	r	−	0.351

* Recombinants from the first cross are labeled "3" and those from the second cross are labeled "4".

least one crossover, or recombinants with a high selective advantage which possibly involve two chromosome breaks. Indeed, on this assumption the last class should be, and is, the most infrequent of the three, for none of this type was recovered in either cross.

Before any further work was done on 60A1, the second fitter type 60B1 was crossed with R4123 and eight recombinants were analyzed (table 4). Upon comparing their slopes (fig. 5) it was seen that only two classes of segregants had appeared, one group (C) consisting of seven isolates with high selective advantage about equal to that of 60B1, and the second group (A) containing only one isolate with an intermediate selective advantage. Recombinants with no selective advantage were not obtained (fig 5). This cross was repeated and yielded nine recombinants with high selective advantage, two intermediates and again none without any selective advantage (table 4 and fig. 5). The results of the two experiments were not significantly different ($\chi^2 = 0.0001$, $P = 0.99$) and could be combined. It is interesting to observe that these results confirm the general conclusions reached concerning the inheritance of selective advantage in 60A1. Furthermore, the simplest hypothesis which can be formulated to explain the proportion of the segregants obtained is the same as that proposed for 60A1, that is, two genes linked to the streptomycin locus. If the genes are allelic the cross 60B1 with R4123 should be the reciprocal of the cross 60A1 with R1131. This assumption is vindicated when the data from the two crosses are compared (table 5) for no significant difference can be demonstrated ($\chi^2 = 2.51$, $P = 0.21$).

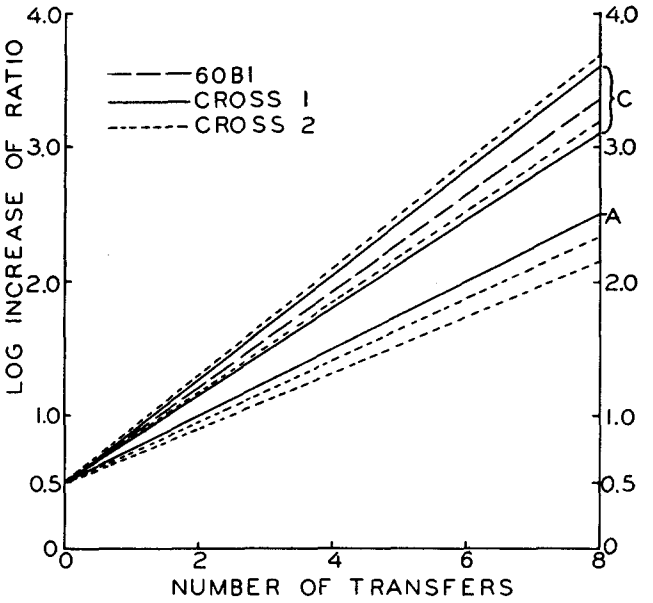

FIGURE 5.—Selection curves plotted from a common point of recombinants from the crosses of 60B1 × R4123. Only the curves at the upper and lower limits of the higher group are drawn. Data are given in table 4.

TABLE 5

A comparison of the segregation of the selective advantage in the two fitter types

Cross	Degree of selective advantage		
	None	Intermediate	High
60A1 × R1131	13	8	0
	High	Intermediate	None
60B1 × R4123	16	3	0

Data in first two columns used for test of independence between segregation ratio of the crosses 60A1 × R1131 and 60B1 × R4123. $\chi^2 = 1.49$, $P = 0.22$.

An additional test of the hypothesis of allelism was possible, for if the two fitter types are crossed with each other all the segregants should have a high selective advantage. Ten segregants from such a cross were analyzed and all possessed a selective advantage about the same as that of 60A1 and 60B1 (table 6). This experiment was repeated and the analysis of ten additional segregants gave similar results (table 6).

The nature of the selective advantage

A study of the nature of the selective advantage in 60B1 was made by comparing the individual growth characteristics of this fitter type with those of the original type R1131. In figure 6 the combined results of two separate determinations of the

TABLE 6

Recombinants from the cross of 60A1 × 60B1

Strain	*S(TL)*	*Xyl*	*Lac*	*Az*	*Tc*	*Mtl*	Slope
60A1	s +	−	+	s	s	+	0.381
60B1	r −	+	−	r	r	−	0.370
5-2	r +	+	−	s	r	−	0.306
5-25	r +	+	−	r	s	−	0.346
5-1	r +	+	+	s	s	−	0.331
5-53	r +	+	+	s	r	−	0.311
5-10	r +	−	+	s	s	−	0.327
5-6	r +	−	+	r	s	−	0.303
5-22	r +	−	−	r	r	−	0.333
5-5	r +	−	−	s	r	−	0.347
5-99	r +	−	+	s	r	−	0.361
5-126	r +	+	−	s	r	+	0.369
6-1	r +	−	+				0.300
6-2	r +	+	−				0.341
6-3	r +	+	+		Markers		0.361
6-4	r +	−	−				0.363
6-5	r +	−	+		not		0.383
6-6	r +	−	+				0.303
6-7	r +	+	−		analyzed		0.320
6-8	r +	−	+				0.400
6-9	r +	−	−				0.350
6-10	r +	+	−				0.333

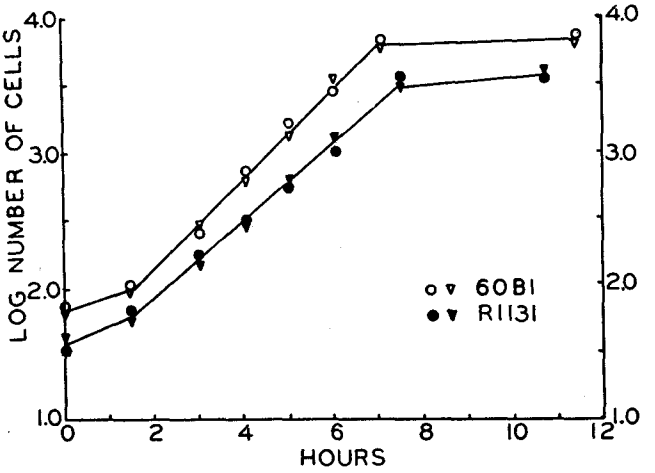

FIGURE 6.—Growth curves of R1131 and 60B1. The combined results of two separate experiments are plotted for each strain.

growth curve are plotted for 60B1 and R1131. In both experiments the generation time of 60B1 was lower (54 and 55 minutes) than that of R1131 (64 minutes) and the final level of growth was higher (5.9×10^8 and 5.6×10^8 per ml) for 60B1 than for R1131 (3.0×10^8 and 4.0×10^8 per ml). The differences in generation time and

plateau are significant and it seems very probable that one or both factors are responsible for the selective advantage of 60B1. The assumption that the critical factor is the difference in generation time can be tested by constructing a theoretical selection curve. This is done by plotting the growth curves of both strains simultaneously, with the initial number of R1131 four times that of 60B1. This of course is the theoretical situation under which the selection curves for all the strains were obtained. When the total number of cells has increased one hundred times this point represents the end of the first ST, and each succeeding one hundred-fold increase marks the end of the following transfer. These points can be estimated from the graph and at each the ratio of 60B1/R1131 is obtained. The log of this ratio plotted as a function of the numbers of transfers produces a straight line with a slope 0.330 which compares favorably with the experimental slopes of 60B1 (0.375 and 0.345). On the other hand, if the plateau difference is used to construct a theoretical selection curve the value obtained for the slope is rather high (0.45 using the lower generation time), and when both plateau and generation time differences are superimposed the value becomes higher still (0.55). It seems probable therefore that in the mixtures the plateau difference does not have the opportunity to manifest itself.

DISCUSSION

A number of interesting comparisons can be made between the results obtained by ATWOOD, SCHNEIDER and RYAN (1951b) and those presented in this paper. They demonstrated that when the selective advantage is used as the comparative measurement, fitter types obtained independently are frequently different from each other, and it is reasonable to suppose that these differences reflect underlying genetic dissimilarity. In this instance the genetic basis of the selective advantage obtained independently in the two strains is apparently the same. It will be necessary to analyze the genetics of many more fitter types before any general conclusions can be drawn, but it does not seem unlikely that parallel fitter types will frequently carry allelic genes. This may be an effect of differences in mutation rates among the genes concerned with selective advantage, for if such differences exist, those mutants which arise more frequently will have a much better chance of establishing themselves during a serial transfer.

Regarding the nature of the selective advantage, the findings of this investigation are in accord with those of ATWOOD and his coworkers (1951b), who demonstrated growth rate increases in several of their fitter types.

A rough estimation can be made of the time needed for the increase of the fitter type mutants after their appearance in the ST, by using the slope of the selection curve for a type with intermediate selective advantage, 0–37 for instance. If such a curve is plotted it can be estimated that a ten-fold increase in the ratio occurs during every four ST. This means that about 32 transfers may have been necessary to increase the mutant ratio from 10^{-8} to 1. Unfortunately it is not possible to estimate by this method the number of transfers necessary in the second cycle for there is no data available yet on the selection curves of mixtures of high and intermediate fitter types. However, it is possible to estimate the growth rate of an intermediate type, and from this a theoretical selection curve of intermediate *vs.* high type can be con-

structed. The slope of this curve indicates a minimum of 44 ST to bring about an increase of 10^{-8} to 1. These values for the number of transfers seem to be rather high when compared with the findings of ATWOOD, SCHNEIDER and RYAN (1951a) who demonstrated that about 30 ST were necessary for their fitter types to dominate the culture. Furthermore, it is particularly interesting to note in the present instance that while 76 transfers is the minimum estimate of the number required to establish the final fitter type, 60B1, only 60 ST were actually carried out. This difference of course may be due to inaccuracies introduced by the approximations, or to a higher mutation frequency than that assumed. A more tempting possibility is that the final fitter type was brought into existence by genetic recombination occurring during the serial transfers. Thus, it is not necessary to assume that cells with both selective advantage genes would begin to appear only after cells with one of the genes had increased through 32 ST. Undoubtedly both single mutations occur quite early in the course of the ST, and if the means exist of bringing them together in a single cell the final fitter type will appear much sooner than expected. In essence, what this means is that recombination will increase the genetic variability upon which the selection process is operating, a fact which is axiomatic in population genetics.

Preliminary experiments indicate that recombination does occur during the course of the serial transfer, and the effect of this factor on the expression of orthoselection is under consideration.

SUMMARY

The results of these experiments indicate that in two strains of *E. coli* allowed to evolve independently, orthoselection has established a pair of genes which confer a high selective value on the cells in the populations. Furthermore, it seems that the genes in the two strains are allelic and linked to the streptomycin locus. The adaptive changes can probably be attributed to an increased growth rate.

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