COMPETITION AMONG GENOTYPES IN TRIBOLIUM CASTANEUM AT VARYING DENSITIES AND GENE FREQUENCIES (THE BLACK LOCUS)[1, 2]

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THE purpose of the experiment reported below was to investigate differences in three biological characters affecting fitness in three genotypes of the red flour beetle, *Tribolium castaneum* Herbst. These biotic properties were investigated at four different densities and in eight different zygotic combinations representing seven different gene frequencies. We hope that these data will contribute to the emerging field of ecological genetics; the findings of this study may also shed some light on the results of a number of experiments in our laboratory (to be published elsewhere) which investigate the ecology of natural selection for or against the black allele.

This study is patterned after an earlier one (SOKAL and HUBER 1963) in which a similar series of experiments was carried out with the sooty mutant in *T. castaneum*. We have since concentrated on the ecological properties of black because we have found that $+/b$ heterozygotes are more easily identified than $+/s$ ones. For convenience we shall refer to the three genotypes employed in the study by their marker locus, black. Thus we shall call them $+/+$, $+/b$ and b/b. In fact, however, it is quite likely that the black and wild-type stocks differ also in their genetic backgrounds and that the $+/b$ beetles are heterozygous for numerous loci. Work in progress at the moment may reveal to what extent the ecological properties observed are those of the black locus (or of genes in its immediate vicinity). This question will be of consequence in elucidating the selection results referred to above. However, in the present study we are mainly concerned with observing the ecological consequences of any genetic differences, and black performs a useful function as an easily visible marker. Extensive genetic manipulation would have been necessary to obtain genotypes differing only with respect to the black locus, and in view of the purposes of our work this did not seem warranted. Since the $+/+$ and the b/b stocks were not isogenic there will also be differences between individuals with identical genotypes at the black locus. However, the large

[1] Contribution No. 1204 from the Department of Entomology, The University of Kansas, Lawrence. This is paper No. 3 in a series on the ecological genetics of Tribolium. Papers 1 and 2 are by SCHLAGER (1963) and SOKAL and HUBER (1963), respectively.

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numbers of eggs employed should produce representative samples of the stocks investigated.

The design of the present study is extended in two important ways beyond that of the earlier study with sooty (SOKAL and HUBER 1963). Records were kept of times of emergence of adults enabling us to compute total developmental periods for the beetles in this experiment. Secondly, the three genotypes were studied under conditions of marked rarity or frequency to see whether such circumstances would produce unusual responses.

This study can also be examined from a comparative point of view. We shall note appreciable differences between the present results and those on sooty (SOKAL and HUBER 1963). A parallel series of experiments has been carried out with house flies (SULLIVAN and SOKAL 1963; SOKAL and SULLIVAN 1963; BHALLA and SOKAL 1964; SULLIVAN and SOKAL 1964). Some generalizations about competition phenomena in these two organisms are made in the discussion below. The general implications of work of this sort have already been discussed (SOKAL and HUBER 1963) and need not be repeated here.

METHODS AND DESIGN OF THE EXPERIMENT

The three genotypes employed singly and in competion were the homozygote black, b/b, the heterozygote $+/b$ and the wild type $+/+$ from the "Foundation" stock. The wild-type strain was obtained from PROFESSOR A. E. BELL's laboratory at Purdue University, and the mutant strain was obtained from DR. A. SOKOLOFF at the University of California. The gene black is an autosomal recessive described by SOKOLOFF, SLATIS, and STANLEY (1960) and located on chromosome III by SOKOLOFF (1962). The heterozygote in the adult is a dark red which has been referred to as "bronze," while the homozygous mutant is purplish black and the wild type is red. The genotypes can also be identified in the immature stages, but this is of no consequence in the present study.

The experiment was carried out in 6-dram shell-vials (diameter 20 mm, height 85 mm) containing 8 g of standard wholewheat flour medium enriched with brewer's yeast in a ratio of 100:5. Both flour and yeast had been finely sifted through No. 7 bolting cloth. The yeast was added before the mixture was sterilized in an oven at 60°C overnight. The prepared medium was stored in the rearing chamber for at least 48 hr before the experiment. The cultures were reared in a walk-in temperature and humidity room controlled at 85.5 ± 1.5°F and at 71 ± 3.5 percent relative humidity (the ± deviations refer to absolute limits). The rearing room was constantly illuminated. The positions of the experimental vials were changed every other day in order to equalize possible microclimatic differences within the rearing room.

Eggs for this experiment were obtained from egg farms in half-pint mason jars containing dense populations of adults in fresh, finely sifted flour. Eggs up to 4 hours old were employed in setting up the vials. Reciprocal crosses were used to obtain heterozygote eggs.

The eggs were placed into rearing vials in pure and mixed culture. Three kinds of pure cultures were set up: wild type, heterozygotes, and black. These are equivalent to gene frequencies $0.0q_b$, $0.5q_b$ and $1.0q_b$, respectively. The mixed cultures were set up to represent Hardy-Weinberg proportions for gene frequency $0.25q_b$, $0.5q_b$ and $0.75q_b$, respectively. Each of these six types of cultures was run at four densities, 5 eggs per gram, 20/g, 50/g and 100/g, corresponding to 40, 160, 400 and 800 eggs per vial, respectively. Ten replicate vials were run at density 5/g, four vials at 20/g, and three vials at 50/g and 100/g. The entire experiment was repeated four times. Series 1 and 2 were run within a week of each other in July 1962, while Series 3 and 4 were also run within a week of each other in December 1962. A few replicates were run at density 200/g. These had extremely high mortalities. Two series of mixed cultures at "extreme" gene frequen-

cies, $0.1q_b$ and $0.9q_b$, in Hardy-Weinberg proportions were run for three densities, 20/g, 50/g and 100/g together with experimental Series 3 and 4.

Once the adults began emerging they were removed from each vial five times at three-day intervals. Adults were removed once more nine days after the last three-day period. Beetles were kept in holding vials containing coarse flour in order to permit them to mature, because the phenotype of the heterozygote cannot be successfully distinguished before the imagines are ten days old. Densities in the holding vials were always below 20 adults per gram and did not seem to have a deleterious effect on the beetles. Adults from identically treated vials were put into separately numbered holding vials for each emergence period permitting the computation of developmental period for these individuals. When they were betwen 10 and 14 days of age, the beetles in a holding vial were removed and etherized, sorted and counted by genotype and placed in an oven at 100°C in order to dry overnight. They were then weighed on an analytical balance in groups representing one genotype from one holding vial. Dead adults in the experimental vials were not transferred to holding vials. Dead adults in holding vials, very few of which were found, were not weighed and counted.

RESULTS

Survivorship: The overall survivorship, not broken down by genotype, for the different gene frequencies of black is illustrated in Table 1. Different gene fre-

TABLE 1

Overall adult survivorship at different densities and gene frequencies

Density	Gene frequency 0.00	0.25	0.50	All +/b 0.50	0.75	1.00
5/g	73.69	79.25	82.31	83.72	84.50	78.01
20/g	75.58	82.70	84.08	89.73	82.72	74.96
50/g	74.44	81.98	79.62	85.17	81.44	77.75
100/g	73.50	77.07	77.68	79.27	77.58	73.99
$\chi^2_{[3]}$	5.24	66.54***	58.18***	188.14***	70.34***	30.62***

Total $\chi^2_{[18]} = 419.05$*** Pooled $\chi^2_{[3]} = 275.35$*** Interaction $\chi^2_{[15]} = 143.70$***

***$= P < 0.001$.
Survivorships are expressed as percentages of the egg input.

quencies vary in their response pattern to increases in the density (interaction χ^2 significant at $P < 0.001$). There appears to be an optimum survivorship at a density of 20/g for all the frequencies except the two highest ($0.75q_b$ and $1.0q_b$). Survivorship of the wild type does not change over the range of densities considered here.

Survivorships of the three genotypes are stated as percentages of the egg input of a particular genotype. This percentage, when expressed as a proportion, may be regarded as the probability of survival of a given genotype under the stated environmental conditions of density and gene frequency. Survivorships, as percentages of input (averaged over the four experimental series) are shown in Figure 1, for the pure cultures, and for mixed cultures at the three gene frequencies and the four densities.

At the left side of the figure are the survivorships of the pure cultures. At density 5/g, survivorships are clearly in the relation $+/b > b/b > +/+$. At the

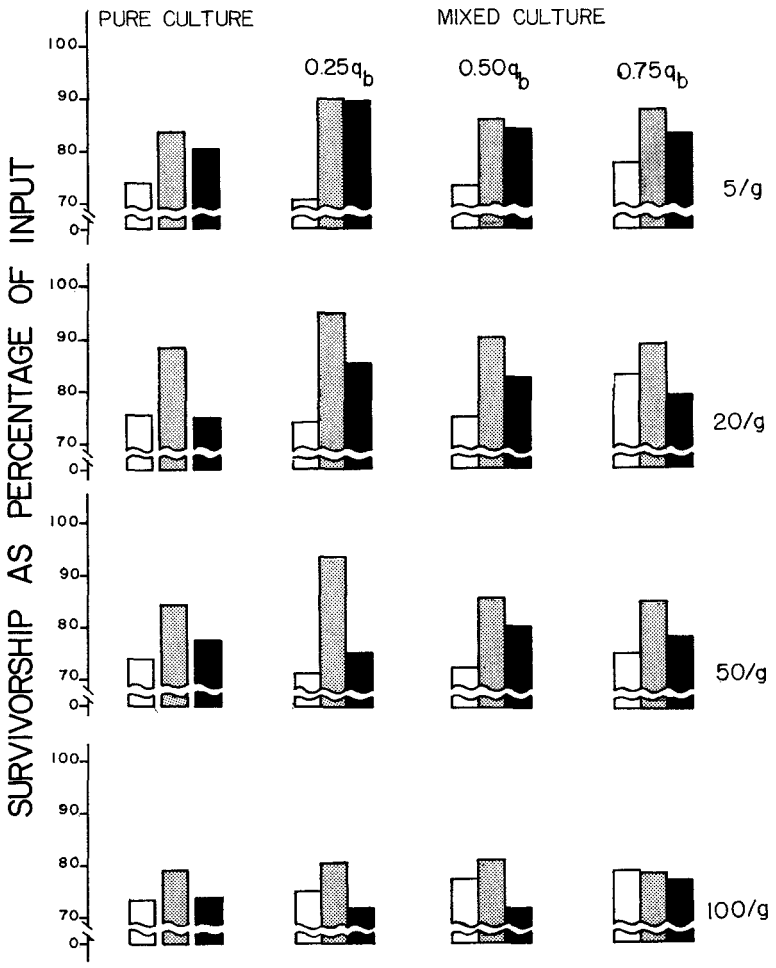

FIGURE 1.—Adult survivorships expressed as percentages of egg input averaged over the replicates and series of the study and shown for the four densities and three gene frequencies employed. Leftmost column with noncontiguous bars represents the results of rearing the beetles in pure culture. White bars represent the +/+ genotype, gray bars +/*b* and black *b*/*b*. Even when the bars are contiguous the percentages expressed for each genotype are formally and logically independent of the percentages of the other genotypes.

higher densities the heterozygotes continue to survive in greater numbers than the two homozygotes, which have about equal survivorship. In mixed cultures, too, the heterozygotes are always better than the homozygotes (except at gene frequency $0.75q_b$ and density 100/g, where the +/*b* emergence is slightly below that of +/+). The heterozygotes in mixed culture are never lower in survivorship than they are in pure culture and at gene frequency $0.25q_b$ we note marked facilitation of heterozygote emergence at densities 5/g, 20/g and 50/g. The black genotype shows similar facilitation at all three gene frequencies for densities 5/g and 20/g, at the two higher gene frequencies for 50/g, and at $0.75q_b$ for 100/g.

Similar moderate facilitation of emergence is shown by the wild type at gene frequency $0.75q_b$ for all densities and for $0.5q_b$ at 100/g.

The responses to density differ among the strains in pure culture. The emergence of wild-type beetles is apparently not responsive to increases in density within the range studied here; the survivorship at 100/g is no less than at 5/g. On the other hand, the heterozygotes decrease in survivorship at 100/g having maintained their emergence at the lower three densities. The emergence of the homozygous mutants decreases immediately as density 5/g is raised to 20/g. This is borne out by the highly significant Density × Genotype interaction in the analysis of variance reported below.

When we examine overall patterns of the three genotypes in mixed culture, we note that these survivorships, and hence adaptive values under these conditions, are both density as well as gene frequency dependent. At density 5/g we find b/b almost as well adapted as the heterozygote, but its survivorship decreases markedly with density 20/g. For gene frequencies $0.25q_b$ and $0.5q_b$, this trend is continued at densities 50/g and 100/g, but at $0.75q_b$ the emergence of $+/+$ is already better than that of b/b at density 20/g. There is an apparent reversal of this trend for $0.75q_b$ at density 50/g. However, this is caused by a single unusually low reading for the wild genotype in Series 2 of the experiment, while in the other three series wild type has a higher emergence than b/b. At density 100/g all three gene frequencies favor wild type over black.

The data graphed in Figure 1 were subjected to a factorial analysis of variance with the square roots of the percentages transformed to arc sines. In spite of the differences in numbers of beetles at different densities, variances within subclasses were approximately the same after the transformation. The results of the analysis are shown in Table 2, column a. Among the main effects we find density, genotypes and the experimental series highly significant. The differences in emergence due to densities and genotypes have already been discussed. The series effect seems to be due to a general lowering of emergence in Series 3. Some shift in the response pattern to density is also evident, causing the Density × Series interaction. However, when examined in detail, these changes are not too drastic. Significance is brought about by the large number of degrees of freedom available for testing. The overall density trend appears to be high emergences at 5/g, 20/g and 50/g with a peak apparently at 20/g and the low point of the study at 100/g. The highly significant Density × Genotype interaction is explained by the fact that at density 100/g the wild type becomes better adapted than black, while at the other three genotypes, black is superior to wild type. The overall effect of gene frequency is significant at $0.01 < P < 0.05$, apparently due to the facilitation in emergence for the $+/b$ and b/b at $0.25q_b$. The Frequency × Genotype interaction is significant and represents the differential response of the three genotypes to changes in gene frequency discussed above.

To summarize the results on survivorship, there is a positive facilitation effect for emergence of the three genotypes in mixed culture. The relative adaptive value of the wild type increases as the gene frequency of black in the culture increases and the relative adaptive value of b/b decreases with density. At the

TABLE 2

Analyses of variance

Source of variation	Degrees of freedom	(a) Survivorship MS	(b) Dry weight MS	(c) Developmental period MS
Main effects				
Densities	3	243.67***	9.76***	32.55***
Genotypes	2	1334.73***	6.41***	35.65***
Frequencies (of *b*)	3	69.82*	.11***	.34
Series	3	226.44***	1.27***	148.10***
First order interactions				
D × G	6	116.44***	.06***	.69***
D × F	9	25.97	.05**	.45**
D × S	9	47.18*	.25***	1.30***
G × F	6	71.01**	.03	.19
G × S	6	56.09*	.12***	.86***
F × S	9	28.45	.26***	.16
Second order interactions				
D × G × F	18	9.04	.13***	.40**
D × F × S	27	17.44	.05***	.29**
D × G × S	18	25.35	.08***	.13
G × F × S	18	19.69	.02	.11
Third order interaction				
D × G × F × S	54	19.33	.014	.122

*$=0.01 < P < 0.05$ **$=0.001 < P < 0.01$ ***$=P < 0.001$.

two lower densities the emergence of black also decreases as its gene frequency increases.

Survivorship percentages partly depend on the hatchability of the egg input. This quantity was investigated and showed appreciable fluctuations between experimental series but no constant differences among the genotypes. Hatchabilities ranged from 80 to 98 percent in different series.

Dry weight of adults: Figure 2 shows the effect of density on the dry weights of adults of the three genotypes reared under the conditions of the experiment. The relations between density and adult weight are clear and comparable to the findings of SOKAL and HUBER (1963). Mean weights for the wild-type strain were comparable to those obtained by those authors, except that at the higher densities the means did not decrease quite as sharply as in the earlier experiment. Beetles of all three genotypes show their highest weight at density 20/g. Density 5/g produces generally a higher weight than density 50/g, while a drastic reduction in weight occurs at 100/g. There are marked differences in weight among the three genotypes. Heaviest are the heterozygotes; the wild-type beetles are intermediate in weight, while the homozygous black adults have the lowest weight. This relation is contrary to the findings for sooty, where relations could be expressed as $s/s > +/+ > +/s$ (SCHLAGER 1963; SOKAL and HUBER 1963).

In their studies with sooty SOKAL and HUBER (1963) found a marked increase in the weights of wild-type and heterozygous beetles, with an increase in the gene frequency of sooty. No such gene-frequency-dependent response could be found

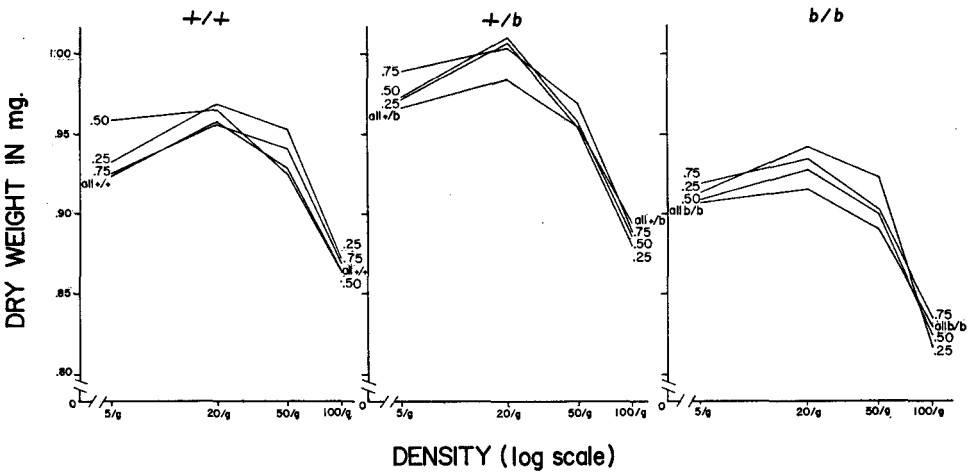

FIGURE 2.—Mean dry weights of adults in milligrams averaged over the replicates and series of the study. The three genotypes are shown in separate panels, each panel representing a graph of dry weight on density of egg input. Separate lines for the three gene frequencies and for pure cultures are shown.

in this study; black does not produce genetic facilitation for weight under these conditions. The only marked changes in weight in response to gene frequency changes are for wild type at densities 5/g and 50/g. Response patterns at these two densities differ markedly and it is hard to interpret these findings.

Although facilitation was not shown in response to gene frequency changes, the facilitation by other genotypes, which was noted for survivorship, is also shown for weight. At least at the lower three densities, the heterozygotes and homozygous mutants are considerably lower in weight when reared in pure culture than when reared in mixed culture at any gene frequency. Their better performance in terms of emergence is also reflected in terms of weight.

When these data were subjected to an analysis of variance, the results shown in Table 2, column b were obtained. Many of the first and second order interactions were significant, making interpretation of the data difficult. However, the overall trends shown in the main effects for density and genotype were of such a magnitude as to be clearly discernible in spite of the interactions. These overall trends can be clearly seen in Figure 2 despite the crossing and nonparallelism of the various lines which graphically mirror interaction. The series main effect is also significant. When this is separated into the pertinent degrees of freedom, it can be shown that all series are significantly different from each other. There is some tendency for beetles in the last two series (3 and 4) to weigh less than in the earlier two. In spite of some interaction involving series, the overall trends are still reasonably parallel among the series.

Length of developmental period: Mean lengths of developmental period were subjected to an analysis of variance summarized in Table 2, column c. In the main effects density, genotype and series are highly significant. The effects of

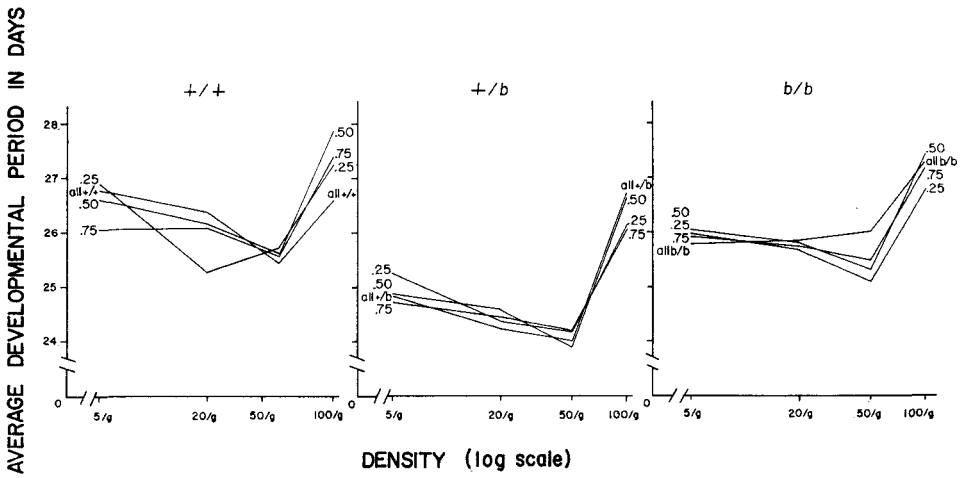

FIGURE 3.—Average developmental period in days as a function of density. Arrangement as in Figure 2.

density appear to be nonlinear (Figure 3). The fastest developmental period is at density 50/g. The developmental period decreases gradually from density 5/g through 20/g to 50/g and then increases steeply between 50/g and 100/g. At this highest density, developmental period is always longest. The significant Density × Genotype interaction reflects differences in the response slope to density among the three genotypes. However, the general reactions are still pretty much the same.

There are marked differences in developmental period among the genotypes. The fastest emerging beetles seem to be generally the heterozygotes, followed by the homozygous mutants which are followed in turn by the wild type. However, when the genotypes are in pure culture (and slightly so at gene frequency $0.25q_b$ in mixed culture) the relative positions of these are shifted at increasing densities. At densities 5/g and 20/g the order of the genotypes is as indicated above ($+/+ > b/b > +/b$), while at density 50/g relations for developmental period are $b/b > +/+ >> +/b$ and at density 100/g the order is $b/b > +/b > +/+$. At gene frequencies $0.5q_b$ and $0.75q_b$ the relative patterns are stable although the means are affected by density. This stability of patterns at higher gene frequencies and the instability of pattern at $0.25q_b$ and in pure culture is shown by the Density × Frequency × Genotype interaction in Table 2, column c.

Significant differences among the series were caused by Series 3 and 4 which took approximately two days longer to go through development than did the first two series. We are not certain how to explain this phenomenon. Series 3 and 4 were run in winter and a minor temperature difference may have existed in the chamber between that time and that of Series 1 and 2, although not clearly indicated in our thermograph charts. Another reason may be that in summer the refrigeration unit of the temperature chamber is operative and the regular oscillations of temperature and humidity brought about by the cooling unit may have

had an accelerating effect as opposed to the straight line temperatures and humidities obtained in winter. Significant interactions were observed between Series × Density and Series × Genotype. These reflected only minor changes in pattern and the overall responses to density and differences in genotype were essentially maintained.

Differences in gene frequency did not have an overall effect on developmental time. However, it would appear that at different densities, varying the gene frequency did affect the developmental period. At density 5/g we find that with increasing gene frequency there is a reduction in the developmental period of wild type and of the heterozygotes, while the black mutants do not change in an appreciable manner. At the higher densities there do not appear to be equivalent trends. An apparent decrease of developmental period in the wild type at density 20/g is largely due to an unusually fast reading in one of the series, which would appear to be atypical. These trends are reflected by the significant Density × Frequency × Series interactions in Table 2, column c. No clear changes are noted on comparing developmental periods in mixed cultures with those of pure cultures. The only exception appears to be a case of negative genetic facilitation for the wild type. The wild-type beetles take considerably longer when in competition with other genotypes than they do in pure culture at density 100/g.

Responses to extreme gene frequencies: Mixed cultures at gene frequencies of $0.1q_b$ and $0.9q_b$ were run together with Series 3 and 4. These resulted in zygotic frequencies of 1 percent and 81 percent for the homozygotes when rare or frequent, respectively. Results from the extreme gene frequency experiments are therefore compared with those from the last two series only, in order to avoid confounding interseries error with gene frequency effects. Figure 4 shows the survivorships for the three genotypes in pure and mixed culture at the three densities at which vials with extreme gene frequencies were run. The new gene frequencies seem to reflect the previously noted trends.

Facilitation for heterozygotes and black homozygotes in mixed culture is still evident, although for the black the phenomenon is no longer appreciable, since density 5/g in which its facilitation was most marked was not used. Perhaps the most interesting feature is the increased emergence of the wild-type homozygotes when they are rare, especially at densities 20/g and 100/g. On the other hand, black when very rare seems to do rather poorer than when it is more frequent. This trend is most noticeable at density 100/g.

The weights present more marked responses to extreme gene frequencies. In cultures at density 20/g, where the wild type was frequent, it weighed less than when not so frequent, but yielded typical weights at higher densities. On the other hand, when wild-type beetles were rare in culture, their weights at 20/g were much higher but their weights at 50/g were somewhat lower than at intermediate frequencies, while at 100/g they were much lower. Comparing the weights of black beetles when rare with their weights when they are more frequent, we find at density 20/g that they have lower weights when rare. Similar comparisons at density 50/g yield no appreciable differences in weight, but at density 100/g weights of the black beetles, when rare, were much lower. A curious relation

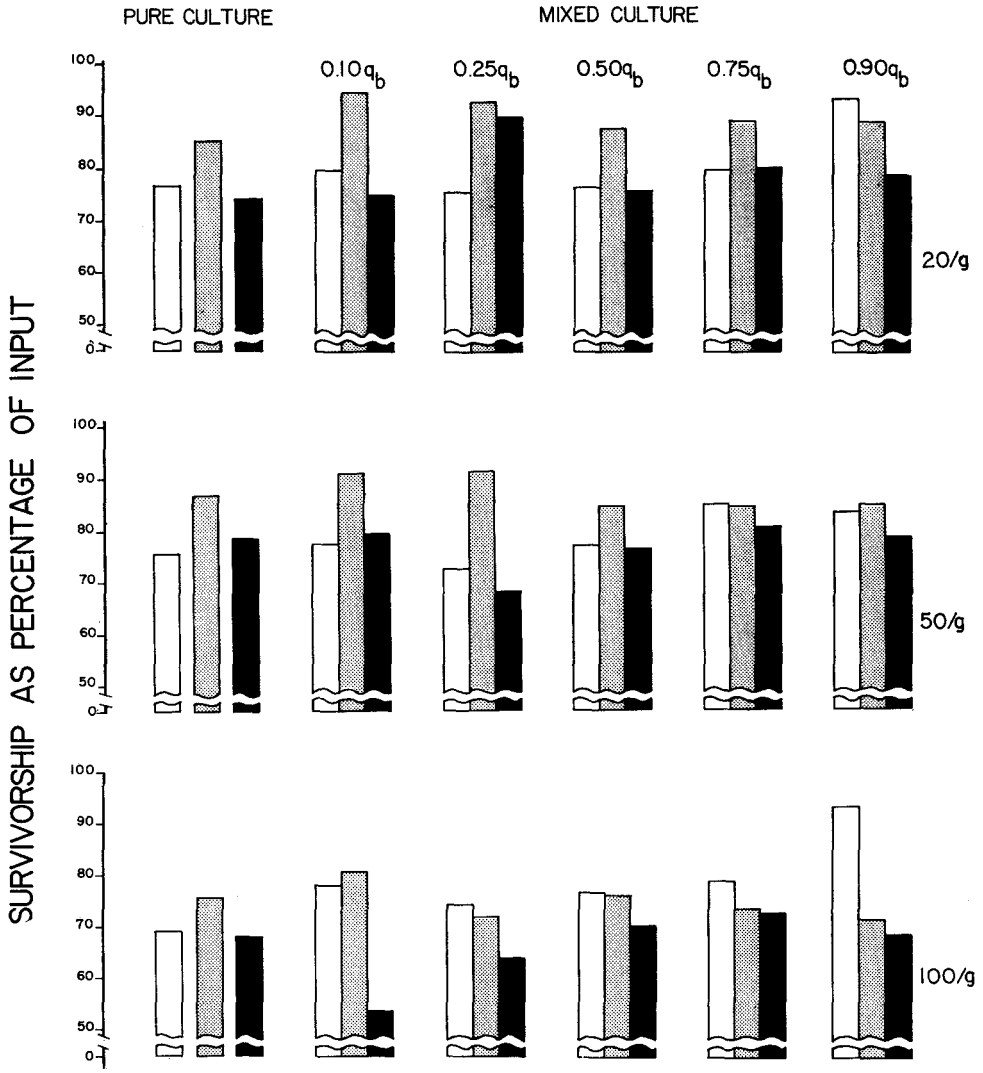

FIGURE 4.—Adult survivorships expressed as percentages of input. This graph is similar in arrangement to Figure 1, but includes "extreme" gene frequencies $0.1q_b$ and $0.9q_b$ and excludes density 5/g. The heights of the bars will not correspond exactly to those in Figure 1, because the present graph is based on Series 3 and 4 only, to make them comparable to the data on the extreme gene frequencies which were run concurrently with these two series.

pertains when the black beetles were frequent in culture. No special differences were found at densities 20/g and 100/g, but weights were considerably higher at density 50/g than when they were less frequent. The heterozygotes gave more or less the same results when at other gene frequencies although this was their lowest zygotic frequency in all the experiments. From these data there emerges a clear trend for weights: wild type does well when it is rare at low density,

poorly when rare at high density and performs normally when it is frequent. The black beetles do poorly when they are rare and dense and do well when they are frequent at all densities (extremely well at 50/g). The data are not consistent enough to permit this conclusion with certainty although most differences cited are statistically significant. However, it is quite clear from these findings that the responses of weight under conditions of extreme frequency or rarity of the genotypes are more variable than they are under intermediate conditions.

No notable trends are found in developmental period at the extreme gene frequencies. This is not surprising since most differences in developmental period occurred at density 5/g, which could not be investigated for the extreme frequencies.

Responses to an extreme density: Two series of pure and mixed cultures at density 200/g were run separately from the other series. Analysis and interpretation of the data is difficult since in most cases very few beetles emerged. The survivorships for the three genotypes were generally quite low. The values ranged from complete mortality in a vial to a survivorship of 33 percent. There were no discernible trends in the survivorship. The most interesting feature is relatively high survivorship for all three genotypes under the two extreme gene frequencies.

The weights also exhibit marked responses to the high density. The average weights are much lower than at any other density studied (range 0.56 to 0.87 mg). Again, owing to the low numbers, clear-cut trends cannot be established.

The developmental period was drawn out greatly among all the genotypes (range 34 to 71 days). The fastest emerging beetles seem to be the heterozygotes, followed by the wild type, which are followed by the homozygous mutant.

DISCUSSION

Density effects. Differences among the three genotypes are evident in their response to density. While the wild type is not at all affected between 5/g and 100/g, the heterozygote and mutant homozygote give some indication that at density 100/g they are beginning their downward trend in percent emergence. SOKAL and HUBER (1963) working with the same wild-type strain at the same densities, also found that survivorship did not decrease at density 100/g. In a selection experiment involving the same strains SOKAL (unpublished) found an average survivorship of 67 percent for the wild type at an average density of 45/g, a value comparable to those obtained here. On the other hand, in that same experiment black homozygotes reared at average densities of 75/g yielded very fluctuating emergences averaging 40 percent. While the densities are similar in the two experiments, the selection experiment is carried out in 40 g of flour contrasted with the 8 g of the present study. Thus the patterns of distribution of the beetles may differ in the two types of cultures. Furthermore, in the selection study emerged adults were collected only once after sufficient time had elapsed for all pupae to eclose, while in the present study adults were removed from rearing vials every third day. Differential cannibalism in the selection experiment may cause this lowering of the emergence.

The apparent lowering of emergence at density 100/g for the heterozygous and homozygous mutants is paralleled by the findings of SOKAL and HUBER (1963) for sooty. There the homozygous mutant *s/s* decreases appreciably at density 100/g, while the heterozygote, which in pure culture has almost 86 percent emergence at density 5/g, has only 59 percent emergence at density 100/g.

At density 200/g emergence of the wild type was only 1.5 percent. Thus, between 100/g and 200/g there lies a critical density for lowering the emergence. Increasing the density to 200/g is not merely an exacerbation of the conditions observed at the lower densities, although this is undoubtedly true as well. Under these very crowded conditions, the medium becomes extremely moist, and in many cases develops molds and bacterial growths and gives off putrid odors. Some of these cultures remain moist through the period of emergence of the adults, while others dry up subsequently and develop into a rather hard, caked up material.

Reactions of weight to density follows the same pattern in all three genotypes and also corresponds to a pattern previously found by SOKAL and HUBER (1963).

In studying the responses to crowding by immature insects, SULLIVAN and SOKAL (1963) distinguished two possible types of responses. In the first type a nearly instantaneous response to an increase in density through a reduction in the number of immatures able to complete their life cycle would result in the fewer emerging adults maintaining their body size. Such a mechanism would presuppose a fairly rapid system of communication among the developing larvae to make the population "aware" of the higher density before a shortage of food develops. Reduction in number must involve the elimination of some immature forms through such processes as fratricide and cannibalism. The second type of response would involve the temporary maintenance of numbers accompanied by reduction in weight. Such a mechanism would require reduction in food intake by the developing immatures. In their work with the housefly, SULLIVAN and SOKAL (1963) found the Type 2 response to prevail, i.e., increases in density produce a decrease in weight considerably before the percentage of emergence is lowered. This situation holds in all published work with cyclorraphous diptera. The present data seem in general to give a response of this type also. While emergence is reduced for at least two of the genotypes by density 100/g, weights of all three genotypes clearly decline from their maximal value by density 50/g. But at the density at which the emergences are beginning to drop, adult weights are not yet at their minimal level. Thus, Tribolium may possess an intermediate type of density response, the survivorship decreasing before a minimal weight level is reached. From our knowledge of the biology of Tribolium some measure of response of the first type is likely, cannibalism being a powerful controlling force in this species. However, under the ecological system devised by these experiments cannibalism is kept to a minimum. It is also interesting that in flies the ratio of maximal to minimal weight is very great, approaching 6:1 in several species (SULLIVAN and SOKAL 1963), while it is less than 2:1 in Tribolium.

Genotypic differences. There are clear differences in emergence among the three genotypes studied in this experiment. These differences vary with the en-

vironmental conditions, yet on the whole remain reasonably stable, the heterozygote apparently being the best adapted genotype as regards emergence. This apparent heterosis of the black heterozygote is contrasted with the inferiority of the sooty heterozygote noted by SOKAL and HUBER (1963). These differences plus the variety of responses to environmental change observed in these studies should underline the complexity of the adaptive process in even as simple an ecogenetic system as the one investigated here. The genotypes differ not only in their responses at a given density, but also in their response curves over the range of densities studied by us. We find that some genotypes such as the sooty heterozygote will lower their emergence in response to an increase in density, while others such as the homozygous wild type are not affected over the present range. Such differences in response patterns were first pointed out by LEWONTIN (1955) in experiments with Drosophila. Although in almost all of these cases the entire response curve is convex in shape, responses may seem to differ in that a limited range of responses for a given genotype may only include one of the limbs of the curve or the central horizontal section. Had we studied undercrowding in these organisms, an optimum emergence would have become evident. A convex response curve is obtained for both weight and developmental periods.

Differences in weight and developmental period among the genotypes have been described. The possible significance of such differences for the overall ecology of the organism should be stressed. Weights may be related to voracity and fecundity of adults, while differences in developmental period will result in early or late emergence and may effect changes in gene frequency in a variety of evolutionary situations. It is obvious that the genotypes of this study will differ in other characteristics as well. We already have evidence that they differ in fecundity (KARTEN 1963; SOKAL, unpublished), in their responses to conditioned flour and in their ability to condition the flour (KARTEN 1963).

Genetic facilitation. The present study provides a striking example of the maxim that the performance of a genotype in a population in association with a variety of other genotypes cannot be predicted from its performance in pure culture, i.e., among like genotypes. As we have seen, the ecological interaction between genetically different individuals produces a situation where the adaptive advantage of a given genotype is often enhanced when it is in competition with genotypes other than its own. This phenomenon has been called *genetic facilitation* (LEWONTIN 1955) following the general concept of facilitation developed by ALLEE, EMERSON, PARK, PARK and SCHMIDT (1949). In thinking about models for facilitation among genotypes, one is easily led to postulate coefficients which indicate the relative competitive advantages of different genotypes, so that when one genotype is in competition with n individuals of another genotype these do not provide an equivalent competitive force as compared with n individuals of the first genotype in pure culture. This idea of different ecological equivalents is fundamental to the classical competition models of LOTKA and VOLTERRA. Whenever competition between two forms or two genotypes benefits one at the expense of the other compared with their respective performance in pure culture, a model of this sort may be invoked. However, in some instances in the present study genetic

facilitation occurs in which all three competing genotypes benefit by their mutual associations when compared with their performances in pure culture; here such a simple model is not sufficient. The occurrence of such overall mutual facilitation (see, for example, in Figure 1 gene frequency $0.75q_b$ at densities 5/g and 20/g and compare with pure cultures at these densities) engenders doubts concerning the validity of a hypothesis of ecological equivalents in simpler situations as well. Genetic facilitation may not involve competitive stresses at all. It is clear from our results that the range of densities investigated does not drastically affect the survival of Tribolium populations. Yet important instances of genetic facilitation and gene-frequency-dependent effects were observed.

At this stage in our work we can only speculate regarding the ecological or physiological nature of the interaction which brings about the observed genetic facilitation. The organisms could give off mutually beneficial substances; their different developmental periods may permit the optimum exploitation of the medium at a given time and avoid mutual jostling of the larvae as they feed. The mixing of genotypes may reduce cannibalism in the cultures. For instance, the heterozygotes may find themselves in much less competition as they pass through the various developmental stages, since there is almost a two-day difference in mean developmental time between them and the other genotypes. On the other hand, if heterozygotes are reared in pure culture, entire cohorts would go through the various developmental stages simultaneously, providing much more actual competition for the members of the cohort. This explanation would not satisfy the finding by SOKAL and HUBER (1963) that the sooty heterozygote is the fastest developing genotype, yet does not do well in mixed culture.

Genetic facilitation is only one possible way of changing relative adaptive values of genotypes in competition. The term is used here to mean the improvement of the performance of one genotype by the presence of a second genotype regardless whether the latter is benefited or harmed. Another change of relative adaptive values would be brought about in cases when the less favored genotype deteriorates in competition with the favored genotype which, however, does no better in mixed culture than it does in pure culture. An example of this sort was found by SOKAL and SULLIVAN (1963) who reared wild-type and homozygous brown body houseflies in competition. At higher densities the homozygous mutant, which performed somewhat poorer than the wild type even under the best conditions, failed drastically, resulting therefore in a higher relative adaptive value of the wild type.

While in terms of emergence only a few environmental conditions yielded overall facilitation in mixed culture, measurements of the biomass obtained in each environmental condition show facilitation to be quite general for exploitation of the medium.

Gene frequency effects. Changes of adaptive value of a genotype with changes in gene frequency are receiving much attention nowadays and a number of cases have been investigated (LEVENE, PAVLOVSKY and DOBZHANSKY 1954; LEWONTIN 1955; SPIESS 1957; LEWONTIN and WHITE 1960; LEWONTIN and MATSUO 1963; SOKAL and HUBER 1963). The present instance adds to this literature. None of the adaptive values in the present situation can be entirely simply expressed, and

it may well be that stating them as functions of a gene frequency is of less use than expressing them as functions of a zygotic frequency (see SOKAL and HUBER 1963).

Extreme frequencies of genotypes. It will be recalled that the wild type did well when it was rare while the homozygous black did especially poorly under these conditions. It is difficult to know whether these reactions are different only in degree along some curve of response to gene frequency that we have not yet discovered or whether these extreme responses are different in kind because of changed ecological conditions under these circumstances. On the assumption that these responses transcend simple functions of gene frequency they become of considerable evolutionary interest. If we can establish that such reactions take place, some of the conventional selection curves discussed in the evolutionary literature would need revision. For example, it is well known that selection against a recessive or semidominant will slow down considerably in the later generations when the frequency of the gene has already been appreciably reduced so that the final loss of a gene in a large population is generally a theoretical concept only. If, however, the adaptive value of a gene when it becomes extremely rare should deteriorate appreciably, then a model for the possible loss or fixation of alleles becomes available. SULLIVAN and SOKAL (1964) have found that extreme rarity in the wild-type OL strain when in competition with the *bwb* mutant enhances the adaptive value of the wild type markedly, paralleling the present findings.

Comparisons with related studies. Comparing the ecogenetic studies on Tribolium and on houseflies (SULLIVAN and SOKAL 1963; SOKAL and SULLIVAN 1963; BHALLA and SOKAL 1964) we note that the recessive mutants in the housefly studied so far all seem to be disadvantageous under conditions of larval competition. On the other hand, the relative adaptive values of the two homozygous mutants of *Tribolium castaneum* are quite high. As can be seen in Figure 1, wild type only rarely had higher emergence values than *b/b*. In view of the high adaptive values of the heterozygote one would expect therefore that Tribolium populations in nature should at least be polymorphic for the black locus. Similar relations are found by SOKAL and HUBER (1963) for the sooty locus. This problem becomes more acute and perplexing when several selection studies carried out in our laboratory are considered. In these studies sooty (SCHLAGER 1963) and black (SOKAL, unpublished) were introduced at various gene frequencies and under a variety of rearing schemes into the population. The gene frequencies were free to follow a course of natural selection. In these cases the mutants did not become lost, but in the case of sooty appeared to approach an equilibrium near 50 percent, while in the current experiments on black it appears to trend toward a very high frequency in the vicinity of 80 percent black. We hope that the concerted analytical studies on the ecology of the black strain currently underway in our laboratory will be able to explain why this gene is not found in appreciable frequencies in nature. A similar apparent equilibrium was found for the pearl locus by KOLLROS (1944).

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SUMMARY

Mixtures of three genotypes (+/+, +/*b*, *b*/*b*) of the autosomal mutant black were reared in eight different combinations and at five densities. The heterozygote +/*b* is distinguishable. The experiment was run in four replicated series employing a total of 205,120 beetles. The numbers, dry weights and total developmental times of emerging beetles were recorded by genotypes.

Adult survivorship is measured as the percentage of egg input for a given genotype. Increase in density reduces survivorship in +/*b* and *b*/*b* but not in the wild type. At density 200/g emergence of adults was extremely low. Marked genetic facilitation is experienced by all three genotypes in mixed culture (most prominently by the heterozygotes). The degree of facilitation for each genotype differs with the gene frequency and density conditions. Heterozygotes have the highest survivorship in almost all combinations of density and gene frequency in the study. At the lower densities black is better adapted than the wild type, while at higher densities and with increasing gene frequencies of black, wild type appears better adapted. Adaptive values of the genotypes are gene frequency dependent. The emergence of +/*b* and *b*/*b* decreases, while the emergence of +/+ increases as the gene frequency of black rises.

Dry weights of adults were highest at density 20/g. Mean weights as a function of density are in the relation 20/g > 5/g > 50/g » 100/g. The weights of the three genotypes are in the following order: +/*b* > +/+ > *b*/*b*. No gene-frequency-dependent effect on the weights could be found.

Developmental period decreases as density increases from 5/g to 50/g but rises rapidly again for density 100/g (100/g » 5/g > 20/g > 50/g). The three genotypes differ in developmental period: +/+ > *b*/*b* » +/*b*. In pure cultures, these relations change somewhat at the highest densities. Changes in gene frequency have no overall effect on developmental period, but some effects at certain densities could be observed.

When a homozygous genotype was extremely rare or frequent in a population (at zygotic frequencies of 1 percent and 81 percent, respectively) genetic facilitation in mixed culture continued as before, but +/+ when rare shows a marked increase of emergence. When rare and dense, black does very poorly. Weights of the wild type are higher when it is rare at low density but lower when at high density. When wild type is frequent its weights are unchanged. When rare, black beetles have lower weights, but when frequent they weigh more at all densities.

Gene-frequency-dependent adaptive values and genetic facilitation appear to be widespread phenomena. Deviant behavior of genotypes under conditions of extreme frequency or rareness may complicate simpler models of selection and appreciably modify conclusions based upon them.

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