GENE FREQUENCY CHANGES AT THE α-AMYLASE LOCUS IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA*

DARRELL G. YARDLEY

Department of Zoology, Clemson University, Clemson, South Carolina 29631

WYATT W. ANDERSON

Department of Zoology, University of Georgia, Athens, Georgia 30602

AND

HENRY E. SCHAFFER[1]

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27607

Manuscript received September 16, 1976
Revised copy received May 20, 1977

ABSTRACT

The frequencies of alleles at the α-Amylase locus of *D. pseudoobscura* were followed in both large and small experimental populations. No evidence for balancing or directional selection was found, although our ability to detect weak selection is limited. The gene frequency changes in our experimental populations were consistent with the hypothesis of selective neutrality and genetic drift due to sampling error.

THE relative roles of selection and drift in bringing about genetic changes in populations remains an open question. Since genetic change is the basic process of evolution, this question is clearly an important one. The fact that it remains unresolved reflects the difficulties in obtaining and properly interpreting data that bear on the issue. Resolution would be simple if either selection or drift alone were responsible for the gene frequency changes and resulting variation that are observed in nature; we could then simply test against one hypothesis or the other. The situation is not "either-or", however, and with a model incorporating both selection and drift, almost any outcome can be explained. Perhaps it is not surprising that none of the many experimental, theoretical, or field studies that have addressed the problems of genetic variation and genetic change have been conclusive (see LEWONTIN 1974).

Several approaches have been pursued. One has involved an examination of statistical properties, such as distributions of heterozygosities and numbers of alleles under various selective and neutral models (EWENS 1972; JOHNSON and FELDMAN 1973; YAMAZAKI and MARUYAMA 1972; and others). Another approach has involved surveys of allozymic variation in natural populations, for which

[1] Paper no. 5029 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, North Carolina. This investigation was partially supported by Public Health Service Research Grant Number GM-11546 from the National Institute of General Medical Sciences.

ALLARD and KAHLER (1972); AYALA (1972); JOHNSON *et al.* (1969); KOEHN, MILKMAN and MITTON (1976); PRAKASH, LEWONTIN and HUBBY (1969); POWELL (1975); SCHAFFER and JOHNSON (1974); and SELANDER (1976) provide summaries and examples. Yet a third approach has been to follow the frequencies of alleles in experimental populations of Drosophila, particularly the frequencies of alleles at loci coding for various enzymes. Such loci should serve as closer approximations to typical natural variation than the visible mutants employed in the past. Some studies of experimental populations seem to implicate selection (AYALA and ANDERSON 1973; BIJLSMA-MEELES and VAN DELDEN 1974; FONTDEVILA *et al.* 1975; GIBSON 1970; POWELL 1973; some results of SING, BREWER and THIRTLE 1973; OHBA 1969; RASMUSON, RASMUSON and NILSON 1967; VAN DELDEN, KAMPING and VAN DIJK 1975; YARBROUGH and KOJIMA 1967), although the evidence has been complicated by such factors as the presence of chromosomal inversions and linkage. Others (MACINTYRE and WRIGHT 1966; YAMAZAKI 1971; and some results of SING, BREWER and THIRTLE 1973) are compatible with drift and little, if any, selection. No single analysis of experimental populations can hope to resolve the central issue, but the accumulated results of many such experiments should provide a perspective on the range of behavior shown by "enzyme loci" in laboratory populations, and hopefully some indication of what might occur in nature. To this end we have studied the frequencies of α-Amylase alleles in experimental populations of *D. pseudoobscura*.

The rationale of our experiment was simple. Genetic drift of neutral or nearly neutral alleles (KIMURA 1968; KING and JUKES 1969) should produce erratic, random fluctuations in gene frequencies, the fluctuations being a function of the sampling error each generation. Persistent selection, even if only moderately intense, should result in directional changes in gene frequencies, whereas fluctuating selection, if sufficiently strong, should show greater gene frequency fluctuations than predicted from genetic drift alone. So that the effects of drift would be apparent, the frequencies of α-amylase alleles in populations of three different sizes, ranging from small to large, were followed. The observed gene frequencies were compared with those expected under drift alone, using a statistical model developed by FISHER (FISHER and FORD 1947). In an effort to eliminate or minimize the effects of chromosomal polymorphism and linkage disequilibrium, the experimental populations were begun with a large number of freshly collected strains polymorphic for the amylase alleles, but chromosomally monomorphic.

MATERIALS AND METHODS

Seven populations were initiated, six monomorphic and one containing a low-level inversion polymorphism. Populations of three sizes were maintained: $N \geq 2{,}000$, referred to as the "large" populations; and $N \approx 200$ and $N = 20$, both referred to as the "small" populations. The N used here and throughout the paper is the number of alleles, or twice the observed number of organisms. The large populations were maintained in wooden cages, while the smaller populations were kept in quarter-pint bottles. All flies were cultured at 25° on cornmeal-agar-molasses medium.

The experimental design is shown in Figure 1. All populations were maintained with discrete

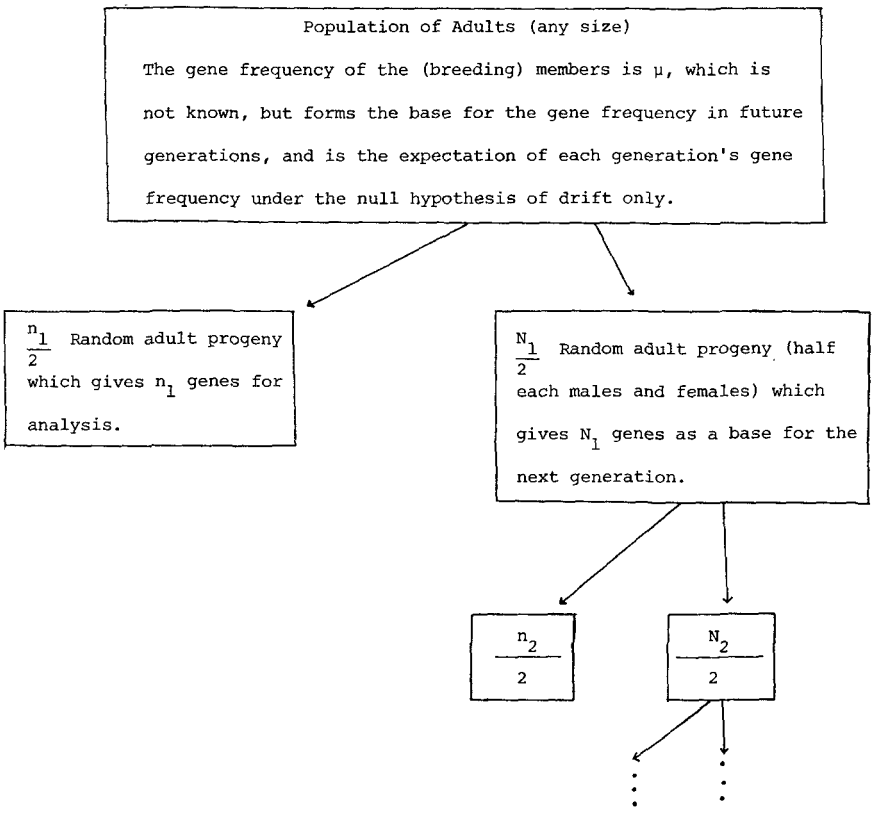

FIGURE 1.—Experimental design for test of selective neutrality. Gene frequencies are observed and n_t and N_t are known for each generation.

generations, large populations on a 30-day cycle and small ones on a 25-day cycle. Each generation was initiated by allowing the $N/4$ females of the previous generation to lay eggs for the last five days of the generation, after which the adults were discarded. In the small populations, a given number of adults, half of each sex, were counted out and transferred to bottles to begin the next generation. In the large populations, fresh food cups were placed in the cages over five days, after which the cups with the next generation's eggs and larvae were transferred to a new cage. A sample of 100 adults was then weighed, the entire population was weighed, and the number of adults in this generation estimated by simple proportion. Egg samples for each generation's amylase allele frequency estimates were taken over the five-day egg-laying period and cultured under nearly optimal conditions (15–19°, extra yeast, minimal density) until adults emerged. Upon emergence, these adults were frozen at —20° or below until they could be examined. Comparisons of the amylase genotypic data with Hardy-Weinberg expectations gave reasonably close results. Consequently, our assumption of "nearly optimal" conditions, implying no significant selection in the rearing process, appears justified.

The strains of *D. pseudoobscura* used in these experiments were collected at Flagstaff, Arizona, in June, 1973, and maintained as isofemale lines. Our experimental populations were begun late in 1973, so that none of them were started with flies kept more than a few generations in the laboratory. These experiments were designed to test for selection on the amylase locus or on closely linked loci, and it was necessary to avoid complications from selection on inversions of the chromosome carrying the amylase locus. In many localities this chromosome, the third, has a rich inversion polymorphism known to undergo powerful selection in nature and in the

laboratory. We chose the Flagstaff population because it is normally almost monomorphic for the Arrowhead (AR) gene arrangement on the third chromosome; of 206 chromosomes studies, 95.1 percent were AR and the remaining 4.9 percent were Chiricahua (CH) (see ANDERSON *et al.* 1975). The chromosomal constitution of each strain was determined by examination of salivary chromosomes from at least eight larvae. At the beginning and near the end of the experiment 400 chromosomes from each of the large populations were examined as a check for contamination; none was found.

The progeny of 38 isofemales lines containing only the AR chromosome were mixed to form a "founder" population, and large numbers of eggs laid by these parents were used to begin later populations. Sample of 10 and 100 of the original progeny mixture, half males and half females, were withdrawn to start populations 3 and 4, respectively. Two small populations (#5, begun with 100 adults; and #6, with 10) and the large population #2 were initiated with samples from the "founder" population after it had been maintained about six generations. Population #7, polymorphic for low levels of CH, was begun with the progeny of 65 isofemales lines; it was started with a chromosomal constitution similar to that of the natural population in Flagstaff.

The amylase electrophoretic technique was modified from DOANE (1966) for horizontal, slab-gel electrophoresis. Flies were homogenized in 20 μl of electrode buffer (0.087 M tris, pH adjusted to 8.9 with boric acid); this homogenate was adsorbed onto 3 × 6 mm strips of Wattman #3 chromatographic paper; and the strips were inserted into tris-borate, 5% acrylamide gels (0.087 M tris, 5% cyanogum, 0.1% tetramethyl-ethylene-diamine, pH adjusted to 8.9 with boric acid, and 1% ammonium persulfate for polymerization). After electrophoretic separation at 200 volts for 15 hours or 400 volts for 6 hours, gels were soaked in starch solution (0.02 M tris, 1.0% soluble potato starch for idometry, pH adjusted to 7.4 with HCl) for 12 to 24 hr, stained for one minute with potassium tri-iodine (0.10 M iodine, 0.05 M potassium iodide), and then rinsed in tap water. Bands appear as clear areas on a dark blue background. Gels were fixed in a mixture of acetic acid, methanol and water (1:5:5, respectively).

The *α-Amy-1* locus in *D. pseudoobscura* has two major alleles, $Amy\text{-}1^{1.00}$ and $Amy\text{-}1^{.84}$, and two minor ones, $Amy\text{-}1^{.74}$ and $Amy\text{-}1^{.92}$, previously reported (PRAKASH and LEWONTIN 1968). We did not observe the $Amy\text{-}1^{.92}$ allele in our populations but did observe a new allele, $Amy\text{-}1^{1.17}$, at low frequencies. This locus was reported by PRAKASH and LEWONTIN (1968) as associated with the third chromosome. Subsequent linkage analysis, to be reported in detail elsewhere, places *Amy-1* approximately 33 may units from the orange locus.

RESULTS AND DISCUSSION

The gene frequencies, numbers of genes sampled, and total numbers of genes in the populations each generation are given in Table 1, and the frequencies of the $Amy\text{-}1^{.84}$ allele are plotted in Figure 2. In the large populations, drift should be of minor importance since the effective population sizes are in the thousands; in these populations there were no noticeable trends in the gene frequencies over ten generations. The frequency of $Amy\text{-}1^{.84}$ fluctuated about a value of 18 percent, not far from the initial values. We attempted to estimate selection coefficients from these data by the maximum likelihood technique of DUMOUCHEL and ANDERSON (1968), assuming selection of constant, or nearly constant, size and direction, and negligible effects of drift. There was no evidence for selection in the large populations; the estimates were entirely consistent with the hypothesis that there was no selection among the three genotypes. The small populations do show movements of gene frequency, as expected under drift, and we cannot attempt to estimate selection coefficients from the gene frequency changes. $Amy\text{-}1^{1.00}$ became fixed in population 3 by the fifth generation. In population 6 the $Amy\text{-}1^{.84}$ frequency rose at first to about 75 percent and then dropped to 42

TABLE 1

Observed percent frequencies of Amy-1^{84} *alleles, number of genes sampled* (n) *and total number of genes* (N); n *and* N, *in that order, are given in brackets below the gene frequencies*

Population	Generation 1	2	3	4	5	6	7	8	9	10
	11.5	19.0	13.5	17.5	19.0	16.0	12.5	18.5	18.5	14.1
1	[400; 1,810]	[200; 9,480]	[200; 15,760]	[200; 7,608]	[200; 8,000]	[200; 13,000]	[200; 9,622]	[200; 4,284]	[200; 7,658]	[198; 9,938]
	20.0	19.0	16.0	14.5	16.5	16.0	11.0	14.5	22.2	21.0
2	[200; 16,000]	[200; 8,004]	[200; 11,372]	[200; 9,710]	[200; 6,816]	[200; 4,656]	[200; 3,456]	[200; 3,204]	[198; 3,008]	[200; 4,340]
	13.5	2.5	1.0	0.5						
3	[200; 20]	[200; 20]	[200; 20]	[200; 20]						
	20.0	22.8	32.8	28.0	22.0	16.0	18.0	13.5	10.4	12.1
4	[200; 200]	[180; 200]	[180; 200]	[200; 200]	[200; 200]	[200; 200]	[200; 172]	[200; 200]	[164; 200]	[140; 200]
	20.0	26.3	31.5	17.2	17.6	9.6	4.5	8.3	8.1	20.3
5	[200; 200]	[160; 200]	[200; 200]	[58; 200]	[176; 200]	[52; 200]	[22; 200]	[36; 200]	[62; 200]	[64;200]
	19.5	29.2	36.2	63.5	76.0	75.0	76.3	75.0	65.8	58.3
6	[200; 20]	[192; 20]	[188; 20]	[200; 20]	[200; 20]	[112; 20]	[80; 20]	[92; 20]	[38; 20]	[48; 20]
	21.0	15.0	20.5	14.5	20.0	19.0				
7	[400; 3,250]	[200; 8,932]	[200; 13,916]	[200; 22,052]	[200; 12,000]	[200; 14,000]				

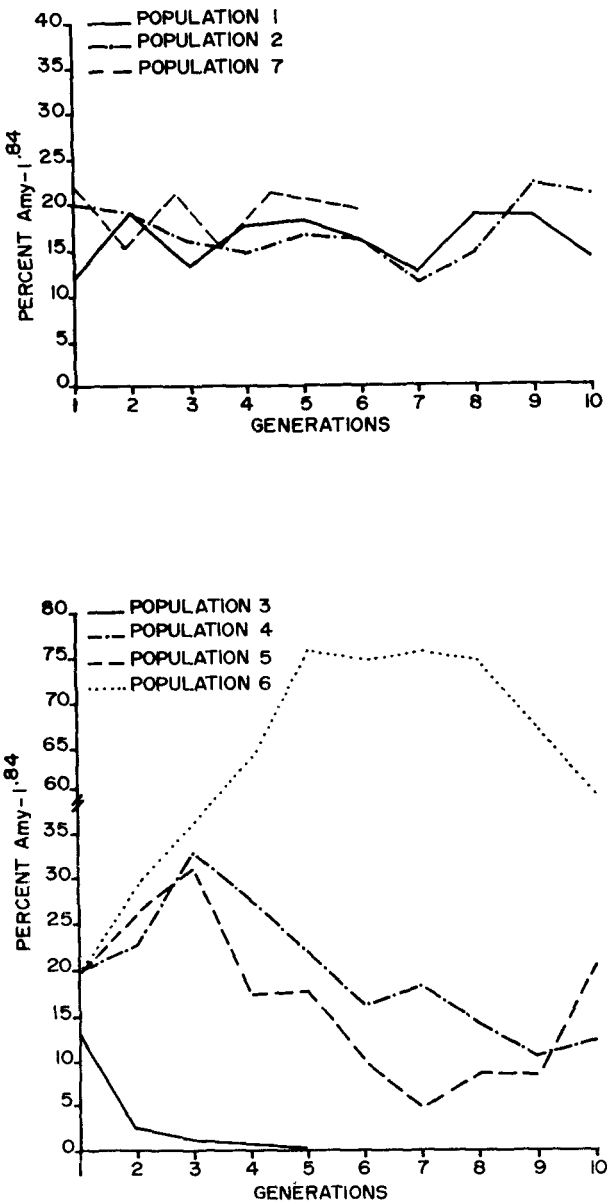

FIGURE 2.—Fluctuations in the frequency of the *Amy-1*^{84} allele in large (top) and small (bottom) experimental populations.

percent by generation 10. In populations 4 and 5 it fluctuated between 5 and 30 percent. If strong directional or balancing selection were involved, the gene frequencies should have shown a consistency in their movements. On the surface, then, the data do not suggest selection on the amylase locus or on loci closely linked with it.

Our ability to detect weak selection is severely limited, since the gene fre-

quency changes that result will not be large enough to distinguish from sampling error. Directional or balancing selection of moderate intensity may be taking place, but may not be apparent because drift is producing larger changes in gene frequency, which mask any selective changes. And it is possible that selection may vary in size and direction, so that any selective changes in gene frequency are erratic. What we should like to do is to determine whether drift alone could produce the gene frequency changes, or alternatively, whether selection of some type must be invoked.

FISHER (in FISHER and FORD 1947) devised just such a test for data on the frequency of a gene controlling pigmentation in the moth *Panaxia dominula.* The frequency of medionigra and dominula alleles, and the size of the natural population of this moth near Oxford, were recorded for the years 1939 through 1946, each year being a separate generation. The question FISHER and FORD asked was whether the gene frequency changes could be accounted for solely by genetic drift due to sampling error, or whether the fluctuations were large enough that some form of selection must be involved. FISHER showed what form the covariance matrix of gene frequencies should have under sampling drift alone, given the size of the population and of the sample taken from it in each generation, and he used it to formulate a test of the hypothesis that drift alone was responsible for the observed changes. The test results in a chi-square that is used to test the null hypothesis. The advantage of this test is that it *specifically* tests the hypothesis that only drift produces the observed gene frequencies in successive generations. In a sense, FISHER and FORD were testing the selection-neutrality question thirty years ago. To our knowledge, however, their test has been used only once since it was first employed, by SHEPPARD (1951) in a further study of the medionigra allele of *Panaxia dominula.* It is ironic that population genetics has come full circle with respect to the drift-selection issue. In the thirties and forties, many population geneticists felt that drift was the most important factor in gene frequency changes and that selection, when it operated, was probably quite weak. Then evidence for rather strong selection was obtained for a wide variety of organisms, and by the sixties selection was assigned the major role. With the advent of the "neutral mutation" theory in the late sixties, the role of genetic drift was reevaluated and accorded more importance. Thus we find ourselves testing the same question as FISHER and FORD, but from a very different perspective.

The details of our husbandry and sampling of the experimental populations are in some particulars different from those of the Panaxia study, and we have consequently modified FISHER's test to fit our situation. It is an unhappy fact that the operation of selection has proved difficult to measure by any known statistical procedures (see, *e.g.*, LEWONTIN and COCKERHAM 1959; DUMOUCHEL and ANDERSON 1968), and as a general rule no such test can be meaningfully applied without a consideration of its power to detect selection of various intensities. Thus we undertook computer simulations to measure its power to discriminate between drift alone and drift with selection. Finally, we have devised a variant of the test which includes a directional change in gene frequency due to selection. These statistical matters are discussed fully in the accompanying

paper (SCHAFFER, YARDLEY and ANDERSON 1977). FISHER's test to detect selection is limited; selection coefficients smaller than 0.1 will usually not be detected. Our test for a linear trend in gene frequency superimposed on drift is more powerful in detecting directional selection, although small selection coefficients again require large population and sample sizes. FISHER's test and our modification of it share with other tests for selection a frustrating weakness in detecting selection of low intensity. Interpreted carefully, the tests are useful in setting bounds on the intensity of selection that could be present, but none can rule out weak selection with the sample sizes that are experimentally feasible.

The analysis of gene frequency changes in our experimental populations by FISHER's test is given in Table 2. The effective number of genes and not the observed ones should be used in the test, and for Drosophila populations the effective number is perhaps one-half to three-fourths that observed (CROW and MORTON 1955). We applied the test with effective numbers equal to, one-half of, and three-fourths of the observed numbers. The latter two estimates are probably closer to the actual situation than the former. Only one of the chi-square values from our modification of FISHER's test for selective neutrality was significant at the usual five percent level, and this one case was with the effective number of genes equal to the observed number, a situation we consider less likely than the other estimates of effective number. In no population would we reject the hypothesis that drift produced all the gene frequency changes, and it is clear that we do not need to invoke selection of any kind. It is important, however, to keep in mind the limitations of the test; the hypothesis of selective neutrality and drift would probably not be rejected unless the selection coefficients were 0.1 or larger. We *can* rule out directional, overdominant, or variable selection of moderate to high intensity, which, given the difficulties of testing hypotheses about selection, is saying a good bit about the gene frequency changes in our populations. We may rule out directional selection of moderate to high intensity with the greatest confidence, because the test we designed for this situation has greater power than for drift alone.

TABLE 2

Results from our modification of Fisher's test for selective neutrality using three estimates of N_e, *the effective number of genes*

Population	Chi-square $N_e = N$	$N_e = 0.75N$	$N_e = 0.5N$	df†
1	13.31	13.00	12.47	9
2	14.33	14.01	13.42	9
3	3.66	2.66	1.96	3
4	12.38	10.50	8.20	9
5	18.82*	16.82	14.11	9
6	10.66	8.12	6.42	9
7	4.15	4.11	4.05	5

N is the observed number of genes.
* Significant at the 0.05 level.
† No. of generations—1.

Our estimates of selection in the large populations constitute a different kind of test, one based on the hypothesis of gene frequency changes under constant selection. The fitness estimates and their covariance matrices may be used to test the hypothesis that the genotypic fitnesses are all alike, that is, that there is no selection (DUMOUCHEL and ANDERSON 1968). This hypothesis could not be rejected; we conclude that there was no selection or, if present, that it was weak.

We have employed two tests that consider the possible causes of the gene frequency changes, each in a different way. On the one hand, our maximum likelihood estimates of the fitnesses give no evidence of selective differences among the genotypes. On the other hand, FISHER's test indicates that the gene frequency changes are within the bounds we expect under drift alone. Our test for drift and a linear gene frequency change by directional selection again failed to isolate a selective component of the changes. It should be kept in mind that the gene frequency fluctuations expected with drift alone in the large populations are quite small, since the effective population sizes were so large. Most of the fluctuations should be simply statistical errors in estimating the gene frequencies from samples of the populations, and the effects of any selection, either constant or variable, should be more apparent and more readily detected than in the small populations.

Our tests *were* sensitive enough to detect selection of the magnitude reported by a number of workers (AYALA and ANDERSON 1973; BIJLSMA-MEELES and VAN DELDEN 1974; POWELL 1973; SING, BREWER and THIRTLE 1973; VAN DELDEN, KAMPING and VAN DIJK 1975; YARBROUGH and KOJIMA 1967). Yet they did not, and in this sense our results join those of MACINTYRE and WRIGHT (1966), YAMAZAKI (1971) and some of those reported by SING, BREWER and THIRTLE (1973).

It is interesting to note that while some experiments do seem to indicate selection, others do not. As pointed out by LEWONTIN (1974) and others, in those cases where selection is indicated, linkage is an important complicating factor. For example, the experiments of POWELL (1973) with *D. willistoni* and of AYALA and ANDERSON (1973) with three species of the *D. willistoni* group may have been complicated by selection on inversions containing the enzyme loci being followed, so that all or a large part of the chromosome was responding to selection, rather than a single locus and genes closely linked to it. This possibility seems particularly unlikely for the latter study, where three different species were involved, but it cannot be discounted. We have taken pains in the present study to avoid any complications from inversion polymorphism. In addition we have taken our stocks from an area where alleles at the α-amylase locus are not normally associated with inversions, so that their association in the laboratory populations will not differ in this respect from the situation in nature.

Linkage disequilibrium for blocks of genes containing enzyme loci is probably the most formidible problem in assessing gene frequency changes in experimental populations. It is a problem which can at best be minimized, for it is nearly impossible to begin a population with an array of chromosomes with

genes at linkage equilibrium. Use of strains maintained for long periods in laboratory isolation undoubtedly worsens the problem. We have tried to minimize this linkage problem by using a large number of freshly collected strains.

The most fruitful approach for studying selection at the allozymic level appears to be ones which combine a biochemical rational with population experiments. GIBSON (1970), BIJLSMA-MEELES and VAN DELDEN (1974), and VAN DELDEN, KAMPING and VAN DIJK (1975) showed that changes in the frequency of alleles at the alcohol dehydrogenase locus could be elicited by addition of various alcohol substrates to the food medium in experimental populations of *D. melanogaster*. Similarly, DE JONG and SCHARLOO (1976), working with an amylase locus of *D. melanogaster*, found strong evidence for viability selection for one of the alleles. In this case, individuals homozygous for one of the alleles had a competitive advantage when the media contained only a small amount of yeast and a high concentration of starch. This evidence was, however, complicated by the presence of an additional amylase locus linked quite closely to the one being examined. These experiments come much closer than any cited above to showing selection at the enzyme locus itself, since the gene frequency changes occurred in response to addition of specific substrates to the food medium.

Our experiments are closest in concept to those of SING, BREWER and THIRTLE (1973), who designed an extensive set of experiments to test specifically for departures from selective neutrality. Their test, like ours, was based on a stochastic model incorporating the effects of drift due to finite population size. Their experimental design and method of analysis, however, were quite different from our own. These authors sampled a natural population of *D. melanogaster* and set up an experimental population from the progeny of 23 inseminated females. They then established 49 sublines of small effective size (10 or less) and followed gene frequencies at seven polymorphic enzyme loci for 21 generations. They compared the proportion of lines segregating and the average proportion of heterozygotes with those expected with and without selection, according to a Markov model of drift due to restricted population size. Their results could not be explained by drift alone; selection was implicated for at least five of the seven loci, or more properly, for the groups of genes segregating with the marker loci.

The available data on gene frequency changes in experimental populations of Drosophila indicate that selection sometimes occurs, and sometimes does not. It is usually not possible, however, to determine whether the selection occurs on the marker locus or on a group of genes including it. Rather more cases of selection than neutrality have been reported, which makes it all the more important to document cases where no selection is apparent, or where selection, if present, must be relatively weak. The amylase locus of *D. pseudoobscura*, like the *Est-5* locus studied by YAMAZAKI (1971), is such a case. We were somewhat surprised by the results of our experiment, for we chose the amylase locus because PRAKASH and LEWONTIN (1968) had shown that specific amylase alleles were associated with inverted gene arrangements of the two major "phylads," or phylogenetic groupings. They suggested that this association was maintained by selection and was evidence for "coadaptation" of adaptive gene complexes on

the third chromosome. The strains we used came from a population polymorphic for amylase alleles but uncomplicated by inversion polymorphism. It is possible that without the reduction in recombination by the inversion polymorphism, the adaptive gene complex which is apparently associated with amylase in certain gene arrangements cannot be maintained. The association of amylase alleles and chromosome type is not complete, however, and NEI and LI (1975) have suggested that neutral mutation and genetic drift could readily account for such observed allozyme-gene arrangement associations. Perhaps greater heterogeneity in the environment than exists in the laboratory culture is required before selective differences among amylase genotypes appear. There are, of course, many other reasons we could advance for our failure to detect selection, but the fact remains we found no evidence for selection. Our perspective on gene frequency changes in experimental populations is probably colored by the many experiments conducted in the past with morphological mutants or inverted chromosomes. These genetic factors often have pronounced selective effects, the magnitude and direction of which may vary with the environment. We have no basis for predicting how often allelic variation at enzyme loci is of adaptive significance. Still, we would not expect to find such differences at all loci in all environments. And, indeed, our results support this view.

We are grateful to RALPH BARGMAN for his help in interpreting FISHER's technique and for his assistance in developing the computer programs for analysis of our data. We also extend our thanks to RICHARD KIMMICH for his excellent technical assistance.

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Corresponding editor: J. F. KIDWELL