INHERITED BIOCHEMICAL VARIATION IN *DROSOPHILA MELANOGASTER*: NOISE OR SIGNAL? I. SINGLE-LOCUS ANALYSES[1]

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ABSTRACT

A study was conducted using small effective population size as an experimental design to test selective neutrality of seven isozyme polymorphisms. Loci varied as to the degree to which the decay of heterozygosity over 21 generations was retarded. Selection for heterozygotes, overdominance, is implicated for at least four of seven loci. Of these ADH gave the largest heterozygote excess in the presence of inbreeding. An interaction between the small population size treatment and excess heterozygosity suggests that (1) the loci studied may be selectively neutral and linked to other loci which are under the influence of selection or (2) the selection coefficients for the loci studied are not independent of the background genotype. In either case four of the seven enzymes studied are signaling the operation of selection. The problem of distinguishing the effect of a single marker from that of a chromosome segment is emphasized. The identification of the genetic unit of selection is crucial to any interpretation of the meaning of enzyme polymorphisms.

MANY population geneticists, until a few years ago, would not have argued with the assertion that selection is necessary to maintain alleles at a locus in polymorphic frequencies (BRUES 1954; WALLACE 1958). Currently, the explanation for the maintenance of all polymorphic loci in terms of a selection hypothesis is being challenged by inferences from two observations. First, the high proportion of enzyme loci estimated to be polymorphic in natural populations requires an excessive genetic load if balanced selection operated independently on each (see CROW 1970, for a recent discussion of load concepts). Second, the rate of amino acid substitution for a number of protein molecules appears to be uniform over geological time (King and JUKES 1969). The second observation has been interpreted by KIMURA and OHTA (1971a and 1971b) to mean that most of the molecular variation among individuals within and between species can be explained by mutation and drift. They argue that genetic polymorphism within a population is only a phase of the dynamics of fixation of a selectively neutral allele. Neither the genetic load of the population nor the cost of gene substitution (HALDANE 1957) need be great if most mutations are neutral, or near neutral.

An alternate analysis of the question states that genetic load has been an unrealistic formulation and the number of loci which can be supported by selection

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is much larger than previous calculations would admit (see SVED, REED and BODMER 1967 and WALLACE 1970). While few would dispute a role for random drift in the evolution of proteins, as CLARKE (1970) points out, the weight of evidence does not support genetic drift as the primary force responsible for amino acid substitutions. The recent work of UZULL and PILBEAM (1971) presents evidence from hominids that the rate of amino acid substitutions may not be as constant as earlier workers suggested. In any event, the very small number of proteins which have been sequenced may not be representative of protein variation in general. Furthermore, failure to reject the hypothesis that proteins with electrophoretically detectable amino acid differences are functionally equivalent is likely a consequence of the insensitivity of our measurements. WILLS and NICHOLS (1971) present an illustration. The polymorphic octonol dehydrogenase phenotypes of Drosophila are apparently equally fit in certain laboratory environments but when specific media were used which stressed the organism, large differences in fitness were found.

For any polymorphic locus it is patent to us that evidence to reject the neutral gene hypothesis is the prime requisite for settling this controversy. Failure to observe the operation of systematic forces only serves as convincing data for the neutral explanation if we have explored all possible ways in which differences could occur. That is, the neutral hypothesis will gain validity only if it holds when we have conducted an adequate number of experiments which have some chance of rejecting it. The study reported here is one such effort to reject the neutral hypothesis for at least some enzymes and to use the results to estimate the nature of the influences of selection in at least one set of environments in one species. We chose to use the design of small effective population size to apply a treatment to a Drosophila population polymorphic at seven loci. Under the neutral hypothesis this treatment produces a predictable dispersion of allele frequencies among a series of small populations sampled from the polymorphic reference population. Failure for allele and phenotype frequency distributions to be modified as expected over time is taken to be evidence to reject the null hypothesis and suggest that some systematic nonrandom forces are operating.

MATERIALS AND METHODS

A survey of various electrophoretic isozyme systems was carried out on a natural population of *Drosophila melanogaster* collected in Ann Arbor, Michigan. The enzyme phenotypes were determined by the method of starch gel electrophoresis (see BREWER 1970). Seven polymorphic enzyme systems were identified. They were phosphoglucomutase (PGM), glucose-6-phosphate dehydrogenase (ZW), 6-phosphogluconate dehydrogenase (Pgd), α-glycerophosphate dehydrogenase (α-GPDH), hexokinase (HEX-3), alcohol dehydrogenase (ADH) and esterase-6 (EST-6). A description of the systems is presented in Table 1. PGM, ZW, Pgd, α-PGDH, and HEX-6 represent a group of loci which determine enzymes which are known to be functionally related, while ADH and EST-6 code for electrophoretic variants which are not known to be involved in the metabolic activities of the others studied here. Each of the three major chromosomes is represented and, within each, various degrees of linkage obtain. Each locus has two codominant alleles in the population of *D. melanogaster* studied. In each case one allele codes for a slowly migrating electrophoretic variant (denoted *S* for convenience of reference in this paper) and the second for a fast variant (denoted *F*). PGM has a third codominant allele which codes for a variant with

TABLE 1

A description of the loci studied

Enzyme	Gene symbol	Variant*	Map unit	References
Alcohol dehydrogenase	ADH	2	2–50.1	GRELL, JACOBSON and MURPHY 1965
Phosphoglucomutase	PGM	3	3–43.4	HJORTH 1970
Esterase-6	EST-6	2	3–36.8	WRIGHT 1963
Glucose-6-phosphate dehydrogenase	ZW	2	1–63	YOUNG, PORTER and CHILDS 1964
6-phosphogluconate dehydrogenase	Pgd	2	1– 0.9	YOUNG 1966
α-glycerophosphate dehydrogenase	α-GPDH	2	2–20.5	GRELL 1967
Hexokinase	HEX-3	2	2–79	FOX and MADHAVEN 1971

* In the Ann Arbor population each enzyme had an electrophoretically slow and fast form. PGM had an additional isozyme of intermediate mobility.

intermediate electrophoretic mobility. For simplification, the frequency of the slowest PGM variant was pooled with the fastest and considered one throughout the initial analyses.

A large reference caged population was established from a sample of 23 inseminated females collected from the natural population surveyed. Samples were taken from the cage periodically for allele frequency assay. After 130 days a regression analysis was conducted to evaluate the stability of allele frequencies in the cage. At this point, 49 finite subpopulations were established by the procedure discussed below. The population cage (and its replicate) was maintained and sampled throughout the study to provide a comparison with changes in the average allele frequency of the subpopulations.

Discrete generations and small effective population size were produced for each of the sublines by starting each generation from five inseminated females of the previous generation. To ensure pair mating and a high degree of insemination we followed a procedure suggested by SPIESS (personal communication). Ten males and ten virgin females were randomly selected and isolated by sex in shell vials with food. After 72 hours of isolation the males were placed with the females for 45 minutes. During a period of this length it is most likely that a male will mate only once. Five of the ten females were randomly chosen and transferred to a fresh ½ pint milk bottle to establish the next generation. Each bottle, representing a subline, was sampled on the eleventh day for parents of the next generation and up to the twentieth day for gel work. Thirty flies were assayed for each enzyme every fifth generation. Only females were assayed for the sex-linked loci, ZW and Pgd. All cultures were maintained at 25° ± 1°. The standard cornmeal and molasses media was used.

The effect of finite population size was measured by studying the distribution among sublines of allele and phenotype frequencies over time. The expected proportion of sublines with a given allele frequency in generation t was computed by powering a probability transition matrix according to a time-homogeneous finite Markov chain system (see EWENS 1969, Chapter 4). The expected proportions from such a system are a function of the initial allele frequency, the effective population size of each subline, and the fitness relationships among the phenotypes. Letting F denote the fast variant and S the slow variant, the fitness model considered is

Phenotype	*FF*	*FS*	*SS*
Fitness	$1\text{-}s_1$	1	$1\text{-}s_2$

where s_1 and s_2 are fitness parameters relating the phenotypes within a subline. The allele fre-

quency *after selection* in a large population of progeny produced by the $N/2$ female and $N/2$ males which established such a subline in any generation is defined as

$$P_i = \frac{i^2(1-s_1) + i(2N-i)}{i^2(1-s_1) + 2i(2N-i) + (2N-i)^2(1-s_2)}$$

where the variable i is the number of fast variants represented in the N parents, before selection. Of course, i may take any of the discrete values, 0, 1, 2 . . . $2N$. The proportion of sublines in the t^{th} generation with parents having i fast variants may be designated f_i^t. The corresponding proportion in the $t+1$ generation, say f_j^{t+1}, may be written

$$f_j^{t+1} = \sum_{i=0}^{2N} f_i^t \times f_{ij}$$

where

$$f_{ij} = \binom{2N}{j} P_i{}^j (1-P_i)^{2N-j}$$

is the general element of the transition probability matrix and j may take any discrete value in the range of i.

When no selection is considered, i.e. $s_1 = s_2 = 0$, P_i takes on discrete values $i/2N$. Rather than increments of $1/2N$, the classes take increments of $2/3N$ for sex-linked loci. Rather than one of these discrete values, the P_i in the transition matrix f_{ij}, for the computation of the expected frequencies for the first generation, f_j^o, was taken to be the continuously varying allele frequency estimated for the cage. A discussion of the choice of estimators for the initial frequency is given in RESULTS.

Two random variables were of major interest. They were the proportion of lines segregating, Ω_t, and the average proportion of heterozygotes, H_t, both in generation t. Comparisons of observed Ω_t and H_t with their expected values using a variety of selection values, s_1 and s_2, were made. The expected Ω_t value for an enzyme was derived from the discrete Markov chain result, f_j^t. It is

$$\Omega_t = 1 - f_0^t - f_{2N}^t .$$

Expected heterozygosity was computed in two ways. First, it was computed using the discrete Markov result as

$$H'_t = \sum_{i=0}^{2N} f_i^t \, 2P_i(1-P_i),$$

where, as defined above, $P_i = i/2N$ for autosomal loci and $\frac{2i}{3N}$ for the sex-linked loci.

To interpret deviations of observed H_t from H'_t as due to differences in fitness among phenotypes within sublines, one must assume that the frequency of sublines of the i class, f_i, is as expected. To examine the consequences of this assumption a second expected value was computed based on the observed distribution of lines among the allele frequency classes. It is

$$H''_t = \frac{\sum_{k=1}^{49} Mkt \, 2\hat{P}_{kt}(1-\hat{P}_{kt})}{\sum_{k=1}^{49} M_{kt}}$$

where t again designates the generation and k the subline. M_{kt} and $\hat{P}_{kt}$ are the number of flies sampled and allele frequency estimate for the k^{th} subline, respectively.

The observed heterozygosity, again weighted by sample size, was taken to be

$$H_t = \frac{\sum_{k=1}^{49} M_{kt} H_{kt}}{\sum_{k=1}^{49} M_{kt}}$$

where H_{kt} is the observed proportion of heterozygotes in the k^{th} subline in the t^{th} generation.

The usual chi-square approximation (with one degree of freedom) for contrasting the experimental outcome in any one generation with expected values was computed with numerator values of

$$[N_t\ (\mathrm{Obs}\Omega_t - \mathrm{Exp}\Omega_t)]^2 + [N_t\ (\mathrm{Obs}(1-\Omega_t) - \mathrm{Exp}(1-\Omega_t))]^2$$

for proportion of N_t lines segregating in generation t and

$$[N_t\ (\mathrm{Obs}\ H_t - \mathrm{Exp}\ H_t)]^2 + [N_t\ (\mathrm{Obs}\ (1 - H_t) - \mathrm{Exp}\ (1 - H_t))]^2$$

for level of heterozygosity among N_t flies in the same generation. Because of serial correlations in the data over time and small sample size, the chi-square was judged inappropriate for testing the goodness-of-fit of the observed values; Ω_t and H_t, to those expected over the entire 21-generation period of the study. As an alternate, three statistics were computed to measure aspects of goodness-of-fit. First, to measure displacement of the trajectory of observations over time, the above chi-square was computed at the 21st generation. Second, the shape of the observed trajectory of either Ω or H was compared to its expected form using the nonparametric Kolmogorov-Smirnov one-sample statistic, D (see BRADLEY 1968). Thirdly, to estimate the lack of parallelism of the data with expectations during the 21-generation study period, the simple linear regression of the variable

$$Y_t = \frac{(\mathrm{observed}_t - \mathrm{expected}_t)}{\mathrm{expected}_t}$$

on time was computed for each Ω_t and H_t. Precise statements of probability concerning significance of the regression coefficients were deemed inappropriate because of the serial correlations in the data over time.

RESULTS

A preliminary study to determine the efficiency of the mating design to produce five inseminated females was conducted. For each of ten replications of the mating procedure, each of the five selected females was isolated in a shell vial with food. In two of the ten replications one female failed to produce progeny. Overall, 96% of the females selected were inseminated.

Average allele frequency: The linear regression of allele frequency on time for the cage populations was computed for each locus from a minimum of 5 samples taken over a period of 130 days after establishing the cage. The slope was not significantly different from zero for any locus. Shortly thereafter, on the 144th day, the sublines were established to begin the study.

An analysis of allele frequency estimates (Table 2) was conducted on samples from the sublines and samples from the cage during the 21-generation period of the study. The linear change in average frequency for the sublines was not significant for any of the seven loci. For the cage used to establish the sublines, there was a significant decrease in the frequency of the fast variant for ADH and a significant increase in the fast variant for α-GPDH. These two effects are sig-

TABLE 2

Analysis of allele frequencies† in the caged population and the sublines over the 21 generations studied

Enzyme	Variant	Subline average, $t=1$	Linear regression on time in days: Intercept		Linear regression on time in days: Slope		T-tests of difference of	
			Sublines	Cage	Sublines	Cage	Intercept	Slope
ADH	F	.5955	.5806	.6090	.000180	—.000700*	.7066	4.2391*
PGM	F+S	.0755	.0466	.0797	.000280	—.000061	.8198	1.7473
EST	F	.4296	.4000	.5275	.000006	—.000861	1.4113	1.9905
ZW	F	.4200	.3710	.4244	.000169	.000293	.7887	.1769
Pgd	F	.7911	.8115	.8202	—.000191	.000198	.1715	1.4890
α-GPDH	F	.8055	.8036	.7450	—.000251	.000709*	1.3558	4.6041*
HEX-3	F	.1500	.1563	.1179	—.000272	.000292	.3363	.9735

* Significant at the .05 level of probability.

† In every case, except PGM, the frequency of the allele coding for the fast electrophoretic variant (F) is presented. For PGM, the fast and slow variant are pooled and the remaining frequency attributed to an allele controlling and electrophoretic variant of intermediate mobility.

nificantly different from the linear regression values estimated from sublines.

The expected distribution of the sublines among the allele frequency classes at generation t using the Markov chain, and assuming equal fitness of electrophoretic phenotypes, is conditional on the initial allele frequency in the cage, $t = 0$, and constancy of the average frequency for the sublines over time. The initial allele frequency for each locus was taken to be the average of all sublines in the first generation (Table 2). This estimate is comparable to the intercept of the linear regression line with the frequency axis at generation zero estimated from the analysis of average allele frequency among sublines or the frequency in the cage during the 21 generations studied. However, the first generation average of the sublines has a smaller expected variance, and will be less biased, than the regression intercept.

Fixation of Sublines (Ω_t): The effect of the small population treatment in the absence of selection is to decrease the proportion of segregating sublines. For any generation, the distribution of sublines among allele frequency classes may be dichotomized into a group which is fixed for either the fast or slow variant and a group which remains polymorphic. The comparison of the proportion of sublines segregating, Ω_t, with the expected proportion based on random fixation of sublines is evaluated in Table 3 and presented graphically in Figure 1. In every instance the proportion of lines segregating after 21 generations was less than expected. This difference, as *number* of lines segregating, was statistically significant for the EST ($Pr(\chi^2 > 4.66) < .05$) and ZW ($Pr(\chi^2 > 10.25) < .01$) enzymes.

The linear change of Y_t based on Ω over t is given as $\hat{\beta}_{Y.t}$ in Table 3. Estimates of $\hat{\beta}_{Y.t}$ indicate a linear divergence over time from the expected Ω_t values in the direction of fewer segregating lines for all except the PGM and HEX-3 enzymes.

TABLE 3

The analysis† of the proportion of lines segregating at generation t, Ω_t

Enzyme	t	Y_t	χ^2 (1 d.f.)	$\beta_{y \cdot t}$	$D(N = 49)$
ADH	1	.00			
	6	—.06			
	11	—.07			
	16	—.17			
	21	—.31	2.84	—1.47	.14
PGM	1	—.19			
	6	—.42			
	11	—.21			
	16	—.06			
	21	—.18	0.19	0.75	.16
EST	1	.00			
	6	.04			
	11	—.20			
	16	—.29			
	21	—.39	4.66*	—2.21	.17
ZW	1	—.02			
	6	—.33			
	11	—.36			
	16	—.68			
	21	—.81	10.25**	—3.85	.30**
Pgd	1	—.14			
	6	—.25			
	11	—.29			
	16	—.66			
	21	—.53	3.08	—2.35	.23*
α-GPDH	1	.02			
	6	—.07			
	11	—.24			
	16	—.34			
	21	—.40	1.48	—2.21	.13
HEX-3	1	—.12			
	6	—.17			
	11	—.28			
	16	—.29			
	21	—.17	0.31	0.43	.12

† Y_t = (observed Ω_t — expected Ω_t)/expected Ω_t, $\beta_{Y \cdot t}$ is the estimate of the linear regression of Y_t on t in generations, χ^2 is a comparison of observed number of lines segregating with expected number in generation 21, and D is the Kolmogorov-Smirnov poorness-of-fit of observed to expected Ω_t, $t = 1, \ldots, 21$. All values computed to four decimals and rounded to two.

* Significant at the .05 level of probability.

** Significant at the .01 level of probability.

The values of Y_t for PGM and HEX-3 enzymes. The values Y_t for PGM and HEX-3 are smaller after 21 generations than for certain intermediate generations.

The form of the observed distribution of Ω_t (Figure 1) was judged by the D statistic to be significantly different from expected for ZW ($Pr(D > .30) < .01$)

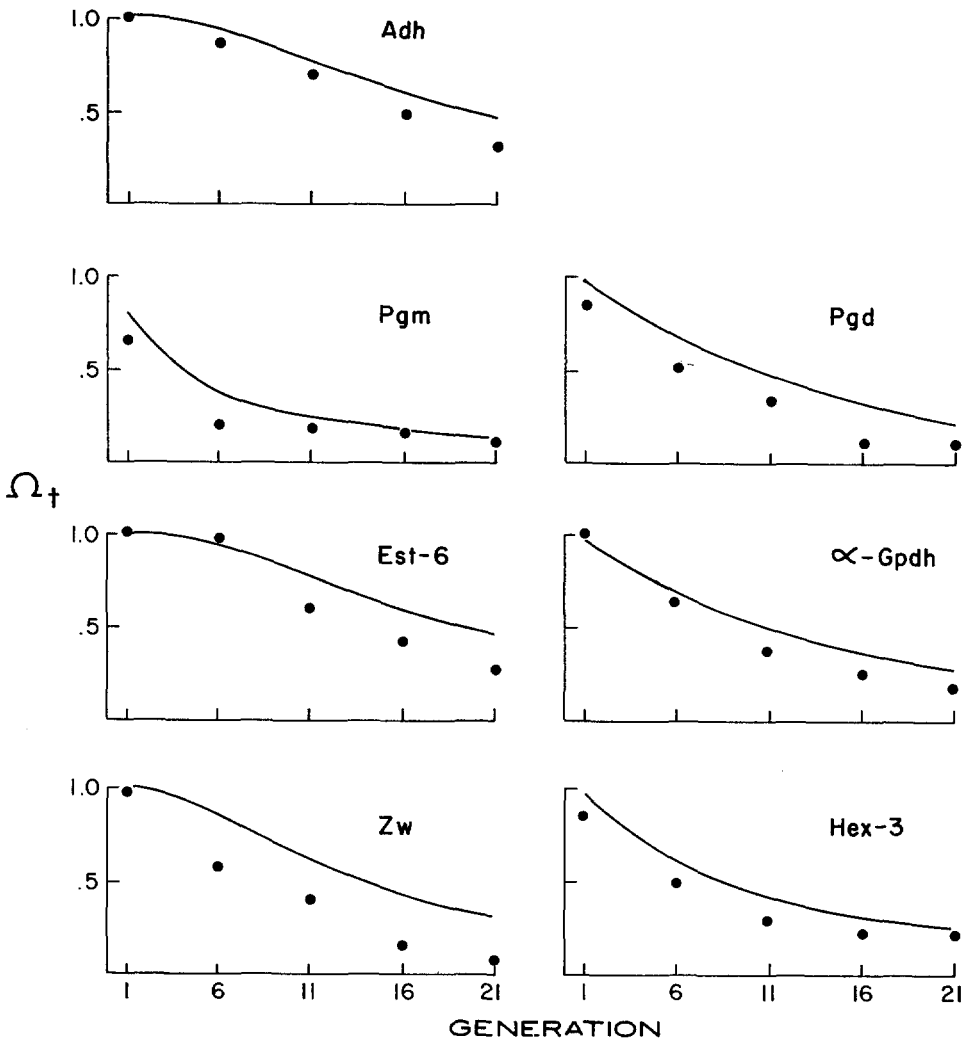

FIGURE 1.—The comparison of the observed proportion of sublines segregating, ●, with the expected, solid line.

and Pgd ($Pr(D > .23) < .05$). These enzymes also have the largest regression coefficients and greatest deviation in proportion of lines segregating at $t = 21$ (Table 3). At $t = 21$ the number of lines fixed for either the F or S variant was not significantly different from the number expected on the basis of its initial frequency ($t = 1$) for any of the enzymes. In summary, PGM and HEX-3 gave the best fit to expected decay in Ω_t whereas ZW and Pgd gave the poorest. As t approached 21 there was a linear increase in the excess of fixed lines for all but the PGM and HEX-3 enzymes.

Average heterozygosity (H_t): The analysis of H_t presented graphically in Figure 2 is given in Table 4. The observed H_t is compared to the two expected

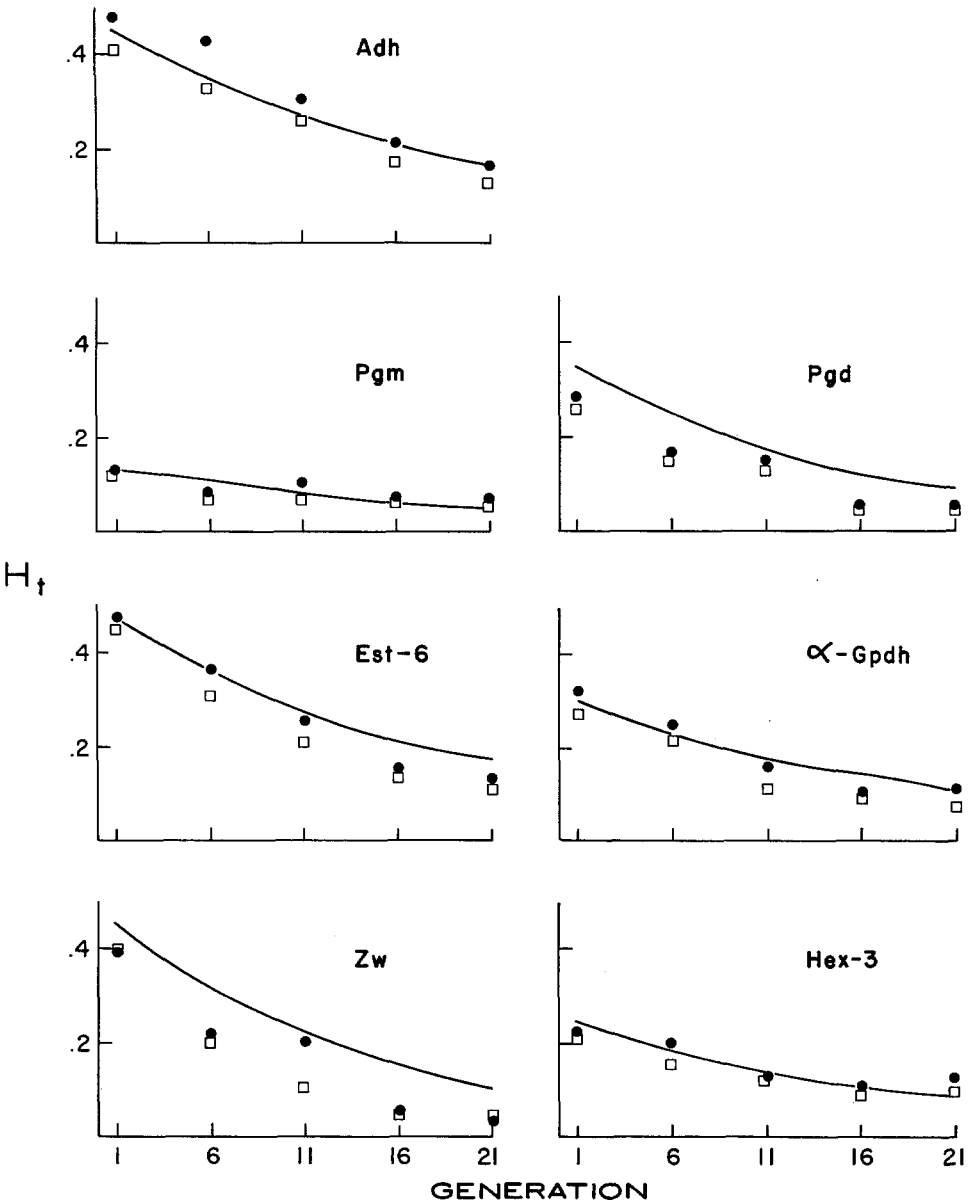

FIGURE 2.—The comparison of observed average heterozygosity, ●, with expected decay based on the Markov result, solid line, and expected based on the observed distribution of alleles among sublines, □.

values, H'_t and H''_t (see MATERIALS AND METHODS for a detailed definition). After 21 generations there is a significant excess, at the .01 level of probability, in average frequency of heterozygotes for the PGM and HEX-3 enzymes when compared to the discrete Markov result, H'_t. For both enzymes there was a linear

TABLE 4

Analysis of average heterozygosity for the sublines in generation t, $\mathbf{H}_t$

		Data†			Fit H'_t				Fit H''_t			
Enzyme	t	Obs. H_t	H'_t	H''_t	Y+	χ^2	$\beta_{Y \cdot t}$	$D(N)$	Y	χ^2	$\beta_{Y \cdot t}$	$D(N)$
ADH	1	.4791	.4549	.4144	.05				.16			
	6	.4278	.3520	.3317	.21				.22			
	11	.3117	.2724	.2587	.14				.20			
	16	.2141	.2104	.1748	.02				.22			
	21	.1601	.1631	.1266	—.02	.03	—.0068	.02	.26	8.39**	.0045	.02
								(1460)				(1460)
PGM	1	.1354	.1385	.1209	—.02				.02			
	6	.0837	.1072	.0667	—.22				.25			
	11	.1025	.0829	.0716	.23				.43			
	16	.0770	.0641	.0659	.20				.17			
	21	.0708	.0496	.0573	.42	8.11**	.0264	.09**	.43	2.62	.0144	.03
								(429)				(429)
EST	1	.4732	.4662	.4544	.02				.04			
	6	.3791	.3607	.3098	.01				.22			
	11	.2492	.2791	.2123	—.11				.17			
	16	.1527	.2159	.1343	—.29				.14			
	21	.1313	.1671	.1099	—.29	7.48**	—.0181	.06*	.19	4.87•	.0050	.03
								(1224)				(1224)
ZW	1	.3894	.4523	.3925	—.14				—.01			
	6	.2240	.3203	.1895	—.30				.18			
	11	.0982	.2268	.1123	—.57				—.12			
	16	.0509	.1606	.0490	—.68				.04			
	21	.0174	.1138	.0403	—.85	68.86**	—.0359	.18**	—.57	8.84**	—.0253	.04
								(727)				(727)

Pgd	1	.2822	.3482	.2635	—.19				.07			
	6	.1711	.2466	.1506	—.31				.14			
	11	.1529	.1746	.1268	—.12				.20			
	16	.0541	.1237	.0445	—.56				.22			
	21	.0496	.0876	.0537	—.43	13.44**	—.0149	.07**	—.08	.19	—.0043	.01
								(647)				(647)
α-GPDH	1	.3242	.3001	.2793	.08				.16			
	6	.2556	.2322	.2225	.10				.15			
	11	.1570	.1796	.1172	—.12				.34			
	16	.0983	.1390	.0904	—.29				.09			
	21	.0941	.1075	.0747	—.12	1.56	—.0161	.07	.26	7.10**	.0027	.02
								(861)				(861)
HEX-3	1	.2264	.2456	.2152	—.08				.05			
	6	.2021	.1901	.1620	.06				.25			
	11	.1289	.1471	.1245	—.12				.04			
	16	.1105	.1138	.0846	—.03				.30			
	21	.1200	.0880	.0966	.36	10.44**	.0159	.04	.24	7.34**	.0088	.03
								(712)				(712)

† H'_i = Expected heterozygosity from Markov chain result and
H''_i = Expected heterozygosity from observed weighted gene frequency. $Y = (0 - E)/E$. See text for further explanation.

* Significant at the .05 level of probability.

** Significant at the .01 level of probability.

increase in the percent excess of heterozygotes with time. $\hat{\beta}_{Y.t}$ for PGM is .0264 per generation and .0159 for HEX-3. Every other enzyme gave a deficiency of heterozygotes at generation 21, when compared to H'_t and a divergence ($\hat{\beta}_{Y.t}$ is negative) of the observed in the direction of fewer than expected as t approaches 21. The deficiency in heterozygosity at $t = 21$ was significant for EST, ZW, and Pgd at the .01 level of probability. The form of the distribution of observed H over time measured by the Kolmogorov-Smirnov D statistic was significantly different from the Markov result, H'_t, for all but the ADH, α-GPDH, and HEX-3 enzymes.

We recognize that H_t is an inappropriate predictor of heterozygosity when the distribution of sublines among allele frequency classes is not as expected. Adjustment of the expected heterozygosity for the observed deficiency of sublines segregating, H''_t, resulted in a better fit to the observed distribution of values over time for every enzyme. D values were each smaller and none were significant at the .05 level of probability when H''_t was considered. Compared to the analysis with H'_t there was an increase in the percent excess heterozygosity, Y_t, for all enzymes for all but a few of the generations. Those with a deficiency of heterozygotes at $t = 21$ compared to H'_t, in particular ZW and Pgd, had a smaller deficiency compared to H''_t.

At generation 21 all but ZW and Pgd had a significant excess of heterozygotes when compared to H''_t. Furthermore, the excess heterozygosity increased for each in the direction of more than expected as t approached 21 ($\hat{\beta}_{Y.t}$ was positive). The similar linear regression values for ZW and Pgd were negative (as they were in the comparison involving H'_t).

In summary, correction of expected heterozygosity for the deviation of the observed allele frequency distribution among sublines from that expected based on the Markov process resulted in two groups of enzymes. For two enzymes, ZW and Pgd, there is a deficiency of heterozygotes in most generations and a negative relationship between percent excess and time. The remaining five enzymes show an excess of heterozygotes in all generations. The excess increases as t approaches 21.

DISCUSSION

Evidence for the role of selection in the maintenance of enzyme polymorphisms has been of two sorts. The first comes from analysis of allele frequency data taken on wild-caught Drosophila (PRAKASH, LEWONTIN and HUBBY 1969, O'BRIEN and MACINTYRE 1969, and LAKOVAARA and SAURA 1971 are examples) and natural populations of Avena (MARSHALL and ALLARD 1970 is an example). One can argue that such data provides a more realistic test of the neutral hypothesis than laboratory studies because there is not the artificiality about the ecology which one is obligated to in the laboratory. However, the interpretation of what constitues necessary evidence to reject the selective neutrality of alleles from such data is moot. Unless one can accurately measure the environment and collect data

suited to a correlation analysis, it will remain improbable that one can assign a role of selection either to the constancy of frequencies or to the heterogeneity of frequencies among interbreeding populations. The recent work of ROCKWOOD-SLUSS, JOHNSTON and HEED (1973) is a step toward a better definition of the relevant environments. One need only compare their study with the arguments of JOHNSON (1971) and those of PRAKASH, LEWONTIN and HUBBY (1969) to realize that there is an element of futility in attempting to reason the role of migration, selection, or effective size in determining lack of variation of allele frequencies among populations on one hand and heterogeneity among populations on the other. Not only is there extremely little information on effective size and migration which can be used to help sort out the causal forces, but it is also very unlikely all loci of a species behave in a similar fashion when subject to the same array of forces. The well defined plant populations studied by MARSHALL and ALLARD (1970) probably provide the best opportunity to establish such a correlation between allele frequencies and environments. The study of JOHNSON *et al.* (1969) of sedentary ant populations sets a precedent for such an analysis but also illustrates the difficulty in the selection of the relevant measures of the environment.

A second kind of study designed to detect a deviation from the neutral allele hypothesis, if in fact it is false, has attempted to show that in at least some laboratory environments enzyme polymorphisms are being maintained by selection (RICHARDSON and JOHNSON 1967; RICHMOND and POWELL 1970; GIBSON 1970; POWELL 1971, and HUANG, SINGH and KOJIMA 1971 are examples). The strength of these studies resides in the possibility of showing that there are at least some environments in which alleles are not neutral. Furthermore, once a selective agent is identified, laboratory studies provide the framework to better define the mode of selection and level of biological organization at which it might operate.

The data reported here suggest that two types of general effects may be detected by the application of the specific treatment of small population size. First, for all enzyme markers there is a deficiency of segregating sublines after 21 generations compared to expectations based on the Markov model with $s_1 = s_2 = 0$. Secondly, after making an adjustment for this effect, there is an apparent excess of heterozygotes for five of the seven enzymes studied. On the average this excess increases for each of the five as inbreeding increases. Four of these five, ADH, EST, α-GPDH, and HEX-3 had a highly significant excess of heterozygotes at the 21st generation. The fifth, PGM, indicates large percent differences of observed from expected for most generations but because frequencies for the alleles pooled to represent the faster migrating variant were very small, the information available for hypothesis testing was inadequate to reject deviations from H''_t at $t = 21$.

The accelerated fixation of lines could be attributable to an improper genetic model, error in assignment of initial allele frequencies, selection for one or the other of the homozygotes, or an effective population size which is truly less than the hypothesized value of ten. Each of these possibilities will be considered below. The Markov model assumes a time-homogeneous process of fixation of sublines. A measure of the change in the shape of the distribution of sublines among allele

TABLE 5

Comparison of observed distribution of sublines among allele frequency classes† with expected based on Markov chain using the D *statistic* (N = 49)

System	Generation 1	6	11	16	21
ADH	.1138	.0479	.0421	.0703	.1027
PGM	.0912	.0105	.0134	.0412	.0590
EST	.1105	.1431	.0918	.0706	.0576
ZW	.2499*	.3715*	.1476	.1209	.0404
Pgd	.2921*	.1567	.0915	.1196	.0522
α-GPDH	.0735	.1014	.0372	.0922	.0399
HEX-3	.0820	.1216	.0463	.0746	.0260

† Seven classes represented; $P_1 = 0$, $0 < P_2 \leq .2 < P_3 \leq .4 < P_4 \leq .6 < P_5 \leq .8 < P_6 < 1.0$, $P_7 = 1.0$.

* Significant at the .05 level of probability.

frequency classes by generation is presented in Table 5. For each generation the form of the observed distribution is compared to the expected Markov result assuming no selection. For every enzyme, except ZW and Pgd, there is no evidence that the fit to the model is poorer in early or later generations. For ZW and Pgd there is a significant deviation from expectations in the first and sixth generations. The deviation in each case diminishes until, in generation 21, they fit as well as any of the enzymes determined by an autosomal locus. This result probably reflects oscillation of allele frequency between males and females in the early generations and not a failure of the Markov model *per se*.

It is unlikely that prediction of number of segregating sublines from the Markov model could be in error due to selection of the improper initial allele frequency. There was little variability among the various estimators considered. Secondly, there was no significant change in the average allele frequency of the sublines for any of the enzymes. The average allele frequency changed least for those enzymes, EST and ZW, which gave the significant deficiencies in observed Ω_t at $t = 21$. Conversely the enzymes PGM and HEX-3, which gave the largest (yet not statistically significant at the .05 level of probability) changes in allele frequency gave the smallest, by a factor of at least five, chi-square values for fit at $t = 21$.

Selection for one or the other of the homozygotes would accelerate the loss of segregating lines. However, the several analyses presented here indicate little evidence for such an effect. The accelerated loss of segregating lines into the two terminal classes was proportional to the initial allele frequency and the average allele frequency among sublines does not change significantly over the 21 generations for any enzyme. Such results are unlikely if directional selection were operating.

The increased rate of allele fixation is likely due to the true effective size, N_e, being smaller than 10. Consideration of a smaller effective population size will be made later in this report as a part of an effort to simultaneously estimate the

selection parameters which could account for the surplus of heterozygotes. But first it is appropriate to discuss the distribution of heterozygosity among the segregating lines.

The average excess of heterozygosity within segregating sublines is evidence to suggest that selection may be operating on the locus being studied or on one or more loci in the chromosome segment marked by the locus. It is our intent here at the outset to treat the closely linked segment of chromosome marked by the locus determining the enzyme as a unit, realizing fully that the behavior of allele variation may be due to properties of the segment and not the enzyme itself. An increase in average heterozygote ($\hat{\beta}_{Y.t}$ for H''_t given in Table 4) for five of the seven enzymes as inbreeding proceeds suggests a progressive increase of heterozygote excess within the segregating sublines. Two possible explanations for this result involving interaction of loci are (1) there is an array of selection values for a locus represented in the initial sample at $t = 1$ due to a nonrandom sample of alleles at interacting loci; inbreeding simply fixes those sublines with a smaller heterozygote advantage sooner than sublines with a larger excess of heterozygotes, or (2) all sublines have a similar degree of heterozygote excess at $t = 1$ but as the genome becomes progressively more homozygous certain sublines become more heterotic than others for the specific loci studied. Both of these hypotheses involve the interplay of the stochastic effects of genetic drift in small populations to fix specific combinations of genes at interacting loci and the effects of a selective advantage of the heterozygote which contributes to the maintenance of heterozygosity.

In the first case the array of selection coefficients could be a consequence of a different sample of alleles within each subline in the initial sample, $t = 1$, for loci which interact with the locus being measured. This possibility becomes more likely as the number of such interacting loci increases. Simply, it would be unlikely that any of the sublines would have been established from ten flies having a similar array of genotypes. The second explanation suggests that a set of polymorphic interacting loci are equally represented in the various sublines at $t = 1$ and as these interacting loci become fixed, due to the pressure of inbreeding, the selection coefficients for the loci being followed are altered. Those sublines which have loci which are fixed for alleles which alter the fitness of the locus being followed in the direction of an increased heterozygous advantage continue to segregate. Whereas in those sublines which become fixed for alleles which tend not to enhance heterozygote fitness, the locus being followed is more likely to be fixed. This latter explanation implies that any observed variability in heterozygote excess among sublines measured in generation one is attributable to sampling of the single-locus phenotypes and one would not expect a decrease in variability of the heterotic effect among sublines to accompany the decrease in mean heterozygosity which results from inbreeding.

To examine the data set to determine if either of these alternative hypotheses may be excluded for any of the loci, the fixation index defined by WRIGHT (1951)

was computed for the k^{th} segregating subline for the l^{th} locus in the t^{th} generation as

$$F_{klt} = 1 - \frac{\text{Observed } H_{klt} \times (M_{klt}-1)}{2\hat{P}_{klt}(1-\hat{P}_{klt})M_{klt} - \frac{b_{klt}}{2M_{klt}}}$$

where $\hat{P}_{ktl}$ is the estimated frequency of the fast electrophoretic variant, M_{klt} is the sample size and b_{klt} is the number of heterozygotes. The correction for finite samples, of the ratio of observed to expected heterozygotes has been suggested by COCKERHAM (1973). The average of this measure, weighted by the number of flies sampled from each subline, is presented in Table 6 for the l and t combinations. The F statistic provides a convenient metric for measuring dispersion of heterozygote excess among sublines which, unlike H_t, involves an attempt to standardize for the effect of variation in allele frequency. Hence, comparisons among generations and, more important, among enzymes may be made. The standard deviation of F among sublines for each l and t combination is also presented in Table 6.

The Kruskal Wallis two-way analysis (BRADLEY 1968) was applied to the F values given in Table 6. This non-parametric analysis was chosen over the standard analysis of variance because of the marked decrease in the standard deviation of F with the increase in number of generations (this outcome is discussed below). The increase in heterozygote excess identified by the analysis of H_t in Table 4 and Figure 2 is represented in F by a somewhat different function of the single locus phenotypes. However, the conclusions are essentially similar. The large average *negative* values for the autosomal loci, $F = -.1447$, compared to the average F of $-.0053$ for the sex-linked loci, emphasizes the heterogeneity of outcome between these two sets of genes. This difference (Kruskal Wallis test for homogeneity of rankings of the two groups within generations) was significant at the .05 level of probability. The test for heterogeneity of the ranks of the five autosomal loci from one generation to another (presented in Table 7) was significant at the 0.01 level of probability. Eighty percent of the Kruskal Wallis χ^2 measure of interaction of enzymes and generations is attributable to the failure of the ranks of α-GPDH and HEX-3, compared to the average of the others, to be consistent among generations. ADH, PGM, and EST showed a consistent rank ordering over all generations. Multiple comparisons among the ranks averaged over all generations resulted in the following summarization

ADH	α-GPDH	HEX-3	PGM	EST

where the error rate per comparison was taken to be 0.05. The multiple comparisons among ranks reflect the obvious groupings of the average F values for ADH, PGM, α-GPDH, and HEX-3 apart from EST. The average F for EST is of the magnitude of that for Pgd. However, unlike the Pgd data, the F is negative in each generation. The rank test failed to distinguish EST as significantly different from PGM, α-GPDH and HEX-3 because of the variability in ranks for the latter two enzymes among generations.

TABLE 6

Analysis of dispersion of heterozygote excess measured by WRIGHT's *fixation index*†

Enzyme	Generation 1 F^*	1 s^*	6 F	6 s	11 F	11 s	16 F	16 s	21 F	21 s	Mean F	Rank	Mean $F_{1:21}$**
ADH	—.1185	.2787	—.2606	.2701	—.1870	.2091	—.1781	.1774	—.2262	.0667	—.1941	1	—.0845
PGM	—.0621	.1311	—.2142	.0752	—.0989	.1487	—.1345	.1217	—.2052	.0663	—.1430	4	—.1246
EST	—.0198	.3043	—.0482	.3601	—.0156	.3553	—.1025	.1760	—.1655	.1182	—.0697	6	—.0202
ZW	.0472	.4266	—.1288	.1717	.1377	.2636	—.0168	.0718	.2852	.0601	.0649	7	—.2841
Pgd	—.0488	.3122	—.1142	.1458	—.1125	.1817	—.1821	.0747	.0798	.1786	—.0756	5	—.1978
α-GPDH	—.1102	.2125	—.1000	.2065	—.1685	.1585	—.0605	.1684	—.3533	.0973	—.1585	2	—.0937
HEX-3	—.0237	.2559	—.1722	.1649	—.1151	.1340	—.2715	.1135	—.2065	.1978	—.1578	3	—.0837
					Average								
All	—.0480		—.1483		—.0799		—.1351		—.1131		—.1049		—.1269
Autosomal	—.0668		—.1590		—.1170		—.1494		—.2312		—.1447		—.0813
Sex-linked	—.0008		—.1215		.0126		—.0994		.1825		—.0053		—.2410

† Using the correction suggested by COCKERHAM (1973) for finite samples.

* F = weighted F for the l^{th} locus in the t^{th} generation = $\sum_{k=1}^{N_t} M_{klt} F_{klt} / \sum_{k=1}^{N_t} M_{klt}$ and s is the standard deviation among the N_t sublines.

** Mean F in generation one of lines segregating at $t = 21$.

TABLE 7

Rank within generation of F *values (given in Table 6) for enzymes determined by autosomal loci*

Enzyme	Generation 1	6	11	16	21	Mean rank
ADH	1	1	1	2	2	1
PGM	3	2	4	3	4	3
EST	5	5	5	4	5	4
α-GPDH	2	4	2	5	1	2
HEX-3	4	3	3	1	3	2

The average increase of the within-subline heterozygote excess is reflected by increasingly more negative values of F as t approaches 21 for the five autosomal loci. Although the test for differences among the ranks of the generation values was not significant, the contrast of generation 1 with generation 21 was significant at the 0.01 level of probability (two-tail).

The standard deviation of estimates of F from sublines (Table 6) for each enzyme in each generation is, without exception, the greatest in the first generation and least in the twenty first. The decreased variance accompanied by an increase in average heterozygote excess as t approaches 21 is consistent for each of the five autosomal loci. This outcome excludes certain aspects of the second hypothesis presented above. That is, if we consider the variance of F at generation 21 to be due to sampling variation, then the additional variability at $t = 1$ may be attributable to biological variation in heterozygote excess. Consequently, the effect of inbreeding within such sublines on the rate of fixation would be inversely proportional to the size of the genetically modified heterozygote excess established for the subline at $t = 1$. Analysis of change in F over generations for the sublines segregating at $t = 21$ further supports the conclusion that for certain of the five autosomal loci the heterotic effect is determined by the initial sample of genotypes rather than evolving as the level of inbreeding increased. The mean F in the first generation, for sublines segregating in the 21st, exceeded the average of all lines in the first generation for three of the five autosomal enzymes (see Table 6, last column). For ADH and α-GPDH the sublines segregating at $t = 21$ had a lower average than the total group of sublines at $t = 1$. On the other hand, because in each case the average F of these sublines for the autosomal enzymes was higher at $t = 21$ than it was at $t = 1$, it is likely that at least a part of the increased heterotic effect resulted from fixation of alleles at interacting loci during the study.

DOBZHANSKY and LEVENE (1951) showed that a heterotic effect for inversions of the third chromosome of *D. pseudoobscura* could develop over a period of time in caged populations. They interpreted their finding as a result of selection for "co-adapted" third chromosomes which were either introduced into their caged study or formed within the cage as a result of recombination. Later, DOBZHANSKY and PAVLOVSKY (1957) conducted a study which implicated the operation of many genes on the heterotic chromosomal effect. To study the relationship between initial small population size in establishing a cage (a so-called bottleneck) and the selection for chromosomal heterozygotes, he chose to follow an

inversion of the third chromosome which was known to be heterotic in natural populations. His results were similar to those reported here. Experimental populations each started from samples of twenty flies varied with respect to the selective fate of the chromosomal inversion. He surmised that genetic drift operated to randomly select various combinations of genes at the outset for each population which modified the outcome of selection in each on the inversion. Although the variable of study was the chromosome and not the enzyme locus, the results of both studies, combined with those presented here, argue that selection for heterozygotes may be influenced by the epistatic effects of the background genotype. MUKAI (1969) has reported a quadratic synergistic effect among mutant polygenes and implicates optimum heterozygosity and recombination (MUKAI and MARUYAMA 1971) as factors which also contribute to the maintenance of polymorphic allelic variation.

CLARKE (1972) has recently suggested that similar results could obtain for a locus if the fitness values for the enzyme phenotypes are dependent on the density of the phenotype. HUANG, SINGH and KOJIMA (1971) have presented experimental results which fit CLARKE's model. They found that the fitness of each of the electrophoretic variants of EST-6 was highest when the medium on which they were grown was preconditioned by first growing variants determined by an alternate genotype in the media. Because the subpopulations were transferred to fresh media each generation and because competition associated with large population sizes could not occur in this experiment, it is unlikely that the role of density-dependent selection could be great. Even if it were a relevant factor in maintenance of the allelic variation in the natural population, the operation of frequency dependency in determining phenotype frequencies would be indistinguishable from heterozygote advantage due to the type of measurements which have been made in this study. Perturbation experiments designed to follow allele frequencies or inbreeding experiments which follow the relationship between measures of fitness and heterozygosity would be necessary.

The analysis of the ranks of the F statistics suggests that the five autosomal loci may be ordered as to the degree to which selection for heterozygotes retards the decay of heterozygosity in this experimental setting. To estimate the selection coefficients, s_1 and s_2, associated with each of the five autosomal loci, a numerical iteration of N_e, s_1 and s_2 using the discrete MARKOV model was conducted to obtain values which lead to the best fit of Ω_t and H'_t. The values of s_1 and s_2 were restricted to those satisfying the relationship

$$s_1 = s_2 \frac{(1 - P)}{P}$$

where P is taken to be the initial allele frequency estimate, $t = 1$. In this way an effective population size is selected and only those values of s_1 and s_2 which lead to a stable average allele frequency over the 21 generations of the study were considered. This restriction seems realistic in view of the observed stability of allele frequencies throughout the study (Table 2). The results of such an analysis are given in Table 8. The value of N_e which simultaneously minimized deviations

TABLE 8

Estimates of N_e, s_1, *and* s_2 *derived by numerical iteration. The values of* s_1 *and* s_2 *which simultaneously minimize deviations from expected* Ω_t *and* H_t *are given for each* N_e

Enzyme		N_e 10	9	8	7	6
ADH	s_1	.0000	.0000	.0250	.0815	.1600
	s_2	.0000	.0000	.0368	.1200	.2355
	χ^2†	.0969	.0521	.0398	.0358*	.0372
	χ^2_Ω†	.0732	.0260	.0110	.0113	.0160
	χ_H†	.0237	.0258	.0288	.0244	.0212
PGM	s_1	.0900	.0400	.0400	.0500	
	s_2	.0073	.0033	.0033	.0041	
	χ^2	.1097	.0896*	.0933	.1434	
	χ^2_Ω	.0857	.0557	.0392	.0538	
	χ^2_H	.0240	.0338	.0540	.0897	
EST	s_1	.0000	.0000	.0000	.0133	.1200
	s_2	.0000	.0000	.0000	.0100	.0903
	χ^2	.2586	.1614	.0865	.0675*	.0890
	χ^2_Ω	.1527	.0833	.0314	.0224	.0361
	χ^2_H	.1058	.0781	.0550	.0451	.0528
α-GPDH	s_1	.0000	.0000	.0000	.1000	.0700
	s_2	.0000	.0000	.0000	.4141	.2899
	χ^2	.1465	.0821	.0398	.0344*	.0859
	χ^2_Ω	.1095	.0554	.0177	.0119	.0567
	χ^2_H	.0370	.0267	.0221	.0224	.0291
HEX-3	s_1	.3000	.2000	.1800	.1800	
	s_2	.0529	.0353	.0318	.0318	
	χ^2	.0777	.0526*	.0542	.1109	
	χ^2_Ω	.0601	.0321	.0184	.0427	
	χ^2_H	.0176	.0205	.0358	.0688	

* Denotes minimimum χ^2.

† $\chi^2_\Omega = \sum_t \frac{(\text{obs}\,\Omega_t - \exp\Omega_t)^2}{\exp\Omega_t}$, $\chi^2_H = \sum_t \frac{(\text{obs}\,H_t - \exp H_t)^2}{\exp H_t}$, and $\chi^2 = \chi^2_\Omega + \chi^2_H$.

from expected Ω_t and H_t is certainly less than ten. For three of the loci—ADH, EST, and α-GPDH—the minimum χ^2 indicates that N_e may be 7. On the other hand PGM and HEX-3 data fit a larger value of $N_e = 9$.

There is no *a priori* basis for ascribing to the minimization based on the sum of χ^2_Ω and χ^2_H. A minimization based on χ^2_Ω only suggests an N_e of 8 for the ADH, PGM, and HEX-3 data, but $N_e = 7$ for the EST and α-GPDH data. This difference in N_e corresponds to the poorness-of-fit of the data (Table 3) to expected Ω_t given the hypothesis of no selection. EST and α-GPDH had a higher rate of fixation of sublines. N_e is certainly constant for all loci and probably lies somewhere between 7 and 9. However, N_e, s_1, and s_2 are interrelated, as N_e decreases s_1 and s_2 must increase to fit a given degree of retardation of fixation. ROBERTSON (1962) uses $N_e(s_1 + s_2)$ from the diffusion equation approximation to dispersion in small populations as a measure of retardation of loss of variability due to se-

lection for heterozygotes. Given our lack of knowledge of the simultaneous effect of N_e, s_1, and s_2 on Ω_t and H_t it appears that one is limited to conditioning statements about selection on the choice of an effective size. This causes a problem in the interpretation of selection coefficients for only one enzyme, α-GPDH. That is, selecting $N_e = 7$ or 8 the value $s_1 + s_2$ for a locus corresponds roughly to the ranking of the average F values, Table 6. As expected, estimates of s_1 and s_2 values for ADH, HEX-3, and PGM are larger than for EST. The s_1 and s_2 values for α-GPDH are perplexing. The great change in values between $N_e = 7$ and $N_e = 8$ are reflected by a relatively small change in χ^2. One can only conclude that the configuration of Ω_t and H_t data was such that the goodness-of-fit was less sensitive to changes in the parameter space.

Except for balanced lethals, selection for heterozygotes in small populations can retard but not prevent fixation of sublines. Theoretical work by ROBERTSON (1962) suggests that the effectiveness of heterotic selection in small populations is related to the ratio of s_1 to s_2. When the expected equilibrium deviates from 0.5 ($s_1 \neq s_2$) the effectiveness of selection to retard decay in heterozygosity diminishes. ROBERTSON's formulations suggest that the rate of fixation may actually increase due to a heterozygote fitness advantage when the equilibrium allele frequencies implied by s_1 and s_2 is less than 0.2 or greater than 0.8. Two enzymes, PGM and HEX-3, fall into the critical region. There is no evidence in the data set reported here that indicates fixation of sublines is accelerated for either of these when compared to the ADH, α-GPDH or EST enzyme loci which have intermediate allele frequencies.

The selection coefficients implicated in Table 8 for the ADH, PGM, α-GPDH and HEX-3 isozymes are large. If we consider each locus as contributing independently to survival, the segregation load as defined by CROW (1970) is obviously large. Two points are apparent, from the analysis of the F statistics, which are relevant to segregation load. First, the negative F values in the first generation are more representative of the random mating population than the average F or the s_1 and s_2 values estimated by the iteration procedure using data from all generations of inbreeding. Inferences about load in the reference population from these data are unjustified because as inbreeding proceeded, load increased and there is evidence that this load may be affected by the random selection of a small set of interacting genotypes, even in the 1st generation. The objective of this experiment was to obtain a comparison among loci and to reject the no selection hypothesis if in fact selection could be operating at certain loci in the reference population. This experiment is *not appropriate* for estimating absolute values of selection coefficients in the reference population. On the other hand, it is important to realize that if selection were not operating we would have not observed an apparent segregation load in these data.

A second point which reflects on the interpretation of load at a locus is the evidence for interaction of the loci studied with the background genotype. This interaction could be a consequence of the effects of linked or unlinked loci. The effect on the excess in heterozygotes of increasing homozygosity at unlinked loci is likely due to epistatic selection. However, the effect of increased homozygosity

at closely linked loci raises an issue we alluded to earlier but chose to ignore until now. Close inbreeding can preserve (increase) linkage disequilibrium among linked loci. In this study it is likely that as inbreeding progressed loci linked closely to the marker gene were simultaneously made homozygous. Consequently, the locus being followed is actually marking the effects of loci in a linked segment of chromosome. The greater the intensity of inbreeding the larger the block of genes will be which forms the unit identified by the locus being studied. This raises the possibility that the excess heterozygosity measured for a locus is due only in part to its own properties. In fact, none of the heterozygote excess at the locus may be attributable to its own contribution to differential fitness. OHTA and KIMURA (1971) have formulated the apparent overdominance which would occur at a locus with selectively neutral alleles because of its linkage to loci under the influence of heterotic selection. They believe that "associative overdominance" may play a role in simultaneously maintaining the large number of polymorphic loci found in natural populations. It is extremely difficult, if not impossible, to formulate a study which could discriminate for a locus between (1) selective neutrality and associative overdominance and (2) selection on the locus itself. Our experimental work (TEMPLETON, THIRTLE and SING, in preparation) with parthenogenetic and sexual *Drosophila mercatorum* indicates that in at least certain biological situations the gene is not the unit of selection. The proper unit of measurement remains obscure. The theoretical computer studies by FRANKLIN and LEWONTIN (1970) have provided insight into how complex the formulation of such a measure might be. Reducing the unit to the gene level by backcrossing is not the answer. In the absence of a measure of the size of the unit of selection one can only conclude from the data presented here that there is evidence for selection in five of seven blocks of genes. On the other hand, regardless of the biological role of the specific enzymes marking these blocks, they do provide a basis for the assertion that a large part of the genome can be predictive rather than simply representing noise in an imperfect biological system.

Until information becomes available on the organization level (the individual, the cellular or the molecular) at which isozymes interact with the selective environments, discussions about the mode of selection can only remain abstract. Knowing information on the level is imperative because it will suggest where to measure in the environment to obtain meaningful correlations of alleles and the selective factors in natural communities. GIBSON (1970) has made some progress in this respect for Drosophila by showing that allele frequencies for ADH do respond to levels of alcohol in the media. The statistical evidence presented in the study reported here suggests that similar work with the PGM, α-GPDH, and HEX-3 enzymes should also be fruitful in our attempts to understand isozyme polymorphisms.

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