DYNAMICS OF CORRELATED GENETIC SYSTEMS. IV. MULTILOCUS EFFECTS OF ETHANOL STRESS ENVIRONMENTS

D. R. CAVENER AND M. T. CLEGG

Department of Botany, University of Georgia, Athens, Georgia 30602

Manuscript received February 17, 1978
Revised copy received April 21, 1978

ABSTRACT

Four replicate populations of *Drosophila melanogaster*, two reared on medium supplemented with ethanol and two reared on standard medium, were electrophoretically monitored for 28 generations. During the first 12 generations, allelic, genotypic and gametic frequencies were determined for eight polymorphic enzymes: GOT, α-GPDH, MDH, ADH, TO, E_6, E_c and ODH. Samples from generation 18 and 28 were electrophoretically typed for ADH and α-GPDH. In addition, samples from generation 27 were analyzed for the presence of inversion heterozygotes.—The experimental results showed rapid gene-frequency divergence between control and treatment populations at the *Adh* locus in a direction consistent with the activity hierarchy of *Adh* genotypes. Gene-frequency divergence between control and treatment populations also occurred at the *α-Gpdh* locus, although the agreement among replicates appeared to have broken down by generation 28. No differential gene-frequency change occurred at any of the six remaining marker loci. Furthermore, values of linkage disequilibria among all linked pairs of genes were initially small and remained small throughout the course of the experiment. Taking these facts into account, it is argued that the gene-frequency response observed at ADH is most probably caused by selection at the *Adh* locus. The gene frequency response at *α-Gpdh* can also be be accounted for in terms of the effect of ethanol on energy metabolism, although other explanations cannot be excluded.

MUCH of population genetic theory is framed in terms of gene-frequency changes at single loci. This theory begins by abstracting to genotypic frequency distributions at a single locus without reference to genes segregating at different loci, a process tantamount to imagining single-locus organisms. The experimental population geneticist is always confronted with vastly more complex genetic systems, usually representing tens of thousands of loci, several thousand of which may be segregating. As a consequence of this complexity, it is uncertain whether the gene-frequency dynamics observed at a particular locus reflect the action of selection at that locus (in a casual sense), or whether the observed behavior reflects a statistical association with other parts of the genome that may be responding to selection. Indeed, LEWONTIN (1974, p. 307) has stated that "the selection of the chromosome as a whole is the overriding determinant of allelic frequencies".

Ideally, complete information on the statistical structure of the entire genetic system and on the functional differences of the gene products of all segregating alleles should be obtained in order to understand the determinants of gene-frequency change. Such a task is, at least at present, technically impossible. Nevertheless, considerable progress can be made by studying the joint behavior of marker genes scattered throughout the genome in experimental populations subjected to specific stress environments, where the alternative genotypes of one of the marker loci (the target locus) can be predicted to respond differentially to the stress factor. The logic of such an experiment is as follows: If marker-gene frequency changes occur in response to the stress environment, and if these changes are produced by fortuitous linkage associations between the marker loci and unobserved loci, then there is no reason to expect a behavior at the target locus different from that at the other marker loci. Conversely, if a response in the predicted direction is observed at the target locus, but not at the other marker loci, then the stress environment must elicit a selective response at the target locus or at a very closely linked gene also involved in the same metabolic process.

One case where a stress environment appears to have elicited a selective response at a target locus concerns the alcohol dehydrogenase locus (*Adh*) of *Drosophila melanogaster* in ethanol environments (GIBSON 1970; BIJLSMA-MEELES and VAN DELDEN 1974; VAN DELDEN, KAMPING and VAN DIJK 1975). These experiments consistently show a differential increase of the Adh^F allele in laboratory populations within a few generations.

However, the question of whether selection acts directly at the *Adh* locus is still open (BIJLSMA-MEELES and VAN DELDEN 1974); none of these investigations considered linkage associations of *Adh* with other chromosome *2* loci or inversions. In addition to this specific issue, none of these investigations have attempted to determine whether the ethanol selection acts only in a localized region around the *Adh* locus or whether the genome responds in a general manner.

The experiments reported in this paper are designed to investigate the question of whether ethanol selection acts only in the neighborhood of the *Adh* locus or whether it also produces a response in other regions of the genome. These experiments involve replicated control and experimental populations of *D. melanogaster*, where the experimental populations had their medium augmented with ethanol. The genetic effect of the ethanol stress environment was evaluated by monitoring the dynamic behavior of four loci on chromosome *2* and four loci on chromosome *3* for 12 generations. Gene frequencies at two of the loci were also determined in generations 18 and 28. In addition, the populations were analyzed for the presence of inversions at generation 27. The results show a rapid gene-frequency response at the target *Adh* locus in the ethanol environments; the direction of gene-frequency change is also in accordance with previous reports in that a marked increase in the frequency of the Adh^F allele occurred. Six of the remaining seven enzyme loci show no gene frequency response to the ethanol stress. The seventh locus, α-glycerophosphate dehydrogenase (α-*Gpdh*), does

exhibit gene-frequency changes in the stress environment, although the *α-Gpdh* behavior is less consistent over replicates than that observed at *Adh*. The linkage disequilibrium data and the lack of second chromosome inversions support the conclusion that the response of these two loci occurred independently. Despite this apparent independence in selection dynamics, the *α-Gpdh* response is consistent with the known metabolic effects of ethanol stress and with the role of *α-Gpdh* in the regulation of energy metabolism.

MATERIALS AND METHODS

Two hundred and nine single pair matings of virgin females and males from the Weymouth 74g stock (collected in Providence, R.I., by M. G. KIDWELL in 1974) were initiated in eight-dram shell vials containing a standard cornmeal-molasses-agar medium. After the flies were allowed to mate and lay eggs for five days in a 25° incubator, their genotype for eight loci coding for the following enzymes were determined: glutamate oxaloacetate transaminase (E.C. 2.6.1.1), *Got* (*2*–4.8); alpha-glycerophosphate dehydrogenase (E.C. 1.1.1.8), *α-Gpdh* (*2*–20.5); malate dehydrogenase (E.C. 1.1.1.37), *Mdh* (*2*–41.5); alcohol dehydrogenase (E.C. 1.1.1.1), *Adh* (*2*–50.1); tetrazolium oxidase, *To* (*3*–32.5); esterase-6, E_6 (*3*–36.8); esterase-C, E_c (*3*–49); and octanol dehydrogenase, *Odh* (*3*–49.2). Matings in which one of the parents exhibited a rare allele at the triallelic E_c locus were eliminated from further consideration since the band produced by this allele interfered with the E_6^F electromorphs. Eleven matings representing 44 genomes, which exhibited intermediate gene frequencies, were used to initiate four cage populations. Five female and five male progeny from each of these matings were distributed to each population cage. The population cages (plastic boxes with dimensions 200 mm × 105 mm × 80 mm) each had eight scintillation vial food containers. Two experimental cages (E_1 and E_2) were maintained on standard cornmeal-molasses-agar medium supplemented with 10% ethanol. The ethanol was added and mixed directly into the standard medium just prior to dispensing into the food vials. A pair of control cages (C_1 and C_2) were maintained under the same conditions as the experimental cages, except that their medium was not supplemented with ethanol. Both control and treatment food vials were allowed to age for at least one day before they were seeded with live yeast and attached to the cages. Aging the treatment media greatly reduced the initial high levels of vaporized ethanol, which can lead to high adult mortality.

Discrete 18-day generations were imposed on the population cages observing the following sequence: Food vials were changed on day zero. Parents were allowed to mate and lay eggs from day zero to day five and then removed (transfer stage). Following the removal of the parental population, 13 days were allowed for the emergence of the next generation. Next, new food vials were introduced and the 18-day sequence was repeated. For each generation a random sample of 60 males was drawn from two of the four cages at the transfer stage. During odd-numbered generations, samples were drawn from cages C_1 and E_1. In even-numbered generations, samples were taken from C_2 and E_2. Sampled males were individually mated to two virgin females from an ebony tester stock that was monomorphic for all of the eight enzyme loci under investigation. (The ebony stock was used as the homozygous tester stock because contamination by wild type could easily be identified.)

After five days of mating, an average of 58 males were electrophoretically typed for the eight enzymes. A single testcross progeny was electrophoretically typed from all males that were determined to be heterozygous for two or more loci on either the second or third chromosome. This progeny test procedure is tantamount to a direct sampling of gametes, provided we assume that the probability of recombination in male *Drosophila melanogaster* is zero. With this assumption, we can deduce the arrangement of marker allozymes on each chromosome received by a tested male by identifying the paternally derived gametes in single testcross progeny. Progeny testing also permits the detection of null alleles in individuals heterozygous for a null allele and a normal allele.

All flies were enumerated from generation two through six and the relative developmental rate was noted. More than 3,000 genomes were sampled during the course of the experiment.

The electrophoretic assays were conducted on horizontal starch gels. The discontinuous Poulik buffer system (POULIK 1957) was used to assay GOT, ADH, ODH, E_c and E_6. The JRP buffer system (AYALA, POWELL and TRACEY 1972) was utilized in the α-GPDH, MDH, and TO assays. Standard histochemical staining methods were employed to identify the enzymatic banding patterns. The two allozymes segregating at each locus in the experimental populations were designated S (slow) or F (fast) corresponding to electrophoretic mobility.

Twenty or more third-instar larvae were drawn from each experimental population at generation 27. Salivary gland squash preparations were made from these samples, utilizing standard techniques and analyzed for the presence of inversions.

RESULTS

A preliminary experiment had shown a significant reduction in the population number of ethanol-treated replicates after one generation. However, the average effect of ethanol on the two experimental cages (E_1 and E_2) over the five generations enumerated revealed no significant reduction in population number relative to the two control cages, C_1 and C_2. The mean adult population sizes for the control cages and the treatment cages were 429 ± 34.0 and 433 ± 68.0, respectively. Significant differences between the two groups were observed within generations for the first three generations enumerated, but the relative population levels were often reversed between generations. A two-day developmental lag was normally observed for the treatment replicates.

TABLE 1

Observed and expected genotypes for Got, α-Gpdh, Mdh, Adh, To, E_c, E_6, Odh *from Weymouth 74 g stock, together with* χ^2 *goodness-of-fit statistics to random mating expectations*

Locus		Genotypes SS	SF	FF	χ_1^2	Observed proportion heterozygotes
Got	Observed	317	86	2	2.01 n.s.	0.21235
	Expected	320	90	5		
α-Gpdh	Observed	136	233	65	4.71*	0.53687
	Expected	147	211	76		
Mdh	Observed	314	112	7	0.69 n.s.	0.25866
	Expected	316	107	9		
Adh	Observed	266	131	20	0.73 n.s.	0.31415
	Expected	264	136	17		
To	Observed	5	69	326	0.31 n.s.	0.17250
	Expected	4	71	325		
E_6	Observed	140	183	74	1.07 n.s.	0.46096
	Expected	135	193	69		
Odh	Observed	0	46	376	1.21 n.s.	0.10900
	Expected	1	43	377		

		SS	SF	FF	SX	FX	XX	
E_c	Observed	152	138	7	83	37	0	0.61871
	Expected	165	119	21	76	27	9	

$\chi_3^2 = 26.74^{**}$

* $p < 0.05$.
** $p < 0.001$.

We report the gene and genotypic differences for the marker loci in the Weymouth 74g stock (Table 1) because the experimental populations represent a selected sample of this population. Both *α-Gpdh* and E_c show a significant deviation from random mating expectations. In each case the deviation is in the direction of excess heterozygosity. All heterozygous genotypes are in excess at the triallelic E_c locus and, in addition no E_c^{xx} homozygotes were observed, although nine were expected, based upon multinominal sampling. As noted above, the experimental populations were initiated from a selected sample of the Weymouth 74g population in order to begin with intermediate gene frequencies at each of the marker loci. Thus, the initial frequencies range from near equality for *α-Gpdh* and E_6 to 0.80 and 0.86 for Mdh^S and To^F, respectively. A further difference between the source population and the experimental populations was the elimination of the E_c^x allele.

Graphical presentation of the gene frequencies from the control and treatment populations over the 12-generation sampling period indicates four patterns. (1) The relative allelic frequencies of the *Got*, E_6 and *Odh* loci appear to be slowly approaching the gene frequencies of the Weymouth 74g stock reported in Table 1 (Figures 1 and 2). (2) the Mdh^S allele has increased in all cages and is approaching fixation in two of the cages (Figure 2). The E_c^S frequencies have actually diverged from the Weymouth 74g stock values (Figure 3). (3) Although

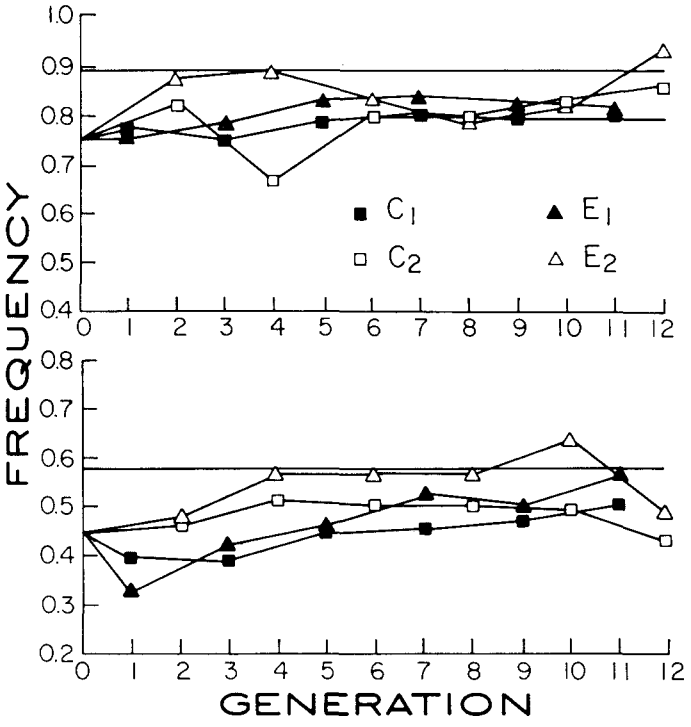

FIGURE 1.—*Got*^{S} (upper figure) and E_6^S (lower figure) frequencies for two control replicates (C_1 and C_2) and two treatment replicates (E_1 and E_2). The horizontal line in each figure represents the Weymouth 74g value.

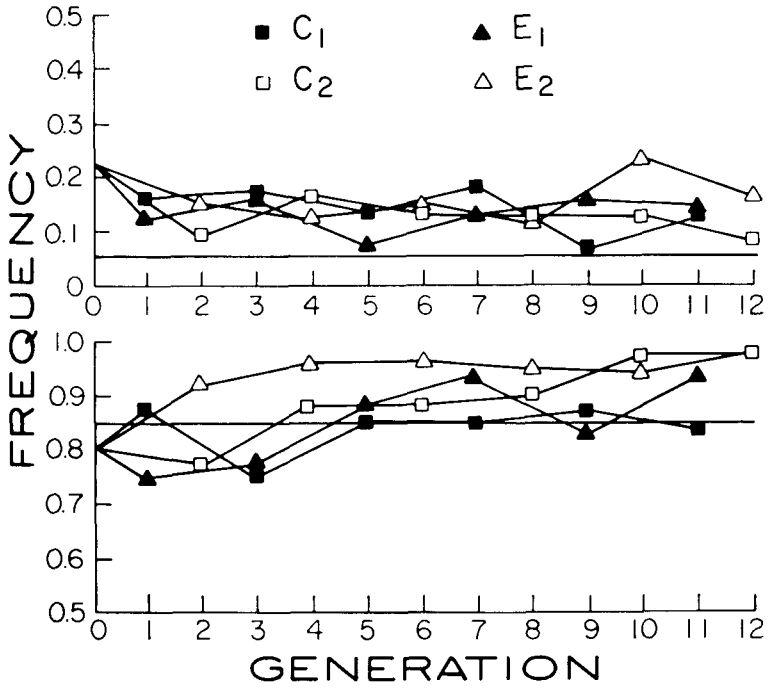

FIGURE 2.—*Odh*^{S} (upper figure) and *Mdh*^{S} (lower figure) frequencies for two control replicates (C_1 and C_2) and two treatment replicates (E_1 and E_2). The horizontal line in each figure represents the Weymouth 74g value.

fluctuations occurred at the *To* locus, this locus apparently maintains a relatively constant frequency (Figure 3). (4) The last pattern is marked by rapid divergence of the treatment allelic frequencies from the control populations for the *Adh* and *α-Gpdh* loci. This divergence first becomes noticeable for *Adh* in replicate E_2 at generation two, but not until generation five for E_1 (Figure 4). By generation seven, the allelic frequencies for the *Adh* locus have diverged appreciably between control and treatment replicates.

Following this rapid increase in the frequency of *Adh*^{F} in the treatment populations, little change is observed until after generation 18. In the interval between generations 18 and 28 the divergence increases, yielding gene-frequency differences between control and treatment populations that range from 0.45 to 0.72 by generation 28 (Table 2). Part of this divergence can be attributed to the increase in frequency of *Adh*^{S} in the control populations that has approached the Weymouth 74g stock value.

α-Gpdh allelic divergence becomes evident after generation three (Figure 5). Clear and replicable differences are observed between the control treatment populations up to generation 12. However, this replication breaks down by generation 28 (Table 2). (These data are not shown in Figure 5.)

Genotypic frequency distributions, which derive from the adult stage, often show significant departures from Hardy-Weinberg expectations, indicating that

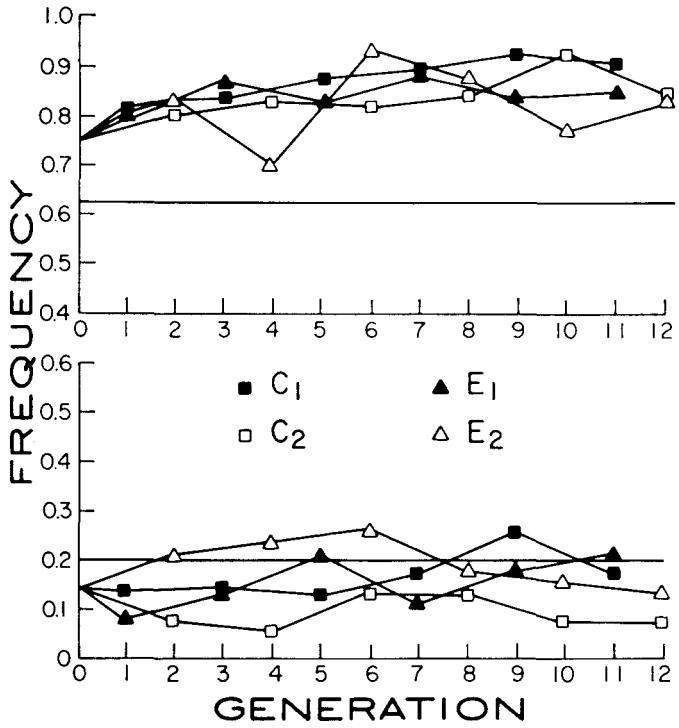

FIGURE 3.—E_c^S (upper figure) and To^S (lower figure) frequencies for two control replicates (C_1 and C_2) and two treatment replicates (E_1 and E_2). The horizontal line in each figure represents the Weymouth 74g value.

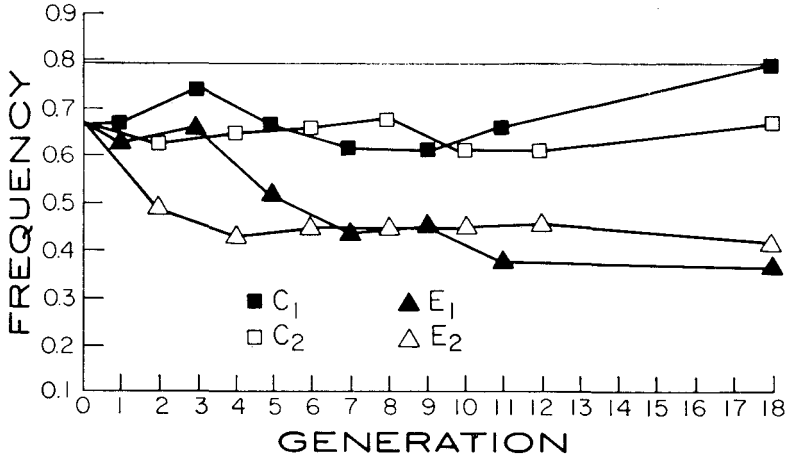

FIGURE 4.—Adh^S frequencies for two control replicates (C_1 and C_2) and two treatment replicates (E_1 and E_2). The horizontal line represents the Weymouth 74g value.

TABLE 2

Gene frequencies for α-Gpdh^S *and* Adh^S *for two control replicates* (C_1 *and* C_2) *and two treatment replicates* (E_1 *and* E_2)

	Gpdh^S				*Adh^S*			
Generation	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2
0	0.538	0.538	0.538	0.538	0.667	0.667	0.667	0.667
	(0.033)	(0.033)	(0.033)	(0.033)	(0.032)	(0.032)	(0.032)	(0.032)
1	0.537		0.405		0.667		0.629	
	(0.048)		(0.046)		(0.045)		(0.045)	
2		0.500		0.529		0.627		0.481
		(0.047)		(0.049)		(0.046)		(0.049)
3	0.492		0.481		0.758		0.664	
	(0.046)		(0.049)		(0.039)		(0.046)	
4		0.608		0.450		0.642		0.425
		(0.044)		(0.045)		(0.044)		(0.045)
5	0.550		0.458		0.658		0.542	
	(0.045)		(0.054)		(0.043)		(0.045)	
6		0.633		0.450		0.658		0.450
		(0.044)		(0.045)		(0.043)		(0.045)
7	0.546		0.470		0.592		0.440	
	(0.045)		(0.047)		(0.045)		(0.046)	
8		0.553		0.359		0.667		0.436
		(0.047)		(0.044)		(0.044)		(0.046)
9	0.544		0.383		0.577		0.461	
	(0.044)		(0.043)		(0.043)		(0.044)	
10		0.460		0.355		0.611		0.460
		(0.047)		(0.043)		(0.046)		(0.045)
11	0.600		0.367		0.658		0.367	
	(0.045)		(0.044)		(0.043)		(0.044)	
12		0.500		0.225		0.600		0.475
		(0.046)		(0.038)		(0.045)		(0.046)
18	0.694	0.458	0.342	0.178	0.798	0.675	0.350	0.408
	(0.041)	(0.047)	(0.043)	(0.035)	(0.036)	(0.043)	(0.044)	(0.045)
28	0.466	0.575	0.593	0.308	0.808	0.642	0.192	0.092
	(0.046)	(0.045)	(0.045)	(0.042)	(0.036)	(0.044)	(0.036)	(0.026)

Standard errors are in parentheses.

most of the loci are affected by selection. However, the departures fluctuate markedly both over time and among replicates. There appears to be little if any identifiable effect of treatment on single-locus frequency distributions. This feature of the data is best illustrated by Wright's fixation index,

$$F = 1 - \frac{H}{2pq}$$

where H is the observed heterozygote frequency and p ($= 1 - q$) is the relative frequency of the slow allozyme. Table 3 reports average values of F over 12 generations. A noteworthy feature of these data is the consistently positive values observed at E_c (indicating a heterozygous deficiency) and the large negative

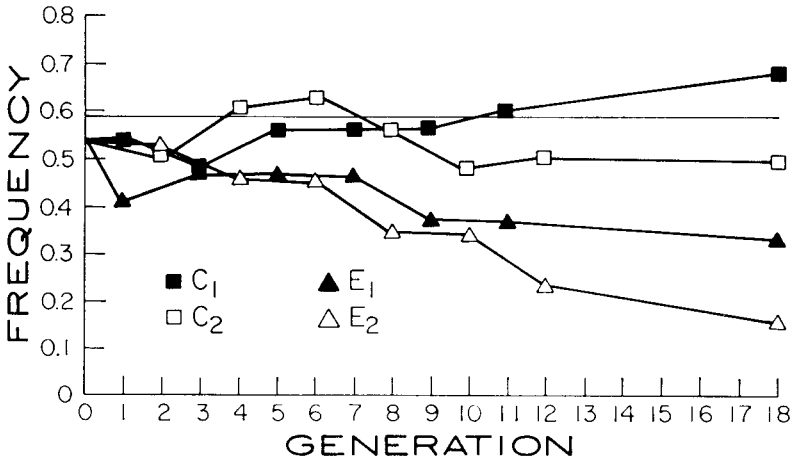

FIGURE 5.—α-*Gpdh*^{S} frequencies for two control replicates (C_1 and C_2) and two treatment replicates (E_1 and E_2). The horizontal line represents the Weymouth 74g value.

values recorded at *Odh* (heterozygotes in excess of random mating expectations), despite the very tight linkage between these two loci ($r = 0.002$). The heterozygous deficiency observed at E_c can not be accounted for by null alleles because no null alleles were detected at any locus, even though progeny testing was extensive. Evidently, the factors influencing the genotypic frequency distribution affect these two tightly linked loci quite differently.

Gametic Frequency Distributions: The scheme employed for sampling gametes permits examination of joint gametic frequency distributions for loci on the same chromosome. Measures of linkage disequilibrium for pairs, triplets and quadruplets of loci can be computed from the joint frequency distributions. The number of disequilibrium statistics is very large when taken over four replicate experiments and 12 generations of data. Consequently, only summaries of the pairwise disequilibria are reported (Tables 4 and 5) and then only for the initial,

TABLE 3

The mean of Wright's fixation index of the Got, α-Gpdh, Mdh, Adh, To, E_6, E_c, *and* Odh *loci for the four experimental populations*

Replicate	*Got*	*αGpdh*	*Mdh*	*Adh*	*To*	E_6	E_c	*Odh*
C_1	0.0136	—0.0060	—0.0455	—0.0006	—0.0336	0.1088	0.1856	—0.1525
	(0.0458)	(0.0521)	(0.0452)	(0.0524)	(0.0458)	(0.0501)	(0.0701)	(0.0278)
C_2	0.0731	—0.0960	—0.0413	—0.1443	0.0136	—0.0223	0.0811	—0.0908
	(0.0549)	(0.0523)	(0.0331)	(0.0499)	(0.0409)	(0.0534)	(0.0575)	(0.0348)
E_1	0.1276	—0.0036	0.0148	—0.0350	—0.0323	—0.1093	0.1406	—0.1723
	(0.0507)	(0.0533)	(0.0571)	(0.0526)	(0.0484)	(0.0529)	(0.0603)	(0.0189)
E_2	—0.0480	—0.0415	—0.0440	—0.1951	—0.1626	—0.1578	0.2243	—0.1820
	(0.0424)	(0.0507)	(0.0162)	(0.0514)	(0.0421)	(0.0517)	(0.0691)	(0.0277)

The empirical standard errors over generations are in parentheses.

TABLE 4

Initial, final, minimum, maximum and mean values of the correlation for each pair of loci (second chromosome)

	Got-α-Gpdh				*Got-Mdh*				*Got-Adh*			
Rep.	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2
Initial	—0.019	—0.019	—0.019	—0.019	0.199	0.199	0.199	0.199	0.229	0.229	0.229	0.229
Final	0.111	0.137	0.221	0.248	0.056	0.101	—0.118	—0.038	—0.035	0.031	—0.182	0.140
Min.	—0.019	—0.210	—0.019	—0.019	—0.067	—0.058	—0.126	—0.083	—0.193	—0.031	—0.182	0.074
Max.	0.202	0.137	0.314	0.414	0.284	0.268	0.200	0.363	0.229	0.229	0.229	0.229
$\bar{X}$	0.093	0.026	0.139	0.214	0.117	0.109	0.045	0.189	0.013	0.076	0.042	0.133
	α-Gpdh-Mdh				*α-Gpdh-Adh*				*Mdh-Adh*			
Rep.	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2
Initial	—0.242	—0.242	—0.242	—0.242	0.153	0.153	0.153	0.153	0.062	0.062	0.062	0.062
Final	—0.115	0.053	—0.189	—0.086	0.201	0.190	—0.184	0.049	0.228	0.131	0.253	0.138
Min.	—0.516	—0.261	—0.274	—0.264	—0.193	—0.063	—0.194	—0.103	0.062	0.062	0.062	0.062
Max.	0.016	0.053	—0.152	—0.086	0.201	0.190	0.153	0.212	0.228	0.303	0.320	0.279
$\bar{X}$	—0.217	—0.111	—0.214	—0.178	0.027	0.077	—0.056	0.088	0.171	0.139	0.206	0.188

TABLE 5

Initial, final, minimum, maximum and mean values of the correlation for each pair of loci (third chromosome)

	To-E$_6$				*To-E$_o$*				*To-Odh*			
Rep.	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2
Initial	0.081	0.081	0.081	0.081	—0.280	—0.280	—0.280	—0.280	0.115	0.102	0.097	—0.078
Final	0.161	0.249	0.152	0.307	0.009	—0.064	0.115	—0.072	0.188	0.096	0.162	—0.052
Min.	—0.172	—0.279	—0.203	—0.070	—0.280	—0.280	—0.280	—0.280	—0.135	—0.004	0.081	—0.078
Max.	0.161	0.081	0.218	0.332	0.096	0.007	0.128	—0.032	0.188	0.112	0.192	0.134
$\bar{X}$	0.063	—0.162	0.021	0.109	—0.018	—0.110	—0.014	—0.100	0.080	0.082	0.154	0.046

	E$_6$-E$_o$				*E$_6$-Odh*				*E$_o$-Odh*			
Rep.	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2	C_1	C_2	E_1	E_2
Initial	—0.094	—0.094	—0.094	—0.094	—0.228	—0.161	—0.046	—0.264	—0.134	—0.123	—0.191	—0.160
Final	0.076	—0.017	—0.242	—0.038	—0.166	—0.076	—0.044	—0.190	—0.043	—0.134	—0.171	—0.154
Min.	—0.144	—0.196	—0.256	—0.134	—0.256	—0.161	—0.331	—0.307	—0.137	—0.208	—0.200	—0.260
Max.	0.098	0.063	—0.071	0.211	—0.089	—0.079	—0.076	—0.037	0.058	—0.115	—0.078	—0.081
$\bar{X}$	—0.045	—0.084	—0.145	0.028	—0.206	—0.088	—0.117	—0.170	—0.066	—0.154	—0.151	—0.153

final, minimum, maximum and average values for each pairwise comparison. The actual statistic reported is the normalized linkage disequilibrium or correlation,

$$r = \frac{g_{11}g_{22} - g_{12}g_{21}}{[(g_{11} + g_{12})(g_{21} + g_{22})(g_{11} + g_{21})(g_{12} + g_{22})]^{1/2}}$$

where g_{11}, g_{12}, g_{21} and g_{22} denote the relative frequencies of gametes bearing the allozymes SS, SF, FS and FF, respectively. Two notable features of these data are: (1) associations are generally weak and/or inconsistent over time and replicates; and (2) no differences in the pattern of association occur between the control and treatment populations.

The most replicable correlations occur between *Mdh* and *Adh* and between *α-Gpdh* and *Mdh*, which are shown in Figure 6. Despite the constant association between *Mdh* and *Adh*, the gene frequency dynamics observed at these loci are quite different. Specifically, Mdh^S exhibits a gradual increase in frequency in all replicates, while Adh^S shows markedly different behaviors between control and treatment replicates (Figures 2 and 4).

Inversion associations: Two third-chromosome inversions were detected in the four experimental populations (Table 6). The more frequent inversion was identified as the cosmopolitan *In(3R)Mo* (BRIDGES and BREHME 1944) between 93D and 98F. The other inversion, found only in C_2, was localized on the left arm between 66C and 71B and may be the same as *In(3L)F* (WATANABE 1967) or *In(3L)E* (STALKER 1976).

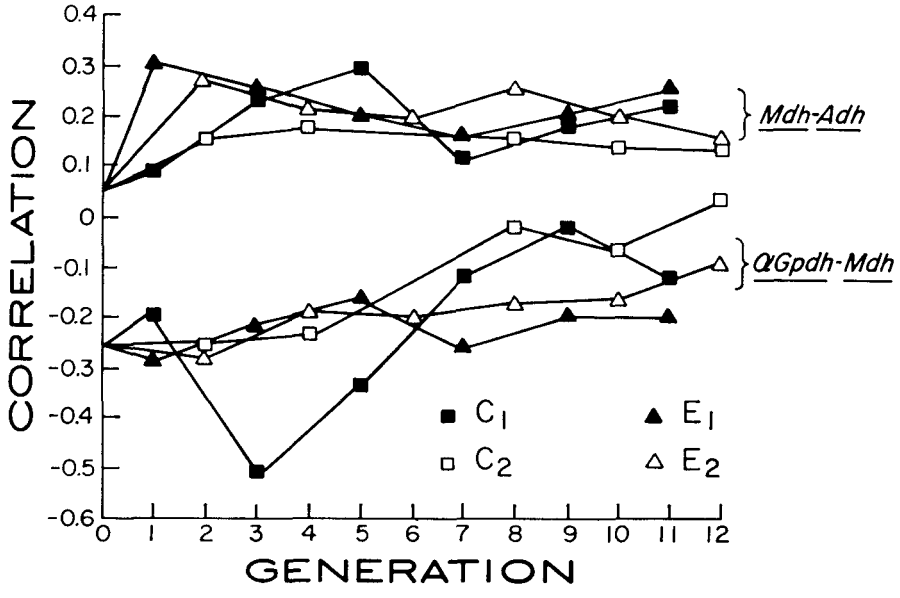

FIGURE 6.—Gametic correlation for *Mdh* and *Adh* (upper figure) and for *α-Gpdh* and *Mdh* (lower figure).

TABLE 6

Number of heterozygotes for two third-chromosome inversions for samples drawn from generation 27 (Sample size = N)

Population	*N*	Number of heterozygotes for *In (3R) Mo*	*In (3L) F*
C_1	20	9	0
C_2	21	5	2
E_1	20	13	0
E_2	23	11	0

No second-chromosome inversion heterozygotes were found among the 84 larvae sampled from the control and treatment populations. With a sample size of 84, the probability of failing to detect an inversion heterozygote with a population frequency of 0.05 is 0.013. Therefore, the gene frequency changes observed for *α-Gpdh* and *Adh* on the second chromosome cannot be ascribed to their association with inversions.

DISCUSSION

The central results of the present experiment can be summarized as: (1) Rapid gene frequency changes occurred at the *Adh* locus in replicate populations subjected to ethanol stress. (2) Linkage disequilibria among the eight marker loci were small. (3) Only two polymorphic inversions were present in the experimental populations and neither of them involved chromosome *2* marker loci. (4) Of the seven remaining marker loci, only *α-Gpdh* appeared to show a differential gene-frequency response to the ethanol stress.

What are the possible explanations for the gene-frequency behavior observed at *Adh*? On several grounds, drift can be eliminated as a cause of the observed gene-frequency change. First, population sizes were relatively large. Second, the pattern of gene-frequency change is very different between control and treatment replicates. And third, the between-replicate variance in gene frequency is much smaller for all the remaining marker loci. Similarly, mutation and migration can be excluded as causal explanations. The only plausible cause for the gene-frequency change is selection.

It is more difficult to establish whether selection is acting directly on the ADH polymorphism or whether other loci, in linkage disequilibrium with *Adh*, are responsible for the behavior observed at *Adh*. The design of the experiment does provide for some information on the latter point. To begin with, six of the marker loci show no differential response to the ethanol stress. *Adh*, on the other hand, shows a replicable response. Evidently, loci responding to the ethanol stress must exist in the neighborhood of *Adh*, but not in the neighborhood of the other six loci. Furthermore, linkage disequilibria are small even among very tightly linked loci (*e.g.*, E_c—*Odh*). Consequently, other selected loci, if they exist, must be very tightly linked to *Adh* to permit substantial linkage disequilibria. In addi-

tion, the gene-frequency changes observed at *α-Gpdh* must result from loci different from those causing the response at *Adh*, because only trivial levels of linkage disequilibrium exist between *Adh* and *α-Gpdh*, which are almost 30 map units apart.

The fact that the Adh^F allele increases in frequency in the alcohol environment strongly suggests that selection is acting directly on the different *Adh* genotypes. Such an increase is consistent with the activity hierarchy of the *Adh* genotypes within populations: FF > FS > SS (GIBSON 1970; DAY, HILLER and CLARKE 1974; MORGAN 1974). Other laboratory experiments have shown an increase in the Adh^F allele under ethanol stress (GIBSON 1970; BIJLSMA-MEELES and VAN DELDEN 1974; VAN DELDEN, KAMPING and VAN DIJK 1975), a lower mortality of Adh^{FF} flies under extreme alcohol stress (MORGAN 1974) and a differential increase in longevity by Adh^{FF} *D. mojavensis* sub-races reared in atmospheric alcohol (STARMER, HEED and ROCKWOOD-SLUSS 1977). Furthermore, surveys of allozyme variation in *D. melanogaster* have shown higher frequencies of the Adh^F allele in flies inhabiting floating mats in fermenting wine (BRISCOE, ROBERTSON and MALPICA 1975), although MCKENZIE and PARSONS (1974) found no differences in allozyme frequencies between vineyard and wine celler populations. Since the stocks utilized in our investigation and in the other ethanol stress investigations were collected from diverse habitats and have diverse genetic backgrounds, the positive Adh^F selection in all investigations is strong evidence that selection is acting directly upon the *Adh* locus.

One further point regarding the Adh behavior requires comment. The gene-frequency changes observed at *Adh* are quite rapid, which implies strong selection in the alcohol environment. Therefore, it appears that it may be possible to induce relatively large selective differences at an enzyme locus through suitable environmental manipulations.

The pattern of frequency change observed at *α-Gpdh* is more difficult to explain. There were no prior grounds for expecting such a frequency change, and the agreement among replicates, while good for the first 18 generations of the experiment, appears to have broken down by generation 28. Nevertheless, the pattern of frequency change at *α-Gpdh* merits consideration. The first point to establish is that selected factors different from those influencing *Adh* must be affecting *α-Gpdh*, because gene-frequency changes at *α-Gpdh* in the treatment population begin in generations six and seven after all the initial gene-frequency change at *Adh* is complete. Secondly, as already noted, the level of linkage disequilibrium between *Adh* and *α-Gpdh* is small. It may very well be that genes that are involved in adaptation to ethanol-containing environments exist in the neighborhood of *α-Gpdh*, and in linkage disequilibrium with *α-Gpdh*. There are, however, biochemical grounds for positing selection directly on the alternative *αGpdh* genotypes due to a secondary effect of the ethanol stress.

The first step in ethanol metabolism involves ADH and a co-factor, NAD^+. The electrons transferred from ethanol to NAD^+ are then transferred to the mitochondria to produce energy *via* one of the electron shuttle systems. The system implicated as being the most critical in insects involves the transfer of

electrons from NADH to α-glycerophosphate, which is able to penetrate the mitochondrion membrane and deliver its electrons to the electron-transport system to produce energy primarily for flight (SAKTOR 1970).

The critical nature of α-GPDH to this shuttle and to energy production is dramatically illustrated by null α-GPDH mutants, which are variable and fertile but cannot sustain flight (OBRIEN and MACINTYRE 1972). The metabolism of large amounts of ethanol may lead to large excesses of NADH, which in turn may have a different effect on α-GPDH allozymes. MILLER, PEARCY and BERGER (1974) have shown that high concentrations of NADH inhibit normal catalysis, but that larger amounts of NADH were required to inhibit α-GPDH^{FF} than those required to inhibit either αGPDH^{SS} or α-GPDH^{FS}. At the moment, such an explanation must remain speculative, but more attention needs to be focused on the biochemical relationships of polymorphic enzymes. Since metabolism is a coordinated and interactive process, the adaptive nature of enzyme polymorphisms can most accurately be assessed by investigating systems of polymorphic enzymes (JOHNSON 1976). Such systems may respond adaptively not only to environmental variability, but also to the drastic variation in energy sources and consumption that accompany the complex life cycle in Drosophila and other genera that undergo metamorphosis.

We are grateful to M. G. KIDWELL and J. F. KIDWELL for the use of their Drosophila laboratory facilities at Brown University and to CAROL HORSCH, JILL BERNSTEIN and DEBORAH CLEVENGER for excellent technical assistance. This work was supported in part by National Science Foundation Grants DEB76-01987 and DEB76-82479.

LITERATURE CITED

AYALA, F. J., J. R. POWELL and M. L. TRACEY, 1972 Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics **70**: 113–139.

BRIDGES, C. B. and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Pub. No. 552.

BIJLSMA-MEELES, E. and W. VAN DELDEN, 1974 Intra- and interpopulation selection concerning the alcohol dehydrogenase locus in *Drosophila melanogaster*. Nature **247**: 369–371.

BRISCOE, D. A., A. J. ROBERTSON and J. M. MALPICA, 1975 Dominance at the ADH locus in response of adult *Drosophila melanogaster* to environmental alcohol. Nature **255**: 148.

DAY, T. H., P. C. HILLER and B. CLARKE, 1974 Properties of genetically polymorphic isozymes of alcohol dehydrogenase in *Drosophila melanogaster*. Biochem. Genet. **11**: 141–152.

DELDEN, W. VAN, A. KAMPING and M. VAN DIJK, 1975 Selection at the alcohol dehydrogenase locus in *Drosophila melanogaster*. Experientia **31**: 418.

GIBSON, J., 1970 Enzyme flexibility in *Drosophila melanogaster*. Nature **227**: 959–960.

JOHNSON, G., 1976 Genetic polymorphism and enzyme function. pp. 46–59. In: *Molecular Evolution*. Edited by F. J. AYALA. Sinauer Associates, Inc., Sunderland, Massachusetts.

LEWONTIN, R. C., 1974 *The Genetic Basis of Evolutionary Change*, Columbia University Press, New York.

MCKENZIE, J. A. and P. A. PARSONS, 1974 Microdifferentiation in a natural population of *Drosophila melanogaster* to alcohol in the environment. Genetics **77**: 385–394.

MILLER, S., R. W. PEARCY and E. BERGER, 1975 Polymorphism at the α-glycerophosphate dehydrogenase locus in *Drosophila melanogaster*. I. Properties of adult allozymes. Biochem. Genet. **7**: 331–345.

MORGAN, P., 1974 Selection acting directly on an enzyme polymorphism. Heredity **34**: 124–127.

OBRIEN, S. J. and R. J. MACINTYRE, 1972 The α-glycerophosphate cycle in *Drosophila melanogaster*. II. Genetic aspects. Genetics **71**: 127–128.

POULIK, M. D., 1957 Starch gel electrophoresis in a discontinuous system of buffers. Nature **180**: 1477.

SAKTOR, B., 1970 Regulation of intermediary metabolism, with special reference to the control mechanisms in insect flight muscles. Adv. Insect Physiol. **7**: 267–338.

STALKER, H. D., 1976 Chromosome studies in wild populations of *D. melanogaster*. Genetics **82**: 323–347.

STARMER, W. T., W. B. HEED and E. S. ROCKWOOD-SLUSS, 1976 Extension of longevity in *Drosophila mojavensis* by environmental ethanol: Differences between subraces. Proc. Nat. Acad. Sci. U.S. **74**: 387–391.

WATANABE, T., 1967 Chromosomal polymorphism in natural populations of *Drosophila melanogaster*. Mem. Fac. Sci. Kyushu Univ., Ser. E. **4**: 159–182.

Corresponding editor: W. W. ANDERSON