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Allozymic Variation in Drosophila melanogaster from Virginia

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ALLOZYMIC VARIATION IN *DROSOPHILA MELANOGASTER* FROM VIRGINIA

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

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ABSTRACT

Populations of Drosophila melanogaster were sampled at six stations in Virginia and West Virginia. Allele and genotype frequencies were examined for both the Esterase 6 and Alcohol dehydrogenase loci. The results of this study indicate that segregation occurred at both loci at all locations sampled. There appeared to be a cline in the frequency of Esterase 6 alleles over distance. Heterosis was indicated at two of the sample sites for Esterase 6.

ALLOZYMIC VARIATION IN *DROSOPHILA MELANOGASTER* FROM VIRGINIA

INTRODUCTION

Although laboratory studies have contributed much to our understanding of evolutionary processes, and will continue to do so, the foundations upon which the theory of natural selection is based should come from studies of natural populations. In the past decade, the study of allozymic variation through gel electrophoresis has become a valuable tool in such research. Investigations performed on many different species reveal numerous genetically polymorphic enzyme systems in natural populations (see review by Stone, Kojima, and Johnson, 1969). The purposes of this paper are to determine and to analyze genotypic variation for the Esterase 6 and Alcohol dehydrogenase loci in samples of Drosophila melanogaster captured at various locations in Virginia.

METHODS AND MATERIALS

Drosophila melanogaster is a cosmopolitan species which is found across the state of Virginia in close association with man. Natural populations of D. melanogaster were sampled at six sites ranging across the state of Virginia into West Virginia. The locations are described in Table 1 and Figure 1. After identification, the flies were frozen and stored at -8°C. until they could be assayed by electrophoresis. Flies were stored no longer than seven months.

Electrophoretic procedure. Each fly was ground in 0.01 ml of distilled water. The homogenate was then absorbed onto a 10 x 2 mm strip of Whatman No. 3 MM filter paper, and each strip was inserted into a vertical slit made in the starch gel. Horizontal gel electrophoresis was performed as described by Beckman and Johnson (1964), using the discontinuous buffer system of Poulik (1957). Gels were prepared with Electro-starch Lot 146 (Otto Hiller) at a concentration of 12.5%. Each gel contained, on the average, 26 samples. An electric current of 13.5 watts was passed through the gel until the buffer front had moved 5 cm past the origin (approximately 2-3 hours). After electrophoresis, the gel was sliced into replicate sections and stained. The

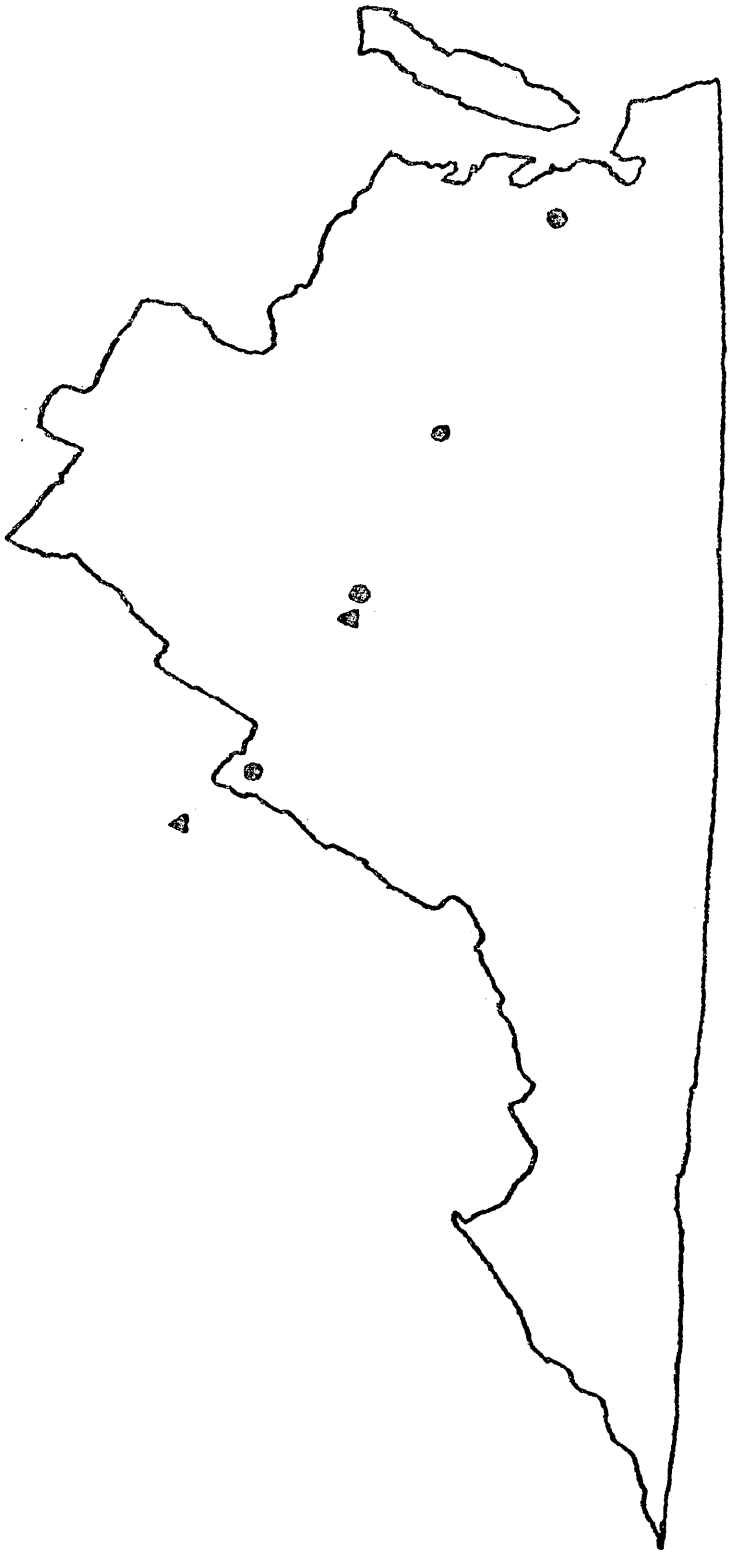
TABLE 1

COLLECTION SITES

Distance*	Site and description
0	Hunt's Farm - orchard in the coastal plain
56	Short Pump - mixed forest in the Piedmont
130	Afton Mountain - mixed forest on the Blue Ridge
135	Waynesboro - fruit market in a mountain valley
182	Monterey Mountain - abandoned orchard on the side of a mountain
219	Elkins - fruit market in a mountain valley

* Approximate air miles from Hunt's Farm.

Figure 1. Collection sites. Circles represent wild-caught samples and triangles represent those samples captured in fruit markets. See Table 1 for a description of these sites.



following enzyme systems were initially stained: Esterase C (EST C), Esterase 6 (EST 6), Alcohol dehydrogenase (ADH), Malic dehydrogenase (MDH), Octanol dehydrogenase (ODH), and α-Glycerophosphate dehydrogenase (α-GDH). Storage of the flies at -8°C. for one month reduced the activity of EST C, MDH, ODH, and α-GDH to the point that they were impossible to score.

Esterase 6 was stained at 22°C. for 90 minutes with the methods described in Johnson, et al. (1966) as modified by Johnson (personal communication): 0.0015 gm α-Naphthyl acetate (dissolved in 1.5 ml 50% acetone), 0.002 gm β-Naphthyl acetate (in 2 ml acetone), 5 ml n-propanol, 0.034 gm Fast Garnet GBC Salt (in 5 ml water), and 100 ml of sodium phosphate buffer pH 6 (made by dissolving 0.536 gm Na_2HPO_4 and 1.39 gm NaH_2PO_4 in water to 100 ml).

Alcohol dehydrogenase was stained as follows (Johnson, personal communication): 50 ml 0.1 M Tris-HCl buffer pH 8.5, 2 ml isopropanol, 0.02 gm NAD^+ (in 2 ml water), 0.006 gm Phenazine methosulfate (in 1 ml water), and 0.02 gm Nitroblue tetrazolium (in 2 ml water). The gel was placed in the stain and then incubated in the dark at 22°C. for 90 minutes.

After staining, the gels were fixed in a 1:5:5 mixture of acetic acid, methanol, and water, respectively.

RESULTS

EST 6 is an enzyme system in which each homozygote has a specific single band and the heterozygotes have two. As first described by Wright (1963), there were two alleles, Fast and Slow. Since then, several rare alleles have been discovered (Johnson, personal communication) and the alleles are numbered with the lowest representing the allozyme which migrates the most anodally. EST 6^4 represents Wright's EST 6^{Fast} and EST 6^6 is EST 6^{Slow}. Both of these alleles were found in all populations sampled in this study; EST 6^5 was found occasionally. Figure 2 presents a diagrammatic representation of the zymograms for each observed phenotype. ADH, as discussed by Johnson and Denniston (1964), has two alleles, but a somewhat more complex pattern of bands than EST 6. The alleles are designated ADH^4 and ADH^6 in the same manner as EST 6. Flies which are homozygous produce two bands, whereas heterozygous flies produce five (see Figure 2). All bands except for one produced by ADH^6 migrate anodally.

The observed numbers of phenotypes and the allele frequencies for each sample site are presented in Tables 2 and 3, along with a χ^2 value and the standard error for p (a given allele frequency). The standard error of p was calculated as: $\sqrt{\frac{p(1-p)}{2n}}$ where n equals the number of individ-

Figure 2. A schematic representation of the zymograms for ADH and EST 6.  represents red bands,  blue-black bands.

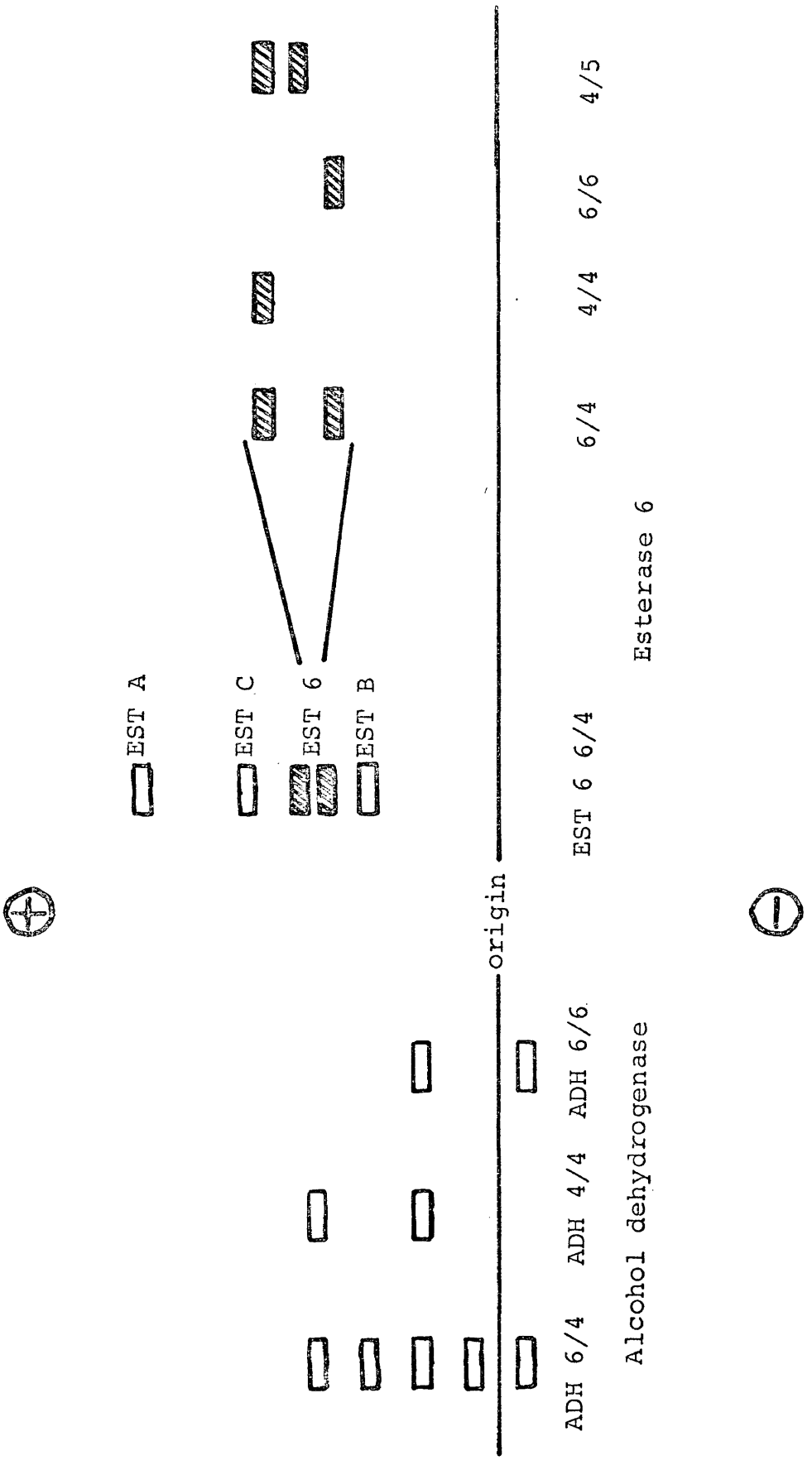

EST A
EST C
EST 6
EST B
origin
EST 6 6/4
6/4
4/4
6/6
4/5
Esterase 6
ADH 6/4
ADH 4/4
ADH 6/6
Alcohol dehydrogenase

TABLE 2

EST 6: PHENOTYPIC DISTRIBUTION AND ALLELE FREQUENCIES

Sample Site	Sample Size	Observed Phenotypes 6/6	6/4	4/4	4/5	Allele Frequency 6	4	5	X^2
Hunt's Farm	50	12	27	11	0	.51±.05	.49	.00	0.32
Short Pump[1]	57	20	23	13	1	.55±.05	.44	.01	3.13
Afton	35	13	21	1	0	.67±.06	.33	.00	4.51*
Waynesboro[1]	76	17	34	23	2	.45±.04	.54	.01	2.15
Monterey	36	16	19	1	0	.71±.05	.29	.00	2.77
Elkins	104	23	67	14	0	.54±.03	.46	.00	9.24**
POOLED[1]	358	101	191	63	3	.549±.018	.447	.004	6.41

[1]- The expected numbers used in calculating the X^2 were obtained through use of Levene's formulae.

* $0.01<p<0.05$
** $0.001<p<0.01$

TABLE 3

ADH: PHENOTYPIC DISTRIBUTION AND ALLELE FREQUENCIES

Sample Site	Sample Size	Observed Phenotypes 6/6	6/4	4/4	Allele Frequency 6	4	X^2
Hunt's Farm	96	36	39	21	.58±.03	.42	2.68
Short Pump	68	37	28	3	.75±.04	.25	1.89
Afton	41	16	16	9	.59±.05	.41	2.55
Waynesboro	86	35	38	13	.63±.04	.37	0.26
Monterey	35	14	12	9	.57±.06	.43	3.15
Elkins	136	52	67	17	.63±.03	.37	0.38
POOLED	462	190	200	72	.63±.01	.37	2.55

uals sampled (Freund, 1967). Chi-square values were calculated comparing the observed phenotypic distribution with that expected on the basis of the Hardy-Weinberg formula.

Polymorphic Loci. The data presented in Tables 2 and 3 make it clear that both EST 6 and ADH are polymorphic at all locations sampled in this study.

Geographic Distribution of Allele Frequencies. Three statistical analyses were employed to determine how allele frequencies changed along the transect.

The z approximation, $\frac{p_i - p_j}{\sqrt{\frac{p_i(1-p_i)}{2n_i} + \frac{p_j(1-p_j)}{2n_j}}}$ was used to test for significant differences in allele frequency between two sample sites. Tables 4 and 5 show the results of these tests. For ADH, there is no apparent pattern as to which frequencies differ significantly among the samples. However, a similar consideration of the EST 6 data indicates the converse. Each allele frequency was not significantly different from the frequency at the adjacent sample, but a comparison of the wild-caught samples across a greater range proved statistically significant.

The data within each enzyme system were pooled (Tables 2 and 3) and compared via χ^2 analysis with the expected Hardy-Weinberg frequencies. If the allele frequencies differ significantly among the populations included in the pooled population, then one would expect a distribution

TABLE 4

Z VALUES TESTING THE HYPOTHESIS THAT $p_i=p_j$, FOR ADH
(See text for description)

	Hunt's Farm	Short Pump	Afton	Waynesboro	Monterey	Elkins
Hunt's Farm	0	3.315 ***	0.154	0.979	0.145	1.089
Short Pump	3.315 ***	0	1.674	2.301 *	2.579 **	2.547 *
Afton	0.154	1.674	0	0.601	0.249	0.649
Waynesboro	0.979	2.301 *	0.601	0	0.861	0.000
Monterey	0.145	2.579 **	0.249	0.861	0	0.910
Elkins	1.089	2.547 *	0.649	0.000	0.910	0

* $0.01<p<0.05$
** $0.001<p<0.01$
*** $0.0001<p<0.001$
**** $p<0.0001$

TABLE 5

Z VALUES TESTING THE HYPOTHESIS THAT $p_i=p_j$, FOR EST 6
(See text for description)

	Hunt's Farm	Short Pump	Afton	Waynesboro	Monterey	Elkins
Hunt's Farm	0	0.586	2.131 *	0.936	2.737 **	0.495
Short Pump	0.586	0	1.645	1.624	2.258 **	0.173
Afton	2.131 *	1.645	0	3.186 **	0.516	1.973 *
Waynesboro	0.936	0.586	3.186 **	0	3.889 ****	1.698
Monterey	2.737 **	2.258 *	0.516	3.889 ****	0	2.675 **
Elkins	0.495	0.173	1.973 *	1.698	2.675 **	0

* $0.01<p<0.05$
** $0.001<p<0.01$
*** $0.0001<p<0.001$
**** $p<0.0001$

which is biased in favor of homozygotes. The χ^2 values were not significant.

Linear regressions were performed by the least squares method using miles from Hunt's Farm as the abscissa and allele frequency as the ordinate. This analysis included all points for each enzyme system and the wild samples only. For EST 6 (Figure 3), the regression on the wild samples suggests a cline, across the state, with b= 0.00117 and r^2= 0.97. For ADH (Figure 4), neither the wild sample regression line nor the regression line on all six samples was significant.

Hardy-Weinberg Ratios. Although the author recognizes that the use of χ^2 may be inappropriate when the expected values are as low as they were for several of these tests, χ^2 was used as a means of comparing the observed phenotypic distribution with that expected on the basis of the Hardy-Weinberg frequencies. In certain cases, Levene's formulae were used to predict the expected numbers. This was only done on those samples which are defined as small (Li, 1955). Considering one locus at a time, only two significant deviations from expected values were found. In the Elkins sample and the Afton sample, the number of EST 6^6/EST 6^4 heterozygotes observed was significantly higher than expected.

These loci are not linked; EST 6 is on the 3rd chromosome (Wright, 1963) and ADH on chromosome number 2 (Grell, Jacobson, and Murphy, 1965). Chi-square tests were performed

Figure 3. Frequency of EST 6^6 vs. miles from Hunt's Farm. Circles represent wild-caught samples and triangles are samples from fruit markets. The vertical lines represent the standard error of the allele frequency. The letter by each point indicates the collection site (see Table 1), and the numeral in parenthesis is the number of flies used in estimating the allele frequency. The solid line is the regression line for the wild-caught samples.

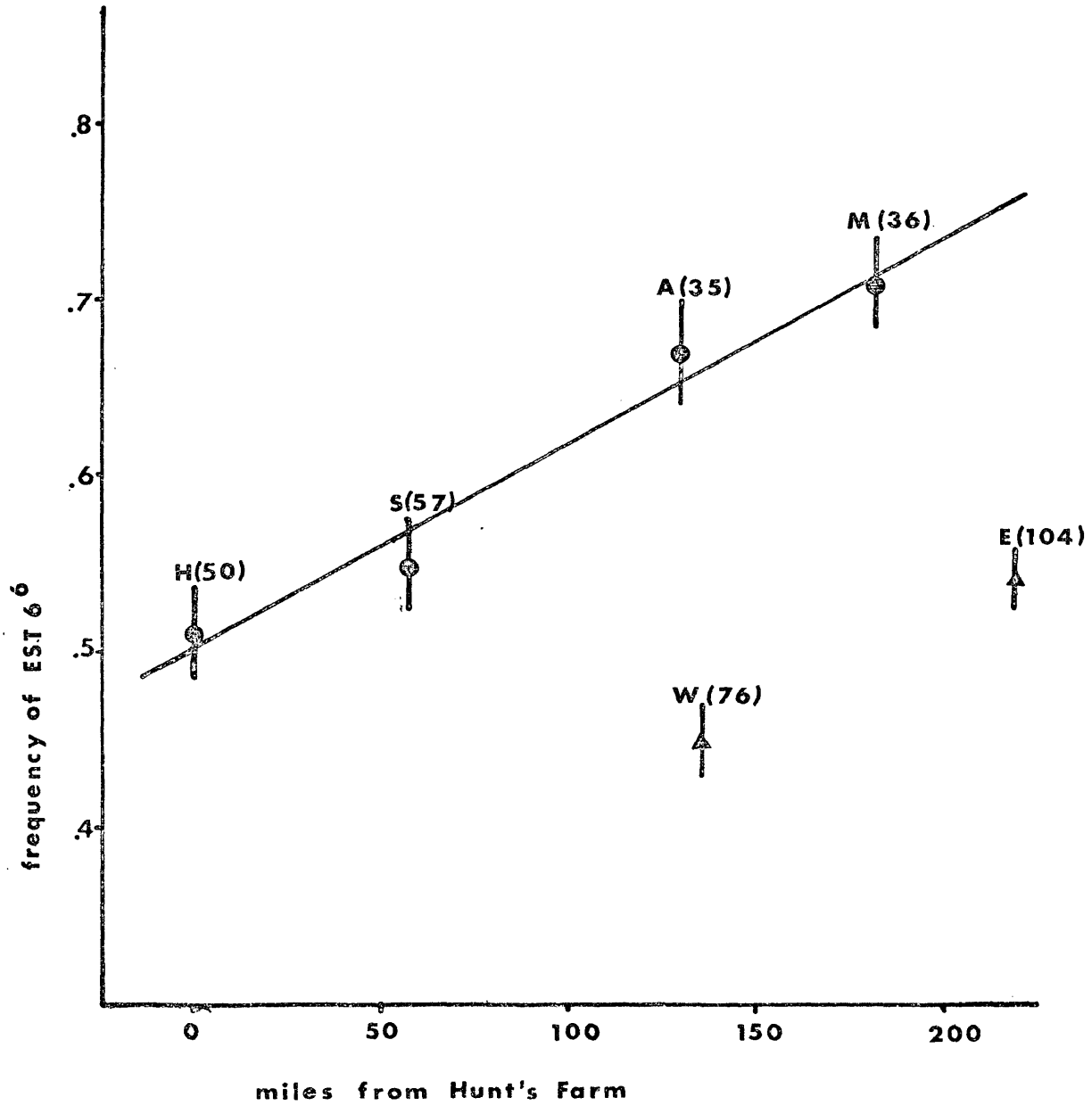

.8
.7
.6
.5
.4
frequency of EST 6 ó
M (36)
A (35)
S(57)
H(50)
E(104)
W (76)
0
50
100
150
200
miles from Hunt's Farm

Figure 4. Frequency of ADH^6 vs. miles from Hunt's Farm. See Figure 3 for explanation of symbols used.

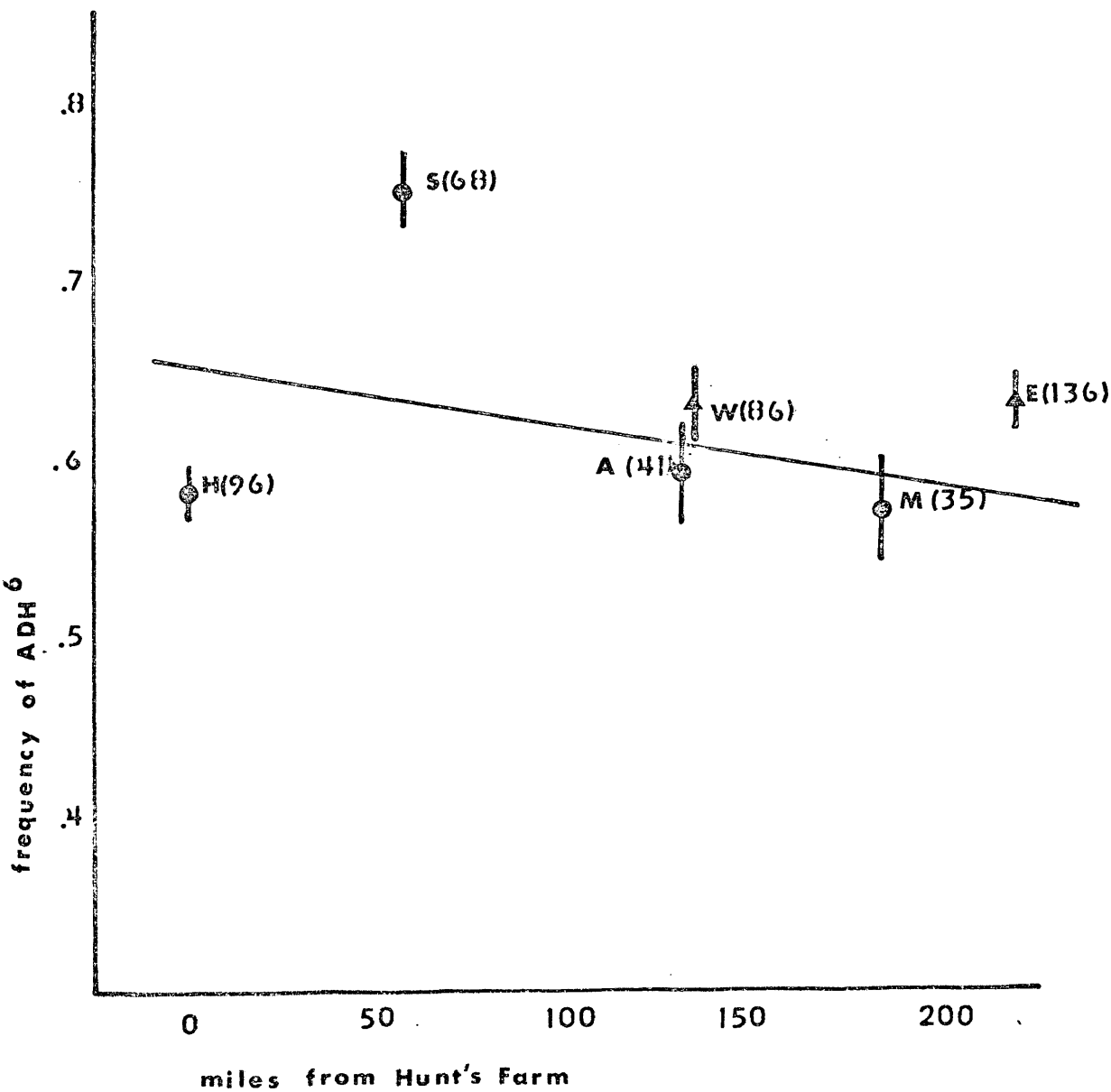

.8
.7
.6
.5
.4
frequency of ADH 6
S(68)
W(86)
E(136)
H(96)
A (41)
M (35)
0
50
100
150
200
miles from Hunt's Farm

TABLE 6

GENOTYPIC DISTRIBUTION CONSIDERING EST 6 AND ADH

Phenotype #	1	2	3	4	5	6	7	8	9	10	11	12			
Sample Site	ADH 6/6 EST 6/6	ADH 6/6 EST 6/4	ADH 6/6 EST 4/4	ADH 6/6 EST 4/5	ADH 6/4 EST 6/6	ADH 6/4 EST 6/4	ADH 6/4 EST 4/4	ADH 6/4 EST 4/5	ADH 4/4 EST 6/6	ADH 4/4 EST 6/4	ADH 4/4 EST 4/4	ADH 4/4 EST 4/5	Total	d. F.	x^2
Hunt' Farm	10	5	5	0	3	12	3	0	2	7	2	0	49	6	11.985
Short Pump	12	13	3	1	8	10	8	0	1	0	1	0	57	14	11.039
Afton	3	8	0	0	5	10	0	0	1	2	1	0	30	6	7.748
Waynesboro	8	16	4	0	5	17	2	1	4	1	1	1	60	14	31.044***
Monterey	5	9	0	0	3	8	0	0	6	2	1	0	34	6	13.272*
Elkins	17	24	7	0	10	39	4	0	4	9	1	0	115	6	15.371**

* $0.0250<p<0.0500$
** $0.0100<p<0.0250$
*** $0.0050<p<0.0100$

to determine whether the populations were in equilibrium considering both loci jointly. Table 6 presents the data and calculated χ^2 values. As some of the gels did not stain well for both EST 6 and ADH for all flies, the sample sizes and allele frequencies differ somewhat from those considered in Tables 2 and 3. The χ^2 calculated for the Waynesboro sample is significant, but this is perhaps a statistical artifact of the extremely low expected number for class #10. The Monterey sample also generated a significant deviation, which is mainly attributable to excesses in classes 2 and 9 and a deficiency in class 5. The Elkins sample again showed significant deviations from expected. The deviation here is caused by discrepancies involving those classes (5, 6, and 7) in which the ADH phenotype is 6/4. Although the total observed in these three classes was close to that expected (53:52.4, respectively), the distribution of EST 6 phenotypes within these classes was not what was expected. More of these were also heterozygous for EST 6 (class 6; 39 observed: 24.49 expected), and deficiencies were found for both of the EST 6 homozygotes (classes 5 and 7).

DISCUSSION

Since the classic paper by Hubby and Lewontin (1966), evolutionary biologists have been aware that natural populations maintain a large amount of genetic variability. This led to considerable controversy in population genetics as to what mechanisms might, in part, account for the maintenance of this genetic heterogeneity. Overdominance in the classical sense of heterozygote superiority was suggested. The possibility that this exists for numerous independent loci in a given population has been seriously questioned on the grounds that it produces a prohibitive segregational load even at equilibrium (Lewontin and Hubby, 1966). Other authors (Sved, Reed and Bodmer, 1967 and Milkman, 1967) disagree, maintaining that it is possible for a natural population to sustain a large number of heterotic loci. Another suggestion was weak selection coupled with high mutation rates. Tobari and Kojima (1972) studied mutation rates at various allozyme loci. For ADH, they observed no mutations in 8.8×10^4 flies and for EST 6 they found none in 5.8×10^4 flies. It would seem doubtful that mutation rates this low could account for the observed variations in the face of even moderate selection. Migration has also been put forward to explain the large numbers of

segregating loci observed in nature. Although melanogaster is a highly mobile species, I do not feel that the area under study represents one panmictic unit. If this were the case, I would expect greater uniformity of the allele frequencies and less significant differences between sample sites, unless selection pressures were sufficiently strong and diverse to account for the observed variation. Selective neutrality and evolution by 'random walk' has also been used as an explanation for the genetic heterogeneity of natural populations, especially for allozymes (King and Jukes, 1969). This explanation may not satisfactorily account for the heterogeneity observed in this study because, at least for EST 6, the variation is too regular. If this variation were accounted for by drift alone, then one would expect random differences in allele frequencies among the populations. Most of the other explanations invoke selection in some manner. The author is of the opinion that the variation observed in this study is not selectively neutral and that the mechanism which maintains this genetic heterogeneity must be some form of balancing selection. Ayala et al. (1972) came to the same conclusion on a study of allozymes in populations of Drosophila willistoni. Several different authors have produced evidence of different types of balancing selection. The work of Kojima and Yarbrough (1967) with EST 6 suggests a means by which variation may be maintained in a natural population. The frequency de-

pendent selection that they describe favors those genotypes below their equilibrium frequency, resulting in little or no load at equilibrium. This phenomenon was also found to occur for the ADH system, again in melanogaster (Kojima and Tobari, 1969). The minority effect in mating (Ehrman et al., 1965) is another form of frequency dependent selection. Still another type of balancing selection may be synthesized from the work of Powell (1971), who suggests that heterogeneity in genotype may be maintained by heterogeneity in the environment. This diversifying selection (i.e., variations in the environment plus non-constant fitness values for a given genotype [discussed in Kojima, 1971]) would offer a strong explanation for the observed variation.

The present study offers some evidence that selection is involved in maintaining allozyme variation. EST 6 seems to show a cline in the natural populations with distance. The sample sites in this study lie on an approximate East-West transect, which passes through several topographic regions. The environmental factors such as rainfall, humidity, temperature, vegetation, etc., change along this transect. There is good reason for considering the wild caught populations apart from the fruit market populations. The selection pressures operating on a population in the field probably would differ from those operating on populations inside buildings (as the fruit market samples). The fact that the Afton sample (wild) and the Waynesboro sample (fruit

market) have significantly different allele frequencies even though these sites are separated by only five miles, lends credence to this contention. Another possible source of differences between the wild populations and those from the fruit markets is migration. Even though D. melanogaster is a relatively vagile species, a sample taken in a fruit market is more likely to include recent migrants, which may have immigrated with a shipment of fruit. The probable presence of the cline for EST 6 supports the contention that selection is responsible for the maintenance of the heterogeneity. This hypothesis is supported by the work of other authors who have described studies which suggest that allozyme genotypes vary in fitness according to their environment. For example, Johnson, Schaffer, Gillaspy, and Rockwood (1969) correlated observed differences in allozyme frequency to various components of weather, vegetation and other environmental factors in the harvester ant (Pogonomyrmex barbatus). In addition, laboratory studies have confirmed that selection can operate on allozymic loci in cage studies and that selection pressures can change with the environment, e.g. Kojima and Yarbrough (1967) did a study using D. melanogaster and different types of media and found that the frequencies of EST 6 alleles approached equilibrium at different rates. Kojima and Tobari (1969) found similar results for ADH in cage populations of D. melanogaster. The data from the present study provide no clear cut evidence for selection

operating on the ADH locus.

The excess of heterozygotes observed for several samples with EST 6 in this study might be explained in several ways: non-random mating, strong selection against one of the alleles, overdominance, or interactions of the EST 6 locus with another locus. This last explanation bears consideration. There is some evidence in this study which points to a possible interaction between EST 6 and ADH. When the two-locus Hardy-Weinberg model was tested, certain deviations were noted in the Elkins sample, which might be explained by hypothesizing that individuals which are doubly heterozygous (for EST 6 and ADH) have a higher fitness than individuals of any other genotype.

Further study is suggested using larger sample sizes and many more sample sites to reduce sampling error and provide a better picture of the genetic structure of these populations.

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