GENETICAL VARIATION FOR ENZYME ACTIVITY IN A POPULATION OF *DROSOPHILA MELANOGASTER*

V. THE GENETICAL ARCHITECTURE, AS SHOWN BY DIALLEL ANALYSIS, OF ALCOHOL DEHYDROGENASE (ADH) ACTIVITY

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SUMMARY

Fifteen highly inbred lines extracted by sib-mating from the laboratory cage population, "Texas", of *Drosophila melanogaster* were crossed in a half-diallel mating design. Female progeny were assayed individually for ADH activity at 25° and 35°C and for total protein. At 25°C there was considerable additive genetical variation and the dominance variation was attributable to specific parents and to specific crosses at random in the diallel table. The character total protein also showed considerable additive variation but less dominance variation. Largely independent gene action was shown by the characters ADH activity and total protein. There were strong genotype-environment interactions for heat-stability. At 35°C most of the genetical variation was additive and mainly due to modifier loci. It was concluded that at 25°C dominance was ambidirectional and almost complete. This genetical architecture was compatible with a past history of stabilising selection for ADH activity in the "Texas" population.

1. INTRODUCTION

NATURAL selection for enzyme activity may have considerable evolutionary importance. A study of enzyme activity can provide the genetical basis of changes in molecular control which have been effected during the course of adaptation to the environment. Alcohol dehydrogenase (ADH) in *Drosophila melanogaster* is a particularly well characterised and amenable enzyme for research. Nonetheless, although studies of natural selection for ADH enzyme activity in allotypes (e.g. Delden, Boerema and Kamping, 1978; Kojima and Tobari, 1969) and in special circumstances of modifier loci (McDonald, Chambers, David and Ayala, 1977) have been described, there have been no attempts to relate the genetical architecture of the gross phenotypic variability in enzyme activity to the mode of natural selection for such enzyme activity in natural or laboratory populations. This approach following Mather (1960) requires at the outset a detailed knowledge of the gene action operating within populations.

Studies of the biometrical genetics of ADH activity leave no doubt that heritable variation in the laboratory population "Texas" can be attributed to the products of the alleles *Adh^F* and *Adh^S* and also to modifier loci (Birley and Barnes, 1973, 1975; Barnes and Birley, 1975, 1978). A diallel cross between four inbred lines, two of the allotype *Adh^{F/F}* and two of the allotype *Adh^{S/S}*, revealed directional dominance for low activity which was attributed

to the heterozygote $Adh^{F/S}$. Gene additivity rather than dominance for ADH activity has hitherto been reported when measured at 25°C (Day, Hillier and Clarke, 1974a; Ward, 1975; Oakeshott, 1976). In a study of genetical architecture it is essential to elucidate the contribution of dominance and other types of non-additive genetical variation to the total phenotypic variance. In this context there are several reasons which may account for discrepancies and point to limitations in the published literature concerned with the genetical control of enzyme activity. These reasons embrace aspects of both experimental design and the genetical nature of the experimental material. They can be summarised as follows:

(a) *Statistical*. Dominance is most readily detected when gene frequencies are equal, the lines are inbred (see (b)) and the degree of dominance is complete and unidirectional. It is possible for a null hypothesis of no dominance to be mistakenly accepted unless very large experimental sizes and an optimal experimental design have been used (Kearsey, 1970).

(b) *Genetical*. Often estimates of dominance are given in relation to the electrophoretically identified allotypes in largely outbred material. As such, any effects of modifier loci which contribute to dominance may be detected only when there is strong associative linkage disequilibrium between the modifier loci and the structural gene locus. Most studies of the genetics of enzyme activity in eukaryotes do not distinguish the two features of dominance variation, potency, the average deviation from the mid-parental value (taking into account the direction and magnitude of dominance in all crosses) and the overall effect of dominance revealed by second-degree statistics (Mather and Jinks, 1971). The value of using both metrics is of course to distinguish between ambidirectional and unidirectional dominance. It is also desirable, particularly if heterosis is anticipated, to use an experimental design which is capable of detecting non-allelic interactions, since this form of non-additive variation rather than dominance may be responsible for the observed gene action (Jinks, 1955). Lastly, gene interactions in metrical characters whether due to dominance or epistasis can exhibit marked genotype-environment interactions (Bucio Alanis, Perkins and Jinks, 1969; Jinks, Perkins and Pooni, 1973).

(c) *The metric*. A range of metrics has been used to study the inheritance of ADH activity, for example, ADH specific activity per fly (Birley and Barnes, 1973; Barnes and Birley, 1975); the specific activity of massed larval (Gibson and Miklovich, 1971) or adult (Rasmuson, Nilson and Rasmuson, 1966) homogenates; ADH activity per mg live weight (Day, Hillier and Clarke, 1974a; Ward, 1975) or simply ADH activity per fly (Barnes and Birley, 1978). The genetical implications of these diverse metrics will be considered in the discussion.

(d) *Populations*. Variations of genetical architecture resulting from natural selection may affect the occurrence and magnitude of any dominance variation in different populations used by different research workers.

(e) *Experimental procedure*. The conditions for the determination of ADH activity, e.g., buffers, temperatures, substrates, etc., vary between laboratories.

Turning from these difficulties to the molecular basis of the genetical control of enzyme activity, the possibility of heterosis has been raised by some authors (e.g., Singh, Hubby and Lewontin, 1974). The mechanism

for heterosis has been considered to be due to increased molecular stability in the non-covalent bonds which form the quaternary structure of the heterodimer and hence is most likely to be the result of amino-acid substitution in the primary structural gene product. This interpretation contrasts with the conclusions obtained from studies of more conventional metrical characters such as plant height and flowering time by Jinks (1955) whose analyses showed that the, albeit *in vivo*, heterosis was due to non-allelic interaction rather than to overdominance at specific loci.

We hope to obtain a comprehensive summary of the genetical control of enzyme activities in eukaryotes using the techniques of biometrical genetics. This paper presents the results of a large half-diallel mating design for the character ADH activity in relation to *in vitro* heat stability with 15 inbred lines derived from the "Texas" laboratory population. We have judged the half-diallel design and the extremely large experimental size to be optimal for a preliminary study of gene action and natural selection in "Texas". The design permits a reliable description of gene action to be made at the ambient life temperature of flies from the "Texas" population as well as a genetical probe of molecular variation in heat-sensitivity. It will be seen that the scope of the experimental design and resulting genetical analysis are pertinent to our criticism of the published small-scale experiments.

2. MATERIALS AND METHODS

Fifteen independently derived inbred lines, extracted by 200 generations of sib-mating from the laboratory population, "Texas" of *Drosophila melanogaster*, were crossed in a half diallel mating design, that is in all possible combinations except that only one of each pair of reciprocal F_1 crosses was raised. Two of the inbred lines were homozygous for the *Adh^F* allele and the remaining 13 were homozygous for the *Adh^S* allele. The origin of the inbred lines has been described by Birley and Barnes (1973) and the half-diallel design was used since previous studies showed evidence of paternal or maternal gene effects for ADH activity in these lines at the 4-day stage of adult development. The 120 crosses were raised in duplicate culture (standard $\frac{1}{8}$ -pt milk bottles) on the standard yeasted oatmeal, agar, treacle food medium, in a single randomised block at 25°C. ADH activity was measured on 4-day old virgin female flies using the methods described by Birley and Barnes (1973) and by Barnes and Birley (1978). At both of the chosen temperatures of 25° and 35°C, two flies were individually assayed for ADH activity from each culture bottle on two separate occasions (blocks). The population, "Texas" is maintained at 25°C, hence 35°C represents an *in vitro* heat-treatment. The flies were individually randomised within every block for determinations of ADH activity. In all, the activities of 1920 flies were measured, there being 480 determinations per block and 960 determinations at each temperature. Total protein per fly was also measured on the fly extracts using slight modifications of the turbidometric technique described by Mejbaum-Katzenellenbogen and Dobryszcka (1959). We found this method to be reliable, reproducible, to give very stable values of optical density and be particularly suitable for large numbers of samples. We are grateful to Drs A. Cornish-Bowden and A. Moir of the Department of Biochemistry for suggesting the method as an alternative to the Folin-Ciocalteu method of protein determination which we found

difficult to apply to large-scale experiments. The standard curve expressed with bovine serum albumen was of the quadratic form $y = 3.39 + 32.22x + 46.17x^2$ and the data for total soluble protein was analysed on the basis of μg protein per fly.

3. RESULTS

(i) *Analysis of variance of the half-diallel table*

Mean ADH activities for the 120 lines (families) determined at 25° and 35°C, together with the Adh genotypes of the parental lines, are shown in table 1. The analysis of variance of these data is shown in table 2. The design of the experiment was such that temperatures and lines were cross-classified items, blocks were duplicated and nested within temperatures and

TABLE 1

Mean ADH activities at 25° (roman) and at 35° (italics) for the half-diallel cross. The electrophoretically identified genotypes Adh^F/F and Adh^S/S are given for each line as FF and SS respectively.

Line	Line														
	27	20	28	5	25	I	15	6	7	16	19	8	10	9	28
	SS	SS	SS	SS	SS	SS	SS	FF	SS	SS	FF	SS	SS	SS	SS
27	4.10	7.81	6.49	5.21	5.23	6.66	7.73	15.19	7.00	6.22	13.83	5.52	7.63	6.52	5.82
	4.01	9.10	7.40	6.25	5.59	8.32	6.56	8.35	7.40	8.78	9.96	5.87	7.32	7.27	7.08
20		8.35	6.22	5.98	8.24	9.23	8.84	15.64	9.27	8.88	18.85	7.78	9.28	7.28	7.97
		10.26	7.07	8.43	10.23	11.45	10.10	10.51	10.75	10.88	13.33	10.57	11.59	9.00	9.81
18			11.15	6.57	7.22	8.33	8.86	13.06	8.50	6.89	19.57	7.03	6.98	8.00	8.23
			7.65	7.13	7.51	9.58	9.46	8.56	10.76	9.20	13.62	8.30	8.01	9.83	8.74
5				8.70	5.78	6.95	8.96	16.78	7.07	7.97	16.81	6.62	5.97	7.36	8.09
				9.88	7.53	8.90	11.41	14.89	8.45	8.71	12.93	8.54	7.20	7.81	9.58
25					3.84	5.56	6.92	8.96	6.32	7.09	11.90	5.49	5.95	7.25	7.50
					5.53	6.14	7.44	8.61	6.77	8.24	9.38	6.28	6.67	8.11	7.47
1						7.21	6.76	13.17	9.02	7.93	14.56	8.23	6.31	7.99	7.11
						7.52	9.54	8.01	7.97	13.51	8.74	7.09	9.38	9.29	9.29
15						7.20		10.41	14.09	6.89	8.48	15.99	6.82	7.72	7.63
								9.61	12.88	7.87	9.33	11.97	8.30	7.25	10.99
6								24.94	13.39	11.97	19.75	11.77	12.70	12.93	12.97
								12.31	8.43	10.91	13.40	8.36	10.41	13.13	10.06
7									8.42	7.92	22.26	7.71	6.67	10.02	8.50
									10.45	10.23	15.27	9.03	8.36	13.38	10.00
16										8.77	14.68	6.95	7.33	7.66	7.43
										8.86	10.34	9.32	6.36	8.25	9.03
19											15.68	14.34	14.07	14.38	16.20
											13.68	13.62	12.45	9.80	12.73
8												7.44	6.74	8.23	8.31
												10.89	8.12	9.47	9.57
10													6.02	6.35	7.16
													7.83	7.80	8.84
9														8.89	8.19
														9.58	9.20
28															10.16
															10.31

the duplicate bottles were nested within lines. The structure of the analysis of variance in terms of expected mean squares is also shown in table 2. A pooled error variance for 1558 degrees of freedom was obtained since the mean squares corresponding to the following items—lines $\times$ blocks within temperatures, temperatures $\times$ bottles within lines, blocks $\times$ bottles within lines and temperatures and replicate flies were homogeneous; $\chi^2_{(3)}$ from a Bartlett test of variance homogeneity was equal to 3.132. Since the application of biometrical genetics to the character enzyme activity is relatively uncommon in the literature the following genetical analysis will be considered in some detail; for a fuller explanation the reader is referred to Mather and Jinks (1971).

In the analysis of the half-diallel table the variation between lines can, following the definitions of Hayman (1954), and the method of Jones (1965),

TABLE 2
Analysis of variance of ADH activity at 25° and 35°C

Item	d.f.	M.S.	Expected mean squares
1. Temperature	1	2.193 n.s.	
2. Lines	119	128.778***	$\sigma^2R + 2\sigma^2BT(E, L) + 4\sigma^2ET(L) + 4\sigma^2LB(E) + 8\sigma^2LE + 480\sigma^2B(E) + 960\sigma^2E$
	14	875.5467***	$\sigma^2R + 2\sigma^2BT(E, L) + 4\sigma^2LB(E) + 8\sigma^2T(L) + 16\sigma^2L$
	1	3.6349 n.s.	
	14	54.8148***	
	90	25.5109***	
3. Blocks within temperatures	2	10.6910 n.s.	
4. Bottles within lines	120	8.3650***	$\sigma^2R + 2\sigma^2BT(E, L) + 4\sigma^2LB(E) + 480\sigma^2B(E)$
5. Lines x temperature	119	27.3414***	$\sigma^2R + 2\sigma^2BT(E, L) + 8\sigma^2T(L)$
	14	166.4420***	$\sigma^2R + 2\sigma^2BT(E, L) + 4\sigma^2LB(E) + 8\sigma^2LE$
	1	7.7410 n.s.	
	14	22.4879***	
	90	6.6763***	
6. Lines x blocks within temperatures	238	5.9620 n.s.	$\sigma^2R + 2\sigma^2BT(E, L) + 4\sigma^2LB(E)$
7. Temperatures x bottles within lines	120	6.4002 n.s.	$\sigma^2R + 2\sigma^2BT(E, L) + 4\sigma^2ET(L)$
8. Blocks x bottles within temperatures and lines	240	5.9908 n.s.	
9. Replicate flies	960	5.1085	$\sigma^2R + 2\sigma^2BT(E, L)$
10. Pooled error	1538	5.2133	σ^2R

Abbreviations: E = Temperature n.s. = not significant Numbers in brackets refer to the appropriate error variance item.

L = Lines
B = Blocks
T = Bottles
R = Replicate flies

P = 5%
**P = 1-0.1%
***P = <0.1%

be partitioned into the following items: a , the additive genetical variation and b , which measures non-additive genetical variation. In the absence of non-allelic interactions, b , measures deviation from non-additivity due to dominance and is itself partitioned into three orthogonal comparisons; b_1 estimating the average deviation of all F_1 crosses from their mid-parental values and thereby measuring the average direction of any dominance, b_2 measuring variation in dominance between the different parents or arrays and b_3 residual dominance attributable to certain crosses rather than to specific arrays or overall unidirectional dominance. These items also partitioned from the significant temperatures $\times$ lines interaction are shown in table 2. They show that both additive and non-additive genetical variation respond to temperature. Whilst there is no evidence for the overall direction of dominance (b_1) to vary with temperature, dominance from

TABLE 3
Analysis of variance of ADH activity at 25° and 35°C

	Item	d.f.	25°C		35°C	
			MS	VR	MS	VR
1. Lines		119	120.078	26.79(7)***	36.038	3.39(3)***
	a	14	871.434	194.39(7)***	205.713	19.34(3)***
	b	105	19.898	4.44(7)***	13.415	1.26(3) n.s.
	b_1	1	9.690	2.16(7) n.s.	0.675	< 1(3) n.s.
	b_2	14	60.612	13.52(7)***	8.429	< 1(3) n.s.
	b_3	90	13.678	3.05(7)***	14.333	1.35(3) n.s.
2. Blocks		1	21.373	4.77(7)*	0.94E-02	1(7) n.s.
3. Bottles within lines		120	4.131	0.92(7) n.s.	10.636	1.82(6)***
4. Lines $\times$ blocks		119	4.675	1.07(6) n.s.	5.847	1.00(6) n.s.
5. Blocks $\times$ bottles within lines		120	5.062	1.16(6) n.s.	4.920	0.84(6) n.s.
6. Replicate flies		480	4.379	—	5.838	—
7. Pooled error		839	4.483	—	5.686 (for 719 d.f.)	—

specific parents (b_2) and from specific crosses (b_3) both vary with temperature. The partition of the lines main effect shows no evidence of directional dominance, but significant overall additive gene action and dominance of types b_2 and b_3 . Also the increase of temperature from 25° to 35°C has produced no overall difference in ADH activity. The significant temperature $\times$ lines interaction therefore is a result of genetical variation in the extent of enzyme denaturation at 35°C. In view of these genotype-environment interactions further analyses (table 3) of the genetical control of ADH activity were carried out separately for each temperature.

Tests of significance at 25°C were carried out with a pooled error variance for 839 degrees of freedom. There is a significant additive genetical variation (a), no evidence of direction dominance (b_1) and significant dominance of types b_2 and b_3 . Deviations of F_1 family means from the mid-parental values (potence) are given in table 4 and support the significance of these latter items since although the direction of dominance is specific to certain parental arrays it can vary markedly in its level. Indeed the overall direction of potency varies for crosses of the two $Adh^{F/F}$ arrays, 19 and 6.

At 35°C the variation between duplicate bottles within families is the appropriate error term for lines in the analysis of variance (table 3). Again

TABLE 4
Potence values at 25°C. The two genotypes Adh^{SS/SS} and Adh^{WF/WF} are given for each line as SS and FF respectively

[illegible]

there is significant additive variation but in contrast to 25°C no evidence for dominance (*b*).

(ii) *Relationships between W_r and V_r*

It will be recalled that non-allelic interactions if present will contribute to variation in the “*b*” items of the Hayman analysis of variance as too will correlated gene distributions and residual heterozygosity in the inbred lines (Jinks, 1954; Dickinson and Jinks, 1956; Hill, 1964). Following Jinks (1954, 1955), the second-degree statistics V_r , the variance of parental arrays and W_r , the covariance of an array with its non-common parental arrays can be used to test for non-additive variation and also test for possible non-allelic interactions, etc. In the presence of non-additive variation the value of $(W_r + V_r)$ will vary between arrays. A joint analysis of variance of $(W_r + V_r)$ with temperature (table 5) shows that although the non-additive variation (arrays) interacts with temperature there is still an overall level of non-additive variation. If the non-additive variation was solely due to dominance the value of $(W_r - V_r)$ will be constant over arrays. The analysis of variance (table 5) shows evidence for a departure from the simple additive-dominance model of inheritance; this departure does not vary between temperatures. Because of the significant interaction between temperatures and arrays further analyses concerning the statistics W_r and

TABLE 5
Analysis of variance of $(W_r + V_r)$ and $(W_r - V_r)$

Item	d.f.	$(W_r + V_r)$	$(W_r - V_r)$
		MS	MS
Temperatures	1	2891.89***	152.49
Arrays	14	69.24***	11.01**
Blocks within temperatures	2	4.72	21.54**
Temperatures $\times$ arrays	14	35.43*	2.75
Arrays $\times$ blocks within temperatures	28	15.13	3.82

Pooled error MS = 3.46 for 42 d.f.

TABLE 6
Analysis of variance of $(W_r + V_r)$ and $(W_r - V_r)$ at 25° and 35°C

Item	d.f.	$(W_r + V_r)$		$(W_r - V_r)$	
		25°C	35°C	25°C	35°C
		MS	MS	MS	MS
Arrays	14	95.03**	9.64 n.s.	11.02 n.s.	2.75 n.s.
Block differences	15	19.97	8.27	5.82	1.32

TABLE 7
Joint regression analysis of W_r on V_r at 25°C

Item	d.f.	MS
Joint regression	1	211.8779**
Heterogeneity of regressions between blocks	1	0.3847 n.s.
Remainder	26	4.4628

Vr were carried out separately for each temperature. It can be seen (table 6) that only at 25°C is there any evidence of non-additive variation. The analyses of (Wr—Vr) only indicate the presence of a departure from the additive-dominance model of inheritance in that the variation between arrays, although not significant at either temperature, but gives probabilities corresponding to the variance ratio's between 12 and 18 per cent. Presumably these effects at 25° and 35°C are reinforced to give the highly significant arrays item in the joint analysis of variance (Wr—Vr) in table 5. At 25°C a comparison of the (Wr+Vr) and (Wr—Vr) analyses shows that

FIG. 1.—The regression of Wr on Vr for ADH activity (block 1) at 25°C.

the magnitude of the dominance variation is by far the greater component of non-additive variance, whilst at 35°C the genetical variation is largely additive.

The relative dominance relationships amongst the lines at 25°C as well as the presence and type of any epistasis can be investigated by regressing Wr on to Vr for all arrays. The joint regression analysis (table 7) gives a joint estimate of slope from the two blocks of 0.61 and this is significantly less than unity ($t_{26} = 4.28$; $P < 0.1$ per cent). Values of Wr and Vr are plotted in figs 1 (block 1) and 2 (block 2) and it can be seen that lines 25, 16 and 9 possess on the whole the most dominant alleles whilst lines 5 and 7 the most recessive alleles in both blocks, for ADH activity. The scatter of points on the Wr/Vr graphs does not indicate any pattern which could be

ascribed to a particular type of epistasis, correlated gene distribution or residual heterozygosity; the deviation of the regressed slopes from unity is probably due to epistasis but its magnitude is small and not readily identified from these data.

(iii) *Second-degree statistics and parameters*

At 25°C both additive and dominance components of genetical variation as well as an environmental variance common to both blocks (c.f. table 3) can be estimated. Of course all types of epistasis will contribute to the additive variation. Nonetheless, as has already been established the effect

FIG. 2.—The regression of W_r on V_r for ADH activity (block 2) at 25°C.

of non-allelic interactions are comparatively small compared to the contribution of dominance variation. Following Jinks (1954) and Hayman (1954), four parameters of genetical interest can be estimated from the second-degree statistics derived from a diallel set of crosses. They are D the additive genetical variance, two statistics H_1 and H_2 concerned with dominance variation and F a measure of unequal allele frequencies. The statistics and their expectations in terms of these parameters and E the environmental component of variation are shown in table 8. For the derivation and further interpretation of these statistics the reader is referred to Mather and Jinks (1971).

At 25° all of these parameters have been obtained as perfect-fit estimates for both blocks (table 9) and show good agreement between blocks. Estimates of D are largest of all. The quantity $\bar{u}\bar{v}$ estimated as $\frac{1}{4}H_2/H_1$ measures the mean gene frequency over all loci and has a maximum value of 0.25 (equal gene frequencies). Since the value of $\bar{u}\bar{v}$ is much less than 0.25 we conclude that the allele frequencies are very unequal. The dominance ratio

TABLE 8
Second-degree statistics and their expectations for a half-diallel cross of size 15

Statistic	Expectation
V_p —variance of the parents	$D + E$
$\bar{V}_r$ —mean variance of arrays	$\frac{1}{4}D + \frac{1}{4}H_1 - \frac{1}{4}F + E$
$V_{\bar{r}}$ —variance of array means	$\frac{1}{4}D + \frac{1}{4}H_1 - \frac{1}{4}H_2 - \frac{1}{4}F + \frac{1}{16}E$
$\bar{W}_r$ —mean covariance of arrays	$\frac{1}{2}D - \frac{1}{4}F + \frac{1}{16}E$

TABLE 9
Second degree statistics, derived parameters obtained as perfect fit estimates and estimates of gene frequency and dominance level at 25°C

Statistic	Block	
	1	2
V_p	116.0208	105.5576
$\bar{V}_r$	39.8508	33.6596
$\bar{W}_r$	43.8232	47.0596
$V_{\bar{r}}$	27.8748	25.2484
E	4.4833	4.4833
Parameter		
D	111.5375	101.0743
H_1	96.8434	48.6698
H_2	31.1663	16.9071
F	48.9777	15.1057
E	4.4833	4.4833
Estimates of dominance and gene-frequency		
$\bar{u}\bar{v}$	0.0805	0.0868
$\sqrt{H_1/D}$	0.9318	0.6939
$\frac{1}{2}F/\sqrt{D(H_1 - H_2)}$	0.2861	0.1333

H_1/D , estimates the average dominance over all loci irrespective of its direction. The ratio is close to unity and hence dominance is almost complete. The value of F is small and consistently positive indicating that dominant alleles are marginally more frequent than recessive alleles. The extent to which dominance (h/d) varies from locus to locus is measured by the ratio $\frac{1}{2}F/\sqrt{[D(H_1 - H_2)]}$. This ratio has a maximum value of unity when dominance (over all loci) is fully directional. Here its value is close to zero and hence the level of dominance varies from locus to locus and is indicative of ambidirectional dominance; this is in agreement with the non-significant b_1 item in the Hayman analysis of variance.

At 35°C analysis of variance of ($W_r + V_r$) showed no evidence of dominance and hence only the components $D(15.41)$ and $E(10.63)$ were estimated as average values for the two blocks. As the estimate of E was obtained from the significant duplicate bottles item (table 3) it may be noted that it is an estimate of $E_2 + \frac{1}{r}E_1$ where E_2 is the duplicate bottles variance, E_1 the environmental variance within families and r the number of replicate observations per family.

In summary, both additive and dominance variation change with *in vitro* temperature. At 25°C dominance is ambidirectional, specific to certain parental arrays and its direction also varies with its *de novo* appearance throughout the diallel table. At 35°C the gene action is mainly additive.

(iv) *Relationship between ADH activity and total protein*

In this experiment we measured ADH activity at 25°C and also total protein on the same individuals. The results of the Hayman analysis of variance for total protein and of analysis of covariance for ADH activity with total protein are given in table 10. The mean squares for ADH activity

TABLE 10
Hayman analysis of variance for total protein, of covariance for ADH activity and total protein, and the correlation coefficient (r) between these two characters

Item	d.f.	Total protein MS	ADH activity and total protein	r
a	14	345.31***	-20.52	-0.04 n.s.
b	105	93.66***	13.63	+0.32***
b_1	1	223.22*	-48.64	—
b_2	14	102.41*	34.15	+0.43 n.s.
b_3	90	90.86**	11.12	+0.32***
Error variance	839	57.74		

(table 3), total protein and covariance for ADH activity with total protein, were used to calculate the correlation coefficients corresponding to the items a , b_2 and b_3 in the Hayman analysis of variance. It is seen that the correlation in additive genetical variance for ADH activity and total protein is effectively zero. Of the non-additive variation only the item b_3 has a significant correlation coefficient. Analysed as a separate character total protein shows extensive additive and some less extensive non-additive genetical variance. Analysis of variance of ($W_r + V_r$) and of ($W_r - V_r$) for total protein showed no evidence of consistent dominance or other types of non-additive variation on the two blocks. It is concluded that the extent of non-additive genetical variation for total protein is much less than that for ADH activity.

4. DISCUSSION

The genetical architectures of a number of metrical traits concerning the gross phenotype, particularly in *Drosophila*, have been investigated, and following Mather (1953, 1960, 1966) inferences drawn of the type of selection

to which the trait had been subjected in the past (Kearsey and Kojima, 1967; Barnes and Kearsey, 1970). Genetical variation in the gross molecular phenotype shown by gel electrophoresis and other separation techniques may contribute to the genetical variance in the enzyme phenotype (e.g. to enzyme activity) but it is not the sole genetical determinant. All of the three major chromosomes of *Drosophila melanogaster* have been shown to contribute either as main effects of interchromosomal interactions (Barnes and Birley, 1975, 1978) to the total phenotypic variance in ADH activity. The present study has shown extensive additive gene action and ambidirectional dominance for ADH activity at 25°C. We believe the "Texas" inbred lines are a random sample of genetical variance in the original cage population (Birley and Barnes, 1973) hence this genetical architecture is consistent with a past history of stabilising selection for an intermediate optimum in ADH activity.

In this diallel cross further partitions of the additive genetical variance at 25°C within lines homozygous for either the *Adh^F* or *Adh^S* alleles and a comparison between all of the *Adh^{S/S}* and all of the *Adh^{F/F}* homozygous lines were as in the study of Birley and Barnes (1975) highly statistically significant. The regression of *W_r* on *V_r* at 25°C demonstrated that lines homozygous for the *Adh^S* allele could have either the most dominant or the most recessive alleles for ADH activity. Inspection of the potency values at 25°C (table 4) shows that dominance was not confined to *Adh^{F/S}* heterozygotes. Indeed a Hayman analysis of variance of ADH activity at 25°C for crosses involving only those parents which were homozygous for the *Adh^S* allele showed statistical significance for two of the partitions of dominance variation, $b_1 - V.R._{1,839} = 6.6$; $P = 1.5$ per cent and $b_2 - V.R._{12,839} = 3.21$; $P = 0.1$ per cent. Hence as for the additive genetical variance differences within an electromorph make a substantial contribution to the total dominance variation. However the most extreme variation in dominance is demonstrated by those crosses involving the two parental arrays (lines 6 and 19) which were homozygous for the *Adh^F* allele (table 4). For line 6 all but one potency value are in favour of low enzyme activity whilst for line 19 all but one value are in favour of high enzyme activity. The overall level of potency (irrespective of its direction) is also largest of all for these two arrays. Whether the dominance variation was due to structural heterogeneity of *Adh^F* or *Adh^S* alleles and/or modifier loci cannot be unambiguously resolved; however multiallelism would be detected in a diallel cross by the presence of non-allelic gene action in the analysis of variance of (*W_r* - *V_r*) (Jinks and Hayman, 1953). Since the dominance variation was by far the greater component of non-additive genetical variance it is quite possible that modifier loci are affecting the extent of dominance in particular crosses.

Directional dominance for low activity largely attributable to *Adh^{F/S}* heterozygotes was obtained by Birley and Barnes (1975) from a diallel cross amongst four inbred lines derived from the "Texas" population. Here the same two *Adh^{F/F}* lines produced in one case, line 6 dominance for low activity and in the other case line 19, dominance for high activity when crossed to the same two *Adh^{S/S}* lines, 15 and 25. In both experiments repeated measures of activity were made on individuals taken from the same culture bottles and crosses, but no genotype-environment interaction was detected because of technical aspects of measuring ADH activity; the result

is best explained by genotype-environment interaction in different culture conditions (Marson and Birley, in preparation) and shows that *Adh^{F/F}* lines are less stable in this respect than *Adh^{S/S}* lines.

Such genotype-environment interaction resulting from small variations in the culture conditions or food substrate is pertinent to the mechanism of selection and resultant genetical architecture. Provided the dominance is ambidirectional over environments, the conditions for stabilising selection are fulfilled; this aspect is being further investigated.

A major problem of comparison in studies of the genetics of enzyme activity are the different units quoted by varying laboratories. Often the rationale of a measure is to standardise enzyme activity to the unit size of tissue sample, that is through body weight or total protein. Previous studies (Barnes and Birley, 1975) showed that the contribution of the X-chromosome to ADH activity could not for two of the "Texas" inbred lines be attributed to genetical variation in total protein. Genetical variation for body weight and total protein has been demonstrated in the 15 "Texas" inbred lines and the genetical correlation between these two characters is approximately unity (Marson and Birley, unpublished). Our data from these 15 inbred lines suggest similar conclusions for enzyme activity to those of Clarke *et al.* (1979) concerning the conventional scaling of ADH protein level by body weight or an associated measure of body size. That the scaling enzyme activity is genetical variation in body weight or total protein is at least partially independent of enzyme activity may give rise to a misleading conclusion about gene action. A detailed study to show that the relationship between enzyme activity and body weight and enzyme activity and total protein were similar when phenotypic correlations of +0.55 and +0.44 respectively (Marson and Birley, unpublished), the measure total protein slightly underestimating body size. Nonetheless in this diallel cross genetical independence of the two characters ADH activity and total protein is very evident. Moreover since both total protein and ADH activity are measured on the same flies, the correlation between these two characters will be inflated by an environmental covariance common to each fly. Hence the true genetical correlation observed for the item b_3 may be lower than the observed correlation. Indeed its value (+0.3) indicates that only some of the random occurrences of non-additivity in ADH activity are in common with some of the random occurrences of non-additivity in total protein. The cause of any such correlation may of course be due to linkage disequilibrium, pleiotropy, co-adapted gene complexes or the environment. Nonetheless it is more likely that the genetical variation in ADH activity is caused by loci controlling activity rather than by secondary effects of body weight or total protein. Hence, attempts to scale ADH activity by either total protein or body weight will lead only to a substantial inflation of error variances in the production of a less precise and different metric. The genetical variance in enzyme activity is most likely caused by structural differences between ADH-F and ADH-S enzymes (e.g., Day *et al.*, 1974b) as well as to loci controlling levels of ADH protein (e.g., Lewis and Gibson, 1978) and post-translational modification of the enzyme.

In this experiment we are concerned with the overall effect of temperature on enzyme activity rather than to any specific mechanisms although the causes may be several, e.g., *in vitro* interactions between the enzyme in question and other proteins (e.g., proteases) and/or other molecules

as well as to the thermal denaturation of the enzyme *per se*. In contrast to 25°C there was no evidence for extensive dominance or non-additive variation at 35°C. It is difficult to compare this result with those of other workers (Gibson and Miklovich, 1971; Vigue and Johnson, 1973; Day *et al.*, 1974b; Oakeshott, 1976) since there is considerable variation in technical methods and the choice of temperature. Tables 1 and 2 demonstrate that the *Adh^F* is more thermally labile in both heterozygous and homozygous states. Assuming the ADH-F enzyme in *Adh^{F/F}* homozygotes continues to lose its reactivity at a rate greater than that of the *Adh^{S/S}* and *Adh^{F/S}* genotypes with increases of temperature then heterosis in the *Adh^{F/S}* genotype should occur; in this sense our results are not inconsistent with those authors who have demonstrated heterosis at higher (+40°C) temperatures (Gibson and Miklovich, 1971; Singh, Lewontin and Hubby, 1974). At 25°C a substitution of *Adh^F* by *Adh^S* alleles will substantially affect ADH activity in this population of inbred lines. Measured at 35°C this effect is small and modifier loci with largely additive gene action will make the greatest contribution to the genetical variation in ADH activity.

This study has demonstrated the potential power of diallel analysis for the study of gene action in enzymes in populations. The design utilising inbred lines derived from the population in question maximises the chance for detection and classification of non-additive variation. Applied to *in vitro* heat-treatment it provides in conjunction with an analysis at the *in vivo* temperature a possible means of screening for novel gene products and action as a preliminary to detailed molecular study.

In summary, the genetical architecture of ADH activity at 25°C shows extensive additive genetical variation and ambidirectional dominance in part attributable to *Adh^{F/S}* heterozygotes and in part to the random occurrence of dominance throughout the diallel table. This dominance was unstable to *in vitro* heat-treatment at 35°C where the genetical control of ADH enzyme activity was largely due to modifier gene action. The genetical architecture at 25°C is consistent with a past history of stabilising selection in the "Texas" population. The experiments of McDonald *et al.* (1977) have demonstrated that regulatory gene products as well as structural changes in enzymes will have evolutionary significance. As yet there are little data about the relationship between Darwinian fitness and enzyme activity in populations, yet theoretical models are being developed (e.g., Gillespie, 1977) to account for evolutionary change in such characters. We are currently investigating the predicted mode of selection, stabilising selection, for ADH activity in the "Texas" population.

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