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

VII. GENOTYPE-ENVIRONMENT INTERACTION FOR ALCOHOL DEHYDROGENASE (ADH) ACTIVITY

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SUMMARY

Genotype-environment interaction was detected for ADH activity amongst a set of 18 highly inbred lines of *Drosophila melanogaster* which had been extracted from the laboratory population, "Texas". The genotype-environment interaction for ADH activity was not wholly associated with genotype-environment interaction for body weight or total protein level. Detailed analyses of the responses of the individual inbred lines in ADH activity in relation to the environmental index, e_i , and following the procedure of Jinks and Pooni (1979), showed substantial diversity in the form of response. Lines homozygous for the Adh^F allele were more environmentally sensitive than $Adh^{S/S}$ lines. Amongst the 16 $Adh^{S/S}$ lines, models of linear, quadratic or two intersecting-straight-lines were used to illustrate the varied responses of genotypes to the environment. The heterogeneity in the response characteristics of the inbred lines was attributed to variations in the conditions of culture media normally present within populations and laboratories. Moreover the non-linear responses shown by some lines to the environment are consistent with a model of genotype-environment interaction for ADH activity mediated by varied genotype-specific sensitivities to different environmental factors.

1. INTRODUCTION

It is now apparent that modifier loci as well as those allelomorphs revealed by gel electrophoresis (electromorphs) contribute to the phenotypic variation in ADH and other enzyme activities in populations of *Drosophila melanogaster* (Ward, 1975; Barnes and Birley, 1978; McDonald and Ayala, 1978; Laurie-Ahlberg, *et al.* 1980; Birley, Couch and Marson, submitted for publication). The possible consequences of varying environmental factors, chosen from those which occur in natural habitats (for example, temperature or substrate concentration) have been explored, in some cases very successfully, in experiments designed to test predictions of the outcome of natural selection from a knowledge of the biochemical properties of electromorphs in selected environments (Gibson, 1970; Morgan, 1975; de Jong and Scharloo, 1976; van Delden, Boerema and Kamping, 1978). Moreover this approach has also shown a possible adaptive significance to loci modifying ADH enzyme quantity in relation to environmental ethanol (McDonald, Chambers, David and Ayala, 1977). A sound knowledge of the genetical control of enzyme activity is critical in such studies.

Except for some studies of *in vivo* thermostability (Gibson and Miklovich, 1971; Oakeshott, 1976) there has been little interest in genotype-environment interaction for ADH activity, or even in relation to enzyme

activity variation in general. A knowledge of the extent of the ability of genotypes or strains with the same electromorph to show genotype-environment interaction is essential for the assessment or prediction of natural selection from a knowledge of enzyme activity and properties. Studies of ADH gene action in highly inbred lines derived from the long established population, "Texas" have already shown implied evidence of genotype-environment interaction (Birley, Marson and Phillips, 1980). The present study examines the response in ADH activity of a set of 18 highly inbred lines derived from the population "Texas" in relation to normal variations in the laboratory environment. The novel feature of this study is the application of a recently published method for the detailed study of genotype-environment interaction, due to Jinks and Pooni (1979), to the character enzyme activity. The method is particularly useful since it allows an assessment of the relative sensitivities of lines to environmental variation of an unspecified or ill-defined nature as may be expected in the ecology of natural or even laboratory populations. Indeed although certain specific environmental variables may affect the genetical control of enzyme activity, in practice, putative selective agents such as temperature, humidity, substrate concentration or pH are likely to be interactive in effect upon ecology creating quite unspecified or unknown forms of environmental variance. The analysis presented here does not rely upon specific environmental effects and has permitted a comparison of environmental sensitivity within and between lines homozygous for different alleles; it is therefore ideal for the investigation of genotype-environment interaction in any population in relation to its complete environment.

2. MATERIALS AND METHODS

Eighteen highly inbred lines derived from the laboratory population "Texas" formed the basis of this experiment. Further details of the origin of these lines are given by Birley and Barnes (1973). Each line was raised as a 10 pair mass-mating in one third pint milk bottles on the standard oatmeal, agar and treacle based food medium. The food medium was the same as that used to maintain the "Texas" population. This food consisted of 15 g Agar (Kobé), 15 ml Nipagin M (10 per cent wv. solution on 98 per cent ethanol), 8.75 g Fowler's black treacle, 180 g oatmeal (Mornflake, fully stabilised) and 395 ml water. A suspension of 0.75 mls of live baker's yeast (250 g compressed yeast creamed in 100 ml water) was added to each bottle one day prior to use. One culture of all 18 inbred lines was raised in a randomised block design at 25° on seven separate occasions (blocks). The seven blocks were all reared within a 9 month period. Live body weight (mg) was recorded with a Cahn electrobalance, model 7550 and flies stored at -20° until they were assayed for ADH activity and total protein, which took place within 3 weeks of storage at -20°. ADH activity was measured in mU at 25° and total protein in µg following calibration with bovine serum albumen. Further technical details of the determination of ADH activity and total protein are given by Barnes and Birley (1978) and by Birley, Marson and Phillips (1980). The three characters ADH activity, total protein and body weight were all measured upon one individual fly. The total experimental size was 378 female flies and this comprised three flies per occasion per line. Measurements of the three characters ADH activity, total

protein and body weight were made upon every one of the 54 individually randomised flies per occasion.

3. RESULTS

Mean values of ADH activity, body weight and total protein are given in table 1 for the inbred lines in the seven blocks of independent culture. This

TABLE 1

Mean ADH activities (bold type), body weight (italics) and total protein (roman type) for the 18 Texas inbred lines when grown on seven occasions

Electromorph line		Block						
		1	2	3	4	5	6	7
<i>Adh</i> ^{S/S}	1	6·86	5·86	4·77	6·55	9·09	9·71	9·35
		<i>1·33</i>	<i>1·14</i>	<i>1·03</i>	<i>1·02</i>	<i>1·40</i>	<i>1·27</i>	<i>1·51</i>
		117·86	75·21	65·31	70·73	89·78	81·87	92·72
<i>Adh</i> ^{S/S}	3	5·01	5·60	3·62	7·11	8·22	6·57	8·77
		<i>1·07</i>	<i>1·22</i>	<i>0·94</i>	<i>1·12</i>	<i>1·30</i>	<i>1·06</i>	<i>1·42</i>
		76·18	59·22	44·63	88·49	85·81	60·15	77·46
<i>Adh</i> ^{S/S}	5	5·70	4·70	4·47	6·82	7·91	8·92	6·33
		<i>1·19</i>	<i>1·11</i>	<i>1·16</i>	<i>1·27</i>	<i>1·41</i>	<i>1·37</i>	<i>1·41</i>
		103·37	68·33	75·81	80·76	70·81	91·22	77·58
<i>Adh</i> ^{F/F}	6	18·35	18·77	21·14	22·39	28·64	32·40	25·72
		<i>1·17</i>	<i>1·19</i>	<i>1·33</i>	<i>1·25</i>	<i>1·31</i>	<i>1·44</i>	<i>1·53</i>
		89·48	68·26	82·03	81·31	80·70	110·07	81·82
<i>Adh</i> ^{S/S}	7	10·08	8·12	8·34	10·71	9·79	8·71	10·49
		<i>1·31</i>	<i>1·33</i>	<i>1·38</i>	<i>1·28</i>	<i>1·33</i>	<i>1·31</i>	<i>1·14</i>
		117·22	72·67	73·08	98·39	115·68	79·87	63·71
<i>Adh</i> ^{S/S}	8	6·47	7·49	6·11	9·34	9·42	11·68	9·48
		<i>1·10</i>	<i>1·53</i>	<i>1·36</i>	<i>1·57</i>	<i>1·31</i>	<i>1·45</i>	<i>1·51</i>
		95·89	130·19	95·30	116·99	104·24	97·01	79·73
<i>Adh</i> ^{S/S}	9	6·90	5·50	5·36	8·02	6·81	8·38	10·45
		<i>1·42</i>	<i>1·19</i>	<i>1·28</i>	<i>1·25</i>	<i>1·35</i>	<i>1·34</i>	<i>1·95</i>
		138·49	81·05	88·90	90·53	95·28	95·91	106·94
<i>Adh</i> ^{S/S}	10	6·56	5·17	4·81	7·44	8·19	8·50	7·32
		<i>1·55</i>	<i>1·33</i>	<i>1·32</i>	<i>1·42</i>	<i>1·45</i>	<i>1·57</i>	<i>1·45</i>
		153·08	81·10	82·13	117·25	102·27	115·93	97·37
<i>Adh</i> ^{S/S}	15	8·24	9·54	8·81	11·17	13·47	14·27	12·00
		<i>1·19</i>	<i>1·51</i>	<i>1·36</i>	<i>1·29</i>	<i>1·36</i>	<i>1·43</i>	<i>1·46</i>
		115·93	85·22	101·34	105·60	108·47	112·60	99·90
<i>Adh</i> ^{S/S}	16	5·74	4·55	4·88	7·35	9·94	11·04	7·25
		<i>1·19</i>	<i>1·25</i>	<i>1·11</i>	<i>1·11</i>	<i>1·43</i>	<i>1·65</i>	<i>1·32</i>
		77·80	76·36	52·11	69·03	116·27	132·35	72·75
<i>Adh</i> ^{S/S}	17	3·57	4·46	3·62	7·77	7·14	5·75	6·41
		<i>1·24</i>	<i>1·24</i>	<i>1·10</i>	<i>1·53</i>	<i>1·47</i>	<i>1·51</i>	<i>1·45</i>
		75·80	73·38	62·76	114·10	81·87	92·72	99·94
<i>Adh</i> ^{S/S}	18	7·82	7·55	4·33	7·82	11·33	8·83	6·88
		<i>1·50</i>	<i>1·35</i>	<i>1·15</i>	<i>1·43</i>	<i>1·51</i>	<i>1·35</i>	<i>1·34</i>
		114·43	92·15	60·86	104·64	112·90	98·84	69·40
<i>Adh</i> ^{F/F}	19	24·83	18·19	17·35	34·51	38·84	43·16	38·77
		<i>1·64</i>	<i>1·43</i>	<i>1·22</i>	<i>1·89</i>	<i>1·79</i>	<i>2·03</i>	<i>2·36</i>
		155·22	91·76	98·37	129·16	143·66	149·57	154·11

TABLE 1—continued

Mean ADH activities (*bold type*), body weight (*italics*) and total protein (*roman type*) for the 18 Texas inbred lines when grown on seven occasions

Electromorph line		Block						
		1	2	3	4	5	6	7
<i>Adh</i> ^{S/S} 20		6.91	7.08	6.10	9.87	9.47	9.39	9.30
		<i>1.29</i>	<i>1.42</i>	<i>1.15</i>	<i>1.56</i>	<i>1.22</i>	<i>1.37</i>	<i>1.45</i>
		87.82	88.04	79.97	121.20	78.75	83.90	94.30
<i>Adh</i> ^{S/S} 22		6.69	7.11	5.75	6.91	10.75	8.37	5.60
		<i>1.21</i>	<i>1.30</i>	<i>1.25</i>	<i>1.42</i>	<i>1.54</i>	<i>1.60</i>	<i>1.20</i>
		84.82	100.40	76.69	88.20	119.92	120.43	71.52
<i>Adh</i> ^{S/S} 25		4.42	3.97	2.86	3.19	4.74	6.20	4.43
		<i>1.42</i>	<i>1.41</i>	<i>1.17</i>	<i>1.21</i>	<i>1.40</i>	<i>1.56</i>	<i>1.07</i>
		94.43	88.76	75.19	77.03	97.74	111.26	65.00
<i>Adh</i> ^{S/S} 27		4.79	2.45	1.79	4.00	3.86	7.13	3.77
		<i>1.19</i>	<i>1.28</i>	<i>1.25</i>	<i>1.35</i>	<i>1.34</i>	<i>1.95</i>	<i>0.88</i>
		72.02	43.52	34.34	73.76	62.61	71.23	37.36
<i>Adh</i> ^{S/S} 28		9.31	7.32	7.31	11.51	12.51	10.45	11.09
		<i>1.51</i>	<i>1.46</i>	<i>1.33</i>	<i>1.30</i>	<i>1.58</i>	<i>1.41</i>	<i>1.48</i>
		145.62	104.73	92.64	98.89	127.14	100.18	97.75

table also depicts the electrophoretically identified phenotype (electromorph) for each of the 18 inbred lines. A preliminary analysis of variance for the three characters described above is given in table 2. The variation between the inbred lines has both in the context of the main effect, between lines and for the interaction of lines and blocks, been partitioned into the three comparisons: between the 16 *Adh*^{S/S} lines, between the 2 *Adh*^{F/F} lines and between the mean of both *Adh*^{F/F} lines and the mean of all *Adh*^{S/S} lines (*Adh*^{F/F} vs. *Adh*^{S/S}). It is immediately apparent (table 2) that all

TABLE 2

Analysis of variance for ADH activity, body weight and total protein for the 18 Texas inbred lines grown on seven occasions (blocks)

Item	d.f.	ADH activity	Mean squares body weight	Total protein
Lines	17	972.99***	0.49***	5522.20***
<i>Adh</i> ^{S/S} lines	15	75.43***	0.29***	3929.21***
<i>Adh</i> ^{F/F} lines	1	498.60***	2.10***	23080.52***
<i>Adh</i> ^{F/F} vs. <i>Adh</i> ^{S/S} lines	1	14910.76***	1.89***	11858.71***
Blocks	6	247.29***	0.45***	7512.31***
Lines × Blocks	102	19.27***	0.07***	728.31***
<i>Adh</i> ^{S/S} lines	90	3.54**	0.06***	717.71***
<i>Adh</i> ^{F/F} lines	6	65.34***	0.14***	698.71 ^{NS}
<i>Adh</i> ^{F/F} vs. <i>Adh</i> ^{S/S} lines	6	209.10***	0.18***	916.98 ^{NS}
Error	252	2.48	0.025	456.16

*** *P* 0.1 per cent

** *P* = 1-0.1 per cent

* *P* = 5-1 per cent

NS = Not significant.

three characters exhibit (disregarding the effect of the *Adh* electromorph), a highly significant interaction between lines and blocks, (102 d.f.). The partitions of the interaction between lines and blocks according to the *Adh* electromorph, as described above, show that in the case of total protein only the variation between *Adh*^{S/S} lines has a significant interaction with blocks. For the characters ADH activity and body weight all of the partitions of the interaction of the between-lines variation and blocks are highly significant. The variation between blocks is highly significant for all three characters.

The partitions of the between-lines variation, as a main effect and according to *Adh* electromorph, were for ADH activity and body weight tested for significance against the correspondingly partitioned and highly significant interactions of lines and blocks. It is seen (table 2) that all of the comparisons of the variation between lines are highly significant. For total protein (after partition) it was only necessary to test the variation between *Adh*^{S/S} lines for statistical significance against its interaction with blocks. For the remaining two comparisons, between *Adh*^{F/F} lines and *Adh*^{F/F} vs. *Adh*^{S/S} lines the replicate fly variance was used as a source of error variation. All of the comparisons between lines for total protein were highly significant. The analysis presented in table 2 shows that whilst there is considerable genetical variation (as shown by the main effects in this analysis) for the three characters ADH activity, body weight and total protein, these characters also show genotype-environment interaction effected by the normal range of conditions encountered in this laboratory.

The observation of genotype-environment interaction in this set of inbred lines is of interest since any heterogeneity of response by line in ADH activity to a standard range of laboratory conditions will considerably influence hypotheses concerned with the maintenance of genetical variation in enzyme activity. A consideration of the source of this genotype-environment interaction in relation to experimental design is given in section 4. Suffice at present to say that the genotype-environment interactions observed in this paper are most likely due to conditions of culture rather than changes of environment associated with the techniques used for the determination of enzyme activity.

Previous studies have already shown for the "Texas" inbred lines that the genetical variation for body weight and total protein is largely independent of the genetical variation for ADH activity (Birley, Marson and Phillips, 1980; Birley, Couch and Marson, submitted). Of course, even if any pair of characters show a perfect genetical association this does not necessarily mean that they are due to the same genetical systems, rather it could also be interpreted through associative linkage disequilibrium of the genes controlling the two characters in question. Nonetheless, statistical evidence of extensive variation which is not held in common by two characters is stronger evidence of some independence in the genetical control of those two characters as is direct evidence from any contrasting associations between mean values of the characters for particular highly inbred lines. It was therefore, of particular interest to investigate the genotype-environment interactions observed for ADH activity in relation to those demonstrated for body weight and also in the case of *Adh*^{S/S} lines, for total protein. The object of the following analysis was to assess the degree of any co-ordinated genetical response in ADH activity due to a common genetical expression of overall tissue size in the environments studied. The

TABLE 3

Regression analyses of ADH activity on body weight and on total protein

Item	d.f.	Body weight MS	Total protein MS
Between $Adh^{S/S}$ lines			
Regression	1	341.98 ^{NS}	476.29 ^{NS}
Remainder	14	56.39***	46.79***
Blocks $\times$ $Adh^{S/S}$ lines			
Remainder	1	107.38***	77.84***
Remainder	89	2.37***	2.70***
Blocks $\times$ $Adh^{F/F}$ lines			
Regression	1	355.91***	247.64*
Remainder	5	7.22 ^{NS}	28.88*
Blocks $\times$ $Adh^{F/F}$ vs. $Adh^{S/S}$ lines†			
Regression	1	649.91 ^{NS}	545.77 ^{NS}
Remainder	5	120.94***	141.77***
Within $Adh^{S/S}$ lines	224‡	1.39	
Within $Adh^{F/F}$ lines	28‡	11.20	

* The error variance used for this item is within $Adh^{F/F}$ lines; see text for further details.

‡ $F_{28,224} = 8.06$; $P < 0.1$ per cent (two-tailed test).

Probability codes as for table 2.

extent to which the genetical variation for ADH activity could be accounted for by genetical variation in body weight or total protein was investigated by a regression analysis of ADH activity on body weight and another regression analysis on total protein. The analysis (table 3) was carried out for those items in the preliminary analysis (table 2) which could be partitioned into a component due to linear regressions and a remainder variance. Hence for the main effect, between lines, our analysis concerns only the variation between $Adh^{S/S}$ lines as the remaining two comparisons concerned with between line variation, $Adh^{F/F}$ lines and $Adh^{F/F}$ vs. $Adh^{S/S}$ lines, each have only 1 d.f. The regression analysis has also been carried out for the interaction of lines and blocks and here all of the comparisons between electromorphs are represented in the analysis in table 3. The error variance for the main effect, $Adh^{S/S}$ lines is as before $Adh^{S/S} \times$ blocks. However, the analysis described in table 2 was essentially of a global or coarse nature aimed at a preliminary assessment of the data and neglected extreme heterogeneity of within-line variances for $Adh^{F/F}$ and $Adh^{S/S}$ lines ($V.R._{28,224} = 8.06$; $P < 0.1$ per cent on a two-tailed test of significance). Hence the error variances in this analysis for the partitions of the blocks $\times$ lines interaction corresponded with Adh electromorph. In the case of the interaction between $Adh^{F/F}$ vs. $Adh^{S/S}$ lines and blocks, the error variance, within $Adh^{F/F}$ lines rather than the smaller within $Adh^{S/S}$ lines, was used so as to give a conservative test of significance. Whenever the variation remaining after accounting for the effect of a linear regression was significant, the remainder item was used as a test of significance for the linear relationship. The analysis (table 3) shows that the remaining variation after accounting for the linear component of the relationship between ADH

activity and either body weight or total protein is highly significant for the items $Adh^{F/F}$ vs. $Adh^{S/S}$ lines $\times$ blocks, $Adh^{S/S}$ lines $\times$ blocks and between $Adh^{S/S}$ lines. There is no indication that the relationship of ADH activity and body weight for the interaction of $Adh^{F/F}$ lines and blocks is other than linear, but for total protein and ADH activity the corresponding remainder item is significant at a borderline level indicating once more that not all of the ADH activity variation shown by the item $Adh^{F/F}$ lines $\times$ blocks is linearly related to the variation in total protein.

For two items, blocks $\times$ $Adh^{F/F}$ lines and blocks $\times$ $Adh^{S/S}$ lines the linear regression is statistically significant for both body weight and total protein. However the linear regression is not statistically significant for the variation between $Adh^{S/S}$ lines or for the interaction of blocks with $Adh^{F/F}$ vs. $Adh^{S/S}$ lines.

It is concluded that the genetical variation in ADH activity is not wholly a direct reflection of the genetical variation in total protein or body weight. This conclusion for the overall main effect, between $Adh^{S/S}$ lines, agrees with earlier studies of the "Texas" inbred lines (Birley, Marson and Phillips, 1980; Birley, Couch and Marson, submitted). The significant remainder items for the interactions of various partitions according to Adh electromorph and blocks indicate a difference in response by the characters ADH activity and body weight or total protein to conditions encountered in the seven blocks or environments. In some cases a linear relationship between the characters in the seven environments is present; however, generally the remainder variance is still statistically significant. Hence further analyses of the genetical response to the environment amongst the 18 inbred lines have been carried out for the character ADH activity without any apparent consideration of genotype-environment interactions for body weight or total protein. Any further appraisal of the reflection of ADH activity variation through body weight or total protein has been left to the discussion of this paper.

The aim of the following analysis was to distinguish between the responses of the inbred lines to variations of the environment and follows the procedures described by Perkins and Jinks (1968) and Jinks and Pooni (1979). The chief attribute of this analysis is that it allows a more detailed description of non-linear responses of the inbred lines to the environment. More generally the analysis is useful whenever the level of environmental factor or difference cannot be ascribed to defined levels of an environmental treatment but rather the environment represents a random or unspecified sequence of events, such as is illustrated by these data, where every environment represents a different occasion upon which the inbred lines were grown. The seven environments are therefore a sample of possible environments encountered in the laboratory. The essence of the procedure is to compare the average ADH activity of each of the inbred lines with the deviation from the average performance of all lines from the overall mean activity in this experiment over the set of j environments. Because of such a definition the average response of all lines compared to the environmental measure, e_j , is of unit slope. As e_j is defined in this way the relative response of any one line is deputed as a comparison with the average responses of the remaining set of inbred lines. Hence the responses of the entire set of inbred lines are not their absolute responses to fixed or defined environments but are relative responses of the complete set of inbred lines to the environment.

TABLE 4

Linear, quadratic and two intersecting-straight-line regression analyses for ADH activity for all 18 "Texas" inbred lines. Lines 6 and 19 are homozygous for the Adh^F allele and the remaining lines are homozygous for the Adh^S allele

Line	Linear regression 1 d.f.	Quadratic regression 1 d.f.	Two-line regression 1 d.f.	Remainder MS 3 d.f.	Error MS 14 d.f.
1	0.7596 ^{NS}	0.0072 ^{NS}	1.8028 ^{NS}	0.4481 ^{NS}	0.5639
3	2.6483 ^{NS}	2.7495 ^{NS}	2.2581 ^{NS}	0.5623 ^{NS}	0.7603
5	2.0413**	0.3243 ^{NS}	0.9511 ^{NS}	0.0305 ^{NS}	0.2107
6	38.1360**	32.1034**	0.6396 ^{NS}	0.6610 ^{NS}	4.5328
7	16.9620**	4.3999**	0.1429 ^{NS}	0.3022 ^{NS}	0.2933
8	0.5676 ^{NS}	0.3077 ^{NS}	0.2251 ^{NS}	0.9040*	0.2154
9	4.8138*	3.3707 ^{NS}	2.8513 ^{NS}	1.2553 ^{NS}	0.8654
10	3.3805**	0.1220 ^{NS}	0.2261 ^{NS}	0.0812 ^{NS}	0.3049
15	0.0078 ^{NS}	2.2214*	0.7825 ^{NS}	0.2190 ^{NS}	0.3626
16	0.2752 ^{NS}	2.4811**	0.7081 ^{NS}	0.1501 ^{NS}	0.1659
17	4.3066**	1.7613 ^{NS}	3.6467*	0.5329 ^{NS}	0.4817
18	2.4004 ^{NS}	0.1541 ^{NS}	3.6591 ^{NS}	3.0193*	0.6489
19	414.4383**	3.1056 ^{NS}	2.9634 ^{NS}	3.0184 ^{NS}	2.9337
20	3.1948**	0.9960 ^{NS}	0.9700 ^{NS}	0.1232 ^{NS}	0.3935
22	6.7840**	1.2211 ^{NS}	1.8409 ^{NS}	2.5389*	0.7489
25	11.0438**	0.6273**	2.3522**	0.1940 ^{NS}	0.0648
27	4.6272*	0.0634 ^{NS}	3.8646(*)	1.4341 ^{NS}	0.8132
28	0.7495 ^{NS}	2.7835*	0.1814 ^{NS}	0.8962 ^{NS}	0.5382

Probability codes: ** $P = 1-0.1$ per cent, * $P = 5-1$ per cent, NS Not significant. A bracketed asterisk indicates not significant when the probability is corrected for possible bias in testing for two pairs of intersecting straight lines (see text for details).

† Adjusted by a factor of $\times \frac{1}{3}$ to be compatible with the analysis of variation between lines which was carried out upon the line means.

The analysis of genotype-environment interaction is presented in a summarised form in table 4. The responses of the inbred lines to the environment are presented as linear, quadratic, two-intersecting-straight-lines and a remainder item. In the analysis of Jinks and Pooni (1979) the quadratic regression for 1 d.f. represents the reduction in the linear remainder SS due to fitting a quadratic regression of the genotypic response on the e_j . Also the statistical test for a model based upon two-intersecting-straight-lines, $Y = a_I + b_I x$ and $Y = a_{II} + b_{II} x$, is based upon 1 d.f. and corresponds to the reduction in the remainder sum of squares of the quadratic regression attributable to fitting two intersecting straight lines rather than a quadratic regression. The only difference from the presentation given by Jinks and Pooni (1979) relates to the item linear regression. As mentioned earlier under conditions of no genotype-environment interaction the regression of line mean onto additive environmental value e_j will yield a slope of unity. Deviation of the regression from unit slope will be caused by genotype-environment interaction, and is given by the relationship $(1 + b_i)$ where b_i is the portion of the variation in the genotype-environment interaction variance which is a linear function of environmental value (e_i) for the i th line. The regression SS presented in table 4 represent the significance of the parameter b_i from unity, tested according to the procedure given by Bucio Alanis (1966) and Perkins and Jinks (1968). Hence the responses shown in this present analysis by the item linear

regression do not also reflect the additive environmental value e_i . In all other respects the analysis is as presented by Jinks and Pooni (1979). The test of significance for an improvement in fit for a model of genotype-environment interaction based upon two intersecting-straight-lines was determined conservatively by adjusting the probability values obtained from the corresponding test of significance for the *a posteriori* procedure of selecting the best pair of intersecting-straight-lines. Hence allowance was made for the two possibilities of obtaining pairs of intersecting straight lines with seven levels of the independent variable e_i and constraining the smallest number of points attributable to any line to be three. There are then two possible pairs of intersecting straight lines. The procedure here, as in the analysis of Jinks and Pooni (1979) is to apply a correction to the probability corresponding to the improvement in fit, whenever it is less than the critical level of 5 per cent, in this case by a multiplication of two. If the probability corresponding to the variance ratio test of significance is still <5 per cent, then a model based upon two intersecting-straight-lines has been accepted. In table 4 significance levels are bracketed whenever $P > 5$ per cent with the application of this more stringent test of significance.

Preliminary analyses have already shown heterogeneity of within line variances, and Bartlett tests of within line variance showed for $Adh^{S/S}$ lines a $\chi^2_{(15)} = 40.8$; $P < 0.1$ per cent as well as an extremely large $\chi^2_{(17)} = 126.5$, $P < 0.1$ per cent for the same comparison amongst all 18 inbred lines. The tests of significance in this detailed analysis of genotype-environment interaction were therefore carried out with the individual within-line variances. The analysis (table 4) demonstrates significant linear regression components (b_i) for many lines (5, 6, 7, 9, 10, 17, 19, 20, 22, 25 and 27). These lines include both of the $Adh^{F/F}$ lines which themselves possess by far the greatest linear components of variance.

The improvement in fit accountable by a quadratic relationship between the line means and the levels of e_i is clearly demonstrated by lines 6, 7 and 25. In addition a quadratic response is a better model for these cases where there is no statistical evidence for a linear relationship (lines 15, 16 and 28), that is a test of b_i being significantly different from unity rather than of the significance of the regression of $(1 + b_i)$ on e_i being different from unity. Application of a regression model based upon two intersecting-straight-lines give a significant improvement in fit over a quadratic response for line 25. For line 17 (adjusted $P = 0.032$) this is again the most suitable of all three models. For line 27, there is a suggestion that the two-intersecting model fits the data; however, application of the stringent test of significance discussed earlier reduces the probability of the variance ratio for 1 and 14 d.f. to 0.094. Lastly there is a significant remainder variance for lines 8, 18 and 22. For lines 8 and 18 none of the regression models fitted the data (b_i was not significantly different from zero but of course the regression of $(1 + b_i)$ on e_i was highly significant in both cases); this suggests a more complex model is needed to describe the response of these lines to the environment. In the case of line 22, b_i was significantly different from zero showing a linear response in genotype-environment interaction but again a different or more complex model may be needed to describe genotype-environment interaction in this line.

The diversity of responses shown by these inbred lines is illustrated in fig. 1. The response of line 5 is given as an example of a linear response to

FIG. 1.—ADH activity response to environmental change. Mean ADH activities are given on the ordinates, e_j values on the abscissae. Where appropriate the equations of best fit are given in Table 6.

the entire range of environments, line 7 shows a clear quadratic response whilst line 25 showed a marked improvement in fit due to the application of the model based upon two-intersecting-straight-lines. For line 18, there was a significant remainder MS and whilst the regression of $(1 + b_i)$ on e_j was significant, $b_i \neq 0$ and the application of neither the quadratic nor the two intersecting-straight-line model produced a significant improvement in fit to the data. The values of e_j produced by this analysis of genotype-environment interaction together with the rank order of the environments (blocks 1 to 7) are given in table 5 should the reader wish to reconstruct these analyses.

TABLE 5

Environments classified by the ranked order of the dependent e_j value. The environments refer to the block numbers given to the mean ADH activities in table 1

Block number or environment	3	2	1	4	5	7	6
e_j	-2.85	-2.18	-1.36	0.55	1.17	2.08	2.60

Inspection of the equations of fit (table 5) further illustrates the heterogeneous response of these lines to the environment. Whilst the linear responses of *Adh*^{S/S} lines (5, 9, 10, 20 and 22) are similar in their slopes (estimated here as $(1 + b_i)$) the response of the *Adh*^{F/F} line 19 is markedly strongest. For the quadratic response, curvature follows two patterns for the *Adh*^{S/S} lines, the values of $|bx|$ and $|cx^2|$ in the general quadratic equation

TABLE 6

Response equations of best fit for ADH activity for the three regression models, linear, quadratic and two intersecting-straight-lines. The table depicts only those statistically significant responses observed in table 4

(a) *Linear response*

Line	Equation of fit
5	$6.41 + 0.73 e_j$
9	$7.37 + 0.58 e_j$
10	$6.86 + 0.65 e_j$
19	$30.81 + 4.88 e_j$
20	$8.30 + 0.66 e_j$
22	$7.37 + 0.50 e_j$

e_j limits are as shown in table 5.

(b) *Quadratic response*

Line	Equation of fit
6	$20.70 - 2.41 e_j + 0.82 e_j^2$
7	$10.64 + 0.13 e_j - 0.30 e_j^2$
15	$10.25 + 1.08 e_j + 0.21 e_j^2$
16	$6.35 + 1.16 e_j + 0.23 e_j^2$
28	$10.87 + 0.77 e_j - 0.24 e_j^2$

e_j limits are as shown in table 5.

(c) *Two intersecting-straight-line response*

Line	Equation A	Equation B
17†	$3.71 - 0.08 e_j$	$7.88 - 0.69 e_j$
25†	$5.94 + 1.03 e_j$	$2.59 + 1.28 e_j$
27†*	$7.36 + 2.04 e_j$	$2.68 + 1.26 e_j$

† e_j limits -2.85 to -1.36 , 0.55 to 2.60 .

* Not a statistically significant fit ($P=0.09$); see text.

$y = a + bx + cx^2$ being of opposite sign in lines 7 and 28 as opposed to lines 15 and 16. The response of line 6 ($Adh^{F/F}$) is altogether greater than that of $Adh^{S/S}$ lines. Also the response forms of both $Adh^{F/F}$ lines (6 and 19) are quite different. Line 19 has a relatively strong linear response throughout the range of e_j , whilst the equation for line 6 shows a strong tendency for a decline in phenotypic value with the highest value of e_j . The response forms of lines 17 and 25 best explained by two intersecting-straight-lines are also perhaps disparate although line 25 is perhaps the only really convincing line which conforms to a model of genotype-environment interaction based on two-intersecting straight lines.

The cause of this diversity of responses must be a complexity in the genetical control of genotype-environment interaction in ADH activity. $Adh^{F/F}$ lines although only small in number are by far the most environmentally sensitive. Nonetheless within each of the electrophoretically distinguished categories there is considerable variation in environmental sensitivity. Any systematic effects of lines with the same electromorph

cannot readily be ascertained for *Adh*^{F/F} lines ($N = 2$); however, within the *Adh*^{S/S} lines there is a tendency for lines with low mean ADH activity to conform to a model based upon two-intersecting-straight lines and for lines with high mean ADH activities to show a quadratic response although slightly varied in form (cf. lines 7 and 15). The linear response ($b_i \neq 0$) is more often observed with lines of intermediate activity.

4. DISCUSSION

This study is the first demonstration of genotype-environment interaction, mediated by normal variations of the environment which are encountered between successive experiments, for the character ADH activity. The genotype-environment interaction could be attributed to both the difference between ADH-F and ADH-S enzymes as well as to variations within an electrophoretically identified class of enzyme. Application of the analysis of genotype-environment interaction described by Jinks and Pooni (1979) has proved to be an invaluable procedure for the classification of the relative forms of response of enzyme activity in true-breeding lines to an undefined or random set of environments. Such environmental variation may well be a component of environmental variance even when fixed treatments form the basis of an experiment and is certainly a factor in the life of population cages of *Drosophila melanogaster*.

Before considering the implications of the genotype-environment interaction, it is of interest to consider its source in relation to experimental design. In this experiment there were three possible sources of environmental variation:

- (a) The seven different occasions upon which flies raised in the seven blocks were assayed for ADH activity; that is, environmental variation which has arisen from the technique of measuring ADH activity.
- (b) The processes of freezing and storage at -20° just prior to the assay of ADH activity.
- (c) The conditions of bottle culture in which flies were raised in the seven blocks.

Possibilities (a) and (b) are considered unlikely. The results of fourteen independent experiments in which duplicate cultures of selected "Texas" inbred lines or derived chromosomal substitution lines were represented, failed to show significant genotype-environment interaction for either the item duplicate bottles within lines $\times$ occasions or for the item lines $\times$ occasions; occasions in this context represent repeated determinations of ADH activity on randomly selected and assayed flies of all lines, from the same set of cultures, on different days. Reagents were prepared fresh daily. Whilst these items also include a component of variance due to storage time at -20° , no interaction of lines with length of storage has been detected under the conditions used in this laboratory, for studies of ADH activity, in specifically designed experiments. It is concluded that the genotype-environment interaction has arisen from differences in culture which occurred in the seven blocks or experiments. The variables responsible are therefore difficult to define and could be attributed to changes in, for example, the incubation temperature, humidity, or culture medium conditions. Even a quite large difference in incubator temperature ($\pm 8^\circ$) failed to

invoke a substantial interaction of lines $\times$ temperature when adult flies were assayed for ADH activity at 25° (unpublished data). Hence the critical environmental variation is most likely to be located in the cultures *per se* rather than in ancillary equipment. Earlier analyses demonstrated non-linear relationships between ADH activity and body weight for the variation between lines and for the interaction of lines $\times$ blocks. This non-linear relationship was also present in most of the partitions between lines which concerned the class of *Adh* electromorph. It might be argued that the relationship between ADH activity and body weight is naturally non linear, *e.g.*, such as is described for the environmental variance between immunologically determined ADH protein level and body weight described by Clarke, Camfield, Galvin and Pitts (1979). In the context of genetical variance, such a relationship could be obtained through gene interaction by homozygous pairs of loci for body weight; the expression of gene activity in such a case being limited by body weight loci *per se*, hence genetical variation in ADH activity would merely reflect non allelic gene action of body weight loci. However two other water-soluble enzyme activities, α -glycerophosphate dehydrogenase and glucose-6-phosphate dehydrogenase (Marson and Birley, unpublished data) are themselves genetically uncorrelated with ADH activity again implying genetical independence in the set of "Texas" inbred lines. Hence all evidence points to genotype-environment interaction in ADH activity being independent of genotype-environment interaction for body weight.

The heterogeneity of response to the environment so clearly demonstrated in this set of 18 inbred lines obviously reflects a diversity of response in gene activity to the environment. The source of such a diversity could of course reside in variation in the structural gene products which have or have not been resolved by gel electrophoresis (variation within *Adh*^{F/F} or *Adh*^{S/S} lines) and/or to a variable expression of modifier gene loci. A feature of the results is the high environmental sensitivity of *Adh*^{F/F} lines relative to that of *Adh*^{S/S} lines. Such environmental sensitivity is also reflected by these inbred lines for *in vitro* heat sensitivity and in relation to the occurrence of dominance in crosses between the "Texas" derived inbred lines (Birley, Marson and Phillips, 1980). This higher sensitivity must in part reflect the greater catalytic efficiency per molecule of the ADH-F enzyme than ADH-S enzyme in these lines (Birley, Couch and Marson, submitted). Of course the control of environmental sensitivity may also be due to variation in the rate of synthesis or even post-translational modification such as is observed in *Adh*^{S/S} lines (Birley, Couch and Marson, submitted).

The application of the analysis of Jinks and Pooni (1979) to variation in the molecular phenotype has been remarkably successful. For most lines the remainder variation was not statistically significant indicating that one of the three models, linear, quadratic and two intersecting-straight-lines adequately describe the relationship between genotype and environment. However, in three instances (lines 8, 18 and 22) the remainder MS was still significant indicating that a more complex or different model of genotype-environment interaction would be more appropriate. It is possible that with a greater set of environments a clearer response by these lines due to for example a model based upon two overlapping straight lines (Pooni and Jinks, 1980) could be tested. Nonetheless the diversity of the form of the responses shown by this set of highly inbred lines is strong evidence for a

multilocus response in genotype-environment interaction within an electromorph class. This conclusion is clarified by reference to the theoretical formulations of Mather (1975) who investigated the relationship between environmental factors effective upon different loci controlling a character in terms of the analysis of genotype-environment interaction. This two-locus model showed the plausibility of two-line regression responses and also included a study of the effect of both unequal gene frequencies in a sample of true-breeding material and the possibility of sampling an incomplete set of the genotype-specific environments. Even in this latter case a varied response to the environment could be demonstrated through a mixture of for example linear, non-linear or two-intersecting-straight-lines, to the environment. Moreover the different forms of response, linear or non-linear have been shown to be inherited by Pooni and Jinks (1980) for strains of *Nicotiana rustica*. The biological significance of these results is therefore to be found in genetically specific or partially specific environmental factors, present in culture, which modify the expression of ADH activity. The physiological genetics of such a mechanism are of course unknown as it is not yet possible to define the environments in physicochemical terms. The heterogeneous form of the responses shown by the inbred lines is evidence that some genotypes are more capable of responding to an overall improvement in the environment than are other genotypes. Such a response could for example be due to genetical variations between inbred lines in the capacity to respond *per se* to the environment or to a control gene which limit or stabilise the response in expression of ADH activity to the environment.

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