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

II. ASPECTS OF THE INHERITANCE OF ALCOHOL DEHYDROGENASE ACTIVITY IN *Adh^{S/S}* FLIES

B. W. BARNES and A. J. BIRLEY

Department of Genetics, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, England

Received 7.i.75

SUMMARY

Two inbred lines of *Drosophila melanogaster* extracted from a laboratory cage population and homozygous for the *Adh^S* allele of alcohol dehydrogenase (*Adh*) showed almost a twofold difference in specific activity. Analysis of generations derived from these lines showed that the genetic variation in ADH activity was controlled by additive gene action. There was no evidence of directional dominance or of non-allelic interactions. Although the *Adh* structural gene is on the second chromosome there was a significant effect of the X chromosome, indicating the action of modifying genes.

1. INTRODUCTION

VARIATION both in the frequencies and the activities of electrophoretically different alcohol dehydrogenase alleles is known to occur within populations of *Drosophila melanogaster* (Gibson, 1970; Gibson and Miklovich, 1971). Furthermore, variation in ADH specific activity of strains which have the same electrophoretic morph was demonstrated by Gibson and Miklovich (1971). In a previous paper Birley and Barnes (1973) estimated the narrow heritability of specific activity for individuals homozygous for the *Adh^S* allele as 20.3 per cent. This genetical variation may result from different *Adh^S* alleles which are not distinguishable electrophoretically, or to the action of genes which modify alcohol dehydrogenase activity, or to a combination of these causes. In this study we have investigated some aspects of the inheritance of alcohol dehydrogenase activity.

2. MATERIALS AND METHODS

A survey of ADH specific activity for 19 inbred lines derived from the "Texas" population was reported by Birley and Barnes (1973). From amongst the 17 lines which were homozygous for the *Adh^S* allele the line with the highest, *P*₁₅, and with the lowest, *P*₂₅, ADH specific activity were selected for further investigation. These two lines together with their reciprocal *F*₁ and *F*₂ generations were raised.

Alcohol dehydrogenase specific activity was measured on individual female flies as described by Birley and Barnes (1973). The experiment was carried out on flies cultured on five separate occasions. The samples of flies from each occasion were divided into two groups (blocks) and in each block four flies were assayed from each non-segregating generation,

while 12 flies were assayed from each of the two reciprocal F_2 crosses. The extract from a single fly was assayed once. In all, the specific activity of 40 individual flies was determined in a single day for each block in a random sequence.

3. RESULTS

The analysis of variance of specific activity for flies from the non-segregating generations is shown in table 1. In this analysis generations, a fixed effect, and occasions, a random effect, are cross-classified while blocks, a random effect, are subsamples within occasions.

TABLE 1
Analysis of variance of ADH specific activity for the non-segregating generations

Item	d.f.	M.S.	P
Lines	3	90.99	<0.1%
15 v. 25	1	268.46	<0.1%
Reciprocal F_1 's	1	1.99	n.s.
F_1 v. P	1	2.55	n.s.
Occasions	4	115.16	1.0-1%
Lines $\times$ occasions	12	3.52	n.s.
15 v. 25 $\times$ occasions	4	4.57	n.s.
Reciprocal F_1 's $\times$ occasions	4	3.84	n.s.
F_1 v. P $\times$ occasions	4	2.16	n.s.
Between blocks within occasions	5	25.47	<0.1%
Blocks $\times$ Lines within occasions	15	2.53	n.s.
Blocks $\times$ 15 v. 25 within occasions	5	1.98	n.s.
Blocks $\times$ Reciprocal F_1 's within occasions	5	2.27	n.s.
Blocks $\times$ F_1 v. P within occasions	5	3.34	n.s.
Replicates	120	3.54	

There are significant differences between occasions and between blocks within occasions. While the former may be due to variation in culture conditions the latter effect is probably the result of differences in the conditions of each block which were beyond our control. The significant variation between lines is attributable to the difference between P_{15} and P_{25} . There is no evidence of directional dominance for specific activity as the F_1 mean is not significantly different from the mean of the two parents nor is there any difference between the reciprocal F_1 generations. None of the comparisons amongst these generation means shows any interactions with occasions and the effects of the lines on each occasion do not interact with blocks. This shows that there are no significant genotype-environment interactions.

The analysis of variance of the specific activity of the reciprocal F_2 generations is shown in table 2. There is again a significant difference between the occasions and some evidence of differences between blocks

within occasions. The difference between the reciprocal F_2 generations is also significant. This shows that some of the variation in specific activity is sex-linked; the structural gene for ADH is located on the second chromosome. Again, the analysis shows no evidence of any genotype-environment interactions.

TABLE 2
Analysis of variance of ADH specific activity for the reciprocal F_2 generations

Item	d.f.	M.S.	P
Reciprocals	1	37.62	1.0.1%
Occasions	4	207.43	<0.1%
Reciprocals $\times$ occasions	4	7.58	n.s.
Between blocks within occasions	5	10.16	7.5%
Blocks $\times$ reciprocals within occasions	5	8.45	n.s.
Replicates	220	4.99	

We may obtain further information on the type of gene action involved in determining the variation between the generation means by carrying out a scaling test (Cavalli, 1952). This test consists of estimating by a weighted least squares procedure the parameters $[d]$, the balance of the additive autosomal effects, $[d_x]$, the balance of the sex-linked additive effects and m , the overall mean. It has already been established that there is no evidence for directional dominance (table 1) and therefore dominance components $[h]$ or $[h_x]$ have not been included in the model. In addition, as there were significant differences in specific activity between blocks, but no genotype-environment interaction, a parameter e_j was estimated as the sum of all the environmental effects within each block. The e_j 's are estimated subject to the restriction that $\sum e_j = 0$. The structure of the model for the j th block is shown below. Line 15 is the most active, but an inspection of the reciprocal F_2 generation means showed that the X chromosome of line 25 has the increasing effect on activity.

$$\begin{aligned}
 P_{15j} &= m + e_j + [d] - [d_x] \\
 P_{25j} &= m + e_j - [d] + [d_x] \\
 F_{1j} &= m + e_j \\
 F_{2(15 \times 25)j} &= m + e_j - \frac{1}{2}[d_x] \\
 F_{2(25 \times 15)j} &= m + e_j + \frac{1}{2}[d_x]
 \end{aligned}$$

The values of the genetical parameters estimated are

$$\begin{aligned}
 [\hat{d}] &= 2.7085 \pm 0.3463 \\
 [\hat{d}_x] &= 0.7919 \pm 0.2887
 \end{aligned}$$

A test of the adequacy of the model, by comparing the observed with the expected value of the generation mean in each block, gave a $\chi^2_{(38)} = 52.83$. This value corresponds to a probability between 0.10 and 0.05. The model fitted to these data in fact accounts for 90 per cent of the total $\chi^2_{(49)}$. Hence the model adequately summarises the data. The genetic variation between the generation means can be accounted for by additive autosomal and additive sex-linked effects. There is no evidence of directional dominance or of non-allelic interactions.

4. DISCUSSION

The measurement specific activity of an extract is the ratio of two variables, enzyme activity and soluble protein content of the extract. The lines used in this study also show genetical variation for soluble protein. It is possible, therefore, that variation in specific activity may be a reflection of variation in activity or in soluble protein or a combination of both. It is, of course, the former that we are principally concerned with. In view of this, analyses of variance were carried out on alcohol dehydrogenase activity $\left(\frac{1}{T} \times 100\right)$ and on the reciprocal of the soluble protein content (1/P). For activity, T is the time taken, in seconds, for the optical density of an extract to change by 0.05 O.D. units at 340 m μ . These analyses are shown in tables 3 and 4.

TABLE 3
Analysis of variance of non segregating generations

Item	Activity			Protein	
	d.f.	M.S.	P	M.S.	P
Lines	3	3.63	$\leq 0.1\%$	13.49	$< 0.1\%$
Occasions	4	1.93	n.s.	30.55	5.1%
Lines $\times$ occasions	12	0.09	n.s.	2.10	n.s.
Between blocks within occasions	5	0.69	$< 0.1\%$	3.21	5%
Blocks $\times$ Lines within occasions	15	0.09	n.s.	1.60	n.s.
Replicates	120	0.12		1.44	

TABLE 4
Analysis of variance of reciprocal F_2 generations

Item	Activity			Protein	
	d.f.	M.S.	P	M.S.	P
Reciprocals	1	1.02	1%	0.52	n.s.
Occasions	4	2.43	1.0.1%	88.84	1%
Reciprocals $\times$ occasions	4	0.22	n.s.	3.33	n.s.
Between blocks within occasions	5	0.64	0.1%	6.79	1%
Blocks $\times$ reciprocals within occasions	5	0.26	n.s.	1.48	n.s.
Replicates	220	0.15		1.82	

We can see (table 3) that there are highly significant differences between lines both for activity and for protein. In this case, therefore, the variation in activity may be a consequence of the differences in soluble protein provided that these differences reflect corresponding variation in the amount of ADH protein. It must be assumed, however, that variation in the amount of ADH protein constitutes only a small part of the total genetical variation in soluble protein. In contrast, there are significant differences between reciprocal F_2 crosses for activity but not for protein (table 4). Hence the difference in the specific activity of the reciprocal F_2 crosses must be due to a difference in enzyme activity as there is no significant difference in

the amount of protein. As the ADH structural gene is located on chromosome II this shows that the X chromosome has a modifying effect on ADH activity.

Acknowledgment.—We wish to thank Nick Martin and Lindon Eaves for helpful discussions.

5. REFERENCES

- BIRLEY, A. J., AND BARNES, B. W. 1973. Genetical variation for enzyme activity in a population of *Drosophila melanogaster*. I. Extent of the variation for alcohol dehydrogenase activity. *Heredity*, 31, 413-416.
- CAVALLI, L. L. 1952. An analysis of linkage in quantitative inheritance. *Quantitative Inheritance*, 135-144. H.M.S.O. London.
- GIBSON, J. 1970. Enzyme flexibility in *Drosophila melanogaster*. *Nature*, 227, 959-960.
- GIBSON, J., AND MIKLOVICH, R. 1971. Modes of variation in alcohol dehydrogenase in *Drosophila melanogaster*. *Experientia*, 27, 99-100.