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

IV. ANALYSIS OF ALCOHOL DEHYDROGENASE ACTIVITY IN CHROMOSOME SUBSTITUTION LINES

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SUMMARY

Chromosome substitution lines derived from two inbred strains of *Drosophila melanogaster* homozygous for the Adh^S allele of alcohol dehydrogenase but differing significantly in ADH activity have been analysed. Variation in activity can be attributed to all three major chromosomes. The effect of the second chromosome, where the ADH structural gene is located, can be modified significantly by the genotype of both the first and the third chromosomes. The most substantial single effect results from homozygous differences between the third chromosomes. In contrast, differences between the X chromosomes are revealed only when the second or second and third chromosomes are heterozygous.

1. INTRODUCTION

HERITABLE variation for ADH activity has been demonstrated amongst lines isolated from a laboratory population of *Drosophila melanogaster* and homozygous for the Adh^S allele (Birley and Barnes, 1973). Allelic heterogeneity which is not detected after electrophoresis and modifier genes can both contribute to this genetical variation. Barnes and Birley (1975) have shown, in respect of modifier loci, that the X chromosome is associated with some of the genetical variation in ADH activity amongst these lines; the *Adh* structural gene is located on chromosome II (Grell, Jacobson and Murphy, 1965). Studies by Hewitt, Pipkin, Williams and Chakrabartty (1974) and Ward (1975) have demonstrated modifier loci for ADH activity on each of the three major chromosomes. In the more detailed study by Ward (1975) chromosome substitution lines were constructed from strains which, although having the same electromorph $Adh^{S/S}$, had been selected for high or for low ADH activity. Chromosomal effects were estimated from lines in which the chromosomes in question, II and III, had been individually substituted into the genome of a third strain, Oregon. In this analysis no information is provided concerning gene interaction either between chromosomes of the selected lines or between selected and Oregon chromosomes. The present study concerning an analysis of a complete set of substitution lines overcomes these difficulties and provides a fuller description of gene action, on a chromosomal basis, for the character ADH activity.

2. MATERIALS AND METHODS

We have been concerned previously with an analysis of ADH activity in a set of inbred lines derived from the " Texas " population of *Drosophila*

melanogaster (Birley and Barnes, 1973 and 1975). From amongst the lines homozygous for the Adh^S allele two were selected for more detailed investigation, P_{15} with the highest and P_{25} with the lowest ADH activity (Barnes and Birley, 1975). The nature of the genetical differences in ADH activity have been investigated further by constructing chromosome substitution lines between P_{15} and P_{25}. If we neglect the fourth chromosome, as it is very small, then there are eight true breeding combinations of the three major chromosomes originating from these two lines. We may represent these as HHH, HHL, HLH, LHH, HLL, LHL, LLH and LLL where, for example, HHL indicates that chromosomes I and II are derived from P_{15} while chromosome III originates from P_{25}. The lines were constructed using standard techniques in which individual chromosomes are manipulated by balancing them against marked inversion stocks. In this way chromosomes from P_{15} and P_{25} are transmitted as units and, ideally, when substituted into different lines retain their identity. The technique will be successful only if the inversions used to suppress crossing over are efficient. In the present scheme the use of triple heterozygotes was avoided as these are known to markedly reduce the efficiency of the inversions (Robertson, 1954).

Alcohol dehydrogenase activity was measured on individual female flies in milliunits (mU) at 25°C using an LKB Reaction Rate Analyser 8600 coupled to an LKB Reaction Rate Calculator 8200. All other technical details are as described by Birley and Barnes (1973 and 1975).

3. Analysis of the substitution lines

(i) *Test for recombination*

Before carrying out a genetic analysis of the substitution lines we need to determine the efficiency of the extraction procedure. If we assume that the inversions have been effective in suppressing crossing over then we would expect lines produced by crossing any of the four complementary pairs of the substitution lines (HHL × LLH, for example) and hence heterozygous for the three major chromosomes to be genetically identical. Conversely, if there has been significant crossing over then it is very unlikely that particular chromosomes extracted independently in different substitution lines would be identical. This recombination will result in significant differences between the multiply heterozygous lines and may be detected in an assay.

The four types of F_1's were produced reciprocally and five females from every cross were individually assayed for ADH activity in each of two randomised blocks. The analysis of variance is shown in table 1. None of the items is significantly greater than the error mean square, in particular the F_1's do not differ and hence there is no evidence for any marked recombination having occurred during the construction of the substitution lines.

(ii) *Homozygous combinations*

Variation in ADH activity between the substitution lines can be partitioned to determine the additive effect of individual chromosomes as well as inter-chromosomal interactions or non-additive gene action. Accordingly, duplicate cultures of each of the eight substitution lines were raised

TABLE 1

Test for recombination in the substitution lines

Item	d.f.	M.S.	P
Between F_1's	3	11·84	20-10%
Blocks	1	0·03	n.s.
Blocks × F_1's	3	3·79	n.s.
Between reciprocals within F_1's	4	11·02	10%
Blocks × reciprocals within F_1's	4	7·91	25%
Replicate error	64	5·78	—
Pooled error	72	6·19	—

and two female flies from every culture were individually assayed for ADH activity in each of six randomised blocks. Altogether 192 individuals were scored.

An analysis of variance of these data is shown in table 2. The highly significant variation between the lines was partitioned into seven orthogonal chromosome effects which are each tested against a pooled error variance for 83 d.f. Both chromosomes II and III show an overall effect upon ADH activity. Turning now to the interactions we can see that although there is no additive effect of chromosome I it is not without influence since the effects of chromosomes II and III are both dependent on the genotype of chromosome I. In addition, chromosomes II and III are not independent in gene action.

The chromosomal effects on ADH activity on an individual fly basis are shown in table 3. The greatest effect, by far, is shown by chromosome III. Since the ADH structural gene is located on chromosome II this,

TABLE 2

Analysis of variance of the substitution lines

Item	d.f.	M.S.	P
Lines	7	107·07	$\ll$0·1%
Chromosome I	1	2·86	n.s.
II	1	82·95	<0·1%
III	1	587·02	<0·1%
I × II	1	20·44	1-0·1%
II × III	1	35·69	<0·1%
I × III	1	19·38	1-0·1%
I × II × III	1	1·17	n.s.
Blocks	5	1·97	n.s.
Lines × blocks	35	1·55	n.s.
Duplicate bottles within lines	8	0·87	n.s.
Bottles × blocks within lines	40	2·76	5-1%
Replicate error	96	1·59	—
Pooled error	83	2·07	—

TABLE 3

Chromosomal effects on ADH activity

I	II	III	I × II	I × III	II × III	I × II × III
—0·122	0·657	1·749	0·326	—0·318	—0·431	0·078

(Tests of significance for each effect are shown in Table 2.)

together with the chromosomal interactions, represents the action of modifier loci.

We should consider, at this point, the apparent discrepancy between the reported sex-linked modification reported by Barnes and Birley (1975) and the results of the present study in which no overall effect of the X chromosome was detected. In our previous work the effect of chromosome I was inferred from a significant difference between reciprocal F_2 females. The genetical expectation of this difference in the absence of non-allelic interactions, is equal to $[d_1]$ the balance of the sex-linked additive effects. When we are able to examine interactions between chromosomes then the expectation of this difference becomes

$$[d_1]+\tfrac{1}{2}j_{1/2}+\tfrac{1}{2}j_{1/3}+\tfrac{1}{4}j_{1/23}$$

Where the j-type interactions occur between homozygous X chromosomes and heterozygous autosomes. The analysis of the substitution lines suggests that it is the homozygous × heterozygous interactions that are leading to the significant reciprocal difference observed in the F_2 generation. If this is the case then in the previous analysis (Barnes and Birley, 1975) the additive sex-linked effect was confounded with the interaction of the X chromosome with the autosomes. We will return to this point in the next section when a more detailed analysis is possible.

(iii) *27 Genotypes*

The genetical analysis of ADH activity may be extended further by producing the 27 female homozygous and heterozygous chromosome combinations from appropriate crosses between the eight substitution lines. Certain genotypes may be obtained from different crosses. LXX (where X represents a heterozygous chromosome), for example, can be obtained by crossing LLL with LHH or LLH with LHL and, of course, from the reciprocal crosses. We have used the procedure outlined by Kearsey and Kojima (1967) in which each of the substitution lines is used with about equal frequency as both male and female parent.

Duplicate cultures of the 27 genotypes were raised and two females from every cross were individually assayed for ADH activity in each of five randomised blocks. In total, the activity of 540 individuals was measured. The results are shown in table 4.

TABLE 4

Mean ADH activity in milliunits of the 27 genotypes derived from the substitution lines

Genotype	Mean	Genotype	Mean	Genotype	Mean
HHH	9·076	XHH	9·311	LHH	8·845
HHX	6·790	XHX	8·290	LHX	7·032
HHL	6·847	XHL	5·643	LHL	5·270
HXH	7·949	XXH	8·871	LXH	9·426
HXX	7·586	XXX	6·950	LXX	6·202
HXL	4·507	XXL	5·549	LXL	4·659
HLH	8·001	XLH	7·527	LLH	9·355
HLX	3·950	XLX	6·665	LLX	7·037
HLL	3·550	XLL	3·218	LLL	4·587

H and L denote the origin of homozygous chromosome combinations while X denotes a genotype heterozygous for H and L chromosomes.

The type of gene action involved in determining the variation between the line means can be examined by estimating the following parameters by a weighted least squares procedure. The notation follows that of Van der Veen (1959) but in this case each parameter refers to a chromosomal effect. A full interpretation of each parameter may be found in Mather and Jinks (1971).

d_i the additive effect of the ith chromosome
h_i the dominance effect of the ith chromosome
i the additive × additive interaction
j the additive × dominance interaction
l the dominance × dominance interaction

The subscripts of each parameter refer to the chromosomes involved. In the case of the j-type interactions, $j_{1/23}$ for example, represents the interaction of a homozygous first chromosome with heterozygous second and third chromosomes. The adequacy of the model can be tested by comparing the observed with the expected generation mean as an approximate χ^2 (Cavalli, 1952).

In the estimation procedure the complete model consisting of 27 parameters is successively reduced by omitting all non-significant parameters and re-estimating the remaining ones. The magnitude and indeed the standard errors of the re-estimated parameters will change unless they are statistically independent of the parameters eliminated from the model. As a consequence some of the remaining parameters may become non-significant when re-estimated. Ideally, we are aiming to find a model in which all the parameters estimated are statistically significant and which adequately accounts for all of the observed variation. This has not proved to be possible in the present case. The χ^2 testing the goodness of fit of the model is of borderline significance when we consider only the significant parameters even though the model accounts for 99 per cent of the observed variation. The goodness of fit can easily be improved but the additional parameters estimated are not statistically significant. We have shown in table 5 two examples of the final models. Model A is an example which adequately summarises the data but contains three non-significant parameters.

TABLE 5

Genetic components of line means

Model A			Model B		
Parameter	Estimate	Standard error	Parameter	Estimate	Standard error
d_2	0·734	0·099	d_2	0·734	0·099
d_3	1·919	0·099	d_3	1·919	0·099
h_3	−0·303	0·201	—	—	—
i_{12}	0·587	0·121	i_{12}	0·587	0·121
i_{23}	−0·338	0·121	i_{23}	−0·338	0·121
$j_{1/3}$	−0·832	0·210	$j_{1/3}$	−0·832	0·210
l_{12}	0·474	0·315	—	—	—
l_{13}	1·044	0·343	l_{13}	0·602	0·257
$j_{1/23}$	1·524	0·364	$j_{1/23}$	1·524	0·364
l_{123}	−1·001	0·603	—	—	—
χ^2_{16}	23·791		χ^2_{19}	30·342	
P	0·094		P	0·048	

If the non significant parameters h_3, l_{12} and l_{123} are removed then we have the example shown in Model B. Here all the parameters are significant but the χ^2 testing the goodness of fit of the model has a probability of 4·8 per cent. In the discussion we will consider Model B in view of the very substantial proportion of the variation for which this model accounts.

We can see that there is an overall additive effect of chromosome II (d_2) which is not surprising, of course, since the ADH structural gene is located on chromosome II and we have deliberately selected for study two lines which, although having the same electrophoretic mobility, differ in their ADH activities. If we look at the evidence for modifying genes we see that chromosome III has a very substantial additive effect, d_3, considerably larger, in fact, than the effect of chromosome II. Turning now to consider the interactions between chromosomes we see that the additive effect of chromosome II, reflecting the effect of the structural gene together with any linked modifiers, depends on the genotype of the first chromosome (i_{12}) and on the genotype of chromosome III (i_{23}). Previously, in the analysis of the substitution lines, we were unable to detect any additive effect of the first chromosome although its influence on ADH activity was shown by the interaction with chromosomes II and III, table 2. In this analysis we can see that the additive effect of the first chromosome depends on having either chromosome III heterozygous, $j_{1/3}$ or by having both chromosomes II and III heterozygous, $j_{1/23}$. The significant difference between females in the reciprocal F_2 crosses indicating the importance of sex-linkage (Barnes and Birley, 1975) is then a reflection of the confounded effect of the parameters d_1, $j_{1/3}$ and $j_{1/23}$. Further evidence for the influence of modifier loci comes from the interaction of heterozygous first and third chromosomes. Finally, we can note that there is no evidence for directional dominance but we must remember that as we are concerned with an analysis of crosses between a set of substitution lines we are, in each case, looking at the *net* effect of whole chromosomes and dominance effects could be ambidirectional.

4. Discussion

A major portion of the total phenotypic variance in ADH activity within the " Texas " population is accounted for by a two-fold difference in ADH-F and ADH-S enzyme activities (Birley and Barnes 1973 and 1975). This difference is also shown in other populations of *Drosophila melanogaster* (Gibson, 1970; Ward, 1975; Oakeshott, 1976). Genetical variation for ADH activity in the " Texas " population is also manifest amongst lines of the same electromorph, indeed there is about a two-fold difference between the highest and lowest ADH activities whether the flies are of the $Adh^{S/S}$ or $Adh^{F/F}$ genotype (Barnes and Birley 1975; Birley and Barnes 1975).

The present study has shown that the phenotypic variation in ADH activity between lines homozygous for $Adh^{S/S}$ can be attributed to the effects of chromosomes I, II and III. Since the structural gene for ADH is located on chromosome II the effect of chromosome III is not only substantial but remarkable. In addition, there was considerable non-allelic variation for the character and the gene interactions involve all of the three major chromosomes in both heterozygous and homozygous states.

A component of the total genetical variation in ADH enzyme activity *i.e.* the difference between ADH-F and ADH-S is adaptive in the presence

of alcoholic substrates in the food medium (Gibson 1970; Bijlsma-Meeles and Van Delden, 1974; Briscoe, Robertson and Malpica, 1975; Morgan, 1975; Oakeshott, 1976) and can be attributed to the catalytic efficiencies of these enzymes (Day, Hillier and Clarke, 1974; Clarke, 1975). The causes and adaptive strategies for the genetical variation at modifier loci are less well understood and may well be manifold. The effects of polymorphic modifier loci on ADH activity could result from variation in, for example, developmental processes, rates of transcription or of translation or molecular interference capable of causing conformational changes in the functional ADH enzyme. The importance of these types of mechanisms in relation to the total variation of the ADH enzyme phenotype obviously requires more detailed investigation.

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