Genet. Res., Camb. (1975), 26, *pp.* 81–93
With 3 *text-figures*
Printed in Great Britain

Alcohol dehydrogenase activity in *Drosophila melanogaster*: a quantitative character

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(*Received* 12 *May* 1975)

SUMMARY

Alcohol dehydrogenase activity in *Drosophila melanogaster* may be considered as a quantitative character, since it shows many features typically associated with such traits. Although strains with the electrophoretically fast phenotype generally have activities greater than those with the slow phenotype, presumably reflecting differences in the nucleotide sequences of the structural alleles, within each electrophoretic class there is considerable variation in activity. The expression of the structural gene, in terms of ADH activity, is to some extent regulated by its genetic background. Strains homozygous for particular structural alleles respond to divergent directional selection for ADH activity. Modifiers have been located to the *X*, second and third chromosomes.

1. INTRODUCTION

The locus coding for the enzyme alcohol dehydrogenase in *Drosophila melanogaster* is frequently found to be segregating for two alleles in natural populations: Adh^{F} and Adh^{S}, coding for the faster and slower migrating allozymes respectively (Johnson & Denniston, 1964). Typically, Adh^{F} homozygotes show approximately twice the activity of Adh^{S} homozygotes (Rasmuson *et al.* 1966; Gibson & Miklovich, 1971; Day, Hillier & Clarke, 1974; Hewitt *et al.* 1974; Ward, 1974), although in strains derived from a wild population an appreciable overlap in the activities of the two allozymes was found (Ward, 1974). Intra-allozyme variation in ADH activity results at least partially from the segregation of modifier genes (Ward & Hebert, 1972), but may also arise from the segregation of different Adh^{F} and Adh^{S} alleles within each mobility class.

It seemed likely that ADH activity could be considered as a quantitative character. Most quantitative characters respond to appropriate selection procedures, and their expression is regulated by a number of genes. It is the purpose of this paper to show that ADH activity in *D. melanogaster* possesses these features.

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https://doi.org/10.1017/S0016672300015871 Published online by Cambridge University Press

2. MATERIALS AND METHODS

Strains

Strains were isolated from a natural population breeding in an orchard a few miles from Cambridge. Each was made homozygous for a single Adh^F or Adh^S allele by a process of inbreeding. Further details of these strains are given in Ward (1974).

Substitution of whole chromosomes onto a Standard Oregon background was carried out using balancer chromosomes. The balancer chromosomes were chosen for their efficiency of cross-over suppression, and are individually distinguishable in all conditions. The dominant markers on the cross-over suppressors are: on the *X*, Bar (*B*); on the second, Plum (*Pm*) and Curly (*Cy*); on the third, Stubble (*Sb*) and Ultrabithorax (*Ubx*); the small fourth chromosome is left unmarked. Further details of these markers and balancers are given in Lindsley & Grell (1968).

Assays

Electrophoretic procedures, utilizing polyacrylamide disc electrophoresis, are given in Ward (1974).

ADH activity was measured by monitoring the increase in absorbance at 340 nm associated with the reduction of NAD and using 2-propanol as substrate. Assays were performed at pH 8·7 and 25 °C using a Perkin-Elmer 124 spectrophotometer. One of three assay procedures was adopted according to the particular needs of the experiment:

(*a*) The majority of assays were carried out on crude extracts of mature third instar larvae. Details of extraction procedures and buffers are given in Ward (1974). Activity is related to the wet weight of the sample.

(*b*) Changes in ADH activity during ontogeny were measured. Flies were allowed to lay eggs on the surface of a nutrient medium for a period of eight hours, after which all flies were removed. Two and three day old larvae were collected by pouring 1·0 M and 0·5 M sucrose respectively into the bottle and agitating. Larvae floated to the surface and were easily harvested. Third instar larvae (present on day 4) and pupae (present on days 5–9) were picked off the sides of the bottle. Adults were aged in 4 in. tubes containing nutrient medium. The number of individuals in approximately 20 mg of the developmental stage to be assayed was noted (or, for two and three day old larvae, estimated from the number in a smaller sample), the sample homogenized in 1 ml ADH buffer, and the homogenate centrifuged. ADH activity in a 0·2 ml aliquot of supernatant was measured, and the relative activity per individual calculated.

(*c*) Adults of ADH-F-1, ADH-F-2, and of the two reciprocal crosses were assayed. After emergence, adults were aged in 4 in. tubes containing nutrient medium. Ten five to nine day old flies were homogenized in 0·7 ml ADH buffer, centrifuged, and a 0·2 ml aliquot of the supernatant assayed for ADH activity. Sexes were assayed separately. Activity is expressed on a per individual basis. This procedure is some-

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what different from that used in (*b*) above, and thus the results are not directly comparable.

Selection procedures

A divergent directional selection regime was employed to select for increased and decreased ADH activity. Forty females (eighty in the case of ADH-S-4c) from the breeding stock of each strain were placed singly in tubes and their adult progeny transferred to bottles. Samples of their progeny were then assayed as mature third instar larvae. Each culture was assayed twice, on successive days, and the mean activity per culture calculated. The remaining larvae were allowed to produce adults. Forty females from the culture with the highest activity and forty from that with the lowest were placed singly in tubes. The progeny of the twenty most fertile cultures in each case were transferred to bottles, and their larvae assayed. The remaining larvae from the culture with the highest activity in the high line and lowest activity in the low line were allowed to produce adults and the process repeated. Selection was carried out in this manner for between four and seven generations. Owing to increasing sterility, frequently less than twenty sets of progeny could be assayed. The actual number of cultures assayed in a given generation is given in the figures.

3. RESULTS

During the course of the present study, three ADH-S and four ADH-F strains were studied, each being homozygous for a particular Adh^{S} or Adh^{F} allele. Third instar larvae activities are given in Table 1 (from Ward, 1974). All experiments were carried out within two years of isolation from a natural population.

Table 1. *The ADH activity per mg of mature larvae of three ADH-S and four ADH-F strains*

Strain	Mean of 5 ADH assays	Standard deviation
ADH-S-4a	4·42	0·73
ADH-S-4c	8·32	0·73
ADH-S-11a	1·71	0·23
ADH-F-1	6·50	0·18
ADH-F-2	2·98	0·10
ADH-F-4b	6·82	0·85
ADH-F-5	4·59	0·26

Disruptive selection lines

Three ADH-S and three ADH-F strains were subjected to selection for high and low ADH activity. The results are given in Fig. 1 and Fig. 2.

The responses of the ADH-S-4a and ADH-S-4c strains were briefly described in Ward & Hebert (1972). These two strains are known to have identical Adh^{S} structural alleles, barring the possibility of mutation, and the differences in

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activity between them are due to modifier genes. It will be seen that the response of ADH-S-4a was immediate and rapid in the first two selected generations, but that subsequent selection gave no further gains. The response of ADH-S-4c was very small, the regression coefficient of the high line being barely significant ($0{\cdot}05 > P > 0{\cdot}02$) and that of the low line non-significant, and in no generation was the mean high selected activity significantly greater than the mean low selected activity. It is likely that ADH-S-4a was segregating for high and low alleles at one or two regulatory loci, and that ADH-S-4c must have been homozygous for the

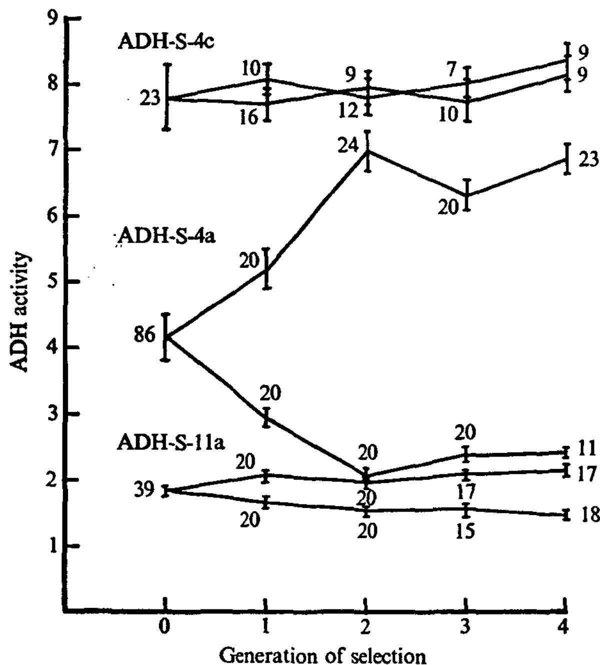

Fig. 1. Response of three ADH-S strains to divergent directional selection for ADH activity. The number of cultures assayed each generation and standard deviations are indicated.

high activity alleles. The high and low lines of ADH-S-4a became fixed for the high and low alleles, respectively. However, the high line of ADH-S-4a stabilized at a level significantly lower than the mean activity of ADH-S-4c ($P < 0{\cdot}001$), and it follows that ADH-S-4c must also have been fixed, or nearly fixed, for high alleles at one or more modifying loci of small effect, and that the high line of ADH-S-4a must have, by chance, become fixed for low alleles at these loci.

ADH-S-11a responded to selection less dramatically than ADH-S-4a, although one generation of selection was sufficient to produce a significant divergence in activity between the high and low lines ($P < 0{\cdot}001$). The response is again due to the segregation of modifier genes.

Each ADH-F strain tested responded to the selection procedure. One generation

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of selection gave a significant divergence between the high and low lines of ADH-F-2 ($P < 0{\cdot}001$) and ADH-F-5 ($0{\cdot}05 > P > 0{\cdot}01$) but not of ADH-F-1. The second generation of selection increased the divergence between the selected lines of ADH-F-5 ($P < 0{\cdot}001$) and now gave a significant divergence in the ADH-F-1 lines ($0{\cdot}01 > P > 0{\cdot}001$). These results cannot be interpreted as certainly as those from the ADH-S strains. The three ADH-F strains were separately isolated, and

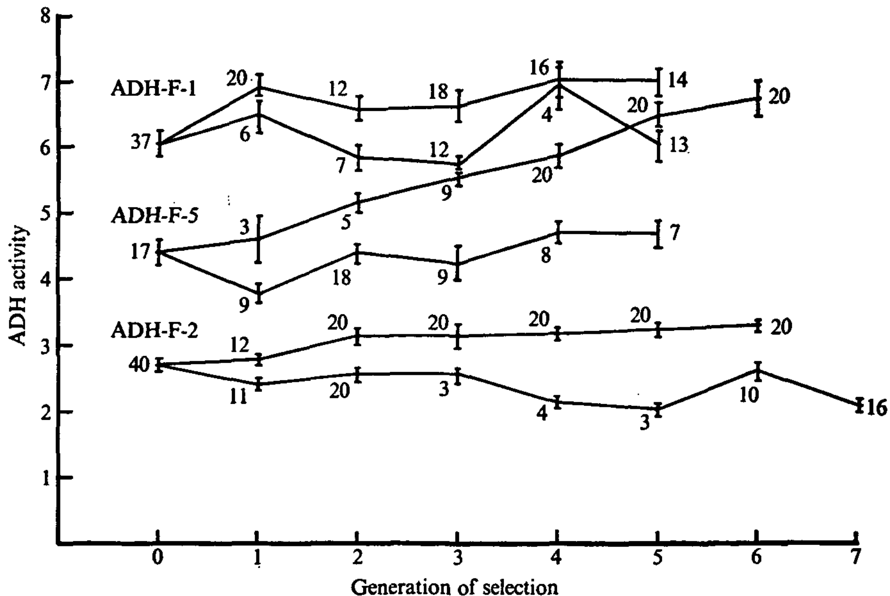

Fig. 2. Response of three ADH-F strains to divergent directional selection for ADH activity.

differences in activity between the base populations of these strains may reflect either structural gene differentiation or modifier gene segregation. The ADH-F strains were rather less fertile than the ADH-S strains, a consequence of the increased number of inbreeding generations required to derive these strains, homozygous for single Adh^{F} alleles, from a natural population where Adh^{F} is the predominant allele, and selection pressures were correspondingly reduced. Nevertheless, the low response patterns suggest that these strains were segregating for minor modifiers rather than the major modifier(s) of ADH-S-4a.

The ADH-S-4 strains

Two ADH-S-4 strains were further analysed and characterized. These are the high activity strain ADH-S-4c, hereafter referred to as ADH-S-4h, and the low line of ADH-S-4a, hereafter referred to as ADH-S-4l. The differences in activity between them are due to modifier genes.

The effect of X-linked genes was tested by assaying larval samples of ADH-S-4h, ADH-S-4l and the two classes of heterozygotes produced by the two reciprocal

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crosses (Table 2). Samples of the four genotypes were assayed together to reduce experimental error. The mean F1 value is not significantly different from the mean mid-parent value, and the mean values of the two F1 classes are not significantly different from one another. Factors on the *X* chromosome have little effect on the difference in activity between ADH-S-4h and ADH-S-4l.

Table 2. *The ADH activity per mg of mature larvae of ADH-S-4h, ADH-S-4l, and the two reciprocal F1 classes*

Genotype	Mean of 7 ADH assays	Standard deviation
ADH-S-4h	8·14	0·33
ADH-S-4l	2·36	0·18
F1 from 4h♀ × 4l♂	5·10	0·28
F1 from 4l♀ × 4h♂	5·03	0·25

Table 3. *The ADH activity per mg of mature larvae of ADH-S-4h (HHH), ADH-S-4l (LLL) and Oregon (OOO), and of the strains isolated by substituting the second and third chromosomes of ADH-S-4h and ADH-S-4l for the corresponding Oregon chromosomes*

Strain	Mean of 9 ADH assays	Standard deviation
HHH	7·85	0·63
LLL	2·26	0·17
OOO	6·47	0·45
OHO	8·30	0·51
OLO	2·99	0·22
OOH	8·35	0·77
OOL	6·91	0·85

The effects of chromosomes 2 and 3 were tested by substituting these chromosomes into an inbred Oregon strain, using the *Cy-Ubx/B-Pm-Sb* stock. The substituted strains are designated OHO and OLO, and OOH and OOL, the chromosomes of the substituted strains being present as homozygotes. The Oregon strain is homozygous for Adh^{F} and has normal ADH-F activity. Fifty individuals from each substituted strain were typed electrophoretically, and in each case the observed phenotype corresponded with that expected. Samples of mature larvae from the three parental strains and four substituted strains were assayed (Table 3). Much of the difference in activity between ADH-S-4h and ADH-S-4l is due to one or more modifiers lying on chromosome 2. The activity ratio of HHH to LLL is 3·46, and that of OHO to OLO is 2·78; isolation of the second chromosomes of ADH-S-4h and ADH-S-4l onto an Oregon background has maintained 80 % of the difference in activity. One or more modifiers are also situated on chromosome 3. ADH activity of OOH is significantly greater than that of either OOO ($P < 0{\cdot}001$) or OOL ($0{\cdot}01 > P > 0{\cdot}001$), which are themselves not significantly different. Factors on chromosome 3 increase OOH activity by about 20 % over OOO and OOL.

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It is concluded that about 80% of the difference in activity between ADH-S-4h and ADH-S-4l results from one or more activity modifiers on chromosome 2, and about 20% from modifiers on chromosome 3. Loci on the *X* chromosome must here be of comparatively minor significance.

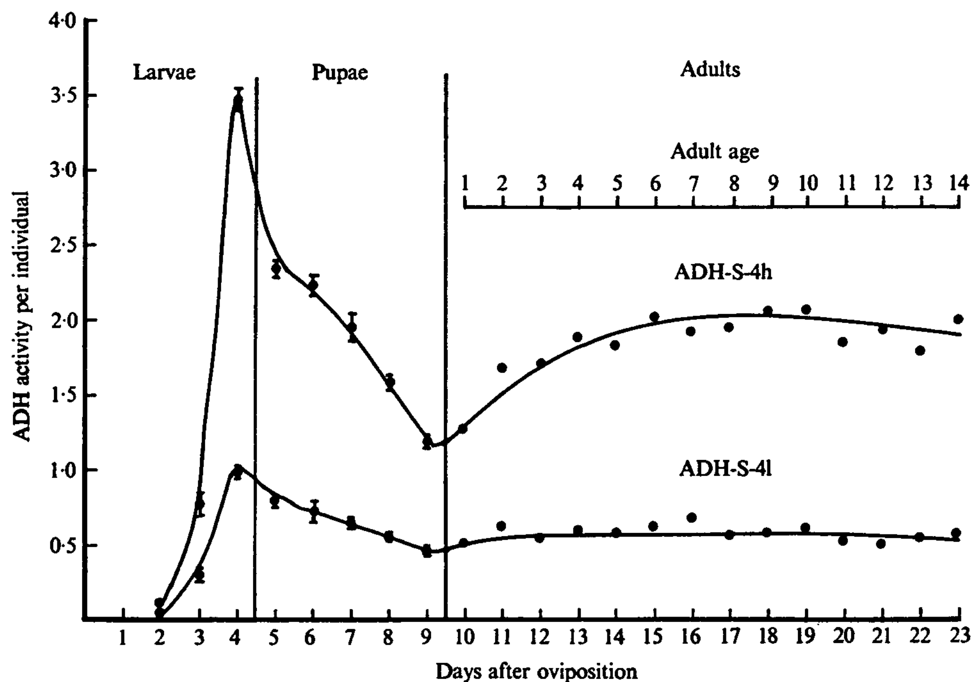

Fig. 3. Developmental changes in ADH activity in strains ADH-S-4h and ADH-S-4l. Standard deviations are indicated for preadult stages. Adult activities are the means of male and female activities.

A preliminary experiment to map the modifier(s) on chromosome 2 showed that the bulk of the activity difference was due to a gene or genes lying at approximately 60 cMs, but fine mapping experiments to determine the exact location of this modifier have not been carried out. It is interesting to note that the ADH structural gene has been located at position 50·1 on chromosome 2 (Grell, Jacobson & Murphy, 1965).

An experiment was carried out to determine whether the two strains maintain their differences in activity throughout ontogeny (Fig. 3). Enzyme activity is expressed in terms of ADH activity per individual. During the preadult stages, four samples per genotype were assayed each day following oviposition (excluding day one), and in the adult stage, four samples per sex per genotype were assayed on each of the first fourteen days of adult life. Adult activities are plotted as the means of the male and female activities in each genotype. Changes in activity during ontogeny are generally similar to previously published reports, although Ursprung, Sofer & Burroughs (1970) indicate that ADH activity per mature adult fly is as great as that of the mature larvae, and Hewitt *et al.* (1974) find a peak of

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post-eclosion activity at day three of adult life. These differences may reflect, for example, differences in culture conditions or genetic differences between strains.

The ratio of activities between ADH-S-4h and ADH-S-4l increases from 2·40 in the early larval stages to 3·54 in mature larvae, and then steadily falls to 2·67 in old pupae and to 2·51 in one day old adults. It then rises again to 3·26 by day four of adult life and to 3·42 by day eight. Thereafter it remained steady for the duration of the experiment. A complete interpretation of these changes is not possible without a fuller knowledge of, for example, the half-life of the enzyme and its exact times of synthesis. Nevertheless, it does seem that the modifier genes, although selected for their effects on ADH activity in third instar larvae, are equally active in adults eight or more days old. Furthermore, the activity ratios in male and female adults are very similar, and the modifiers must be equally active in both sexes of the adult (Table 4).

Table 4. *The ADH activity per individual of adults of ADH-S-4h and ADH-S-4l. Days 1 to 14 of adult life*

	Males			Females			Overall
Genotype	n*	$\bar{x}$	S.D.	n*	$\bar{x}$	S.D.	$\bar{x}$
ADH-S-4h	56	1·77	0·34	56	1·96	0·32	1·87
ADH-S-4l	56	0·54	0·09	56	0·62	0·17	0·58
Activity ratio: 4h/4l		3·28			3·16		3·22

n* Number of samples assayed.

Reciprocal crosses between ADH-F strains

These experiments arose from an original observation that larvae from the cross between ADH-F-2 females and ADH-F-1 males had ADH values significantly greater than both the mid-parent value and the larvae from the reciprocal cross, which had ADH activities not significantly different from the mid-parent value. Subsequently, all possible reciprocal crosses were made between ADH-F-1, ADH-F-2, ADH-F-4b and ADH-F-5, and third instar larvae assayed. Two independent trials were carried out, the second group of larvae being reared and assayed approximately one week after the first. Four runs per trial were made, each run consisting of sixteen assays, one from each of the four parental classes and the twelve reciprocal crosses. This allowed calculation of the expected mid-parent value for each cross in each run. The data were analysed by a set of ANOVARs. The overall mean activities of the genotypes are given in Table 5. ADH-F-1 now has an activity just significantly greater ($0·05 > P > 0·01$), by about one activity unit, than when it was first assayed. This may reflect a small change in the genetic constitution of this strain from the time of its isolation. Other strains showed mean values not significantly different from the initial assays.

ANOVARs showed that when, and only when, ADH-F-2 is one of the parents, the mean F1 value is significantly greater than the mean mid-parent value. It can

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be seen from Table 5 that this partial dominance effect is almost completely associated with crosses where the female parents are from ADH-F-2. Progeny of the cross ADH-F-4b females by ADH-F-2 males have a mean activity just significantly greater than expected.

Table 5. *ADH activity per mg of mature larvae from ADH-F-1, ADH-F-2, ADH-F-4b and ADH-F-5, and from the reciprocal crosses. The overall mean activities only are given, values per trial and standard deviations being omitted for the sake of clarity. MP is the mid-parent value*

Parental activities

ADH-F-1	7·45
ADH-F-2	2·98
ADH-F-4b	7·25
ADH-F-5	4·45

Comparison of reciprocal crosses with MP value

	Cross	Reciprocal	MP
1♀ × 2♂	5·48	5·82**	5·22
1♀ × 4b♂	7·15	7·13	7·35
1♀ × 5♂	5·94	5·82	5·97
2♀ × 4b♂	5·90***	5·55*	5·15
2♀ × 5♂	4·14***	3·95	3·72
4b♀ × 5♂	6·00	5·81	5·85

Probability levels: * $0·05 > P > 0·025$, ** $0·025 > P > 0·01$, *** $0·01 > P > 0·001$.

It can be postulated that, relative to the *X* chromosomes of ADH-F-1, ADH-F-4b and ADH-F-5, the *X* chromosome of ADH-F-2 carries one or more regulatory genes giving a slight increase in ADH activity, and that the plus modifiers in a female heterozygous for these two types of *X* chromosome are fully or partially dominant.

If the *X* chromosome effect is also expressed in adults, then male adults from the cross ADH-F-2 females by ADH-F-1 males should have higher activity than those from the reciprocal cross. Females would be expected to have identical activity. The results of a study investigating this hypothesis are given in Table 6, ADH activities being expressed per individual rather than per unit wet weight. There are no significant differences between the mean parental value and the mean F1 value, nor are there any differences between the two reciprocal crosses. The mean ADH activity of males from the cross ADH-F-2 females by ADH-F-1 males is no higher than that of males from the reciprocal cross. The *X* chromosome effect has disappeared in the adults.

Sexual differences in activity

For both ADH-S-4h and ADH-S-4l, the mean ADH activity per female adult is significantly greater than that per male (Table 4, $0·01 > P > 0·001$ for both genotypes). The ratio of male to female activity is 0·90 and 0·87 respectively. An

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ANOVAR of Table 6 shows that for these ADH-F flies the mean female activity (2·37) is just significantly greater ($0·05 > P > 0·025$) than the mean male activity (2·12). Here, the ratio of male to female activity is 0·89.

Table 6. *ADH activity per individual of adults of ADH-F-1, ADH-F-2, and the two reciprocal crosses. (These values are not directly comparable with those of Table 4.)*

Genotype	Sex	No. of samples assayed	Mean ADH activity	Standard deviation
ADH-F-1	Males	5	2·66	0·66
	Females	5	2·93	0·43
ADH-F-2	Males	5	1·50	0·25
	Females	5	1·63	0·14
F-1♀ × F-2♂	Males	10	2·17	0·50
	Females	10	2·53	0·39
F-2♀ × F-1♂	Males	10	2·11	0·38
	Females	10	2·29	0·52

4. DISCUSSION

Adult male *Drosophila melanogaster* have less ADH activity than adult females. Three sets of results gave male/female ratios of 0·90, 0·87 and 0·89. This sexual dimorphism is probably related to body mass differences, since these values are of the same relative magnitude as ratios calculated for various body size dependent characteristics: body weight, 0·77 (Lewis & Lewis, 1963); body length, 0·83, wing length, 0·87, and the number of bristles of the fourth and fifth abdominal segments, 0·85 (Keller & Mitchell, 1962). The ratio of male to female xanthine dehydrogenase activity is 0·84 (Keller, 1964), but for the *X*-linked enzyme glucose-6-phosphate dehydrogenase it is 1·5 (Komma, 1966).

Before going on to discuss the nature of the control of ADH activity, one point concerning the extraction of the ADH-S strains from the natural population should be made. It has been assumed that the occurrence of Adh^F/Adh^F and Adh^F/Adh^S genotypes in the progeny of an Adh^F/Adh^F female taken from the wild resulted from insemination of that female by a single Adh^F/Adh^S male, and that therefore ADH-S strains derived from this mating would be homozygous for a particular Adh^S allele. The ADH-S strains used in the present study were derived in this manner. However, it has been recently shown that in at least some populations of *D. melanogaster* double insemination of females is common (Milkman & Zeitler, 1974). Thus matings of Adh^F/Adh^F females giving rise to Adh^F/Adh^F and Adh^F/Adh^S progeny may in fact be a variety of types, some of which allow for the presence of two different Adh^S alleles in the progeny (for example, insemination by two different Adh^F/Adh^S males). Assuming the frequency of double insemination to be 50% (the value estimated by Milkman & Zeitler), and knowing the genotype frequencies in the Cambridge populations (FF = 0·868, FS = 0·129, SS = 0·003), the probability that two or more Adh^S alleles might be present in the progeny of an

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assumed ♀FF × ♂FS mating can be estimated: it is 0·060. If the genomes of the two males of a doubly inseminated female do not contribute equally to the progeny, this figure is reduced. Thus responses to selection of the three ADH-S-4 strains, which were isolated from a single assumed ♀FF × ♂FS mating, are far more likely to result from selection at modifying loci than from selection for alternative Adh^S structural alleles. Furthermore, the factor responsible for most of the activity difference between ADH-S-4h and ADH-S-4l is located approximately ten map units from the structural gene. Responses to selection of these strains must result primarily from modifier gene segregation.

The selection responses of the three ADH-F strains resulted entirely from selection at modifying loci, since these strains were each derived from single pair cultures of the type SS × FS, and each must be homozygous for a particular Adh^F allele. These responses become even more striking when it is remembered that these strains were derived by several generations of inbreeding, a procedure which must have resulted in the loss of appreciable amounts of genetic variability.

The results detailed here implicate modifiers on the *X* chromosome, chromosome 2 and chromosome 3 in the regulation of ADH activity. A major modifier, located quite close to the ADH structural gene on chromosome 2, accounted for the bulk of the activity difference between two ADH-S strains with a 3·5 fold difference in activity. The high activity allele of this modifier must be assumed to be at low frequency in most natural populations, since high activity ADH-S strains are rare. Most modifiers affect activity to a smaller extent. Modifiers of ADH activity in *D. melanogaster* have also been detected by Hewitt *et al.* (1974).

ADH activity may be considered a quantitative character, and shows many features typically associated with such traits. There is considerable variation in activity within both ADH-F and ADH-S strains (Hewitt *et al.* 1974; Ward, 1974) and some overlap between them (Ward, 1974). Variation in activity between ADH-S strains has also been described by Birley & Barnes (1973). In populations segregating for Adh^F and Adh^S alleles, the upper region of the activity distribution will be occupied primarily by ADH-F strains and the lower regions by ADH-S strains, heterozygotes showing intermediate or nearly intermediate levels of activity. The nucleotide sequence of the structural gene is almost certainly of primary importance in determining levels of ADH activity, and there may exist multiple Adh^F and Adh^S alleles, but there is now much evidence that other loci have a regulatory function. Like most quantitative characters, the expression of ADH activity is controlled by a number of genes.

There are, of course, many ways in which regulator genes can influence ADH activity. Rates of transcription or translation of ADH mRNA could be altered, activators or inhibitors of ADH activity could be synthesized, substrate availability or the rate of product removal could be controlled. In this context, it is interesting to note that Gibson (1970) recorded increased ADH activity in larvae of Adh^F/Adh^F, Adh^F/Adh^S and Adh^S/Adh^S genotypes when reared on food supplemented with 6% ethanol; ADH levels rose between 20 and 45%. Larvae from ADH-S-4h and ADH-S-4l respond similarly (Ward, unpublished). Alcohol levels

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in the environment may well be reflected in ADH activity through the mediation of regulator genes.

A number of experiments have now been reported showing that *Adh*^{F} and *Adh*^{S} alleles are differentially affected by the presence of alcohol in the nutrient medium. Addition of ethanol favoured an increase in the frequency of *Adh*^{F} in segregating populations (Gibson, 1970; van Delden, Kamping & van Dijk, 1975) and preferential survival of *Adh*^{F} homozygotes relative to *Adh*^{S} homozygotes (Morgan, 1974; Briscoe, Robertson & Malpica, 1975). The strains used in these studies possessed typical ADH-F or ADH-S activities, the ADH-F strains having two or three times the ADH activity of the ADH-S strains. It would be interesting to see how strains with low ADH-F or high ADH-S activities respond to such treatment, for it might then be possible to disentangle selection operating upon activity differences from selection operating on some aspect of those differences that are detectable electrophoretically. It seems probable that high activity ADH-S strains would be more resistant to the effects of alcohol than low activity ADH-F strains. The overlap in activity between ADH-F and ADH-S strains may be expected to thwart any simple explanation for the maintenance of the electrophoretically detectable polymorphism.

I am particularly grateful to Dr P. D. N. Hebert for many stimulating discussions on this and allied work and to Dr J. B. Gibson for his help and advice. This work was supported by an S.R.C. Research Studentship.

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