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Variation in activity and thermostability of alcohol dehydrogenase in *Drosophila melanogaster*

J. McKAY*

Institute of Animal Genetics, West Mains Road, Edinburgh EH9 3JN, Scotland

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SUMMARY

The activity and thermostability of alcohol dehydrogenase (ADH) from 247 strains of *Drosophila melanogaster* were studied by spectrophotometric assay. The strains, in which second chromosomes had been made homozygous in a standard genetic background, were derived from five natural populations from diverse geographical and ecological sites. Evidence is presented that the majority of variation in ADH activity is attributable to the presence, in all five populations, of two electromorphs of the enzyme. However, some variation does exist between strains carrying the same electromorph, to some extent associated with variation in body weight. Two strains showed atypical ADH activities. Variation in ADH thermostability was almost wholly attributable to the presence of two electromorphs; only two strains had enzymes with thermostabilities atypical of their electromorph. In the four strains with abnormal ADH properties the locus (loci) responsible map in the region of the *Adh* locus. The relatively low level of heterogeneity within electrophoretic classes at this locus is discussed in view of recent findings at other enzyme loci in *Drosophila*.

1. INTRODUCTION

The enzyme alcohol dehydrogenase (ADH) in *Drosophila melanogaster* has been the subject of intense study concerned with the molecular basis of mutations affecting the enzyme (Day *et al.* 1974*a*, *b*; Thompson, Ashburner & Woodruff, 1977) and the population genetics of these mutations (Vigue & Johnson, 1973; Clarke, 1975; McDonald & Ayala, 1978). Genetic changes at the *Adh* locus have been described in terms of changing frequencies of the two common electrophoretic forms of alcohol dehydrogenase, ADH-Fast and ADH-Slow.

Evidence is now available for a number of proteins that, as predicted by models of protein variation (Ohta & Kimura, 1974; Marshall & Brown, 1975), extensive heterogeneity of protein properties exists within electrophoretic classes (electromorphs) in natural populations (Johnson, 1977; Cochrane & Richmond, 1979). The main techniques used to study this heterogeneity have been modified conditions of electrophoresis and thermostability. Sampsell (1977), reviewing the use of thermostability differences in detecting variants of *Drosophila* enzymes, has

* Present address: Department of Genetics, Trinity College, Dublin 2, Ireland.

argued that, although the technique is sensitive, such variation is relatively rare at the *Adh* locus.

A survey of the activity and thermostability of ADH in strains derived from five natural populations of *Drosophila melanogaster* was undertaken. The objective of this survey was to determine the extent to which variation in the properties of ADH is attributable to the segregation of the two common electromorphs. In this way it may be possible to determine whether descriptions of the *Adh* locus in terms of electromorph frequencies are adequate measures of genetic change in populations under artificial or natural selection.

2. MATERIALS AND METHODS

Isogenic strains

Second chromosomes of males from five natural populations were made homozygous in a common genetic background using a stock containing the balancer chromosones *Cy*° (curly of Oster) and *TM-3* and the multiply marked chromosomes *Bl-all* and *ruPrica* on an Oreogon-K inbred strain background (Lindsley & Grell, 1967). A total of 247 such strains were made from four newly captured samples of males and males from a population, Dahomey, which had been kept in population cages in this laboratory for ten years.

Culture conditions

Flies were grown at 25 °C on agar-molasses-killed yeast medium (11 per cent killed yeast). Adults of specific ages were collected at emergence and de-yeasted by daily transfer to fresh sterile medium. Flies for experimental purposes were raised in half-pint milk bottles containing approximately 60 ml of medium on which 20 females were allowed to lay eggs for five days to reduce larval competition.

Electrophoresis

A Tris-versenè-borate buffer system (pH 8) of horizontal starch gel electrophoresis was used for ADH (Shaw & Prasad, 1970). Staining of gels was performed at room temperature for 30 min using ethanol as substrate.

Preparation of extracts

Ten six-day-old adult males of known weight were homogenized in 1 ml of 0·1 M-Na_2HPO_4/KH_2PO_4, pH 8, at 4 °C. The homogenate was centrifuged at 30000 *g* for 30 min at 4 °C and the supernatant kept at this temperature until assayed (within 4 h).

ADH assay

The rate of reduction of NAD to NADH was measured by changing absorbance at 340 nm over 5 min at 30 °C in an automatic rate analyser. The reaction mixture consisted of:

Table 1. *Genetic variation in five natural populations*

Population	Origin	Number of strains made	Electrophoretic variation[a]		Lethal analyses[b]		Body weight[c]	
			Proportion of loci polymorphic	$\bar{H}$	Lethal second chromosomes %	Rate of allelism %	Mean (mg/20 males)	C.V. (%)
Prevosti	Canary Islands	79	5/9	0·17	28	0·9	17·2	6·4
Chapingo	Mexico	77	6/9	0·20	24	0	16·9	7·5
Macomb	Illinois, U.S.A.	34	5/9	0·17	36	0·8	16·1	5·8
Riverside	California, U.S.A.	28	6/9	0·21	26	0	16·4	8·4
Dahomey	West Africa	29	5/9	0·18	28	3·6	15·9	4·2

[a] Nine loci were studied in cage populations (*Esterase-C*, *Esterase-6*, *Phosphoglucomutase*, *Acid phosphotase*, *Aldehyde oxidase*, *Alcohol dehydrogenase*, *Octanol dehydrogenase*, *α-glycerophosphate dehydrogenase* and *Tetrazolium oxidase*). Loci are described as polymorphic if two or more electromorphs occur each at a frequency of at least 1 %. $\bar{H}$ equals average heterozygosity. Sample sizes were 70–100 individuals per population per locus.

[b] Chromosomes which could not be made homozygous were classified as lethal. Allelism rates are based on the intercrossing of all lethals within populations.

[c] Body weight measurements and the coefficient of variation are based on data from ten inbred strains established from each population and subject to full sib mating for ten generations.

0·1 ml enzyme extract
0·8 ml 0·125 M isopropanol in 0·1 M-Na_2HPO_4/KH_2PO_4, pH 8
0·1 ml 20 mM NAD in 0·1 M-Na_2HPO_4/KH_2PO_4 adjusted to pH 8, prepared daily.

Under these conditions ADH activity is proportional to enzyme concentration over a wide range of concentrations. [100 units of activity = 0·0175 O.D. units. min^{-1} or 2·81 μmoles of NADH.min^{-1}; 1 unit of specific activity = 3·5 O.D. units per min per g of body weight or 0·536 mmoles of NADH per min per g of body weight.]

Enzyme thermostability estimation

Extracts were heated in a Grant water bath (accurate to ± 0·05 °C over the range of temperature used) and two samples assayed at each of four time points (0, 15, 30 and 90 min). Half-life of enzyme activity ($t_{\frac{1}{2}}$) was estimated by linear regression of *ln* (activity) against time.

3. RESULTS

(i) *Genetic variation in the five populations.*

Three measures of genetic variation were used; electrophoretic variation at nine enzyme loci, the frequency and allelism of recessive lethals on chromosome II and variation in body weight. Results are presented in Table 1.

Average heterozygosity for electromorphs in *D. melanogaster* is typically between 14 and 20 % (Kojima *et al.* 1970; Band, 1975). All five samples therefore have levels of electrophoretic variation consistent with their being from large outbred populations. Genetic distances between populations (see Table 2), as estimated by the formula of Nei (1976), are generally higher than is typically found between populations of *Drosophila* (Lewontin, 1974; Nei, 1976). This may be due to the deliberate choice of populations from diverse origins and using a small number of particularly variable loci.

Table 2. *Genetic distances between five natural populations*

Populations	Prevosti	Chapingo	Macomb	Riverside	Dahomey
Prevosti	0	0·038	0·071	0·058	0·055
Chapingo	—	0	0·044	0·031	0·018
Macomb	—	—	0	0·012	0·056
Riverside	—	—	—	0	0·033
Dahomey	—	—	—	—	0

The frequencies of lethals and their rates of allelism are typical of large outbred populations (Ives, 1945). Although there are serious statistical problems in estimating effective population size from such data (Prout, 1954; Nei 1968) it is clear that no recent population bottlenecks have occurred in these populations. In 1500 tests for allelism between lethals from different populations no case of allelism was found. These data imply clear genetic differentiation between populations.

Coefficients of variation for body weight suggest that each population contains considerable genetic variation for that trait. The body weights of strains from different populations were significantly different and those from Dahomey and Prevosti correspond well with their expected values based on the cline of body weight with latitude studied by David and Bocquet (1975).

Overall, the levels of genetic variation within and between populations indicate that we are dealing with five large, clearly differentiated populations. The sample from Dahomey retains considerable variation despite a long period in the laboratory.

(ii) *Electrophoretic variation in ADH.*

Under our conditions of electrophoresis 246 of the 247 strains were unambiguously assigned to two classes on the basis of ADH electromorph, ADH-F and ADH-S. Strains from different populations were run together and with standards from inbred laboratory stocks Kaduna-F and Kaduna-S. One strain from the Prevosti population, P79, showed no detectable staining of ADH activity after electrophoresis. The frequencies of ADH electromorphs in the remaining 246 strains are shown in Table 3.

These results are typical of surveys of electrophoretic variation at the *Adh* locus. Although other electromorphs are known each is rare and local in occurrence.

(iii) *Variation in ADH activity and thermostability.*

Each of the 247 strains was assayed for ADH activity and thermostability on three separate occasions. Strain P79 again showed no detectable ADH activity. In the other 246 strains the majority of activity in crude extracts was associated with the ADH-5 isozyme. Only this isozyme is unstable at 40 °C, the temperature at which the thermostability of ADH was determined. Results are presented in Fig. 1 and Table 3.

Three strains have unusual combinations of properties. These strains, two ADH-S strains from the Chapingo population and one ADH-F strain from the Prevosti population, are excluded from the following analyses and will be discussed in Section (iv). Among the remaining strains it is clear from Fig. 1 and analyses of variance (Table 3) that most of the variation in ADH properties within and between populations is associated with the segregation of the electromorphs ADH-F and ADH-S.

No significant heterogeneity in half-life was found among strains of the same electrophoretic class, excluding the two ADH-S strains from Chapingo. This was true within populations and also between populations ($F^4_{490} = 1{\cdot}15$, $P > 0{\cdot}25$ for ADH-F strains and $F^4_{230} = 1{\cdot}20$, $P > 0{\cdot}25$ for ADH-S strains). The twelve strains with most extreme values of ADH half-life (three highest and lowest of each electromorph) were assayed twice more. On a total of five replicates for each strain none of these strains had an ADH half-life significantly different from their class means.

In all but the smallest sample significant differences were found in ADH

Table 3. *Analysis of variation in ADH activity and half-life.*

			ADH activity				ADH half-life		
Population	Electromorph	Numbers	Specific activity (units/mg body wt.)	Variance[a]	P^b	Ratio F:S	Mean $t_{\frac{1}{2}}$ (mins)	Variance[a]	P^b
Prevosti	ADH-F	67 (+1)	4·83	0·07	**	2·14	9·00	0·70	$P > 0·10$
	ADH-S	10	2·26	0·04	**		14·02	2·42	$P > 0·25$
Chapingo	ADH-F	55	4·74	0·07	**	1·96	8·93	0·67	$P > 0·25$
	ADH-S	20 (+2)	2·42	0·02	**		13·84	1·60	$P > 0·25$
Macomb	ADH-F	10	4·88	0·04	*	2·05	8·82	1·05	$P > 0·25$
	ADH-S	24	2·40	0·05	**		13·96	1·98	$P > 0·25$
Riverside	ADH-F	10	4·64	0·05	*	2·04	9·06	1·07	$P > 0·25$
	ADH-S	18	2·27	0·04	**		14·04	2·28	$P > 0·25$
Dahomey	ADH-F	23	5·18	0·08	**	2·10	8·92	0·90	$P > 0·25$
	ADH-S	6	2·51	0·03	NS		14·23	3·32	$P > 0·10$
Overall,	ADH-F	166	—	—	—	—	8·96	0·79	$P > 0·25$
	ADH-S	80	—	—	—		13·98	2·15	$P > 0·25$

[a] Variance between strain means estimated from analyses of variance.

[b] Level of significance on F test of variation between and within strains. (** $P < 0·01$; * $P < 0·05$; NS, $P > 0·05$)

activity among strains of the same electrophoretic class. Differences between populations in the activity of both electromorphs were also significant (F^4_{161} = 3·5, $P < 0·01$ for ADH-F and $F^4_{79} = 2·7$, $P < 0·05$ for ADH-S). However, in all five populations ADH-F strains had approximately twice the activity of ADH-S strains.

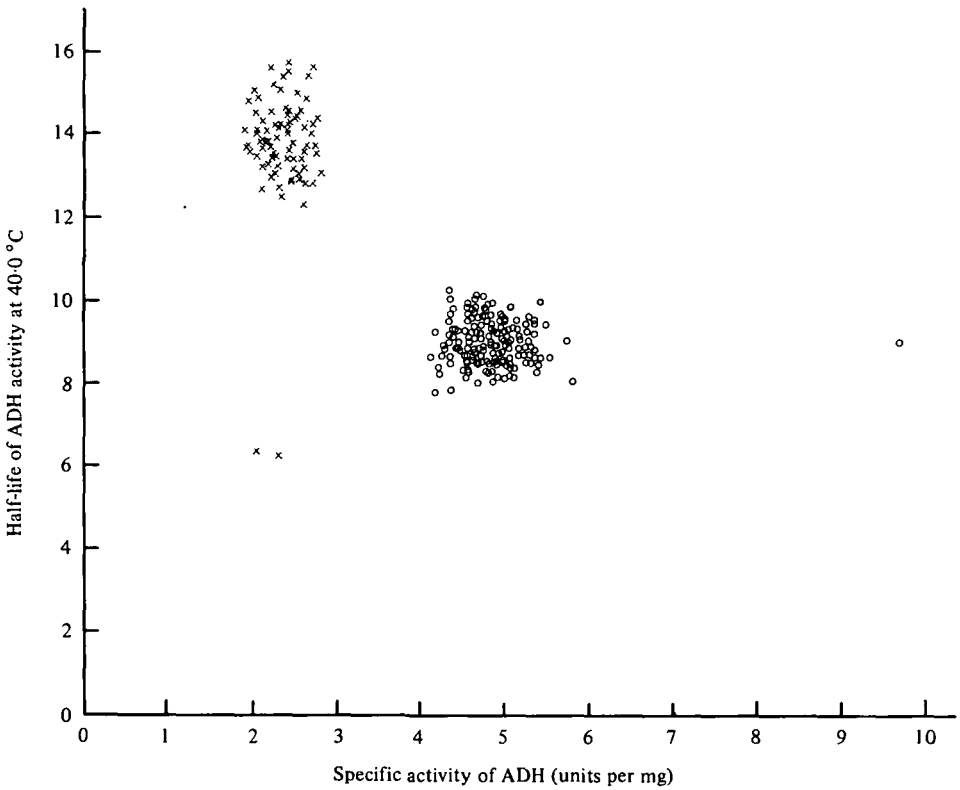

Fig. 1. Distribution of ADH activity and heat stability in (×) 80 ADH-S, and (○) 166 ADH-F strains.

The differences in ADH activity can only be due to loci on chromosome II in these experiments. Since loci affecting ADH activity are known to occur on all three major chromosomes (Ward, 1975; McDonald & Ayala, 1978) differences between these strains need not adequately reflect the pattern of variation in natural populations. However in surveys of natural populations (Birley & Barnes, 1973; Vigue & Johnson, 1973; McDonald & Ayala, 1978) a major proportion of total variation has been associated with the segregation of the two electromorphs and ratios of activity are generally similar.

Within populations a significant positive relationship between enzyme activity and body weight was found (see Table 4); no such relationship was present between populations (regression coefficient = $-0·046 \pm 0·058$). A similar relationship was reported by Hewitt *et al.* (1974) and the relationship between body weight and

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ADH activity within strains has been shown to be allometric by Clarke *et al.* (1979). The relationship between strains implies that loci affecting body weight may contribute to variation in ADH activity. No differences in body weight were detected between ADH-F and ADH-S strains of any population.

In summary, although there are significant differences in ADH activity between strains of the same electrophoretic type, the classification of strains by ADH

Table 4. *Regression of ADH activity per fly on body weight*

Population	Electromorph	Number	b[1]	P[2]
Prevosti	ADH-F	67	3·8	**
	ADH-S	10	0·6	NS
Chapingo	ADH-F	55	3·5	**
	ADH-S	20	1·7	*
Macomb	ADH-F	10	3·8	NS
	ADH-S	24	0·7	NS
Riverside	ADH-F	10	3·4	NS
	ADH-S	18	2·6	NS
Dahomey	ADH-F	23	2·4	*
	ADH-S	6	1·0	NS

[1] Regression coefficient: (units of activity per mg fresh weight).
[2] Significance level of b (**, $P < 0.01$; * $P < 0.05$; NS, $P > 0.05$).

electromorph accounts for the major portion of the variation in activity. Only two of the 247 strains had ADH thermostabilities atypical of their electrophoretic classes. These data represent strong evidence that little heterogeneity exists within electromorphs at the *Adh* locus.

(iv) *Genetic Analysis*

Four strains with unusual combinations of properties were subjected to genetic analysis; one Prevosti strain (P79) with no detectable ADH activity, one Prevosti strain (P44) with approximately twice the normal level of ADH activity, and two Chapingo ADH-S strains (C32 and C65) in which the enzyme was unstable (see Fig. 1). The map positions of the factors causing these differences were determined by crossing to a tester stock carrying the recessive markers *b* (black; II, 48·5), *pr* (purple; II, 54·5) and *cn* (cinnabar; II, 57·5). For each of the strains the properties of ADH activity, thermostability and electrophoretic mobility segregated together and the factor responsible was located between the markers *b* and *pr*. Estimated map positions are presented in Table 5. The map position of the *Adh* locus is 50·1 (Lindsley & Grell, 1967).

In all assays the enzymes from the Chapingo ADH-S strains C32 and C65 were indistinguishable in properties. It is therefore probable that these strains share an unstable form of ADH-S. Although no direct comparison was made, the ADH-S from these Mexican strains has similar properties to the ADH-S reported by Sampsell (1977) in American populations. It is not known whether the zero ADH activity of strain P77 or the two-fold ADH-F activity of strain P79 are mutants

of the *Adh* structural locus or a closely linked regulatory locus as described by McDonald *et al.* (1977) and Thompson *et al.* (1977). The possibility of their being a deletion and duplication of the *Adh* locus was not confirmed by cytological examination (unpublished observations).

Table 5. *Genetic analysis of strain differences*

Strain	ADH electrophoretic mobility	ADH activity	ADH thermo-stability	Map position ± 2 S.E.
P44	Fast	2 × normal	Normal	50·0 ± 0·6
P79	Unknown	Zero	Unknown	50·1 ± 0·3
C32	Slow	Normal	Low	50·6 ± 0·6
C65	Slow	Normal	Low	50·3 ± 0·5

4. DISCUSSION

The five populations studied were shown to have levels of genetic variation typical of large outbreeding populations of *Drosophila melanogaster* and to have considerable genetic differences between them. However, the vast majority of variation in ADH properties between second chromosomes was attributable to the presence of two electromorphs of the enzyme.

Thermostability studies revealed little heterogenetiy within electromorphs. Although the populations studied are derived from a wider geographical range, these results are broadly in agreement with those of Sampsell (1977) who found that three new alleles are rare but widespread in populations in the United States (overall frequency 1·7% in 4436 strains). The spectrophotometric assay system used here is more sensitive than the technique of heating whole gels after electrophoresis used in a number of laboratories studying other *Drosophila* enzyme loci, e.g. *Xdh* in the *D. virilis* group (Bernstein, Throckmorton & Hubby, 1973) *Odh* in *D. pseudoobscura* and the *D. virilis* (Singh, Hubby & Throckmorton, 1975; Singh, Lewontin & Felton, 1976) and *Pgm* and *Est-6* in *D. melanogaster* (Trippa, Loverre & Catamo, 1976; Cochrane & Richmond, 1979). In these cases the heterogeneity within electromorphs is extensive and considerably increases heterozygosity. Although the genetic basis of this variation has not been fully studied in all cases, it is clear that there are large differences between *Adh* and the other loci in the level of variation in thermostability.

It is possible that variation does exist within ADH electromorphs and would be detectable by other methods such as modified conditions of electrophoresis (Johnson, 1977; Coyne, Felton & Lewontin, 1978). This must be considered, especially since it has been argued that environmental temperature and enzyme stability are important factors in the dynamics of the *Adh* polymorphism (Vigue & Johnson, 1973; Malpica & Vassallo, 1980). The range of thermostabilities of *Adh* alleles may be restricted by natural selection.

Alternatively the *Adh* locus may have fewer alleles than other *Drosophila* enzyme loci. The rate of production of new protein variants will vary between loci with differences in mutation and intragenic recombination rates. Since these rates

may increase with increasing molecular weight of the enzymes (but see Voelker, Schaffer & Mukai, 1980) it should be noted that the ADH protomer is relatively small (24,000 Daltons) compared with most other *Drosophila* enzymes (e.g. ODH 55000 and XDH 140000).

The available evidence suggests that descriptions of genetic change at the *Adh* locus in terms of changes in frequency of electromorphs account for most of the differences in activity and thermostability due to the locus. Other loci, some closely linked, may affect the level of ADH activity but there is no evidence of extensive heterogeneity within electromorphs. Thus using electromorphs to interpret geographical patterns or effects of artificial selection on *this* locus may be an accurate reflection of genetic change.

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