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An Electrophoretically Cryptic Alcohol Dehydrogenase Variant in *Drosophila melanogaster*. I. Activity Ratios, Thermostability, Genetic Localization and Comparison with Two Other Thermostable Variants

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Abstract

The alcohol dehydrogenase (ADH) variant ADH-FCh.D. has a secondary alcohol/primary alcohol activity ratio characteristic of ADH-S although it has an electrophoretic mobility inseparable from ADH-F. ADH-FCh.D. is distinguished from these two common ADH variants by being much more thermostable.

Genetic analysis suggests that ADH-FCh.D. is specified by an allele at the *Adh* locus. Biochemical comparisons show that ADH-FCh.D. has the same electrophoretic mobility, activity ratio and thermostability as the two other heat-resistant variants which have been reported, ADH-F71K in Europe and ADH-Fr in North America. The geographically widespread distribution of a thermostable ADH variant within the ADH-F electrophoretic class indicates that it should be considered in attempts to explain the *Adh* polymorphism in natural populations.

Introduction

It is clear that allozymes characterized by their electrophoretic mobility are not necessarily a homogeneous class when other properties of the enzyme are investigated (see Johnson 1977*a* for a review). Thus theories which aim to explain the distribution of allelic variety in natural populations must take account of electrophoretically cryptic forms of variation. It is, therefore, important to quantify the extent of this variation, to characterize biochemically some of the variants, and to test the hypothesis that the variants are alleles at the structural gene locus rather than closely linked modifiers of its product.

Variation within electrophoretically homogeneous classes of alleles derived from natural populations of *Drosophila* spp. has been demonstrated for enzyme activity (e.g. Doane 1969; Collier and MacIntyre 1977), amount of enzyme protein (Lewis and Gibson 1978), thermostability (Wright and MacIntyre 1965; Gibson and Miklovich 1971; Bernstein *et al.* 1973; Singh *et al.* 1975, 1976; Trippa *et al.* 1976), and conformation (Johnson 1977*b*).

In particular the two common electrophoretic variants of alcohol dehydrogenase (ADH) (EC 1.1.1.1) in *Drosophila melanogaster* have been examined for cryptic variation; Milkman (1976) and Sampsell (1977) have described thermostability variants and Ward (1974), Thompson and Kaiser (1977), Barnes and Birley (1978) and MacDonald and Ayala (1978) described variation in ADH activity within both ADH-F and ADH-S electrophoretic types.

Lewis and Gibson (1978) isolated a number of single-allele strains of Adh^F and Adh^S from a natural population and described their properties in terms of the amount

of enzyme protein produced and the ADH activities with 2-propanol and with ethanol as substrates. Of the nine homozygous Adh^F strains which they tested one had an ADH specific activity with ethanol and with 2-propanol characteristic of strains homozygous Adh^S. In this strain, which was denoted 14k12j1a, the ratio ADH activity with 2-propanol to ADH activity with ethanol was much lower than for the other Adh^F strains and was close to the value found for all of the seven Adh^S strains. We have subsequently shown (Wilks *et al.* 1980) that this ADH-F variant occurs in 19 of the 34 natural population samples which have been assayed from a geographically wide range of sites in Australasia. This paper describes some of the biochemical characteristics of 14k12j1a and compares them to those of two other electrophoretically cryptic ADH variants, of which one was isolated from a laboratory stock (Thörig *et al.* 1975) and the other from natural populations in North America (Sampsell 1977).

Materials and Methods

The variant 14k12j1a and the Adh^F and Adh^S alleles used as controls in these experiments were isolated from fertilized females taken in 1975 on a waste dump of grape pressings at the Chateau Douglas winery at Segenhoe in New South Wales (Lewis and Gibson 1978). These single-allele strains were subsequently maintained by mass culture in the laboratory as homozygous lines.

The thermostable putative allele Adh^{Fr} (for 'Fast resistant') was isolated by Sampsell (1977) from a natural population in Cedar Rapids, Iowa, in 1975 and maintained as a homozygous line.

The thermostable putative allele Adh^{71K} was identified by Thörig *et al.* (1975) in a laboratory stock N^8/Δ^{49} containing Notch (*N*, 1–3·0, Lindsley and Grell 1967). We found that a sample of this stock sent to us by Dr Thörig was segregating heat-sensitive ADH-F as well as the thermostable ADH-71K. From this stock we isolated seven single-allele strains each homozygous for an electrophoretically fast variant which had much higher thermostability than the common ADH-F; this variant allele is denoted Adh^{F71K} hereinafter.

Df(2L)64j/CyO^{nA} is a deficiency strain which expresses no ADH activity and which was derived by Gerace and Sofer (1972) from *Df(2L)64j/CyO* (Lindsley and Grell 1967) by mutagenesis with ethyl methanesulfonate.

Genetic Mapping

The ADH-F variant 14k12j1a was localized in the second chromosome by crossing flies from the single-allele stock to a multiply-marked, second-chromosome stock carrying black (*b*, 2–48·5), purple (*pr*, 2–54·5), and cinnabar (*cn*, 2–57·5) which we showed by electrophoresis was homozygous Adh^F (*Adh*, 2–50·1, Lindsley and Grell 1967). Wild-type female progeny were back-crossed to *b* Adh^F *pr cn* males and recombinants for *b* and *pr* and parental-type offspring were typed for the 14k12j1a variant with our post-electrophoresis, heat-treatment technique (Wilks *et al.* 1980).

This post-electrophoresis technique was also used to classify the F_{14} progeny derived from reciprocal crosses between 14k12j1a and Adh^F, and between 14k12j1a and Adh^S, to identify any recombinants between electrophoretic mobility and thermostability which had accumulated during the 14 generations of mass culture.

A further experiment investigated ADH activity in strains heterozygous for 14k12j1a and the second chromosome deficiency *Df(2L)64j*. This deficiency extends from band 34E5 to band 35D1 (Lindsley and Grell 1967) and spans the *Adh* locus (within bands 35B1–3, Woodruff and Ashburner 1979); *Df(2L)64j* is lethal in males and in homozygous females.

The evidence from these experiments, which is described below, suggests that the variant 14k12j1a is produced by an allele at the *Adh* locus; we have designated this putative allele $Adh^{FCh.D.}$ for 'Fast Chateau Douglas', and this nomenclature is used below.

Electrophoresis and Enzyme Assays

The electrophoretic mobilities of the strains used were determined on cellulose acetate sheets with a continuous buffer system as previously described (Lewis and Gibson 1978).

To prepare extracts for ADH assays newly emerged flies were collected from the progeny of cultures initiated with 20 pairs of flies. In all experiments flies were collected from at least four separate cultures of each of the lines to be assayed, and replicate samples of each extract were assayed. The male flies for assay were aged for 7 days on new culture media before extracts were prepared. The flies were anaesthetized with ether and kept on ice before being counted, weighed and homogenized by hand in ground-glass tissue grinders at 10 mg live weight per millilitre ice-cold 100 mM sodium phosphate buffer, pH 7·5. The crude homogenates were centrifuged at 10 000 rpm in a Sorvall SS-3 centrifuge with a SM-24 head (12 350 *g*) for 30 min to remove debris. The pale orange supernatants were taken and stored on ice until assayed. Extracts showed no appreciable decline in activity during the course of the separate experiments.

ADH activity was measured in a 1-ml reaction mixture which included 150 mM ethanol or 2-propanol and 2 mM NAD^+ in 100 mM sodium phosphate buffer, pH 7·5, with 10–80 μl extract. The assay conditions were chosen to give the largest activity ratios for a variety of primary and secondary alcohols. The ratio ADH activity with 2-propanol to ADH activity with ethanol is hereinafter referred to as the activity ratio. The reaction was monitored at 340 nm to record NADH production for 2–3 min with a Unicam SP1800 spectrophotometer. 1 unit of activity is defined as an increase in absorbance at 340 nm of 0·001/min at 25°C, (i.e. $1{\cdot}61\times10^{-4}$ μmoles NADH produced/min). The reaction rate was proportional to enzyme concentration for the three control lines over the range of activities and protein concentrations used. In none of these experiments was there any evidence for a non-linear relationship between ADH activity and body weight (see Clarke *et al.* 1979). Blank assays, carried out without the addition of alcohol to the reaction mixture, showed a small non-linear decrease in absorbance; since this never amounted to greater than 3% of the recorded activity no correction was made. Enzyme activities are given in the tables as units per milligram live weight.

To investigate ADH thermostability in crude extracts 200-μl samples were transferred to small test tubes and heat treated in a water-bath for 5 min at either 40 or 44°C. Thermal denaturation was terminated by plunging the tubes into ice-cold water and ADH activity was assayed as above with 2-propanol as substrate.

Culture conditions and chemicals were as used by Lewis and Gibson (1978).

Table 1. Mean values for ADH activity in control strains and, in progeny derived from crosses between them

Standard errors in parentheses. There were no significant differences in ADH activity in assays of the progeny of reciprocal crosses

Strain	No. of cultures assayed	ADH activity with 2-propanol as substrate (A)	ADH activity with ethanol as substrate (B)	Activity ratio (A/B)
Adh^F/Adh^F control	12	366·7 (12·08)	53·2 (1·71)	6·9 (0·08)
Adh^S/Adh^S control	14	114·1 (6·22)	25·0 (1·36)	4·6 (0·05)
$Adh^{FCh.D.}/Adh^{FCh.D.}$ (14k12j1a)	14	134·2 (6·80)	30·1 (1·36)	4·4 (0·05)
Adh^F/Adh^S	7	227·8 (9·13)	36·4 (1·61)	6·3 (0·12)
$Adh^{FCh.D.}/Adh^F$	21	212·8 (7·64)	34·8 (1·21)	6·1 (0·05)
$Adh^{FCh.D.}/Adh^S$	21	129·4 (6·78)	28·5 (1·37)	4·5 (0·07)

Results

Substrate Specificity and Thermostability of ADH-FCh.D.

ADH assays using ethanol and 2-propanol as substrates were carried out on the progeny of crosses between $Adh^{FCh.D.}$ and Adh^F, $Adh^{FCh.D.}$ and Adh^S, Adh^F and Adh^S, as well as on the parental lines homozygous for these three alleles. These data (Table 1) show that for all genotypes ADH activity is higher with 2-propanol than with ethanol as substrate and that this activity ratio is higher for the homozygous Adh^F strain

than for the homozygous Adh^S strain. The $Adh^{FCh.D.}$ strain has an ADH activity ratio not significantly different from that of the Adh^S strain but significantly lower than that of the Adh^F strain. These results are consonant with those of Lewis and Gibson (1978). The results of these assays (Table 1) also show that the activities and the activity ratios of extracts of heterozygotes between any two of the three alleles are between those of the extracts of the respective homozygous strains.

Thermostability studies were carried out on samples of the same extracts and these data (Table 2) reveal that relative thermostability at 40 and 44°C is a second distinctive property of $Adh^{FCh.D.}$. After incubation at 40°C for 5 min extracts of $Adh^{FCh.D.}$ retain over 90% of their initial activity with 2-propanol as substrate, whereas extracts of both Adh^F and Adh^S strains only retain about 36% of their starting activity. Treatment at 44°C for 5 min emphasizes the thermostable nature of $Adh^{FCh.D.}$ extracts, for over 38% of the activity remains compared to only 2–4% for Adh^S or Adh^F.

Table 2. Percentage ADH activity remaining after heat treating the extract for 5 min at 40 and 44°C with 2-propanol as substrate

Standard errors in parentheses

Strain	No. of cultures assayed	ADH activity remaining in heat-treated extracts (%) 40°C	44°C
Adh^F/Adh^F	12	37·6 (2·58)	3·2 (0·32)
Adh^S/Adh^S	14	36·1 (3·33)	2·4 (0·31)
$Adh^{FCh.D.}/Adh^{FCh.D}$	14	92·9 (1·62)	38·9 (1·99)
Adh^F/Adh^S	7	39·5 (2·53)	4·1 (0·38)
$Adh^{FCh.D.}/Adh^F$	21	62·4 (0·94)	10·1 (0·87)
$Adh^{FCh.D.}/Adh^S$	21	66·5 (0·95)	11·2 (1·71)

Analysis of the data in Tables 1 and 2 shows that, in $Adh^{FCh.D.}/Adh^F$ heterozygotes, the properties of activity with 2-propanol, activity ratio and activity after 5 min at 44°C all have a significant dominance component which, with the exception of the activity with 2-propanol, is in the direction of the properties of Adh^F (Table 3). However, the dominance effects are numerically small and by far the largest proportion of the variance in all characteristics measured is additive.

Table 3. Proportion of variance in ADH activity attributable to additive and dominance effects in the $Adh^{FCh.D.}$ and Adh^F control strains and in reciprocal crosses between them

*** $P<0·001$

Effect	Activity with 2-propanol	2-Propanol/ethanol ratio	Activity after 5 min at: 40°C	44°C
Additive	0·95***	0·96***	1·0***	0·86***
Dominance	0·05***	0·04***	—	0·14***

The properties of ADH-FCh.D., ADH-F and ADH-S have also been investigated in purified extracts and these experiments, which will be described in detail elsewhere, show unequivocally that the ADH produced by $Adh^{FCh.D.}$ has the same activity ratio as the ADH produced by Adh^S but that it is much more thermostable at 40 and 44°C than the ADH from either Adh^S or Adh^F homozygous strains.

Assays for ADH activity, both before and after heat treatment, were also made on crude extracts of male progeny from crosses between Adh^F or Adh^S or $Adh^{FCh.D.}$ males and $Df(2L)64j/CyO^{nA}$ females. In these assays the ADH activity with either substrate was between 40 and 53% of the activity of the appropriate homozygote (Table 4) and in no case was this level of activity significantly different from half the activity of the relevant homozygote. However, the activity ratios of the $Adh^{FCh.D.}$, Adh^S and Adh^F hemizygotes remained similar to those characteristic of the respective homozygotes. The thermostability properties of $Adh^{FCh.D.}$ hemizygotes were similar to those of $Adh^{FCh.D.}$ homozygotes. At 44°C extracts from all three hemizygotes were slightly less heat stable than extracts from the corresponding homozygotes, but this effect was not present in the ADH-FCh.D. extracts at 40°C. These data show that even when only half the amount of ADH enzyme is produced the properties of the ADH-FCh.D. enzyme are essentially unchanged compared to that produced in homozygotes, although there is a suggestion that different genetic backgrounds affect the heat stabilities of ADH-F and ADH-S.

Table 4. ADH activity before and after heat treatment in the male progeny derived from crosses between the control strains and $Df(2L)64j/CyO^{nA}$

Four cultures of each cross assayed. Control lines homozygous for the alleles tested were also assayed and the data were not significantly different from those shown in Tables 1, 2 and 7. Standard errors in parentheses

Cross	ADH activity with 2-propanol	Activity (%) compared with homozygotes on substrates: 2-Propanol	Ethanol	Activity ratio	Activity (%) remaining after 5 min at: 40°C	44°C
$Adh^F/Df(2L)64j$	174·5	47·6	46·2	6·9	21·1	2·1
	(6·63)	(4·32)	(4·13)	(0·12)	(2·31)	(0·11)
$Adh^S/Df(2L)64j$	59·8	52·4	49·6	4·0	22·3	1·1
	(1·61)	(4·61)	(3·14)	(0·08)	(2·44)	(0·12)
$Adh^{FCh.D.}/Df(2L)64j$	61·5	45·8	47·4	3·8	90·2	29·2
	(1·82)	(3·25)	(2·82)	(0·11)	(2·67)	(2·31)
$Adh^{Fr}/Df(2L)64j$	45·9	40·1	39·6	4·2	82·6	15·7
(see p. 485)	(1·58)	(5·16)	(4·21)	(0·13)	(3·35)	(1·62)

Localization of $Adh^{FCh.D.}$

To test the hypothesis that $Adh^{FCh.D.}$ is an allele at the *Adh* locus male flies homozygous for $Adh^{FCh.D.}$ were crossed to the *b* Adh^F *pr cn* stock and the female progeny were backcrossed to males homozygous for *b* Adh^F *pr cn*. From these crosses recombinant progeny *b* + +/*b pr cn* and + *pr cn*/*b pr cn* were scored for the presence of $Adh^{FCh.D.}$ on cellulose acetate sheets by post-electrophoresis heat treatment (15 s at 43°C, Wilks *et al.* 1980), and flies of each parental type were also checked.

These results (Table 5) provide an estimate of the relative distance of $Adh^{FCh.D.}$ from *b* in the *b-pr* region of 0·19, which on the basis of the published marker locations (see Materials and Methods) locates $Adh^{FCh.D.}$ to within 0·5 cM of the *Adh* locus.

Further information on the genetics of $Adh^{FCh.D.}$ was derived from reciprocal crosses between the variant and the Adh^F and the Adh^S control strains. These four lines were subsequently maintained by mass transfer for 15 generations. All of the flies transferred from F_{14} to F_{15} in each line were typed after laying eggs in the new

cultures. If the properties of ADH-FCh.D. were due to modification of the *Adh* structural gene product the modifier(s) might change either ADH-F or ADH-S to produce ADH-FCh.D. The properties of such modifiers are illustrated in Table 6 together with the consequences of recombination between the modifier(s) and the

Table 5. Classification of recombinants between *b* and *pr* produced in the cross between $Adh^{FCh.D.}/b\ Adh^{F}$ *pr cn* females and *b* Adh^{F} *pr cn* males

Marker genotype[A]	ADH types detected		Total No. of flies scored
	ADH-F	ADH-FCh.D. and ADH-F	
b + +/*b pr cn*	84	14	98
+ *pr cn*/*b pr cn*	22	73	95

[A] Forty flies of each parental marker genotype were also scored and no recombinants for ADH types were detected.

Adh locus. For example, in the F_{14} progeny of reciprocal crosses between Adh^{F} and $Adh^{FCh.D.}$ we might detect Adh^{S} recombinants if the modifier changed ADH-S to ADH-FCh.D., and Adh^{F} recombinants in the progeny of reciprocal crosses between Adh^{S} and $Adh^{FCh.D.}$ if the modifier changed ADH-F to ADH-FCh.D. None of these recombinant types were detected amongst the F_{14} flies of any of the crosses (Table 6).

Table 6. Model of modification of ADH protein to produce ADH-FCh.D.

M represents the modifier, + is a wild-type allele at the modifier locus and *S* and *F* represent *Adh* structural genes

Variant modified	Properties modified	F_1 genotype in cross to Adh^{F}	F_1 genotype in cross to Adh^{S}
ADH-S	Electrophoretic mobility and thermostability	$\frac{+\ F}{M\ S}$	$\frac{+\ S}{M\ S}$
Recombinants detectable in later generations		$\underline{+\ S}$ and $\underline{M\ F}$	None
ADH-F	Activity ratio and thermostability	$\frac{+\ F}{M\ F}$	$\frac{+\ S}{M\ F}$
Recombinants detectable in later generations		None	$\underline{+\ F}$ and $\underline{M\ S}$
Experimental results:	No. of flies typed at F_{14}	334	319
	Adh frequency	$F = 0{\cdot}69$	$S = 0{\cdot}51$
	Recombinants detected	None	None

These experiments will be described in more detail elsewhere, but we can use their data to calculate the maximum map distance between a modifier and the *Adh* locus consistent ($P < 0{\cdot}01$) with our experiment *not detecting* a recombinant, i.e. if the map distance was greater than the calculated value there would be a probability $> 0{\cdot}99$ of detecting a recombinant (Crow and Kimura 1970, pp. 47–50). These analyses

(which admittedly assume large population sizes) show that if the modifier changes Adh^S to $Adh^{FCh.D.}$ the modifier must be closer than 0·0042 cM to the *Adh* locus and if the modifier changes Adh^F to $Adh^{FCh.D.}$ it must be closer than 0·0047 cM to the *Adh* locus. Thus if the properties of ADH-FCh.D. are due to a modifier its linkage with the *Adh* locus is less than the distance of 0·01 cM which Thompson *et al.* (1977) found to separate an ADH activity modifier from the *Adh* locus.

Comparison of the Properties of ADH-FCh.D. with other Thermostable Variants

The discovery that ADH-FCh.D. is more thermostable than either ADH-F or ADH-S suggested to us that the activity ratios of other thermostable ADH variants should be investigated, although the ones previously described had been detected solely on the basis of their higher thermostabilities compared to the common *Adh* electrophoretic alleles. For example Sampsell (1977) isolated a putative allele which she denoted Adh^{Fr} for 'Fast resistant' from a natural population of *Drosophila melanogaster* in Iowa, using post-electrophoretic treatment of cellulose acetate sheets for 20 s at 43°C. She localized Adh^{Fr} to position 50·8 on the second chromosome and argued that it was probably an allele at the *Adh* locus.

Table 7. Comparison of the activity ratios and thermal stabilities of ADH from $Adh^{FCh.D.}$ and Adh^{Fr} strains

Eight cultures assayed for each strain. ADH assays of Adh^F and Adh^S control strains gave results similar to those in Tables 1 and 2. Standard errors in parentheses

Strain	ADH activity with 2-propanol as substrate	Activity ratio	Activity (%) remaining after 5 min at: 40°C	44°C
$Adh^{FCh.D.}/Adh^{FCh.D.}$	143·9 (1·56)	4·4 (0·09)	90·1 (1·87)	50·3 (1·59)
Adh^{Fr}/Adh^{Fr}	114·6 (1·67)	4·2 (0·11)	88·8 (1·53)	44·9 (2·71)
Paired *t*-tests (t_7)	14·79 $P<0·001$	2·01 n.s.	1·13 n.s.	2·16 n.s.

We assayed ADH in a stock homozygous Adh^{Fr} and compared the results with assays of the $Adh^{FCh.D.}$ strain. These data (Table 7) show that although ADH-FCh.D. has significantly higher ADH activity than ADH-Fr (which we suspect is due to the variants having different genetic backgrounds) the activity ratios and heat stabilities of the two strains after 5 min at 40 or 44°C are the same. We also assayed Adh^{Fr}/*Df(2L)64j* heterozygotes and these had significantly less than half the ADH activity but the same activity ratio and thermostability properties as the Adh^{Fr} homozygotes (Table 4). We conclude that ADH-FCh.D. and ADH-Fr probably differ from ADH-F by the same amino-acid substitution or by the same modification of the *Adh* gene product; our evidence suggests that they are produced by alleles at the *Adh* locus and may indeed be the same *Adh* allele.

Thörig *et al.* (1975) described an ADH variant, 71K, which had the same electrophoretic mobility on both polyacrylamide and agar gels as the product of the Adh^F allele but which differed from ADH-F and ADH-S in being more heat stable in

crude whole fly extracts at 40°C. Genetic analysis of the variant suggested that it was an allele at the *Adh* locus.

We isolated seven separate Adh^{F71K} alleles from the N^8/Δ^{49} stock and made them homozygous. The electrophoretic mobility of all seven single allele strains was indistinguishable on cellulose acetate sheets from Adh^F, $Adh^{FCh.D.}$ and Adh^{Fr} (Fig. 1).

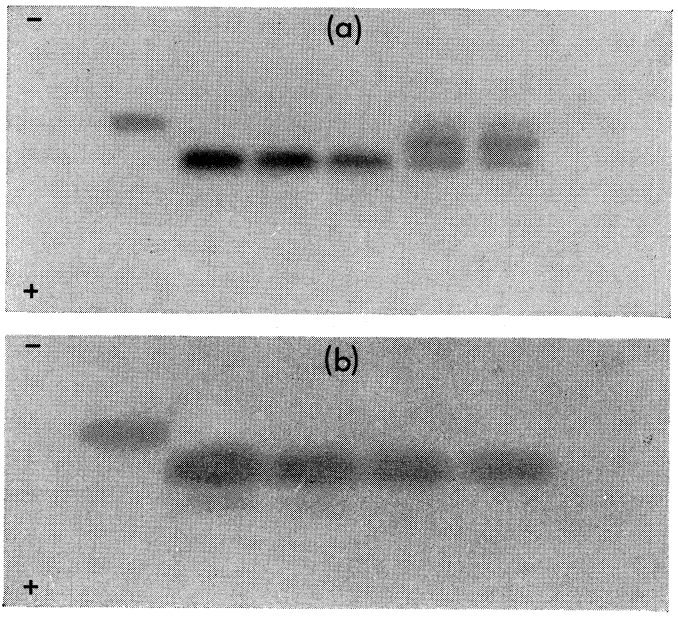

Fig. 1. Electrophoretic phenotypes of ADH variants on cellulose acetate sheets: (*a*) (from left to right) Adh^S/Adh^S, Adh^F/Adh^F, $Adh^{FCh.D.}/Adh^{FCh.D.}$, Adh^{Fr}/Adh^{Fr}, Adh^F/Adh^S, $Adh^{FCh.D.}/Adh^S$; (*b*) (from left to right) Adh^S/Adh^S, Adh^F/Adh^F, $Adh^{FCh.D.}/Adh^{FCh.D.}$, Adh^{Fr}/Adh^{Fr}, Adh^{F71K}/Adh^{F71K}.

ADH activities in three of the single-allele strains of Adh^{F71K} were assayed concurrently with assays of the $Adh^{FCh.D.}$ strain. One of the Adh^{F71K} strains has significantly lower ADH activity than $Adh^{FCh.D.}$ (Table 8) but the activity ratios are all very similar to each other and to that characteristic of ADH-S. The heat stabilities at 40°C and at 44°C of all three Adh^{F71K} strains are lower than those for $Adh^{FCh.D.}$ but they are clearly much more similar to $Adh^{FCh.D.}$ than they are to control Adh^F or Adh^S strains (*cf.* Tables 2 and 8). These data show that ADH-F71K has the same activity ratio as ADH-FCh.D. and on the basis of the characteristics which we are scoring they suggest that they are produced by the same allele.

Discussion

The variant ADH-FCh.D. has a secondary alcohol/primary alcohol activity ratio characteristic of ADH-S even though it is electrophoretically inseparable from ADH-F on polyacrylamide gels or cellulose acetate sheets. Our results have further shown that this electrophoretically cryptic ADH variant is distinguishable from both common ADH forms, ADH-F and ADH-S, in being very much more resistant to heat treatment at 40 or 44°C.

We have also shown that in terms of the properties of electrophoretic mobility, activity ratio and thermostability, ADH-FCh.D. is very similar to, if not identical with, the thermostability variants ADH-Fr isolated by Sampsell (1977) and ADH-F71K isolated by Thörig *et al.* (1975) (see also Scharloo *et al.* 1977). Thus probably the same variant has been found in *D. melanogaster* populations on three different continents and we have data (Wilks *et al.* 1980) which show that the same variant occurs in many *D. melanogaster* populations sampled over a wide area of Australia.

Table 8. Comparison of the activity ratios and thermal stabilities of ADH from $Adh^{FCh.D.}$ and from three Adh^{F71K} homozygous strains

Six cultures were assayed for each strain except when material was insufficient, in which case number of cultures assayed (*n*) was as indicated. Isolates (i), (ii), (iii) indicate three separately isolated Adh^{F71K} alleles. ADH assays of Adh^{F} and Adh^{S} control strains gave results similar to those in Tables 1 and 2. Standard errors in parentheses

Strain	ADH activity with 2-propanol as substrate	Activity ratio	Activity (%) remaining after 5 min at: 40°C	44°C
$Adh^{FCh.D.}/Adh^{FCh.D.}$	135·8 (2·20)	4·5 (0·15)	93·0 (2·02)	37·8 (3·11)
Adh^{F71K}/Adh^{F71K}				
Isolate (i)	143·5 (6·48)	4·5 (0·15)	86·2 (5·72) $n = 4$	34·5 (4·43) $n = 2$
Isolate (ii)	117·7 (5·42)	4·4 (0·11)	85·2 (1·22)	25·25 (4·26) $n = 4$
Isolate (iii)	131·7 (6·83)	4·3 (0·08)	94·7 (1·45) $n = 3$	27·0 (1·01) $n = 2$

The question arises as to whether $Adh^{FCh.D.}$, Adh^{Fr} and Adh^{F71K} are alleles at the *Adh* locus or whether their ADH properties are due to closely linked modifiers. It is suggested that thermostability and electrophoretic mobility of ADH allozymes can be changed by binding of small molecules (Jacobson *et al.* 1972; Schwartz and Sofer 1976). Thörig *et al.* (1975) and Sampsell (1977) both localized their thermostable variants to the region of the *Adh* locus, but none of their analyses had sufficient resolution to detect modifiers as closely linked to the *Adh* structural gene as the one found by Thompson *et al.* (1977) which decreased the number of ADH molecules at equilibrium. We have shown that the purified ADH-F, ADH-S and ADH-FCh.D. proteins which are isolated from their genetic backgrounds, retain the same relative properties, suggesting that if a modifier is present it must act by directly changing the ultimate structural gene product.

In view of the current controversy (Johnson 1977*a*) about the relevance of post-translational modification of enzyme protein to questions concerning amounts of allelic variation in natural populations it is important to summarize our evidence in support of the hypothesis that $Adh^{FCh.D.}$ is an allele at the *Adh* locus:

(1) Genetic analysis using the *b pr cn* marker stock shows that $Adh^{FCh.D.}$ maps to the region of the *Adh* locus.

(2) Heterozygotes between $Adh^{FCh.D.}$ and Adh^{F} or Adh^{S} produce ADH enzymes with properties intermediate between those of the relevant homozygotes; $Adh^{FCh.D.}$ behaves as a co-dominant allele.
(3) Heterozygotes $Adh^{FCh.D.}/Df(2L)64j$ have half the ADH activity of, but similar activity ratios and thermostability properties to, $Adh^{FCh.D.}$ homozygotes.
(4) Finally, in the F_{14} progeny of crosses between $Adh^{FCh.D.}$ and Adh^{F} and between $Adh^{FCh.D.}$ and Adh^{S} strains we have not detected segregation of electrophoretic mobility and thermostability.

In toto our evidence supports the hypothesis that $Adh^{FCh.D.}$ is either a structural allele at the *Adh* locus or is so tightly linked that it behaves as an allele in terms of the genetics of natural populations. Proof that $Adh^{FCh.D.}$ is a structural *Adh* allele awaits the results of amino acid sequence analyses which are being carried out on purified ADH-FCh.D., ADH-F and ADH-S proteins from the single allele strains used in these experiments.

Whether or not $Adh^{FCh.D.}$ is an allele at the *Adh* locus the variant occurs over a wide geographic area, is relatively easy to identify after post-electrophoresis heat treatment (even in heterozygotes), is much more thermostable at 40 or 44°C than ADH-F and ADH-S and, as we show later (Wilks *et al.* 1980), it is often polymorphic in natural populations. These facts mean that the presence of this electrophoretically cryptic ADH variant must be taken into account in any discussion of the mechanism of the maintenance of the *Adh* polymorphism.

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