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An Electrophoretically Cryptic Alcohol Dehydrogenase Variant in *Drosophila melanogaster*. II.* Post-electrophoresis Heat-treatment Screening of Natural Populations†

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Abstract

The two common genetic variants of alcohol dehydrogenase in *D. melanogaster*, ADH-F and ADH-S, differ in substrate specificity and electrophoretic mobility. A third inherited variant, ADH-FCh.D., has a substrate specificity like ADH-S, an electrophoretic mobility like ADH-F, but much greater thermostability than either of the others. ADH-FCh.D. can be identified after post-electrophoresis heat treatment (15 s at 43°C) on cellulose acetate sheets. The $Adh^{FCh.D.}$ allele has been found in 19 of 34 natural populations in Australasia but its frequency in these populations does not exceed 0·06. The partial correlation between $Adh^{FCh.D.}$ frequency and a maximum temperature variable is significant and positive among the Australasian populations although different climatic associations are found for a thermostable form of ADH-F in North America.

Introduction

Investigations of the maintenance of the alcohol dehydrogenase (ADH, EC 1.1.1.1) polymorphism in natural or laboratory populations of *Drosophila melanogaster* have focused on the properties of genotypes for the two common electrophoretic variants at the *Adh* locus, Adh^F and Adh^S (see for example Gibson 1970; Vigue and Johnson 1973; Day *et al.* 1974; Oakeshott 1976). However, it has been shown that there is genetic variation for properties of ADH within these two common electrophoretic forms (Gibson 1970; Thörig *et al.* 1975; Milkman 1976; Sampsell 1977; Lewis and Gibson 1978).

Lewis and Gibson (1978) discovered in a natural population of *D. melanogaster* an ADH variant with electrophoretic mobility like ADH-F but substrate specificity like ADH-S. Gibson *et al.* (1980) showed that this variant, ADH-FCh.D., which is specified by an allele at the *Adh* locus, is more thermostable than either ADH-F or ADH-S. Comparison of their properties suggests that $Adh^{FCh.D.}$ is probably identical to both the thermostable Adh^{Fr} allele isolated from a natural population in Iowa by Sampsell (1977) and the thermostable Adh^{F71K} allele isolated by Thörig *et al.* (1975) from a laboratory population in the Netherlands (Gibson *et al.* 1980). These results suggest that natural populations of *D. melanogaster* might generally be polymorphic for at least three distinguishable alleles at the *Adh* locus. Therefore, we have screened 34 Australasian population samples to quantify this variation and to investigate relationships between the frequencies of $Adh^{FCh.D.}$ and climatic variables.

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†This paper is dedicated to Emeritus Professor M. J. D. White, F.A.A., F.R.S. on the occasion of his seventieth birthday.

Materials and Methods

Origin of Strains

The single-allele stock 14k12j1a was isolated by Lewis and Gibson (1978) from gravid females collected on a pile of grape pressings outside the Chateau Douglas winery at Segenhoe, near Scone, N.S.W. in 1975. Gibson *et al.* (1980) provided evidence that 14k12j1a is an allele at the *Adh* locus and named it $Adh^{FCh.D.}$ for 'fast Chateau Douglas'.

The geographical locations of the 34 Australasian populations which we have screened for *Adh* variants are included in Table 3 and Fig. 2 below. These population sites are all located on the Australian mainland except one on Melville Island (Snake Bay) off the northern coast of Australia and one in Papua New Guinea (Sogeri). The locations cover 34° of latitude (from 8°S. to 42°S.) and 37° of longitude (from 116°E. to 153°E.). All of the collections were made between December 1978 and April 1979 with the exception of those at Mount Molloy and Innisfail, which were made in November 1979.

Ten of the populations were each initiated in the laboratory by over 200 wild-caught flies. The other 24 populations were represented by between 4 and 77 single female lines. For each of these 24 populations the flies typed for *Adh* alleles were taken in equal numbers from each of the single female lines which represented a population. The number of single female lines and the total number of genes scored in each population are also given in Table 3. All populations were scored for *Adh* alleles within six generations of the laboratory cultures being established and most were scored within three generations. For some populations mass cultures as well as single female lines were maintained and in these there was close agreement in the estimates of gene frequencies derived from the single female lines and the mass cultures.

Climatic Data

Climatic data for weather stations close to the collection sites were obtained from *World Weather* (1967; H.M.S.O.: London) and *Climatic Averages Australia* (1975; Aust. Govt Publ. Serv.: Canberra). The average distance between a collection site and the closest weather station was 13 ± 2 km in Australia and 34 ± 3 km in the United States of America. Of the temperature and rainfall measurements available at each station four were selected because they were the least intercorrelated and because they were likely to be ecologically significant. The four variables averaged over 20 years (except for Sogeri, Papua New Guinea, where there were data for only 1 year) were:

(1) maximum temperature (average daily maximum in °C for the hottest calendar month at the station);

(2) minimum temperature (average daily minimum in °C for the coldest calendar month at the station);

(3) maximum rainfall (total rainfall in millimetres for the wettest calendar month at the station, square root transformed);

(4) minimum rainfall (total rainfall in millimetres for the driest calendar month at the station, square root transformed).

In addition, the longitude, latitude and elevation (log transformed) for each collecting site were known. The gene frequencies were angularly transformed and standardized for sample size (Mulley *et al.* 1979).

The results of analyses of the relationships between the climatic variables and the frequencies of Adh^F and Adh^S will be described elsewhere.

Electrophoresis and Enzyme Assays

Electrophoresis on cellulose acetate sheets, ADH enzyme assays and measurements of thermostability were carried out as previously described (Gibson *et al.* 1980). ADH activity was assayed both with ethanol and 2-propanol as substrate and the ratio of ADH activity with 2-propanol to ADH activity with ethanol is referred to below as the activity ratio (after Gibson *et al.* 1980).

Thermostable ADH variants were detected by following the standard electrophoresis procedure and then sealing the cellulose acetate sheets in transparent plastic wrapping film (Glad-wrap) and immersing them for 15 s in a water-bath maintained at 43°C, prior to activity staining.

Our standard practice in screening populations was to apply each single fly extract to two cellulose acetate sheets, subject the extracts to electrophoresis under standard conditions, and heat-treat one of the sheets prior to staining.

After electrophoresis on untreated cellulose acetate sheets the isozyme ADH-5 is most heavily stained and ADH-3 is also visible for each of the six genotypes; ADH-1 is not observed (nomenclature of Ursprung and Leone 1965; Schwartz and Sofer 1976). Additionally, a heterodimer band is observed in heterozygotes between electrophoretic variants (Fig. 1). After heat-treatment of the cellulose acetate sheets ADH-3 is the only band of activity remaining in Adh^F/Adh^F but in $Adh^{FCh.D.}/Adh^{FCh.D.}$ the prominent band is ADH-5; in $Adh^F/Adh^{FCh.D.}$ heterozygotes bands ADH-5 and ADH-3 are visible. The little activity remaining in Adh^S/Adh^S is its ADH-3. The ADH-3 band of Adh^F is the most prominent in Adh^F/Adh^S heterozygotes. In $Adh^S/Adh^{FCh.D.}$ heterozygotes the prominent band of activity contains ADH-5 of $Adh^{FCh.D.}$ superimposed on the activity remaining in ADH-3 of Adh^S.

Comparison of the heat-treated and untreated cellulose acetate sheets thus identifies six genotypes at the *Adh* locus. In addition, the occurrence of heat-resistant Adh^S variants was noted where ADH-5 remains strongly stained; such variants were present in some populations at frequencies less than 1 % but they have not yet been investigated in more detail.

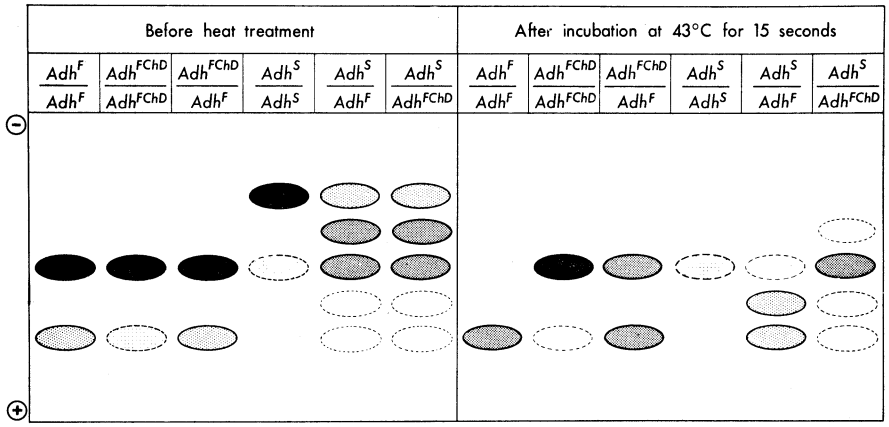

Fig. 1. Diagrammatic representation of ADH staining regions on cellulose acetate sheets before and after incubation at 43°C for 15 s. The different shadings give an indication of the intensity of staining. The most cathodal band for each extract on the untreated cellulose acetate sheets is ADH-5 and the most anodal band is ADH-3.

Results

Re-isolation of $Adh^{FCh.D.}$ from Chateau Douglas

Lewis and Gibson (1978) first identified $Adh^{FCh.D.}$ as one of two electrophoretically fast single-allele strains isolated from the Chateau Douglas population. A further nine separately extracted single-allele Adh^F strains isolated at the same time from Chateau Douglas were also assayed but none of these produced ADH-FCh.D. on the basis of their activity ratios or thermostabilities. These few data suggest either that $Adh^{FCh.D.}$ occurs at a frequency of about 0·09 amongst electrophoretically fast alleles in the Chateau Douglas population or that it arose by mutation during the course of the breeding program which was used to produce the homozygous single-allele lines.

In order to distinguish between these possibilities we used the post-electrophoresis heat-treatment technique described in the Materials and Methods section to screen for $Adh^{FCh.D.}$ in a further collection of flies taken at Chateau Douglas in April 1979. At that time the waste dump outside the winery had dried out and neither larvae nor adult flies were found. Inside the winery, however, 35 adults together with larvae were collected from wine leakages around barrels. These 35 adult flies together with

190 adults which emerged from the collected larvae were typed for *Adh* alleles with the post-electrophoresis heat-treatment technique. Results obtained were as follows:

Genotype	*F/F*	*F/S*	*S/S*	*FCh.D./FCh.D.*	*F/FCh.D.*	*FCh.D./S*
Trapped adults	13	14	2	–	3	3
Emerged adults	76	79	22	2	8	3

In the trapped adult flies six heat-resistant alleles were detected among 49 electrophoretically fast alleles and in flies that emerged from the collected larvae 15 heat-resistant alleles were detected among 254 fast alleles. On the presumption that the heat resistant fast alleles were all $Adh^{FCh.D.}$ the overall frequencies of detectable alleles at the *Adh* locus were 0·33 Adh^S, 0·63 Adh^F and 0·04 $Adh^{FCh.D.}$. There were too few flies of each genotype to critically detect departures from Hardy–Weinberg proportions but the $Adh^{FCh.D.}$ genotypes were found in approximately the proportions expected.

To test whether heat-resistant fast alleles were indeed $Adh^{FCh.D.}$, we investigated their activity ratios and thermostabilities in crude extracts.

Table 1. Mean values for ADH activity in 10 separately extracted single-allele lines classified as 'heat sensitive' (HS) and 'heat resistant' (HR) electrophoretic fast alleles by the post-electrophoresis heat-treatment technique

Standard errors are given in parentheses

Strains	No. of cultures assayed	ADH activity with 2-propanol as substrate (A)	ADH activity with ethanol as substrate (B)	Activity ratio (A/B)
Chateau Douglas				
(1) HS	8	333·1 (8·94)	49·1 (1·01)	6·8 (0·12)
(2) HS	8	304·4 (18·68)	45·5 (2·72)	6·7 (0·17)
(3) HR	8	173·1 (4·55)	36·7 (0·56)	4·7 (0·15)
Mudgee				
(1) HS	8	291·6 (16·14)	42·2 (2·15)	6·9 (0·11)
(2) HS	8	382·7 (12·79)	56·1 (2·12)	6·8 (0·12)
(3) HR	8	115·9 (8·73)	26·4 (1·91)	4·4 (0·06)
Hunter Valley				
(1) HS	8	282·9 (10·47)	41·6 (1·80)	6·8 (0·11)
(2) HS	8	383·5 (12·89)	54·9 (2·10)	6·9 (0·08)
(3) HS	8	369·6 (4·32)	55·1 (1·30)	6·7 (0·11)
(4) HR	8	137·1 (9·90)	31·0 (1·31)	4·4 (0·14)
Controls				
Adh^F/Adh^F	8	292·4 (10·92)	43·4 (2·35)	6·8 (0·17)
$Adh^{FCh.D.}/Adh^{FCh.D.}$	8	148·9 (7·59)	32·2 (1·37)	4·6 (0·11)
Adh^S/Adh^S	8	85·4 (2·41)	19·6 (1·12)	4·4 (0·23)

Biochemical Properties of Newly Isolated Heat-resistant Fast Lines

The progenies of this collection of flies from inside the Chateau Douglas winery were used to set up a number of single-pair matings from which single-allele lines were derived. This was done as previously described (Lewis and Gibson 1978), except that the post-electrophoresis heat-treatment technique was used to detect heat-resistant fast alleles. Similarly, from collections from other natural populations of *D. melanogaster* at Mudgee (140 km west of Chateau Douglas) and in the Hunter

Valley, N.S.W. (80 km south-east of Chateau Douglas), representatives of heat-resistant fast alleles and heat-sensitive fast alleles were isolated and maintained as single-allele lines.

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

Abbreviations as in Table 1. Standard errors given in parentheses

Strain	No. of cultures assayed	ADH activity remaining in heat-treated extracts (%) 40°C	44°C
Chateau Douglas			
(1) HS	8	38·0 (2·99)	5·0 (0·27)
(2) HS	8	39·2 (3·18)	3·9 (0·55)
(3) HR	8	95·4 (1·40)	50·0 (2·79)
Mudgee			
(1) HS	8	31·9 (3·61)	2·5 (0·27)
(2) HS	8	43·0 (2·49)	5·7 (0·47)
(3) HR	8	92·4 (1·34)	30·4 (4·60)
Hunter Valley			
(1) HS	8	24·4 (2·92)	1·9 (0·23)
(2) HS	8	37·5 (2·88)	4·5 (0·50)
(3) HS	8	40·6 (4·28)	4·7 (0·47)
(4) HR	8	90·4 (2·17)	31·2 (5·70)
Controls			
Adh^F/Adh^F	8	31·1 (3·55)	2·0 (0·27)
$Adh^{FCh.D.}/Adh^{FCh.D.}$	8	92·4 (0·91)	43·2 (3·93)
Adh^S/Adh^S	8	33·4 (4·22)	1·4 (0·43)

Flies from heat-sensitive and heat-resistant single-allele fast lines in each of these three geographically separate samples were assayed for ADH activity and heat sensitivity. Duplicate assays were made from each of eight separate cultures of these 10 single-allele lines and of the control lines Adh^F/Adh^F, Adh^S/Adh^S and $Adh^{FCh.D.}/Adh^{FCh.D.}$ used by Gibson *et al.* (1980). These assays (Tables 1 and 2) show that each of the fast alleles classified as heat-resistant following post-electrophoresis heat-treatment of cellulose acetate sheets has the ADH activity ratio and the thermostability after treatment at 40°C that distinguishes ADH-FCh.D. from ADH-F. At 44°C there are significant differences in thermostability between the three newly extracted heat-resistant variants and ADH-FCh.D. However, it is clear that these variants are far more similar to ADH-FCh.D. than to ADH-F or ADH-S and the differences are likely to reflect the effects of the genetic backgrounds. All three heat-resistant, fast, single-allele lines had lower ADH activities than the other fast, single-allele lines derived from the same population. This result agrees with Lewis and Gibson's (1978) data in which the electrophoretically fast allele with the ADH activity ratio characteristic of ADH-S had lower ADH activity than the other fast, single-allele lines assayed. In combination with the electrophoretic survey of the Chateau Douglas population these results show that the $Adh^{FCh.D.}$ allele isolated by Lewis and Gibson (1978) does occur in natural populations and did not simply arise by mutation during the extraction breeding program.

Geographical Distribution of $Adh^{FCh.D.}$

Using the post-electrophoresis heat-treatment technique 34 natural populations of *D. melanogaster* were screened for Adh^S, Adh^F and $Adh^{FCh.D.}$ alleles (Table 3). The Innisfail sample was fixed for Adh^S but in the other 33 populations the *Adh* locus was

Table 3. Geographical locations and *Adh* frequencies for 34 Australasian populations of *D. melangoaster*

Location[A]	Lat. (°S.), Long. (°E.)	No. of female lines	Genes scored	Adh^S	Adh^F	$Adh^{FCh.D.}$
Sogeri, PNG	8·8, 148·3	22	220	0·94	0·06	—
Snake Bay	11·4, 130·7	12	224	0·71	0·29	—
Darwin	12·5, 130·8	13	208	0·62	0·33	0·05
Mount Molloy	16·7, 145·6	24	144	0·92	0·08	—
Derby	17·3, 123·6	4	104	0·49	0·51	—
Innisfail	17·5, 146·0	6	100	1·00	—	—
Townsville	19·3, 146·7	8	190	0·62	0·33	0·05
Nambour	26·6, 153·0	52	208	0·92	0·08	—
Brisbane	27·5, 153·0	77	224	0·69	0·29	0·02
Stanthorpe	28·7, 152·0	22	176	0·58	0·37	0·05
Sandalford*	31·9, 116·0	27	298	0·37	0·60	0·03
Westfield*	31·9, 116·0	14	208	0·31	0·67	0·02
Chateau Douglas*	32·1, 150·9	m.c.[B]	450	0·33	0·62	0·05
Mudgee						
Craigmoor*	32·6, 149·6	m.c.	432	0·35	0·62	0·03
Linden*	32·6, 149·6	m.c.	212	0·29	0·67	0·04
Montrose*	32·6, 149·6	m.c.	280	0·35	0·64	0·01
Hunter Valley						
Bellevue*	32·8, 151·3	m.c.	940	0·35	0·60	0·05
Elliotts*	32·8, 151·3	m.c.	494	0·38	0·58	0·04
Lindemans*	32·8, 151·3	m.c.	434	0·40	0·56	0·04
Rothbury*	32·8, 151·3	m.c.	580	0·32	0·64	0·04
Tyrrells*	32·8, 151·3	m.c.	208	0·40	0·56	0·04
Saint Hallet*	34·3, 138·9	58	224	0·23	0·77	—
Willsford*	34·3, 138·9	15	210	0·30	0·70	—
Saint Peters	34·9, 138·6	21	210	0·08	0·90	0·02
Colonel Light Gardens	34·9, 138·6	3	78	0·10	0·90	—
Coriole*	35·0, 138·5	38	224	0·47	0·53	—
Wirra Wirra*	35·0, 138·5	19	200	0·25	0·74	0·01
Canberra	35·3, 149·2	44	342	0·27	0·69	0·04
Araluen	35·5, 149·8	53	318	0·38	0·62	—
Rutherglen*	36·1, 146·5	6	60	0·38	0·62	—
Chateau Tahbilk*	36·4, 145·4	m.c.	166	0·30	0·68	0·02
Great Western*	37·1, 142·8	14	360	0·32	0·68	—
Melbourne	37·7, 144·8	38	224	0·17	0·80	0·03
Cygnet	43·0, 147·3	21	226	0·49	0·51	—

[A]Asterisks indicate winery populations. [B]m.c., mass culture.

polymorphic with the frequency of Adh^F varying from 0·06 in the Sogeri sample to 0·90 in populations sampled near Adelaide (Fig. 2). There was a general latitudinal cline with Adh^S frequency decreasing with increasing distance from the equator (see Table 3), as has been found in the Northern Hemisphere (Vigue and Johnson 1973).

The thermostability variant $Adh^{FCh.D.}$ was found in 19 of the populations at frequencies up to 0·06 (Table 3) and over a wide range of Adh^{F} frequencies (0·33–0·90). It is, of course, possible that $Adh^{FCh.D.}$ occurs at very low frequencies in populations where we have not detected it. However, $Adh^{FCh.D.}$ was not detected in four of the 11 populations in which the Adh^{F} frequency was greater than 0·66 and over all

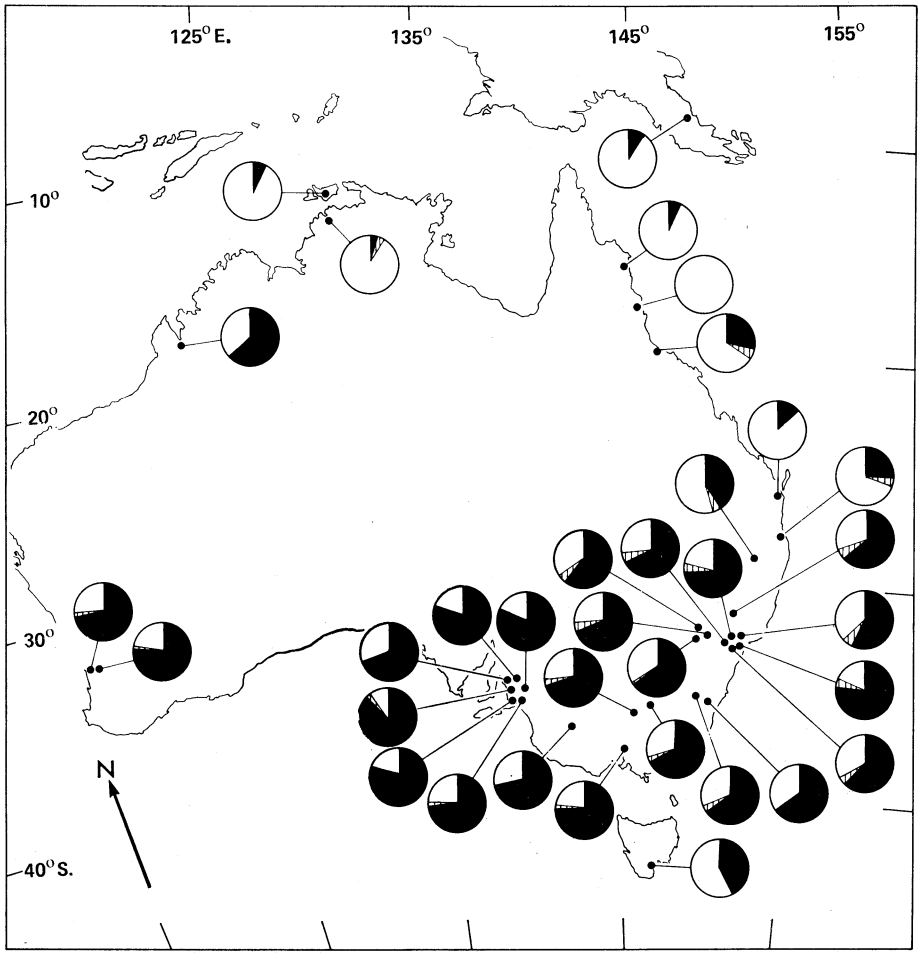

Fig. 2. Geographical location and frequencies of Adh^{F} (black), $Adh^{FCh.D.}$ (hatched) and Adh^{S} (open) in Australasian population samples.

collections the regression of $Adh^{FCh.D.}$ frequency on the number of single female lines was not significant. Also, when populations are considered in three categories based on the Adh^{F} frequency, there is a suggestion that the frequency of $Adh^{FCh.D.}$ is highest in populations with an Adh^{F} frequency between 0·34 and 0·66 (Table 3). However, this observation might be biased by the five Hunter Valley population samples which have similar Adh^{F} frequencies and are relatively close together.

Climatic Associations with $Adh^{FCh.D.}$ *Frequency*

Gibson *et al.* (1980) have previously shown that the heat-resistant variants ADH-F71K (Thörig *et al.* 1975) and ADH-Fr (Sampsell 1977) have the same ADH

activity ratio and thermostability properties as ADH-FCh.D. and they argued that the three separately discovered variants probably represent the same *Adh* allele. Whether or not $Adh^{FCh.D.}$, Adh^{Fr} and Adh^{F71K} are the same *Adh* allele, their properties are extremely similar relative to those of other *Adh* alleles and they can be considered collectively in analysing their geographic distribution. Scharloo *et al.* (1977) noted that alleles with properties similar to Adh^{F71K} occurred in natural populations in the Netherlands but did not describe their distribution. Sampsell (1977) screened populations from 19 different locations in North America for heat-resistant *Adh* fast alleles. She found Adh^{Fr} in 13 populations with an overall frequency of 0·016 but varying between zero and 0·068 in individual populations. The average frequency of the heat-resistant fast allele in North America is not significantly different from the mean $Adh^{FCh.D.}$ frequency of 0·021 ($P > 0·2$) in the Australasian populations.

Table 4. Partial correlations between the frequency of heat resistant *Adh* fast alleles and the climatic variables together with longitude and latitude

The correlations shown are those which remain when all the other variables have been controlled. None of the other possible partial correlation coefficients were significant. Number of populations in parentheses. *P<0·05; **P<0·01; n.s., not significant

Variable	Australasia	North America[A]
Maximum temperature	0·40* (29)	−0·79** (8)
Minimum temperature	−0·26[n.s.] (29)	0·76* (8)
Minimum rainfall	−0·01[n.s.] (29)	−0·76** (8)

[A]Data from Sampsell (1977).

We have analysed the relationships of four climatic variables to $Adh^{FCh.D.}$ frequency in our 34 Australasian samples and to Adh^{Fr} frequency in Sampsell's 19 North American populations. None of the simple correlations between the frequency of $Adh^{FCh.D.}$ or Adh^{Fr} with latitude, longitude, log elevation or with the four climatic variables is significant for either the Australasian or North American data. However, analyses of partial correlations between the frequency of $Adh^{FCh.D.}$ and the spatial and climatic variables (Table 4) show that, for the Australasian populations, maximum temperature is significantly and positively correlated with $Adh^{FCh.D.}$ frequency when the effects of the other variables have been taken into account. Although the same partial correlation is significant in the North American data the sign is negative. The partial correlations for minimum temperature and maximum rainfall are also significant for the North American frequencies but not in the Australasian data. Overall these analyses do not provide evidence of any consistent relationships between the frequencies of electrophoretically fast, heat-resistant *Adh* alleles and climatic or spatial variables on the two continents.

Discussion

Our experimental results show unequivocally that the post-electrophoresis heat-treatment technique using single fly homogenates on cellulose acetate sheets can be used to identify $Adh^{FCh.D.}$ in samples of adults from natural populations. The technique was used to isolate heat-resistant ADH variants indistinguishable in electrophoretic mobility from ADH-F. All such variants that were assayed had

activity ratios similar to those characteristic of ADH-S but greater thermostability than either ADH-S or ADH-F.

It seems very likely that many, if not all, heat-resistant, fast ADH variants isolated by this technique will be ADH-FCh.D. We have now used the technique to isolate 14 heat-resistant, electrophoretically fast *Adh* alleles from *D. melanogaster* populations on three continents and all of them have properties characteristic of ADH-FCh.D. (see also Gibson *et al.* 1980).

The geographic survey shows that $Adh^{FCh.D.}$ occurs at frequencies up to 0·06 in 19 of 34 Australasian populations which is similar to the results of Sampsell (1977) who found a heat-resistant, fast variant at frequencies up to 0·07 in 13 of 19 North American populations. *In toto* the data indicate that natural populations of *D. melanogaster* can be polymorphic for at least three detectable alleles at the *Adh* locus.

Table 5. Summary of results of studies on the thermostability of ADH-F, ADH-S and ADH-F/S

Heat treatment	Life cycle stage of extract	Relative thermostability	Absolute ADH activities after heat-treatment	Reference
10 min at 40°C	Third-instar larvae	S>F/S>F	F/S>S>F	Gibson (1970)
10 min at 40°C	Adults	S>F/S>F	F/S≡S>F	Vigue and Johnson (1973)
10 min at 40°C	Adults	S>F>F/S	F>F/S>S	Day et *al.* (1974)
10 min at 40°C	Adults and larvae	F≡F/S>S	F>F/S>S	Oakeshott (1976)
5 min at 40°C or 44°C	Adults	F≡F/S≡S	F>F/S>S	Gibson *et al.* (1980)

In populations in which $Adh^{FCh.D.}$ is found its frequency is higher than is generally explained by recurrent mutation balanced by weak selection although, of course, it is possible that the mutation rate to $Adh^{FCh.D.}$ is higher than normal or that some other generative process, such as intragenic recombination, is involved (Watt 1972). However, the amino-acid sequence data argues against the formation of $Adh^{FCh.D.}$ by intragenic recombination between Adh^{F} and Adh^{S}, since they differ from each other by a single amino acid replacement (Fletcher *et al.* 1978; Thatcher, personal communication).

It is possible that $Adh^{FCh.D.}$ is a relatively new allele at the *Adh* locus [sequence analyses of purified enzymes show that ADH-FCh.D. differs from ADH-F by at least one amino acid and from ADH-S by at least two and, therefore, if $Adh^{FCh.D.}$ is newly arisen, it is more likely to derive from Adh^{F} (our unpublished data)] but without knowing whether the $Adh^{FCh.D.}$ frequencies are at equilibrium we are unable to draw inferences about its relative fitness. The consistently low $Adh^{FCh.D.}$ frequency in populations is compatible with hypotheses that invoke non-selective processes or selective processes, such as frequency-dependent selection, for its maintenance. Nevertheless two of the biochemical properties of ADH-FCh.D., considered in conjunction with the population distribution, suggest possible mechanisms by which $Adh^{FCh.D.}$ might be selectively maintained.

Firstly, the frequency of $Adh^{FCh.D.}$ tends to be highest in populations with intermediate frequencies of Adh^F and Adh^S, in which intermediate levels of ADH activity might be optimal (Oakeshott *et al.* 1980). $Adh^{FCh.D.}/Adh^{FCh.D.}$ genotypes have low levels of ADH compared with Adh^F/Adh^F but relatively high levels compared with Adh^S/Adh^S (Table 1 and Lewis and Gibson 1978). Therefore Adh^F/Adh^S heterozygotes and all genotypes which include $Adh^{FCh.D.}$ will be of intermediate ADH activity in the absence of large dominance effects (Gibson *et al.* 1980).

Secondly, the thermostability which distinguishes ADH-FCh.D. from both ADH-F and ADH-S might have a selective advantage in some circumstances, in which case $Adh^{FCh.D.}$ would be a conditionally neutral allele in Thoday's (1975) terminology. Previous studies on the thermostabilities of ADH-F, ADH-S and ADH-F/S have suggested that adaptation to different temperatures might be a selective factor. However, evidence on their differential thermostabilities is conflicting and certainly does not suggest that one of the common alleles would have a consistent advantage over the other (see summary in Table 5; Gibson 1970; Vigue and Johnson 1973; Day *et al.* 1974; Oakeshott 1976 and Gibson *et al.* 1980). The ADH-F and ADH-S assayed as controls in the present study do not differ in thermostability at 40°C or at 44°C (Table 2).

The thermostability of ADH-FCh.D. is much greater than either ADH-F or ADH-S in both crude extracts and purified enzyme preparations (Chambers *et al.*, unpublished data). However, it might be expected that the absolute activity remaining after heat shock, rather than relative activity, has more effect on fitness. In absolute amounts $Adh^{FCh.D.}$ genotypes have the highest ADH activity in extracts incubated for 5 min at 44°C (Tables 1 and 2) but at 40°C Adh^F/Adh^F retains the highest activity. Although the frequency of $Adh^{FCh.D.}$ is positively related to maximum temperature in the Australasian populations, the relationship is opposite in sign in Sampsell's (1977) North American data on what we have concluded is $Adh^{FCh.D.}$. This argues against the simple causal relationship between the *in vitro* thermostability of ADH and the fitness of flies exposed to temperature stress which has been suggested by Johnson and Powell (1974). Experiments in progress comparing the fitnesses of the thermostable *Adh* variants with those of Adh^F and Adh^S under a variety of environmental conditions should provide data pertinent to these contrasting results.

Whatever the adaptive significance of $Adh^{FCh.D.}$, however, its distinctive array of biochemical properties and consistent patterns of occurrence in natural populations imply that screening for this electrophoretically cryptic variant will be essential in any future investigations of the *Adh* polymorphism.

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