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Persistence of an alcohol dehydrogenase thermostable variant in a natural population of *Drosophila melanogaster*

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Summary

A temporal survey of the alcohol dehydrogenase locus in a natural population of *Drosophila melanogaster* from the Canary Islands has revealed the existence of an electrophoretic Fast thermostable variant, mapped within 0.8 units of the structural *Adh* locus, and which persists over a 6-year period with a frequency ranging from 0.8 to 0.5%.

1. Introduction

Two sources of information support the idea that the Alcohol Dehydrogenase (*Adh*) polymorphism in *Drosophila melanogaster* is maintained by selection. The first are studies of natural populations showing the existence of geographically widespread clines for this locus (David, 1982; Oakeshott *et al.* 1981; Singh, Hickey & David, 1982), and the second are experimental gene frequency changes induced in the laboratory (Gibson, 1970; Johnson & Powell, 1974; Bijlsma-Meeles & Van Delden, 1974; Oakeshott *et al.* 1980). This evidence depends on the assumption that the two main electrophoretic variants *Adh-Fm* and *Adm-Sm*, found in nearly all populations, are genetically homogeneous classes. Nevertheless, heat-denaturation studies in *Adh* have revealed the existence of a third widespread variant of the same electrophoretic migration as *Adh-Fm* but with a greater thermostability than either of the two common phenotypes. Frequencies for this allele (*Adh-Fr*) average 1.6% in North American populations (Sampsell, 1977) and 4.9% in Australian populations (Gibson, Wilks & Chambers, 1982). Previous experimental results could now be questioned if this new variation is considered. When frequencies of the thermoresistant variant are taken into account, there is significant heterogeneity among some Australian populations which were before considered similar (Gibson *et al.* 1982). This reinforces the importance of screening more natural populations in order to test the ubiquity and persistence of this heat-stability variant.

In this paper we report the existence of an electrophoretically Fast and thermostable variant *Adh-Fr* in a natural population of the Canary Islands, and its persistence over a 6-year period.

2. Material and Methods

(i) *Control strains and population samples*

In a previous screening for thermostability variants at the *Adh* locus of *D. melanogaster* (Cabrera *et al.* 1978) 19 laboratory stocks homozygous F/F and 12 homozygous S/S were examined. No differences in thermostability were recognized among the S/S strains but two F/F strains (*st*, scarlet, provided by the Genetics Department of the Valencia University of Spain and *b-310*, w&+.=, from the Department of Biology of the Bowling Green State University of U.S.A.) proved to be homozygous for a more thermostable variant (*Fr/Fr*). From segregation backcrosses it was deduced that these thermostable variants are due to changes at the structural *Adh* gene or at genes no farther than 0.8 map unit apart, (95% confidence limits), (Cabrera *et al.* 1978). It was also demonstrated that after heat treatment there are clear-cut differences in the stain intensity of the major cathodal bands (*Adh-5* and *Adh-3* following the nomenclature of Grell, Jacobson & Murphy, 1965; 1968) among homozygous individuals for the same electrophoretic fast migration but with different thermostable phenotypes. Likewise heterozygous F/S can be distinguished from heterozygous Fr/S for the post-heat-treatment stain intensity of the band F-5 and the hybrid band FS-5. In some cases it is not easy to discriminate between homozygous F/F with great enzymatic activity (the F-5 band does not completely disappear after heat treatment) and heterozygous Fr/F (the F-5 band has approximately half the activity of homozygous (Fr/Fr). In these cases, instead of comparing the F-5 band intensity alone, we used the relative activity of the F-3 band to the F-5 band. This

measure was in all cases significantly greater in the F/F individuals with higher activity. Measures of the isozyme activities were obtained with a *Quick Scan* densitometer, the maximum peak height of each band being the measure used for the estimation of the ratios.

The procedure for detecting the existence of thermostable variants in a natural population was as follows: In the vintage period (1977, 1980 and 1983) samples were taken from the locality of Guímar (Tenerife Island, in the Canarian Archipelago). Isolines were founded with females, and males were crossed with a *Cy*(Curly), *Adh-Fm/Pm*(Plum), *Adh-S* strain. After this, wild parents were tested for electrophoretic mobility and thermostability. In order to facilitate their phenotypic assignation the following marker stocks were run with the wild flies: *st* as *Fr/Fr*, 'Oregon' as *Fm/Fm*, *G-21* as *Sm/Sm*; F_1 from the cross *st* × 'Oregon' as *Fr/Fm* and F_1 from the cross *st* × *G-21* as *Fr/Sm*.

(ii) *Electrophoresis*

Electrophoresis was carried out on large horizontal slab 5% acrylamide gels (45 × 12 × 0.45 cm). The gel and electrode buffer were 0.1 M Tris-borate (pH 8.9). Single flies were homogenized in 30 μl of the same buffer. After electrophoresis for 4 h at 400 V (100 mA) and 0–2 °C, the gel was cut horizontally into three sections (1.5 mm wide) each of which was subjected to a different heat treatment.

(iii) *Heat treatment*

For detection of allozymes with different heat stabilities we used the comparative loss of activity, after heat treatment, of the most cathodal and thermosensitive *Adh-5* band, the comparative loss of activity of the more thermoresistant *Adh-3* band and, when necessary, the rate of loss of the *Adh-5* band compared to the *Adh-3* band in the control and heat-treated sections. Three post-electrophoretic heat treatments (Cabrera *et al.* 1978) were used: (1) 20 min at 37 °C in order to detect alleles more heat sensitive than those of the control strains 'Oregon' (*Fm/Fm*) and *G-21* (*Sm/Sm*), neither of which are affected by this treatment. (2) 15 min at 60 ± 0.5 °C in order to detect alleles more heat stable than those of the 'Oregon' and *G-21* strains whose *Adh-5* are almost undetectable after this treatment, whereas the *Adh*-5 band of the thermostable stock, *st*(*Fr/Fr*), shows approximately half of its activity. (3) 20 min at 60 ± 0.5 °C to detect alleles with equal, higher or lower heat stability than those of the *st*(*Fr/Fr*) strain which, although they lose the *Adh-5* band activity, still show enzymatic activity at the *Adh-3* band. Both bands of the moderately sensitive strains ('Oregon' and *G-21*) are degraded after this treatment. The general procedure for all three treatments was as follows: After electrophoresis each of the three sections of gel were placed in trays with 150 ml of 0.05 M Tris-HCl buffer, pH 8.5, and preheated at the above-mentioned temperatures. Trays were wrapped in tinfoil and gently shaken during the heat treatment in an incubator, so as obtain homogenous conditions for all the samples inserted along the gel. After heat treatment all slides were stained for 15 min at 37 °C in the following solution: 4 ml 2-propanol, 40 mg NAD, 30 mg MTT, 4 mg PMS in 150 ml of 0.05 M Tris-HCl buffer, pH 8.5. They were then fixed in H_2O-metanol-acetic (3:3:1), and scanned for stained intensity of the bands.

(iv) *Genetics*

Once the *Adh* phenotype of the wild flies was determined, 15 F_1 flies of the uncommon phenotypes were tested to confirm the existence of variants in descendants. If that was the case, sib-mated crosses were set up. The parents were tested to establish their phenotypes and the expected Mendelian segregation of each cross tested. For each variant a minimum of 350 offspring were assayed. If there were too few flies in a line for analysis, homozygous flies for the rare variant were crossed with a homozygous stock with the normal heat stability and with different electrophoretic mobility, and the segregation of the two characteristics tested in the back-cross.

3. Results and Discussion

Figure 1 illustrates the heat-treatment described above. In Table 1 the scanning values of bands 5 and 3 for the phenotypes *Fm/Fm*, *Fm/Fr*, and *Sm/Sm*, in each treatment, are given. These values are means obtained from a minimum of 65 individuals scored. At the first tretament (37 °C/20′) band 5 shows greater activity than band 3 for the three phenotypes, the enzymatic activity of both fast variants being similar and greater than those of the slow variant. The same occurred in the 60 °C/15′ treatment, but in this case the activity of band 5 disappears in the *Sm/Sm* phenotype and is greatly reduced in *Fm/Fm* when compared with *Fr/Fr*. Curiously enough band 3 for both fast variants shows more activity than in the previous treatment; this can be seen in Fig. 1. In the third treatment (60 °C/20′), both bands are drastically reduced in the *Fr/Fr* phenotype although they are both visible, but only band 3 shows residual activity in the *Fm/Fm* and *Sm/Sm* phenotypes. The rate of loss in band 3, is less in the *Sm/Sm* phenotype than in the *Fm/Fm* phenotype throughout the treatment (Table 1). This is in agreement with earlier results of enzymatic activity, on crude extracts determined spectrophotometrically, which indicate that the *Adh-F* enzyme is more active but less heat-stable than the *Adh-S* enzyme (Day, Hillier & Clarke, 1974). In this temporal survey we have found three variant types in *Adh*: The two common electrophoretic variants (*Adh-Fm* and *Adh-Sm*), and the thermostable variant *Adh-Fr*, with

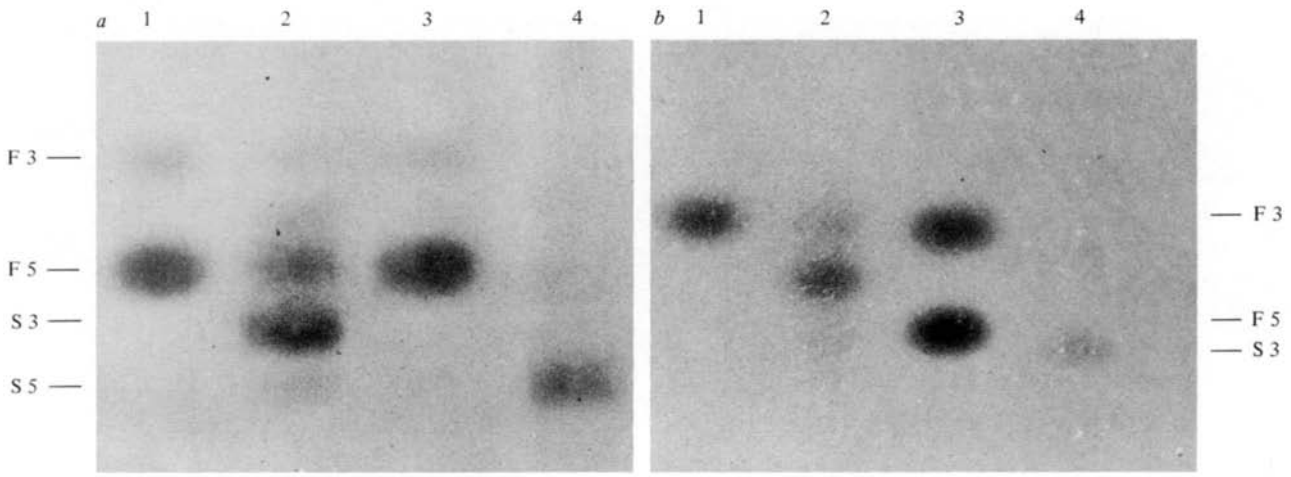

Fig. 1. Photograph of polyacrylamide slides from the same gel showing the patterns of *Drosophila melanogaster* under control conditions (*a*) and after heat-treatment at 60 °C during 15 min (*b*). (1) *Fm/Fm*; (2) *Fm/Sm*; (3) *Fr/Fr*; (4) *Sm/Sm*.

Table 1. *Means* (S.E.) *of the densitometric values of bands 5 (F5 or S5) and 3 (F3 or S3) for different phenotypes at three heat-treatments*

		Treatments		
Phenotypes	Band	37 °C/20′	60 °C/15′	60 °C/20′
Fm/Fm	3	12.75 (0.3)	21.18 (0.14)	2.2 (0.8)
	5	77.64 (0.7)	2.4 (0.10)	0.58 (0.6)
Fr/Fr	3	11.91 (0.6)	30.1 (0.67)	17.4 (0.26)
	5	83.51 (0.5)	23.2 (0.22)	8.05 (0.17)
Sm/Sm	3	8.51 (0.4)	3.04 (0.36)	2.85 (0.24)
	5	36.50 (0.81)	—	—

Table 2. *Frequency of* Adh *alleles in samples from a temporal survey of a natural population from Güimar*

Year	Number of alleles tested	*Adh-Sm*	*Adh-Fm*	*Ahd-Fr*
1977	174	37(0.213)	136(0.782)	1(0.005)
1980	268	38(0.142)	228(0.850)	2(0.008)
1983	560	175(0.313)	381(0.680)	4(0.007)

the same electrophoretic migration as *Adh-Fm* but with greater activity in both bands after heat-treatment (Fig. 1). In addition, interstrain variation in enzymatic activity was found in certain lines. These variations, however, do not seem to be allelic as they do not obey Mendelian segregation and presumably reflect differences in modifying loci (Ward, 1975; Maroni, 1978; Maroni & Laurie-Ahlberg, 1983). This type of variation has not been considered here. The allelic frequencies found for the discrete variants and the number of genomes sampled in each temporal survey are given in Table 2. These frequencies fall quite well within the range observed by other authors in previous geographic samples for the same locus and species in North America (Sampsell, 1977) and Australia (Wilks *et al.* 1980). The *Adh-Fr* variants behaved as simple Mendelian characters, this can be deduced by the mean and standard error values estimated of each phenotype *F-5* band, after heat-treatment. A sample of 30 males of each phenotype obtained from crosses between *Fm/Fr* parents shows the following discrete values: *Fm/Fm*, 0.92 ± 0.02; *Fm/Fr*, 2.29 ± 0.03; *Fr/Fr*, 8.56 ± 0.02. Furthermore the segregation ratios from the F_2 and back-crosses of heterozygous individuals *Fr/Fm* were close to the expected mendelian proportions. In addition, the evidence that this thermoresistant variant is an allelic form of the structural *Adh* locus itself, is supported by the lack of segregation for the parental characteristics of electrophoretic migration and heat-stability in seven independent crosses, in each of which a minimum of 350 offspring were tested. The allelism of other *Adh* heat-stable variants has also

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been demonstrated by genetical (Sampsell, 1977) or structural analysis (Chambers *et al.* 1981).

Although other rare variants have been occasionally detected, the available data suggests that the *Adh* locus polymorphism of *D. melanogaster* is supported by a tri-allelic system (Gibson *et al.* 1982) consisting of the two common alleles (*Adh-Fm* and *Adh-Sm*) and the rare allele *Adh-Fr*. The latter has been observed consistently at low frequency in the screening of laboratory stocks (Thöring, Schoone & Scharloo, 1975; Milkman, 1976; Cabrera *et al.* 1978) and in natural population surveys (Sampsell, 1977; Wilks *et al.* 1980; McKay, 1981; This report). Furthermore, structural (Thatcher, 1980; Chambers *et al.* 1981; Kreitman, 1983) and functional analysis (Sampsell & Steward, 1983; Chambers *et al.* 1984) suggest that these three widely distributed alleles could be derived from a unique origin. If this important assumption is confirmed it will provide strong support to the role of natural selection in the maintenance of the *Adh* polymorphism. Meanwhile the persistence of the rare thermostable variant, found in the natural population of Güímar, over a 6-year period at a stable frequency in spite of the significant fluctuation of the more abundant alleles (Table 2: $X^2 = 29.81$; D.F. $= 2$; $P < 0.001$) is consistent with the interpretation that the *Adh-Fr* allele is maintained by natural selection, although mutation, other than point-mutation, could also explain its persistence.

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