Variation in alcohol dehydrogenase activity *in vitro* in flies from natural populations of *Drosophila melanogaster*

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Abstract

Alcohol dehydrogenase (ADH) activity variation in male flies taken directly from seven natural populations of *Drosophila melanogaster* is largely accounted for by segregation of alleles at the *Adh* structural gene locus. There was little overlap in the ADH activities of Adh^F and Adh^S homozygotes. Body weights varied only slightly between *Adh* genotypes and contributed little to ADH variation. Between and within population variation in ADH activity and ADH protein in flies in the wild is mainly due to the relative frequencies of Adh^F and Adh^S.

Introduction

The debate on the adaptive significance of enzyme polymorphisms has recently been focussed more on the problem of identifying the phenotypes on which selection might act than on the geographical distribution of alleles. This shift in emphasis has pointed to the possible role of regulatory variation in the processes of adaptation to environmental heterogeneity. In this respect it has been argued that regulatory variation may be at least as relevant as that produced by allelic variation at structural gene loci (McDonald & Ayala, 1978; Ayala & McDonald, 1980).

Genetic variation affecting enzyme activity levels has been detected in natural populations of many species and a well studied example is alcohol dehydrogenase (ADH) encoded by the *Adh* structural gene in *Drosophila melanogaster* (see reviews in Gibson, 1982 and Van Delden, 1983).

There are three main groups of factors influencing the population variation in alcohol dehydrogenase activity. First, there are three electrophoretically detectable alleles at the *Adh* locus (Adh^F, Adh^S and Adh^{FChD}) and it is now generally agreed (Gibson, 1972; Lewis & Gibson, 1978; Maroni, 1978; McDonald, Anderson & Santos, 1980) that Adh^F homozygotes produce more ADH protein at equilibrium than either Adh^{FChD} or Adh^S homozygotes. (The study by Day *et al.*, 1974 reported some conflicting results for lines extracted from the Kaduna cage population.) These differences in ADH amounts are consistent for alleles extracted from different populations and the difference, at least between some Adh^F and Adh^S strains, is caused by ADH synthesis rate variation correlated with differential amounts *in vivo* of cytoplasmic *Adh* mRNA (Anderson & McDonald, 1983).

A second source of variation is due to the effects on ADH activity of segregation of linked and unlinked modifier genes. The presence of such modifiers has been inferred in material from a number of *D. melanogaster* populations (Ward & Hebert, 1972; Thompson *et al.*, 1977; McDonald & Ayala, 1978; Laurie-Ahlberg *et al.*, 1980; Birley *et al.*, 1980; Maroni *et al.*, 1982) but none have yet been analysed in sufficient detail to indicate their modes of function. Maroni & Laurie-Ahlberg (1983) have reported finding a variety of modifiers in three Adh^F and three Adh^S lines extracted from a natural

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population. They showed that the differences in enzyme activity between the Adh^F lines were due to polygenic trans acting factors but that cis-dominant tightly linked factors distinguished the activities produced by the Adh^S lines.

Modifiers could influence ADH activity via differential rates of transcription, mRNA processing, translation, degradation and via post-translational changes, tissue distributions etc. At least one, located by Birley *et al.* (1981) on the third chromosome, influences ADH activity without apparently changing the amount of ADH protein. It is also known that ADH activity varies markedly between life cycle states, between the sexes and with the age of adult flies (Van Delden, 1983).

There are fewer data on the third group of factors influencing ADH activity levels which encompass environmental heterogeneity in nutritional factors (Schwartz & Sofer, 1976; Papel *et al.*, 1979) or climatic factors. There is evidence that variation in the amounts of yeasts in the culture medium can bring about fourfold changes in the amount of ADH protein (Clarke *et al.*, 1978). Bijlsma-Meeles (1979) found that exposure of eggs to ethanol induced an increase in ADH activity in the eggs and a similar increase was observed by Gibson (1970) in larvae and by Harikawa *et al.* (1967) in embryonic cells exposed to ethanol.

These sources of variation have been identified in laboratory stocks under laboratory conditions of culture but there is no information about the levels of activity variation in flies in their natural habitats. It follows that the relative contributions of the different sources of ADH variation in nature are unknown, and it is possible that the ADH activities of the *Adh* genotypes overlap to a considerable extent providing little variation for differential selection between genotypes based on ADH activity.

The data described here were obtained to extend the laboratory findings to more natural conditions by assessing the relative effects of segregation at the *Adh* locus on *in vitro* ADH activity in flies in the wild. The aim was to see whether the distributions of ADH activities were such that any selection based on the level of ADH activity would discriminate between flies of different *Adh* genotypes. The populations chosen for study were selected because they spanned ten degrees of latitude, lived in a variety of habitats and encompassed a range of *Adh* frequencies.

Materials and methods

The seven populations sampled were all located in Australia (see Table 1) and included three populations in Tasmania. The Tasmanian material was included to test whether the frequency of Adh^S, which in general decreases with increasing distance from the equator in both hemispheres (Vigue & Johnson, 1973; Wilks *et al.*, 1980; Oakeshott *et al.*, 1982), varied over the island. The collections were all made between mid-April and mid-May 1982.

At each location adult flies were net swept and transported back to the laboratory in culture vessels with standard food. Male flies were sorted under CO_2 anaesthetisation and identified as *Drosophila melanogaster*. From each population, within four days of capture, about one hundred male *D. melanogaster* were individually weighed, frozen in liquid nitrogen in a microcentrifuge tube and macerated with a perspex pestle. Buffer was added in proportion to the fly's weight (200 μ/ mg) and the samples were centrifuged at 10 000 g for 3 min. The

Table 1. Adh frequencies in seven geographically separate Australian population samples.

Population	Location Lat°S Long°E	Breeding habitat	Adh^S	Adh^{FChD}
Griffith (N.S.W.)	34.3/146.0	leakage from wine barrel inside winery	0.32	0.03
Fernhill (S.A.)	35.2/138.7	grape pressings in vineyard	0.21	0.01
All Saints (VIC)	36.1/146.6	grape pressings in vineyard	0.34	0.03
St. Huberts (VIC)	37.8/145.0	grape pressings in vineyard	0.24	0.01
Pipers Brook (TAS)	41.1/147.1	grape pressings in vineyard	0.29	-
Cradoc (TAS)	43.2/147.0	decaying fruit in orchard	0.41	-
Cygnet (TAS)	43.2/147.0	grape pressings in vineyard	0.45	-

supernatant was assayed for ADH acivity as previously described (Gibson *et al.*, 1980). (Preliminary studies had shown that keeping wild caught adult flies on laboratory media for four days did not affect ADH activity.) Each pellet, and the remaining supernatant, were mixed to prepare a sample for electrophoresis on cellulose acetate membranes to classify the flies into *Adh* genotypes (Lewis & Gibson, 1978; Wilks *et al.*, 1980). In some samples the amount of ADH protein was assayed immunologically using the method and antisera described by Lewis & Gibson (1978). At each locality the gene frequency data from the single fly assays were supplemented by electrophoresing between two and four hundred male flies and these provided the data shown in Table 1. As the frequency of Adh^{FChD} was never higher than 3% it was not scored in the single fly assays.

Results

Adh *frequencies*

The data in Table 1 show that in these populations the highest frequency of Adh^{FChD} was 3% and that it was not detected in any of the Tasmanian samples (each greater than 350 flies). The geographical variation in the frequency of Adh^{S} was expected, given the range of latitude of the samples (Wilks *et al.*, 1980). However, the Tasmanian populations were surprisingly heterogeneous, with higher frequencies of Adh^{S} in the two most southern populations. This heterogeneity will be discussed elsewhere but it provides additional information on the form of the *Adh* cline (Oakeshott *et al.*, 1981).

ADH activity

Within each of the seven populations the mean ADH activities of flies of the three *Adh* genotypes (Table 2) are significantly different; on average Adh^{F}/Adh^{F} homozygotes have more than twice the acivity of Adh^{S}/Adh^{S} homozygotes. There was no consistent pattern to the activities of the heterozygotes in comparison with the mean of the two homozygotes. In two populations the activity of the heterozygotes was higher, in three populations lower, and in two populations not significantly different from the mean of the homozygotes (Table 2).

There were significant differences between populations in mean ADH activity ($F_{6/619} = 28$, $p < 0.01$) and in the differences in activity between genotypes ($F_{12/619} = 2$, $p < 0.05$). The major part of the variation in ADH activity overall was associated with differences between the three genotypes ($F_{2/619} = 349$, $p < 0.001$). The extent of the contribution of *Adh* genotypes to this variation can be gauged by the range of activities encompassed by each genotype (Fig. 1). In three of the populations (All Saints, Pipers Brook and Cygnet) there is no overlap in activity between flies of the two homozygous genotypes and in each of the other four populations the overlap is caused by the data on only a few flies.

Fly weight and ADH activity

In contrast with the data on ADH activity there is much less variation in fly weight within and between the seven populations (Table 3). Overall, there is no evidence for variation in weight between

Table 2. ADH activity (units/mg live weight) and standard errors in flies of different *Adh* genotypes. The number of single male flies assayed of each genotype is given in parenthesis. One unit of ADH activity is defined as an increase in absorbance at 340 nm of 0.001/min (equivalent to 1.61×10^{-4} moles NADH produced per min).

Population	*Adh* genotypes		
	FF	FS	SS
Griffith	163 ± 6 (43)	118 ± 5 (39)	65 ± 4 (18)
Fernhill	170 ± 5 (62)	113 ± 5** (29)	74 ± 7 (4)
All Saints	166 ± 6 (46)	114 ± 4 (45)	56 ± 3 (9)
St. Huberts	143 ± 6 (59)	98 ± 5 (39)	46 ± 5 (7)
Pipers Brook	218 ± 5 (33)	152 ± 5* (26)	73 ± 8 (11)
Cradoc	151 ± 10 (32)	113 ± 5** (50)	56 ± 5 (10)
Cygnet	209 ± 6 (22)	132 ± 4* (43)	68 ± 5 (13)

The significance of the difference between the activities of the heterozygotes compared with the mean of the two homozygotes is indicated, * $p < 0.05$, ** $p < 0.01$.

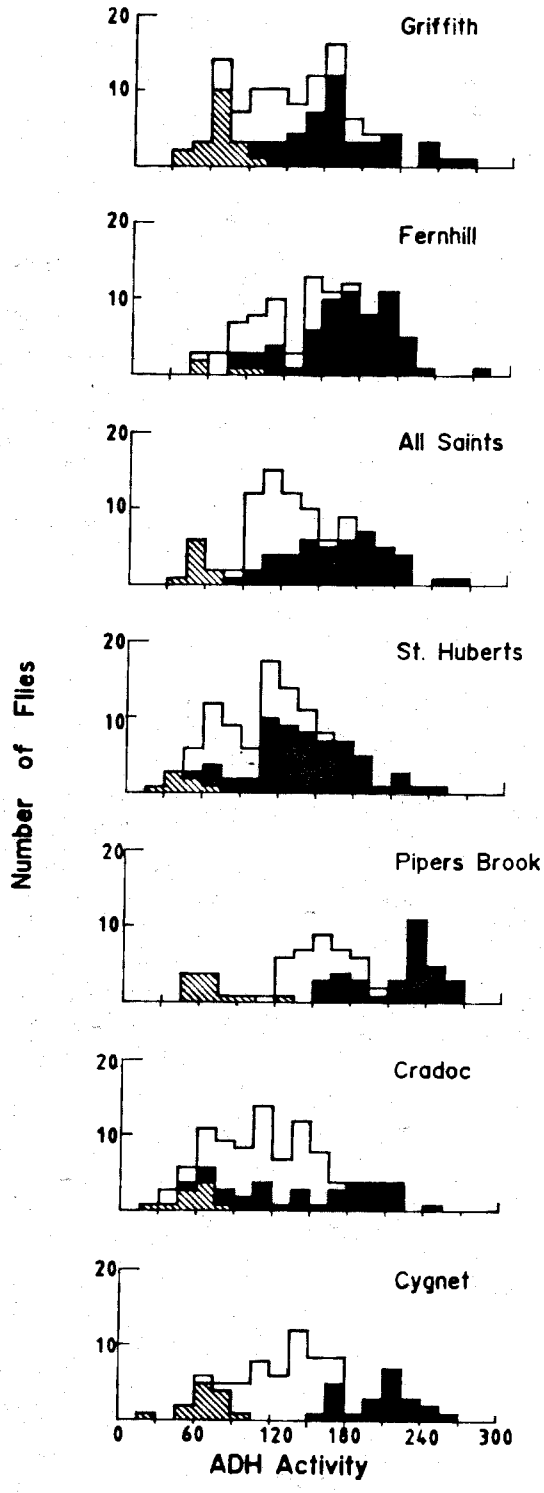

Fig. 1. Variation in ADH activity (assayed in single fly extracts) among *Adh* genotypes in seven Australian populations. Adh^S/Adh^S hatched, Adh^S/Adh^F open, Adh^F/Adh^F solid shading. ADH units are as defined in the legend to Table 2.

Table 3. Mean body weights (10^{-5} g) and their standard errors of flies of different *Adh* genotypes. The sample sizes are as given in Table 2.

Population	*Adh* genotypes		
	FF	FS	SS
Griffith	72 ± 2	73 ± 2	74 ± 4
Fernhill	62 ± 2	63 ± 3	57 ± 7
All Saints	75 ± 2	75 ± 2	69 ± 4
St. Huberts	71 ± 2	69 ± 3	76 ± 6
Pipers Brook	89 ± 1	88 ± 2	89 ± 3
Cradoc	79 ± 3	76 ± 2	84 ± 4
Cygnet	82 ± 2	81 ± 2	81 ± 3

the *Adh* genotypes ($F_{619/2} = 6$, $p > 0.1$) and most variation in weight is between populations ($F_{6/619} = 29$, $p < 0.001$), with a tendency for flies from the most southerly localities to be heavier.

With two exceptions, there is no evidence for a correlation between the weights of flies used to make the extracts and ADH activity for any *Adh* genotype in any population (Table 4). The exceptions are heterozygotes in Griffith and Adh^F/Adh^F homozygotes in Cradoc, in which ADH activities are positively correlated with body weight; in Cradoc, this gives rise to a significant positive correlation over all genotypes.

ADH protein amounts and ADH activity

For All Saints samples data were obtained on the amount of ADH protein measured immunologically in radial immunodiffusion experiments on single fly extracts. The correlation between ADH activity and ADH protein amount overall was 0.87.

Table 4. Correlations between body weight and ADH activity in assays of single male flies.

	Over all genotypes	*Adh* genotypes		
		FF	FS	SS
Griffith	0.11	0.13	0.31*	0.31
Fernhill	0.02	−0.09	0.28	0.02
All Saints	0.01	−0.03	−0.21	0.05
St. Huberts	0.11	0.17	0.09	0.29
Pipers Brook	0.02	0.05	−0.02	0.08
Cradoc	0.28**	0.60***	0.19	0.47
Cygnet	0.06	−0.01	0.09	−0.03

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

These experiments show that the vast majority of *in vitro* variation in ADH activity in *D. melanogaster* in the wild segregates with the two common electrophoretic alleles at the *Adh* structural gene locus. Even though the assayed males in each population were likely to have a wide age distribution, and to have developed on a variety of nutritional resources, there was little overlap in ADH activity between Adh^F and Adh^S homozygotes. This result was consistent over all populations assayed, although the magnitude of the difference varied, as did the mean activities of the populations. There was no evidence to support the suggestion that heterozygotes were generally dominant in activity. It remains possible that there is variation between populations, and between *Adh* genotypes, in the tissue distribution of ADH (e.g. fat body compared with Malpigian tubules) and this should be investigated in future studies.

We conclude from these data that even if modifiers of ADH activity are segregating in these populations, their contribution to ADH variation is slight compared with the effects of the two variant alleles at the *Adh* locus. It is pertinent that data collected in the same way on 3rd instar larvae and adult females from the All Saints locality gave similar results.

The Pipers Brook and Cygnet populations had higher ADH activities, in all genotypes, than the third Tasmanian population from Cradoc (Table 2). It is interesting that the Cradoc samples came from decaying fruit in an orchard whereas the Pipers Brook and Cygnet flies came from discarded must at wineries. However, the level of ADH in the Cradoc flies is not lower than that observed in the mainland samples, which all came from winery habitats. It has been shown (Gibson *et al.*, 1981) that average ethanol levels in winery habitats usually do not exceed those in decaying fruits or vegetables. Thus it is unlikely that the heterogeneity in mean ADH activities between the three Tasmanian populations is a reflection of different levels of ethanol in the habitats in which the flies developed.

Notwithstanding our lack of information on the conditions under which the flies developed the absence of an allometric relationship between fly weight and ADH activity in the assays deserves comment. An allometric relationship between ADH activity and body weight has been demonstrated in laboratory material, when conditions of culture have been varied with different amounts of yeasts (Clark *et al.*, 1979; Van Dijk, 1981). It has also been observed in a biometrical analysis of ADH activity in flies from the Texas cage population (Birley *et al.*, 1981) where factors increasing ADH activity but not ADH protein amount, were identified. However, Birley *et al.*, 1980, 1981) emphasised that it is likely that genetical variation in ADH activity is caused by loci controlling activity rather than by secondary effects of body weight. The overall view from our results is that although the assayed flies were undoubtedly exposed to a variety of nutritional conditions, and were of a variety of ages, any effects on ADH activity (and ADH protein amount) were relatively slight compared with differences ascribable to segregation at the *Adh* locus. This suggests that in populations along the *Adh* cline the major factor affecting the level of ADH activity is likely to be the relative frequencies of Adh^F and Adh^S (Anderson, 1982).

These results therefore pose two further questions. First what is the cause of the difference in ADH activity between the different genotypes at the *Adh* locus? And second, do these differences in *in vitro* activity have any biological relevance? Neither question can yet be answered with any assurance. There is consistent evidence for ADH protein amount differences between Adh^F and Adh^S strains and this is consistent with demonstrated differences in the rates of ADH synthesis and of the relative abundance of *Adh* coding sequences in cytoplasmic RNA transcripts (Anderson & McDonald, 1983). Whether these differences between Adh^F and Adh^S are related to the single amino acid substitution distinguishing ADH-F and ADH-S (Thatcher, 1980; Chambers *et al.*, 1984), to enhancer sequences or to other nucleotide variation in, or close to, the Adh^S transcription unit (Langley *et al.*, 1982; Kreitman, 1983; Benyajati *et al.*, 1983; Maroni & Laurie-Ahlberg, 1983) remains to be determined.

The biological relevance of these differences in ADH activity is also open to question. Interpretation of the laboratory and field data on the role of ADH activity variation in ethanol tolerance is controversial because of differing results from inbred and outbred material (Gibson, 1982; Gibson & Oakeshott, 1982; Van Delden, 1982). Of particular

importance to this debate are the results of Middleton & Kacser (1983) on the *in vivo* effects of ADH variation. They demonstrated that, in terms of ethanol metabolism, differences in activity *in vivo* between Adh^F and Adh^S homozygotes gave non-significant differences in metabolic flux and were physiologically ineffective.

If the results of Middleton & Kacser (1983) have any generality it is possible that the population variation in ADH activity *in vitro* is irrelevant to variation in rates of ethanol metabolism and to variation in ethanol tolerance. Nevertheless, fitness differentials between *Adh* genotypes could depend on the catalytic activity of ADH on substrates other than alcohols (Jeffrey & Jornvall, 1983). The *in vitro* ADH differences we have observed in flies from natural populations might persist in the same way with other substrates and be important *in vivo*. Thus segregation at the *Adh* locus potentially provides population variation in ADH activity upon which selection could act to maintain the polymorphism.

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References

Anderson, D., 1982. Alcohol dehydrogenase activity and ethanol tolerance along the ADH cline in Australia. In: Advances in genetics, development and evolution of Drosophila (ed. S. Lakovaara) pp. 263–272. Plenum, New York.

Anderson, S. M. & McDonald, J. F., 1983. Biochemical and molecular analysis of naturally occurring Adh variants in Drosophila melanogaster. Proc. natn. Acad. Sci. U.S.A. 80: 4798–4802.

Ayala, F. J. & McDonald, J. F., 1980. Continuous variations: possible role of regulatory genes. Genetics 52: 1–15.

Benyajati, C., Spoerel, N., Haymerle, H. & Ashburner, M., 1983. The messenger RNA for alcohol dehydrogenase in Drosophila melanogaster differs in its 5′ end in different developmental stages. Cell 33: 125–133.

Bijlsma-Meeles, E., 1979. Viability in Drosophila melanogaster in relation to age and ADH activity of eggs transferred to ethanol food. Heredity 42: 79–89.

Birley, A. J., Marson, A. & Philips, L. C., 1980. Genetical variation for enzyme activity in a population of Drosophila melanogaster. V. The genetical architecture, as shown by diallel analysis of alcohol dehydrogenase (ADH) activity. Heredity 44: 251–268.

Birley, A. J., Couch, P A. & Marson, A., 1981. Genetical variation for enzyme activity in a population of Drosophila melanogaster. VI. Molecular variation in the control of alcohol dehydrogenase (ADH) activity. Heredity 47: 185–198.

Chambers, G. K., Wilks, A. V. & Gibson, J. B., 1984. Variation in the biochemical properties of the Drosophila alcohol dehydrogenase allozymes. Biochem. Genet. 22: 153–168.

Clarke, B., Camfield, R., Galvin, A. & Pits, C., 1979. Environmental factors affecting the quantity of alcohol dehydrogenase in Drosophila melanogaster. Nature 280: 517–518.

Day, T. H., Hillier, P. C. & Clarke, B., 1974. The relative quantities and catalytic activities of enzymes produced by alleles at the alcohol dehydrogenase locus in Drosophila melanogaster. Biochem. Genet. 11: 155–165.

Gibson, J. B., 1970. Enzyme flexibility in Drosophila melanogaster. Nature 227: 959–961.

Gibson, J. B., 1972. Differences in the number of molecules produced by two allelic electrophoretic enzyme variants in Drosophila melanogaster. Experientia 28: 975–976.

Gibson, J. B., 1982. The alcohol dehydrogenase polymorphism in Drosophila melanogaster: patterns of variation for three alleles. In: Advances in genetics, development, and evolution of Drosphila (ed. S. Lakovaara) pp. 273–284. Plenum, New York.

Gibson, J. B., Chambers, G. K., Wilks, A. V. & Oakeshott, J. G., 1980. An electrophoretically cryptic alcohol dehydrogenase variant in Drosophila melanogaster I. Activity ratios, thermostability, genetic localisation and comparison with two other thermostable variants. Aust. J. biol. Sci. 33: 479–489.

Gibson, J. B., May, T. W. & Wilks, A. V., 1981. Genetic variation at the alcohol dehydrogenase locus in Drosophila melanogaster in relation to environmental variation: ethanol levels in breeding sites and allozyme frequencies. Oecologia (Berl.) 51: 191–198.

Gibson, J. B. & Oakeshott, J. G., 1982. Tests of the adaptive significance of the alcohol dehydrogenase polymorphism in Drosophila melanogaster: paths, pitfalls and prospects. In: Ecological genetics and evolution. The cactus-yeast-Drosophila model system (eds. J. S. F. Barker & W. T. Starmer) pp. 291–301. Academic Press, New York.

Horikawa, M., Ling, L. N. L. & Fox, A. S., 1967. Effects of substrates on gene-controlled enzyme activities in cultured embryonic cells of Drosophila. Genetics 55: 569–583.

Jeffrey, J. & Jornvall, H., 1983. Enzyme relationships in a sorbitol pathway that bypasses glycolysin and pentose phosphates in glucose metabolism. Proc. natn. Acad. Sci. U.S.A. 80: 901–905.

Kreitman, M., 1983. Nucleotide polymorphism at the alcohol dehydrogenase locus in Drosophila melanogaster. Nature 304: 412–419.

Langley, C., Montgomery, E. & Quattlebaum, W., 1982. Restriction map variation in the Adh region of Drosophila. Proc. natn. Acad. Sci. U.S.A. 79: 5631–5635.

Laurie-Ahlberg, C. C., Maroni, G., Bewley, G. C., Lucchesi, J. C. & Weir, B. S., 1980. Quantitative genetic variation of enzyme activities in natural populations of Drosophila melanogaster. Proc. natn. Acad. Sci. U.S.A. 77: 1073–1077.

Lewis, N. & Gibson, J. B., 1978. Enzyme protein amount variation in natural populations. Biochem. Genet. 16: 159–170.

Maroni, G., 1978. Genetic control of alcohol dehydrogenase levels in Drosophila. Biochem. Genet. 16: 509–523.

Maroni, G., Laurie-Ahlberg, C. C., Adams, D. A. & Wilton, A. N., 1982. Genetic variation in the expression of ADH in Drosophila melanogaster. Genetics 101: 431–446.

Maroni, G. & Laurie-Ahlberg, C. C., 1983. Genetic control of ADH expression in Drosophila melanogaster. Genetics 105: 921–933.

McDonald, J. F. & Ayala, F. J., 1978. Genetic and biochemical basis of enzyme activity variation in natural populations. I. alcohol dehydrogenase in Drosophila melanogaster. Genetics 89: 371–388.

McDonald, J. F., Anderson, S. M. & Santos, M., 1980. Biochemical differences between products of the Adh locus in Drosophila. Genetics 95: 1013–1022.

McKenzie, J. A., 1974. The distribution of vineyard populations of Drosophila melanogaster and Drosophila simulans during vintage and non-vintage periods. Oecologia (Berl.) 15: 1–16.

Middleton, R. J. & Kacser, H., 1983. Enzyme variation, metabolic flux and fitness: alcohol dehydrogenase in Drosophila melanogaster. Genetics 105: 663–650.

Oakeshott, J. G., Gibson, J. B., Anderson, P. R., Knibb, W. R., Anderson, D. G. & Chambers, G. K., 1982. Alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase clines in Drosophila melanogaster on three continents. Evolution 36(1): 86–96.

Papel, J., Henderson, M., Herrewege, J., David, J. & Sofer, W., 1979. Drosophila alcohol dehydrogenase activity in vitro and in vivo: effects of acetone feeding. Biochem. Genet. 17: 553–563.

Schwartz, M. & Sofer, W., 1976. Diet-induced alterations in distribution of multiple forms of alcohol dehydrogenase in Drosophila. Nature 263: 129–131.

Thatcher, D. R., 1980. The complete amino acid sequence of three alcohol dehydrogenase alleloenzymes (Adh^{N-11}, Adh^{S} and Adh^{UF}) from the fruit fly Drosophila melanogaster. Biochem. J. 187: 875–886.

Thompson, J. N., Ashburner, M. & Woodruff, R. C., 1977. Presumptive control mutation for alcohol dehydrogenase in Drosophila melanogaster. Nature 270: 363.

Van Delden, W., 1983. The alcohol dehydrogenase polymorphism in Drosophila melanogaster. Evol. Biol. 15: 187–222.

Van Dijk, H., 1981. The relationship between ADH activity and body weight in Drosophila melanogaster. Drosoph. Inf. Serv. 56: 150–151.

Vigue, C. L. & Johnson, F. M., 1973. Isozyme variability in species of the genus Drosophila. VI Frequency-property-environment relationship of allelic alcohol dehydrogenase in D. melanogaster. Biochem. Genet. 9: 213–227.

Wilks, A. V., Gibson, J. B., Oakeshott, J. G. & Chambers, G. K., 1980. An electrophoretically cryptic alcohol dehydrogenase variant in Drosophila melanogaster. II. Post-electrophoresis heat-treatment screening of natural populations. Aust. J. biol. Sci. 33: 575–585.

Ward, R. D. & Hebert, P. D. N., 1972. Variability of alcohol dehydrogenase activity in a natural population of Drosophila melanogaster. Nature 236: 243–244.

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