

Genetic Variability of Alcohol Dehydrogenase Among Australian *Drosophila* Species: Correlation of ADH Biochemical Phenotype With Ethanol Resource Utilization

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ABSTRACT Alcohol dehydrogenase (ADH) activities, electrophoretic phenotypes, and the extent of ethanol resource utilization are compared for three groups of species distinguishable on ecological criteria: 1) the cosmopolitan species *D. melanogaster*, a frequent inhabitant of wineries; 2) fruit-baited species of the typically Australian subgenus *Scaptodrosophila*: *D. lativittata*, *D. nitidithorax* and *D. howensis*; and 3) *Scaptodrosophila* species not attracted to fermented-fruit baits being collected by sweeping in temperate rain forests (*D. inornata*, *D. collessi*) or from *Hibiscus* flowers (*D. hibisci*). *D. melanogaster* showed the highest levels of ADH activity and an electrophoretic polymorphism with two active allelic forms, while group 2) species showed intermediate ADH activities and polymorphisms, which were consistent with "high activity" and "low activity" allelic forms in natural populations of these species, and group 3) species showed only "low activity" forms. Ethanol resource utilization follows the same sequence, being $1 > 2 > 3$ (*D. howensis* and *D. collessi* were not tested). Therefore the species considered show an association of ADH biochemical phenotype, laboratory ethanol utilization, and resources utilized.

Alcohol dehydrogenase (ADH; E.C.1.1.1.1.) in *Drosophila melanogaster* has been extensively investigated in recent years, particularly for allelic and activity variation, and for the biological processes maintaining this variability in natural populations (Clarke, '75; David, '77). ADH in *D. melanogaster* is encoded by a single structural locus (*Adh*) at position 50.1 on the second chromosome (Grell et al., '65). Natural populations of *D. melanogaster* are usually polymorphic for two electrophoretic variants, designated ADH^F and ADH^S (O'Brien and MacIntyre, '69). In polymorphic populations kept on food supplemented with different alcohols, a considerable rise in F allele frequency has been often found (Gibson, '70; Bijlsma-Meeles and Van Delden, '74; Van Delden et al., '75, '78); this has been interpreted as selection for the higher specific activity ADH^F allozyme (Day et al., '74). The relationship between ADH activity and mortality has been further confirmed by Thompson and Kaiser ('77) and Kamping and Van Delden ('78), who

reported a negative correlation between ADH activity and mortality among strains of identical *Adh* genotype, which differed in ADH activity. Several studies have shown, however, that the level of ADH activity can be modulated by other loci (Ward and Hebert, '72; McDonald et al., '77), some of which are on the third chromosome and regulate activity by altering the number of enzyme molecules in natural populations (McDonald and Ayala, '78).

McKenzie and Parsons ('74) and McKenzie and McKechnie ('78) have shown that ethanol tolerance in a natural population of *D. melanogaster* is polygenically determined, involving loci largely independent of the *Adh* locus on both chromosomes 2 and 3; i.e., there are parallels with the ADH activity results. Using the approach of ethanol tolerance assessments, McKenzie and Parsons ('72; '74) investigated tolerances of various strains of *D. melanogaster* derived from wild populations collected in the vicinity of a vineyard and observed microdifferentiation, whereby those strains derived

from flies collected in the cellar were more resistant than those collected outside the cellar. Apparently there is direct selection for ethanol resistance by the presence of environmental ethanol. Starmer et al. ('77) developed a method for assessing the extent of ethanol utilization by examining *Drosophila* longevity when exposed to atmospheric ethanol in the absence of other food; an increase in longevity over the control implies ethanol utilization and a decrease means a stress. Their studies demonstrated that the extent of ethanol-mediated longevity increase for adults of the cactus-breeding *D. mojavensis* depends upon the population, and suggested that these differences are controlled by the *Adh* locus and other loci including the octanol dehydrogenase locus and regulatory genes. Parsons et al. ('80) modified this method when examining the effect of environmental ethanol upon the longevity and development of the sibling species, *D. melanogaster* and *D. simulans*. Both showed longevity increases at low ethanol concentrations (0.5–3.0%), with *melanogaster* always exceeding *simulans* and exhibiting much higher ethanol threshold values at which ethanol becomes a stress. These results agree with the field situation, since *D. simulans* larvae and adults may coexist with *D. melanogaster* at low ethanol concentrations, while *D. melanogaster* only is found at high ethanol concentrations (McKenzie and Parsons, '72; McKenzie and McKechnie, '79). Parsons ('80) examined ethanol resource utilization levels among a number of other cosmopolitan and endemic Australian *Drosophila* species attracted to fermented-fruit baits, and compared them with species not so attracted being collected by other procedures. Species attracted to fermented-fruit baits use gaseous ethanol as a resource, whereas the remaining species either have a very low ethanol threshold or apparently do not utilize ethanol over the range of ethanol concentrations tested.

In this communication, several Australian endemic *Drosophila* species are examined and compared with *D. melanogaster* for 1) electrophoretic phenotype, and activity variation of ADH as determined by electrophoretic techniques; 2) the specific activity of ADH using spectrophotometric methods; and 3) the utilization of ethanol as a resource by observing the longevity with ethanol vapor as the only available food resource. The results suggest that ADH activity variations at the interspecific level provide one of the major determining factors in the response of a variety of *Drosophila* species to environmental ethanol.

MATERIALS AND METHODS

Drosophila populations

Mass bred populations of *D. melanogaster* (Townsville), *D. lativittata* (Fairfield, Melbourne), *D. nitidithorax* (Perth), and *D. howensis* (Lord Howe Island) were obtained from fermented-fruit baits set out in the localities indicated in brackets. *D. inornata* and *D. collessi* were collected by sweeping foliage in Kinglake National Park, Victoria, and *D. hibisci* was aspirated from flowers of endemic *Hibiscus* species, especially *H. heterophyllus*, at the University Farm, Camden, NSW. (See Cook et al., '77; Bock and Parsons, '78; Parsons and Bock, '79 for basic biological information on these species).

Ethanol tolerances

Ethanol resource utilization and tolerances were assessed by exposing adults to vapor over various concentrations of ethanol in water, following the procedure of Starmer et al. ('77) as modified by Parsons et al. ('80). *D. inornata* and *D. hibisci* were tested 1 day after collecting flies from the field. For these two species, five replicates of ten flies per sex were tested for each of the points plotted in Figure 2. The data are expressed as LT_{50} 's calculated by linear interpolation at the various ethanol concentrations used.

Homogenization of flies

Individual *Drosophila* of various species were extracted in a multiple sample homogenizer (Roberts, '71) in 50 μ l of 50 mM Tris-0.1% Triton X-100-HCl buffer, pH 8.0 (extraction buffer), and centrifuged at 15,000 g \times 15 minutes prior to electrophoretic examination. Extracts for spectrophotometric analysis of *Drosophila* ADH activity were made by homogenizing 20 flies in 2 mls of extraction buffer using an Ultra-Turrax homogenizer and subsequently centrifuging at 15,000 g \times 15 minutes.

Spectrophotometric analysis of ADH activity

ADH activity was measured with a Varian Model 634 spectrophotometer at 25°. The absorbance at 340 nm was recorded after addition of 100 μ l of extract to 3 ml of reaction mixture containing 100 mM ethanol, 0.4 mM NAD⁺, and 50 mM Tris-HCl buffer, pH 8.0. Units of specific activity of ADH are expressed in terms of International Units (μ moles/minute) per gm wet weight of *Drosophila*.

*Cellulose acetate electrophoresis and
ADH staining*

Homogenate supernatants were subjected to zone electrophoresis on Titan III cellulose acetate plates (60 by 75 mm) (Helena Labs., Texas) with tris-glycine buffer (25 mM tris, 192 mM glycine, pH 8.5 at 25 V/cm for 20 minutes. Three 0.5 μ l applications of extract were placed at the origin, situated approximately 3 cm from the cathodal edge of the plate, prior to electrophoresis. The plates were then stained for activity, washed, dried, and photographed.

ADH was stained by an agar overlay technique described in detail elsewhere (Holmes, '78). The final concentrations in the agar-overlay solution were 100 mM tris-HCl (pH 8.0), 50 mM or 100 mM ethanol, 0.9 mM methyl thiazolyl blue (MTT), 0.3 mM phenazine methosulphate (PMS), and 0.4 mM NAD⁺. Control stains containing 100 mM tris-HCl (pH 8.0), 0.9 mM MTT, and 0.3 mM PMS were also used to reveal aldehyde oxidase activity in the absence of coenzyme (100 mM ethanol incorporated) and dehydrogenase activity in the absence of substrate (0.4 mM NAD⁺ incorporated).

RESULTS

Figure 1 illustrates the phenotypic variation of ADH extracted from individual flies of the species above. The results for *D. melanogaster* conform with many previous studies showing a single *Adh* locus with two major allelic forms in natural populations: *Adh*^a (A₃ or ADH-S isozyme) and *Adh*^b (A₂ or ADH-F isozyme), giving a 3 allelic isozyme phenotype in hybrid animals (A₃, A^aA^b, A₂) (O'Brien and MacIntyre, '69; David, '77). Additional multiple forms are also observed for each allelic isozyme of *melanogaster*; however, these represent epigenetic products arising from the differential binding of the coenzyme, NAD⁺, to ADH (Schwartz et al., '79). In contrast to this electrophoretic heterogeneity, variation in activity of a single form of ADH was observed in *nitidithorax* and *lativittata* individuals. Three distinct phenotypes were observed: "high" activity, "intermediate" activity and "null" activity for ADH, with the genotypes being designated *Adh*^a*Adh*^a, *Adh*^a*Adh*^b and *Adh*^b*Adh*^b respectively. This proposal assumes that the observed activity is regulated by two codominant alleles at a single locus, which is supported by a close fit to a Hardy-Weinberg distribution in each case (Table 1). It is of course not known whether this is the structural or "regulator"

locus for ADH. *D. collessi* individuals exhibited a uniformly single "low" form of ADH activity. The remaining species, *D. inornata* and *D. hibisci*, gave "null" patterns of ADH activity when examined electrophoretically (Fig. 1). However, staining of the "null" variants of *D. lativittata*, *D. nitidithorax* and *D. howensis*, and the high frequency "null" variants of *D. inornata* and *D. hibisci*, using 100 mM ethanol (compared with 50 mM for Fig. 1), revealed some ADH activity migrating in the same position as the "higher activity" variants. Apparently the ADH "null" variants in these species are actually "low activity-higher Km" variants, which appear as light bands at lower concentrations of substrate.

Table 1 gives ADH specific activities and allelic frequencies, from which the species can be placed into three groups for both categories: 1) *D. melanogaster*, 2) the fruit-baited *Scaptodrosophila* species, and 3) the non-fruit-baited *Scaptodrosophila* species. Similarly, ethanol resource utilization thresholds and the $\frac{LT_{50}}{LT_{50 \text{ control}}}$ values (Fig. 2) are in the sequence, *D. melanogaster* >> *D. lativittata* ~ *D. nitidithorax* >> *D. inornata* > *D. hibisci*, showing that the *D. melanogaster* population has an extremely high ethanol utilization threshold compared with the two other fruit-baited species, which in turn are able to use ethanol more effectively than the nonfruit-baited species, *D. inornata*. *D. hibisci* presents a special case since 1% ethanol is a stress and 0.5% ethanol does not increase longevity, although the possibility of ethanol utilization at extremely low concentrations has not of course been eliminated.

DISCUSSION

As documented in the introduction, selection for ethanol resistance in *D. melanogaster* may occur in the presence of environmental ethanol. The molecular basis of this phenomenon is under extensive investigation.

In this paper, we extend considerations from the intraspecific level usually studied to the interspecific level with a comparative electrophoretic and spectrophotometric analysis of ADH from Australian species, and have attempted to correlate these results with ethanol resource utilization studies and ecological information. In general, ADH specific activity is associated with ethanol tolerance and resource utilization, ranging from *D. melanogaster* at the high extreme, to *D. hibisci*, where evidence for ethanol utilization was not found. Low to undetectable use of ethanol as a food resource

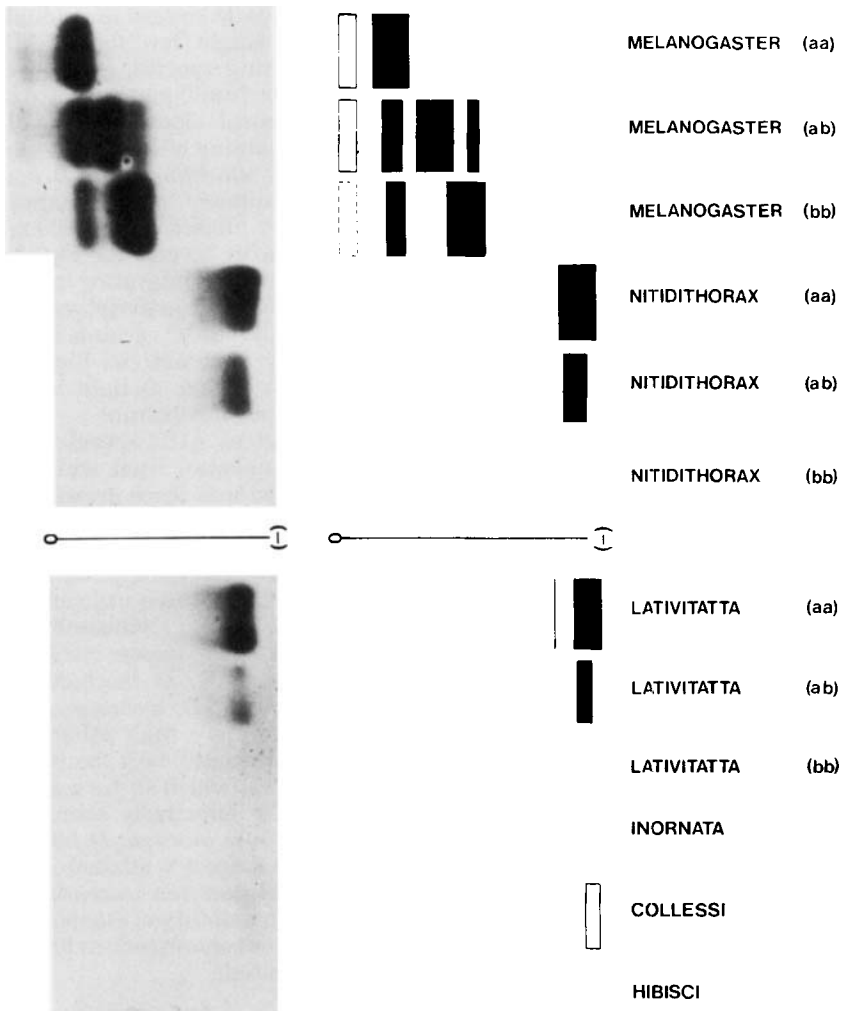


Fig. 1. Cellulose acetate zymogram and diagrammatic illustration of electrophoretic and activity variants of alcohol dehydrogenase (ADH) from various endemic Australian species of *Drosophila*. Proposed genotypes encoding electrophoretic variants of *melanogaster* ADH and those determining the activity of ADH in other *Drosophila* species are given. Ethanol concentration used was 50 mM. "Null" variants for *nitidithorax*, *lativittata*, *inornata*, and *hibisci* showed low activity on the zymogram when stained using 100 mM ethanol as substrate. Anodal migrating zones of aldehyde oxidase activity (which stain in the absence of NAD⁺) have not been included in these zymograms.

as in *D. inornata* and *D. hibisci* may well be quite common in the genus *Drosophila* as a whole given the number of species in the Australian fauna not attracted to fermented-fruit baits (Parsons, '77, '80). Compared with these species, *D. lativittata* and *D. nitidithorax* show a much increased capacity to utilize ethanol, which may reflect their adaptation to the availability of fermented fruits and other ethanol-containing resources in their habitats. They are commonly found in orchard/urban habitats in the southwest and east (*lativittata*)

and southwest (*nitidithorax*) of Australia respectively (Bock and Parsons, '78) and have presumably spread into such habitats following European settlement from temperate and subtropical forests where fermented-fruit resources are not common (Parsons, '77). At the intraspecific level the three species not attracted to fermented-fruit baits did not exhibit detectable activity variation, and it would appear that the "low" *Adh* allele found in these species is either fixed or in a very high frequency. By comparison, in the three *Scaptodro-*

TABLE 1. Alcohol dehydrogenase specific activities and allelic frequencies among endemic Australian *Drosophila* species

Species	Subgenus	Collection method	Sex	Adh specific activity ¹	Allelic frequencies ²
<i>melanogaster</i> (Townsville)	<i>Sophophora</i>	fermented fruit baits	♀	7.3 ± 0.4	n.d.
			♂	8.8 ± 0.7	n.d.
<i>lativittata</i> (Fairfield)	<i>Scaptodrosophila</i>	fermented fruit baits	♀	2.6 ± 0.5	a 0.57 b 0.43 ³
			♂	2.5 ± 0.3	
<i>nitidithorax</i> (S.W. Australia)	<i>Scaptodrosophila</i>	fermented fruit baits	♀	3.4 ± 0.9	a 0.63 b 0.37 ³
			♂	2.9 ± 0.3	
<i>howensis</i> (Lord Howe Is.)	<i>Scaptodrosophila</i>	fermented fruit baits	♀	2.1 ± 0.6	a 0.51 b 0.49 ³
			♂	1.8 ± 0.5	
<i>inornata</i> (Victoria)	<i>Scaptodrosophila</i>	sweeping forest habitats	♀	1.1 ± 0.3	a 0.00 b 1.0
			♂	0.9 ± 0.2	
<i>collessi</i> (Victoria)	<i>Scaptodrosophila</i>	sweeping forest habitats	♀	0.8 ± 0.2	a 0.00 b 1.0
			♂	0.4 ± 0.1	
<i>hibisci</i> (Sydney)	<i>Scaptodrosophila</i>	sweeping Hibiscus flowers	♀	0.7 ± 0.1	a 0.00 b 1.0
			♂	0.6 ± 0.1	

¹Micromoles NADH produced per minute per gm of *Drosophila*.

²♂ and ♀ results combined; n.d.—not determined; a allele has a high activity ADH phenotype; b allele—"null" or low activity phenotype; based on data for 64 ♂ and 64 ♀ flies.

³Population data consistent with two dominant alleles at a single locus in Hardy-Weinberg equilibrium; *D. lativittata* ($\chi^2 = 0.26$; 2df; $p > 0.8$); *D. nitidithorax* ($\chi^2 = 0.17$; 2df; $p > 0.9$); *D. howensis* ($\chi^2 = 0.19$; 2df; $p > 0.9$).

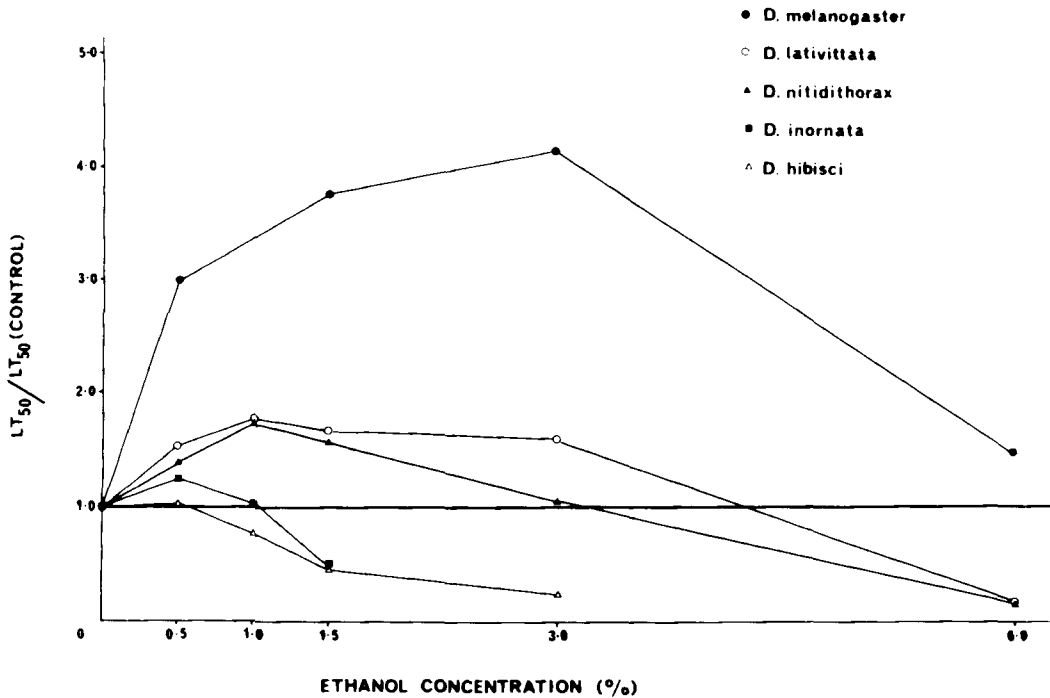


Fig. 2. Adult survivorship expressed as the ratio $\frac{LT_{50}}{LT_{50} \text{ Control}}$ for various *Drosophila* species plotted against ethanol concentration. The thresholds for each species are indicated by the intercept of the line plotted for a ratio of one with the survivorship curves (summarized from Parsons, '80).

sophila species attracted to fermented-fruit baits, three ADH phenotypes were observed: "high," "intermediate," and "low" ADH activities. Assuming the "low" activity allelic form of ADH is representative of the ancestral phenotype, then it is possible that the "high" activity allele is favored in these three species in their new orchard/urban environment, so permitting the species to spread. On this argument, the ADH specific activity and *Adh* alleles of *D. melanogaster* form a response to the almost totally domesticated array of habitats occupied by this species, which of course includes wineries.

The argument that "low" activity alleles for ADH are ancestral gains support from Throckmorton's ('75) review of the phylogeny, ecology, and geography of *Drosophila*. He argues that originally the Drosophilidae were probably associated with slowly fermenting leaves and other fleshy plant parts on the forest floor, as well as sap and broken and damaged plant parts of living plants themselves. This was a relatively austere existence since resources exploited were not rich in carbohydrates. But it provided a step towards the exploitation of the fermentation mode of existence. It may be that in comparison with *D. inornata* and *D. hibisci*, such species as *D. lativittata* and *D. nitidithorax* respond to natural selection by altered genetic constitutions in relation to ADH genotypes. Thus, these species would be better able to exploit the normally richer resources of the orchard/urban environments into which they have spread."

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