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The epistasis of *Adh* and *Gpdh* allozymes and variation in the ethanol tolerance of *Drosophila melanogaster* larvae

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Summary

The role of epistatic interaction of allozymes in the determination of variation in larval ethanol tolerance of *Drosophila melanogaster* was examined. Isofemale lines from the Tahbilk Winery were made homozygous for different common alleles of alcohol dehydrogenase (*Adh*) and sn-glycerol-3-phosphate dehydrogenase (*Gpdh*). When fed 6% ethanol, all the lines had reduced survival and, in the survivors, reduced body weight and lengthened development time. A strong positive correlation between tolerance and development time suggested that alleles responsible for slowing development on ethanol also increased ethanol tolerance. Analysis of larval ethanol tolerance over four generations showed that larvae of the Adh^{FF} $Gpdh^{FF}$, and Adh^{SS} $Gpdh^{SS}$ allelic combinations were more tolerant than larvae with the other combinations. However, these genotypes were not associated with the slowing of development nor the weight loss on ethanol. Hence, larvae with certain combinations of *Adh* and *Gpdh* allozymes may have a greater capacity to metabolize ethanol and be more tolerant to its toxic effects.

1. Introduction

Drosophila melanogaster is extremely tolerant of environmental alcohol (David & van Herrewege, 1983); a trait that has evolved over the last 3–9 Myr (Easteal & Oakeshott, 1985). Although both larvae and adults may occupy natural habitats which contain ethanol (Oakeshott *et al.* 1982; McKechnie & Morgan, 1982), there is considerable genetic variation in the level of ethanol tolerance (McKenzie & Parsons, 1974; David & Bocquet, 1975). More than 90% of the ethanol consumed by *D. melanogaster* is degraded by the metabolic pathway represented by alcohol dehydrogenase ADH (EC 1.1.1.1) (Geer *et al.* 1985), and normal ADH activity is needed for a normal level of ethanol tolerance (David *et al.* 1976). However, efforts to link variation in ethanol tolerance to ADH allozyme variation in *D. melanogaster* populations have met with only limited success.

sn-Glycerol-3-phosphate dehydrogenase (GPDH; EC 1.1.1.8), is a component of the glycerol-3-phosphate cycle that facilitates efficient regeneration of NAD^+ in adults and larvae, and also functions to provide the glycerol moiety of lipid (O'Brien & MacIntyre, 1972; Geer *et al.* 1983). GPDH has been implicated in ethanol tolerance. Ethanol induces higher tissue activity levels of both GPDH and ADH in larvae (Geer *et al.* 1983). This suggests that GPDH and ADH may be parts of the same metabolic process. Cavener & Clegg (1981) and Sanchez & Rubio (1986), studying laboratory populations of *D. melanogaster*, and van Delden (1984), examining more natural populations, noted an epistatic interaction between the electromorphs encoded at the *Adh* and *Gpdh* loci. Interaction between these two enzymes may be partly responsible for natural variation in tolerance. There is a need to characterize the 'fit' allelic combinations, and to define the conditions which produce these interactions. In this report we assess the involvement of *Adh* and *Gpdh* polymorphisms in the determination of variation in larval ethanol tolerance by examining three different fitness components for associations with genotypes in strains established from the Chateau Tahbilk Winery population of Victoria, Australia.

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2. Methods and materials

(i) Drosophila *lines*

Eighty lines of *D. melanogaster* were each derived from a single wild female collected from the Tahbilk wine cellar and adjacent orchard between December, 1982 and April, 1983. Within lines, single-pair matings were set up among F_1 progeny, and the *Adh* and *Gpdh* genotype of parents and progeny samples determined by Cellogel electrophoresis (Chemetron, Milano, Italy). Lines were selected if they gave rise to a family which was homozygous at both loci or at one locus only. The latter families were again single-pair mated, some for 2 further generations, monitored for genotype and selected until 42 lines remained. All four

homozygous combinations of 2 common electromorphs at each locus were derived at about equal frequencies (10 or 11 lines of each combination) with a minimum of inbreeding. A two-gel heat treatment technique (Wilks *et al.* 1980), testing 10 adults from each of the 22 Adh^F lines, indicated the absence of any heat-stable alleles. The derived lines were maintained by mass culture at 25 °C in bottles on standard semolina-treacle medium (McKechnie & Geer, 1986) and their genotypes were checked every second generation. The *Adh* and *Gpdh* loci are both on chromosome 2 (positions 50.1 and 20.5, respectively).

The lines were not screened for the presence of In(2L)t, reported to be in positive linkage disequilibrium with Adh^S near the equator, but present only at low frequency (3%) at Tahbilk where Adh^S occurs at a frequency of 30% (Knibb *et al.* 1981). Since In(2L)t is rapidly lost from laboratory strains of *D. melanogaster* (Inoue, 1979), especially at 20 °C (van Delden, 1984), it is unlikely that the inversion was a factor in this study.

(ii) *Larval ethanol tolerance test*

All lines were tested for survival using a test slightly modified from that of McKenzie & Parsons (1974). For each line, three replicates of 25 larvae (0–3 h old) were transferred to standard medium with either 0 or 6% ethanol (3 vials each) at 20 °C. To score development times the number of adults to eclose each day was recorded for each vial. This test was repeated on the whole set of 42 lines for four consecutive generations (Tests 1–4). Tolerance was scored as the total number of surviving adults from the ethanol vials expressed as a percentage of the total number of surviving adults from the non-ethanol vials. Weighted mean development time in days was determined for the three ethanol vials and is expressed as a ratio of the development time for larvae on ethanol to that of larvae fed the non-ethanol medium.

We measured the body weights of adult males and females from all vials of Test 4 only. Newly eclosed adults of each sex were collected each day and housed on unyeasted standard medium. These were counted and weighed as a group within 5 days of the first eclosion from each vial. Lines with less than 5 adults were excluded from the analysis. Weighted mean weights were obtained for the replicate vials and a ratio of the mean weight of flies on ethanol to those given a non-ethanol medium was calculated for each sex. There was a significant correlation between male and female values of this statistic ($r = 0{\cdot}69$, $P < 0{\cdot}001$), but there was no difference between sexes in the means over all lines. The analysis was conducted on the mean of the two sexes.

3. Results

Forty-two isofemale lines of the following homozygous genotype combinations were derived: $Adh^{FF}\,Gpdh^{FF}$, 11 lines; $Adh^{FF}\,Gpdh^{SS}$, 11 lines; $Adh^{SS}\,Gpdh^{FF}$, 10 lines and $Adh^{SS}\,Gpdh^{SS}$, 10 lines. The first of four tolerance tests (over consecutive generations) was carried out on these lines three months after field collection of the original females. Although there were some changes in ranking of lines

Table 1. *Analysis of fitness tests*

(*a*) ANOVA of tolerance and development time by line and test number

	Tolerance			Development time		
Source of variation	D.F.	Mean square	*F*	D.F.	Mean square	*F*
Lines[a]	40[c]	700·6	2·33***	40[c]	0·023	3·16***
Error	121	300·3		121	0.007	
Test[b]	3	612·9	1·27	3	0·05	4·88**
Error	162	433·7		153[c]	0·01	

(*b*) Correlations between fitness characters using line means (above diagonal) and partial correlation coefficients (below diagonal) (Test 4)[d]

	Tolerance	Development time	Weight[e]
Tolerance	1	0.42**(38)[f]	−0.42**(38)
Development time	0·33*(37)	1	−0·34(38)*
Weight	−0·33*(37)	−0·20(37)	1

[a] Four tests used as replicates.
[b] Lines used as replicates.
[c] Line OB4 omitted from analysis.
[d] Lines with less than 5 survivors on ethanol omitted from analysis.
[e] Mean value of sexes.
[f] Degrees of freedom.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

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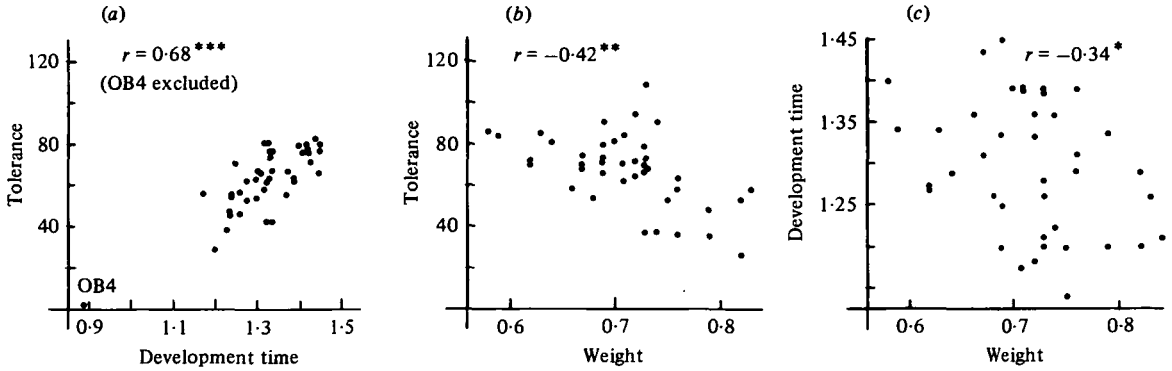

Fig. 1. Pairwise comparison scattergrams of fitness related characters. (*a*) Plot of the line means over 4 tests. (*b*), (*c*) Plots of data from Test 4 in which adult weight was estimated. Lines were excluded from analysis if less than 5 adults eclosed on ethanol medium. Significant correlation coefficients (r) are indicated: * $P < 0{\cdot}5$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

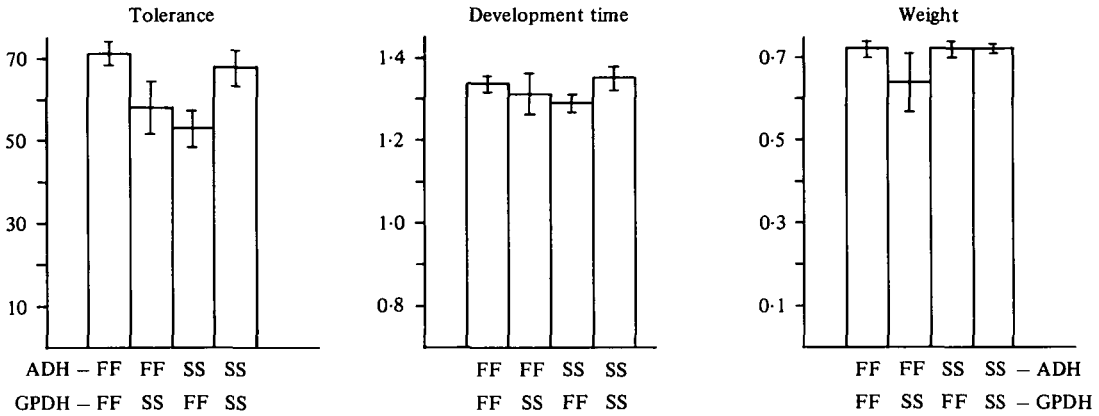

Fig. 2. Comparison among the 4 dihomozygous (ADH/GPDH) genotype groups of the means (±S.E.M.) of three fitness estimates. (*a*), (*b*) bar heights are the means of the line means over 4 tests. (*c*) Line means of Test 4 in which adult weight was estimated.

in their survival on ethanol over generations, a strong genetic component to the variation was indicated by an analysis of variance, using the four tests as replicates (Table 1*a*). Here 'line' made a highly significant contribution to the overall variation. This was supported by the finding that one line, OB4 (see Fig. 1*a*), was always ranked last and was always conspicuously low in tolerance. There is a strong genetic basis for variation in larval tolerance (McKenzie & McKechnie, 1978). The mean tolerance of all lines was 68.4% and did not vary significantly from one test to the next (Table 1*a*).

In the analysis of development time on ethanol medium, a genetic component to the variation among lines was indicated by analysis of variance using the 4 tests as replicates (Table 1*a*). Lines contributed significantly to the overall variation. The mean development time across all lines and 4 tests was 1·32. As has been found previously (McKechnie & Geer, 1984), surviving larvae took longer to develop to the adult stage on ethanol-supplemented medium. This ratio varied significantly from one test to another (Table 1*a*).

There was a strong positive correlation between tolerance and development time (Table 1*b*; Fig. 1). The lines with higher tolerance took longer to develop to the adult stage when fed ethanol. There was a negative correlation between weight and tolerance, and between weight and development time. Partial correlations on these Test 4 data indicated that the weight/development time association is likely to be the consequence of a more direct relationship between tolerance and each of these (Table 1*b*).

To assess the possible contribution of ADH and GPDH allozymic variation to variation in fitness characters, the means of the four genotype classes were compared (Fig. 2). The most interesting result was for tolerance. Comparison of the mean survival of the 22 lines homozygous for Adh^F with that of the 20 lines homozygous for Adh^S indicated no significant difference ($t = 1{\cdot}78$, $P = 0{\cdot}08$; the tolerance of each line being the mean over four tests). This was also true when homozygous *Gpdh* genotypes were compared, without regard to *Adh* genotypes ($t = -0{\cdot}86$, $P = 0{\cdot}39$). However, a marked epistatic interaction in the determination of larval tolerance occurred when both loci were considered together. Factorial analysis of variance (Table 2) gave a highly significant two-way

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Table 2. *Factorial ANOVA of fitness traits by* Adh *and* Gpdh *genotype*

Source	D.F.	Mean square	*F*
		Tolerance	
Adh	1	179·14	0.77
Gpdh	1	2·30	0·01
Adh × *Gpdh*	1	1948·50	8·34**
Error	38	233·58	
Total	41	268·44	
		Development time[a]	
Adh	1	0.001	0.08
Gpdh	1	0·001	0·10
Adh × *Gpdh*	1	0·018	1·68
Error	38	0·011	
Total	41	0·010	
		Weight[b]	
Adh	1	0·002	0·42
Gpdh	1	0·002	0·49
Sex	1	0·002	0·13
Adh × *Gpdh*	1	0·000	0·03
Adh × sex	1	0·003	0·59
Gpdh × sex	1	0·000	0·06
Adh × *Gpdh* × sex	1	0·000	0·03
Error	72	0·005	
Total	79	0·005	

[a] On line means over 4 tests; lines as replicates.
[b] On Test 4 with lines as replicates.
** $P < 0·01$.

interaction. The $Adh^{FF}\,Gpdh^{FF}$ and $Adh^{SS}\,Gpdh^{SS}$ genotype combinations were the more tolerant of the four genotype groups. No such effects of genotype at these two loci considered separately, or in combination, were evident for development time or weight (Fig. 2; Table 2). Separate analyses (not shown) of the number of adults, mean development time and mean weight per adult fed on ethanol-free medium gave no significant main effects or interactions.

4. Discussion

Little is known about the exposure to ethanol of natural populations of *D. melanogaster* or about the relative importance of different fitness components. These experiments included a variety of ethanol fitness measures. All test lines fed 6% ethanol had lower survival, a prolonged larval period and a decreased adult weight (usually associated with lower fecundity) compared to control groups. All these ethanol-induced changes are likely to be significant in nature. We find strong evidence that alleles of the *Adh* and *Gpdh* loci interact in an epistatic manner in the determination of one of the fitness traits, larval survival, in an ethanol environment; $Adh^{FF}\,Gpdh^{FF}$ and $Adh^{SS}\,Gpdh^{SS}$ being the most fit. Surprisingly, in two other studies the $Adh^{SS}\,Gpdh^{SS}$ genotype was found to be at a disadvantage compared to the other allelic combinations. In one study (Cavener & Clegg, 1981) adults were allowed to oviposit on 10% ethanol medium, so that both adult and larval fitness factors were at play. In our study no adult fitness components were estimated. The test temperature was different in a study by Sanchez & Rubio (1986), 17 °C in the study by these authors compared to 20 °C here, and ethanol was not a component of the medium on which fecundity was assessed. Temperature has previously been implicated as a component of selection at both the *Adh* and *Gpdh* loci (van Delden, 1984). It would be useful to examine the epistatic interaction of the *Adh* and *Gpdh* loci at different life stages and at different temperatures.

Our observations imply that there are alleles that increase ethanol tolerance and at the same time slow development and reduce weight gain when ethanol is encountered. A partial dependence of ethanol related fitness components has also been reported by van Herrewege & David (1980) and Dorado & Barbancho (1984). Although some loci probably affect both tolerance and development time, others probably influence only one of these fitness components. *Adh* and *Gpdh* fall into the latter category, influencing tolerance only.

There is little evidence to suggest that established differences in enzyme activity among polymorphic allozymes influence flux through pathways and fitness (Koehn *et al.* 1983; Middleton & Kacser, 1983; Dykhuizen *et al.* 1987). However, flux is a systematic property of a pathway influenced by all contributing

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enzyme loci (Kacser & Burns, 1981). Hence these two-locus allozyme combinations involved in the ethanol breakdown pathway have the potential to result in flux changes. Also, recent data on larvae subjected to particular feeding conditions indicate that ADH activity differences do cause flux changes (Heinstra *et al.* 1987; Geer *et al.* 1988). Although it is premature to speculate on any biochemical mechanism underlying fitness variation in this system, it is possible that optimal fitness results when there is a particular balance between glycolysis, the glycerol-3-phosphate cycle, and lipogenesis.

Does epistatic selection by ethanol occur in nature? Our investigation suggests that it may occur in habitats with ethanol concentrations high enough to cause larval mortality. If so, the presence of *Gpdh* variation could obscure any effect of ethanol selection on frequencies at the *Adh* locus. This could help to explain the difficulties encountered in associating ethanol tolerance variation to *Adh* allozyme frequencies in natural populations of *D. melanogaster*. Field sampling of *Adh*/*Gpdh* genotype combinations from larval habitats where ethanol concentrations are high, such as in the grape pile at the Chateau Tahbilk Winery (McKenzie & McKechnie, 1979), in Spanish wine vats (Briscoe *et al.* 1975), or in seepage from wine casks (Gibson *et al.* 1981), may give information about the significance of such selection. Although the lack of information on ethanol exposure in nature makes the ecological significance of laboratory measurements of survival, development rate and body weight difficult to assess, the information on associations of fitness measurements and their genetic bases sheds light on the genetic mechanisms by which *D. melanogaster* has adapted to environmental ethanol.

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