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Strains of *Drosophila melanogaster* differ in alcohol tolerance

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The influence of environmental ethanol on different fitness components and the larval activities of some enzymes were studied in three strains of *Drosophila melanogaster*. All three strains carried the Adh^S-$\alpha Gpdh^F$ allele combination on their second chromosomes while they had unique allele combinations at the *Odh* and *Aldox* loci on their third chromosomes (strain 1: Odh^S-$Aldox^F$; strain 2: Odh^F-$Aldox^S$; strain 3: Odh^{S*}-$Aldox^S$). Normal lines and exposure lines, kept on 5 % ethanol supplemented medium for at least 20 generations, were established from each strain and the responses of the two lines to different ethanol concentrations were compared. Two survival components were estimated in the juvenile life history stages. In addition, the weights of the emerging adult males were measured at various concentrations of ethanol. The changes in the activities of two enzymes (ADH and αGPDH) were also surveyed in the larvae after the different ethanol treatments. Strain-specific differences were observed in the responses of all investigated traits to ethanol. Odh^S-$Aldox^F$ larvae seemed to be more tolerant to ethanol than the larvae of the other two strains while the utilisation of ethanol as energy source appeared to be the least effective in this strain. Larvae of the exposure lines had significantly higher tolerance to ethanol, and the adult males were heavier than the ones from the normal lines. The enzymatic responses of the two lines to the ethanol treatments were also different. ADH activity, fresh male weight, and pupa-to-adult survival seemed only to be associated under short-term exposure to ethanol. Ethanol tolerance appeared to be independent of the utilisation of ethanol in the larva-to-pupa stage.

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Alcohol tolerance of *Drosophila melanogaster* is a model system in studies involving the relationship between molecular variation and adaptation to environment. The most abundant natural breeding substrates of *D. melanogaster* are fermenting fruits, the alcohol content of which can be quite high (GIBSON et al. 1981; MCKECHNIE and MORGAN 1982; DAVID 1988). The fruitfly tolerates both the toxic effect of different environmental alcohols (DAVID and BOCQUET 1976; KERVER and VAN DELDEN 1985) and can also use ethanol as a food source (VAN HERREWEGE and DAVID 1980; MCKECHNIE and GEER 1984).

Natural populations of *D. melanogaster* exhibit considerable genetic variation in ethanol tolerance. Clinal patterns of variation have been documented in Europe (DAVID and BOCQUET 1976), in Australia (PARSONS 1980), and on the eastern and western coasts of US (COHAN and GRAF 1985). On a microgeographic scale, flies have higher alcohol tolerance inside wineries compared with the surrounding areas (BRISCOE et al. 1975; MCKENZIE and MCKECHNIE 1979; HICKEY and MCLEAN 1980; GIBSON and WILKS 1988). In laboratory experiments, ethanol tolerance increased when flies were grown on ethanol supplemented medium (KERVER and VAN DELDEN 1985; SANCHEZ-CANETE et al. 1986; BARBANCHO et al. 1987, KERVER and ROTMAN 1987).

Alcohol dehydrogenase (ADH) is the key enzyme in the detoxification of ethanol (DAVID et al. 1976) and ADH allozymes do show different in vitro enzyme activities (GIBSON 1970), yet the relation between the level of polymorphism at the *Adh* locus and ethanol tolerance does not seem to be straightforward. There is evidence suggesting that natural populations with higher Adh^F allele frequency (ADHF is the high activity allozyme) exhibit higher level of ethanol tolerance (BRISCOE et al. 1975; HICKEY and MCLEAN 1980). In addition, increased Adh^F allele frequency has been observed when polymorphic populations were selected for higher ethanol tolerance on ethanol supplemented media (CAVENER and CLEGG 1978; VAN DELDEN et al. 1978). Other evidence, however, indicates that increased ethanol tolerance is not invariably associated with increased Adh^F allele frequency (MCKENZIE and PARSONS 1974; OAKESHOTT et al. 1984; BARBANCHO et al. 1987). Hence, tolerance to environmental ethanol cannot be explained by genotypic differences at the *Adh* locus exclusively. In natural populations, considerable activity variation can be detected among flies homozygous for *Adh* presumably due to the effects of polymorphic modifier genes (BARNES and BIRLEY 1978; LAURIE-AHLBERG et al. 1980; ANDERSON and GIBSON 1985). A positive correlation has been demonstrated between larval ADH activity and egg-to-adult survival on ethanol supplemented media (THOMPSON and KAISER 1977; KAMPING and VAN DELDEN 1978). In the adults, however, ethanol tolerance was not related to ADH activity (MERCOT and MASSAAD 1989).

Even though the biochemical and physiological background of larval ethanol tolerance is not yet fully understood, accumulating evidence shows that the entire energy metabolism is influenced and the activities of several enzymes are altered when larvae are exposed to environmental ethanol (GEER et al. 1983; MCELFRESH and MCDONALD 1983; MCKECHNIE and GEER 1984; GEER et al. 1990). Several authors have found epistatic interaction between *Adh* and α-glycerophosphate dehydrogenase (*αGpdh*) genotypes (CAVENER and CLEGG 1981; VAN DELDEN 1984; MCKECHNIE and GEER 1988; IZQUIERDO and RUBIO 1989). In an earlier survey we have demonstrated that the enzymatic responses to exogenous ethanol depended on the *Odh-Aldox* two-locus genotype of the flies (PECSENYE et al. 1994a). Larval survival on ethanol supplemented medium has been reported to correlate with the GPO activity of the larvae (MCKECHNIE and GEER 1986; GEER et al. 1991). Accordingly, ethanol tolerance in *D. melanogaster* is a complex metabolic trait determined by several enzyme and regulatory loci.

The aim of this work was to provide further evidence on the influence of the *Odh-Aldox* region of the third chromosome. Different responses (changes in two survival components, adult male weights and enzyme activities) of three strains were compared with various concentrations of ethanol. All of our strains carried the Adh^S-$\alpha Gpdh^F$ allele combination on their second chromosomes while they had unique allele combinations at the *Odh* and *Aldox* loci on their third chromosomes. We have also studied the influence of long-term ethanol exposure on the investigated traits. Two lines were established from each strain: normal lines (NL) were kept on regular food medium while exposure lines (EL) were kept on 5 % ethanol supplemented medium for at least 20 generations. The responses of the two lines to the different ethanol treatments were analysed in all three strains.

MATERIALS AND METHODS

Strains

Three strains of *Drosophila melanogaster* were constructed from the offspring of a single female of an isofemale line (CARDWELL Australia 1986) and maintained at the Umeå Drosophila Stock Center. Given the past history of the female from which all three strains originated, the genetic composition of the strains can be assumed to be similar. The original genetic variation of the Cardwell population was considerably depleted by three bottle necks: (i) in 1986 or earlier, an isofemale line was established in Cardwell; (ii) in 1987, a few (4–5) females of this isofemale line were sent to the Umeå Drosophila Center and a stock was founded; (iii) in 1988, the strains were constructed from a single female of this stock. Genetic variation may have decreased further after the second bottle-neck due to inbreeding when the stock was kept in Umeå for about 30 generations.

Strain 1 was monomorphic for the Odh^S-$Aldox^F$ allele combination, strain 2 had the Odh^F-$Aldox^S$ two-locus genotype and strain 3 was monomorphic for the Odh^{S*}-$Aldox^S$ allele combination. These two loci are closely linked on the third chromosome with 7.5 map units between them. All strains carried the *In(2L)t* inversion, which is fixed for the Adh^S-$\alpha Gpdh^F$ allele combination on their second chromosomes. Two lines were established from each strain: one line was kept on normal cornmeal molasses medium (NL) while the other line on 5 % ethanol supplemented cornmeal molasses medium for at least 20 generations (EL).

Culture conditions

Prior to all experiments, the strains were kept in mass cultures at 18°C and approximately 70–80 % relative humidity. 1 l of normal cornmeal molasses medium contained 72 g maize flour, 10 g agar, 6 g dead yeast, 60 g sucrose, and 4 ml propionic acid. Ethanol supplemented media were prepared by adding the appropriate volume of 96 % ethanol to freshly cooked medium with rigorous stirring after it had been cooled to 50°C. Ethanol concentrations are given as percentages by volume.

Alcohol tolerance

Two survival components were studied in both lines of the three strains: larva-to-pupa and larva-to-adult survival. Adults were put to fresh media and were allowed to lay eggs for four days, and then second instar larvae (approximately 4 days old) were collected. 50 larvae were put into vials containing 5 ml normal or ethanol supplemented cornmeal molasses medium. Six ethanol concentrations were used for the normal lines while seven for the exposure lines (0, 5, 7.5, 10, 12.5, 15, and 17.5 %) with 10 replicates per concentration. 10–20 days later the number of pupae and adults were counted in each vial.

Weight determination

When counting the adults, 10 seven day old males were collected from each vial, and they were measured together. Consequently, fresh weight obtained for each vial was the mean weight of 10 flies. Male weights were measured at three ethanol concentrations (0, 5 and 7.5 %) for all strain and line combinations.

Enzyme studies

The activities of alcohol dehydrogenase (ADH) and α-glycerophosphate dehydrogenase (αGPDH) were measured on starch gels after the separation of the proteins by gel electrophoresis.

Flies raised on regular medium were allowed to lay eggs for four days, and then second instar larvae (approximately 4 days old) were collected. 20 larvae were then subjected to 5 ml medium containing different concentrations of ethanol (0, 5, 7.5 %). The sampling procedure, the conditions of electrophoreses, and the enzyme assays were carried out as described by PECSENYE et al. (1996). Six estimates of activities (replications) were collected for each enzyme, strain, and food combination.

Data analyses

The data from the survival experiments were analysed in two parts. The larva-to-pupa and larva-to-adult components were analysed as the proportions of pupae and adults dying out of the original 50. Death rates were analysed using generalized linear models with binomial errors and logit link function in both cases (FRANCIS et al. 1994; CRAWLEY 1993). The model (Table 1) was analysis of co-deviance with ethanol concentration (E) as independent variable and strains (S) and lines (L) as main factors. The model also contained the two- and three-way interactions among main factors and the error term, which was the variation among replicate vials. The terms were included sequentially, i.e., the effect of any term was conditional on all those fitted above it in the table. Differences in the degrees of freedom from those appropriate to complete models were consequences of missing values. Tests of significance were performed by comparing the changes in deviance with a chi-square distribution. The slopes of the regression lines were used in the comparison of the alcohol tolerance of the strains and lines. We also estimated the initial survival in the absence of ethanol (IS = 1 − (exp(intercept/slope))) and the ethanol concen-tration which killed 50 % of the individuals (LD_{50} = 1/(1 + (1/exp(intercept))))) using the regression equations predicted by the models for all line and strain combinations.

For the other responses (fresh weights and enzyme activities), the data were analysed by generalized linear models (MCCULLAGH and NELDER 1989) assuming constant coefficient of variation which can be approximated by specifying gamma error distribution coupled with a reciprocal link function. All models constructed to analyse the weight data and the enzyme activity data (Table 5) had three main factors (ethanol concentrations: E, lines: L, and strains: S), all two- and three-way interactions and the error terms. Tests of significance were performed by comparing the mean changes in deviance with the F distribution. All arithmetic was performed using GLIM, release 4.

Table 1. *Analyses of co-deviance for larva-to-pupa (L-P) and larva-to-adult (L-A) death rate data. Independent variable: E—ethanol concentration. Factors: L—lines; S—strains; df—degrees of freedom; Error—residual mean deviance; ns—not significant; *—significant at 0.05 level; **—significant at 0.01 level; ***—significant at 0.001 level*

Factor	df	L-P	L-A
E	1	1023.0***	1062.0***
L	1	266.6***	387.9***
S	2	30.5***	16.1***
E × L	1	8.9**	23.8***
L × S	2	18.2***	3.4 ns
E × S	2	7.6*	0.4 ns
E × L × S	2	0.2 ns	3.6 ns
Error	372+	355.2	387.6

+: Difference in the degrees of freedom for the error term from those appropriate to a complete model is the consequence of missing values

Table 2. *Parameters of the regression lines obtained in the analyses of co-deviance for the two survival components in both lines of all three strains.*
NL: normal lines; EL: exposure lines; L-P: larva-to-pupa survival; L-A: larva-to-adult survival; slope: slope of the regression lines; intercept: intercept of the regression lines; SE: standard error

Line	Strain	L-P		L-A	
		Slope (SE)	Intercept (SE)	Slope (SE)	Intercept (SE)
NL	1	0.3758 (±0.0286)	−2.681 (±0.2589)	0.3979 (±0.0211)	−1.554 (±0.0630)
	2	0.4693 (±0.0232)	−2.306 (±0.2567)	0.3979 (±0.0211)	−1.485 (±0.0627)
	3	0.4090 (±0.0301)	−1.885 (±0.2329)	0.3979 (±0.0211)	−1.123 (±0.0619)
EL	1	0.2795 (±0.0411)	−2.986 (±0.2690)	0.2796 (±0.0134)	−2.662 (±0.0651)
	2	0.3730 (±0.0284)	−4.075 (±0.3315)	0.2796 (±0.0134)	−2.593 (±0.0647)
	3	0.3127 (±0.0247)	−2.894 (±0.2663)	0.2796 (±0.0134)	−2.231 (±0.0628)

RESULTS

Ethanol tolerance

All regression lines for the two lines of the three strains were significant in the survival analyses (Table 1). As we counted the number of pupae and adults for each vial we could analyse larva-to-pupa (L-P) and larva-to-adult (L-A) survival in parallel. Comparing the results obtained for these two components we could also draw certain inferences for pupa-to-adult (P-A) survival.

Larva-to-pupa survival. — Strain specific differences were observed in the initial death rates of the normal lines (Table 1 L-P: S and L × S), specifically strain 3 (Odh^S*-$Aldox^S$) had the highest initial survival rate (Table 3: IS). Significant differences were also found in the IS values between the exposure and control lines (Table 1 L-P: L and Table 2 L-P: intercept), namely long-term ethanol exposure decreased initial death rates considerably (Table 3: IS). Nevertheless, the least difference in the IS values between the two lines was detected in strain 1 (Odh^S-$Aldox^F$: 25 %).

Significant differences were observed in the slopes of the regression lines among the normal lines of the strains (Table 1 L-P: E × S and Table 2: slope). Strain 1 (Odh^S-$Aldox^F$) appeared to be the most tolerant to ethanol in the larval stages (Table 2 and Table 3: LD_{50}). Although strains 2 (Odh^F-$Aldox^S$) and 3 (Odh^S*-$Aldox^S$) seemed to be similarly sensitive to ethanol on the basis of their LD_{50} values (Table 3), the significant difference between the slopes of these two strains implies that strain 3 had higher level of tolerance to ethanol than strain 2 (Table 2: slope). Long-term ethanol exposure improved larval alcohol tolerance in all three strains (Table 1: L-P: E × L and Fig. 1); the slope of the regression lines decreased considerably (20–25 %) and the LD_{50} values increased to a great extent. Strain-specific differences were not observed in the ethanol tolerance of the exposure lines (Table 1: L-P: E × L × S).

Table 3. *Predicted values of initial survival in the absence of ethanol (IS) and the LD_{50} values (ethanol concentration which killed 50 % of pupae and adults) in the larva-to-pupa (L-P) and larva-to-adult stage (L-A). NL: normal lines; EL: exposure lines*

Line	Strain	L-P		L-A	
		IS	LD_{50}	IS	LD_{50}
NL	1	0.936	7.13	0.825	3.91
	2	0.909	4.91	0.815	3.73
	3	0.868	4.61	0.754	2.82
EL	1	0.952	10.68	0.935	9.52
	2	0.983	10.92	0.930	9.27
	3	0.947	9.25	0.903	7.98

Larva-to-adult survival. — Strain-specific differences were also detected in the initial survival rate in the larva-to-adult stage (Table 1: L-A: S). Similarly to the larva-to-pupa stage, strain 3 (Odh^S*-$Aldox^S$) had the highest IS values (Table 3). Long-term ethanol exposure had a significant effect on initial survival (Table 1 L-A: L); exposure lines had considerably higher IS values in all strains (Table 3: IS).

Both indices of ethanol tolerance (slope of the regression lines and LD_{50} values) indicated that the effect of ethanol on larva-to-adult survival was similar in the three strains (Table 2: slope and Table 3). Long-term ethanol exposure has a significant effect on alcohol tolerance (Table 1: L-A: E × L); exposure lines of all strains were considerably more tolerant than the normal lines. It is remarkable that this difference between the two lines was much higher in case of the larva-to-adult survival than in the larva-to-pupa survival (Table 4).

Pupa-to-adult survival. — Although our experimental design was not appropriate to analyse pupa-to-adult survival in detail, certain inferences could be drawn for this survival component as well when the results of the analyses for the larva-to-pupa and larva-to-adult stages were compared. The results indicated that pupa-to-adult survival had two interesting features. (i) In the larva-to-pupa stage, the normal line of strain 1 (Odh^S-$Aldox^F$) had much lower slope value than the normal lines of the other two strains (Table 2). At the same time, the normal lines of all three strains had similar slope values estimated for the larva-to-adult stage (Table 2). It implies that the ethanol tolerance of the pupae was also different in the three strains, namely strain 1 (Odh^S-$Aldox^F$) pupae must have been the most sensitive to ethanol. (ii) Long term ethanol exposure improved alcohol tolerance in all investigated life history stages. It is, however, quite interesting that the difference between the normal and exposure lines was slightly higher for the larva-to-adult stage than for the larva-to-pupa stage (Table 2: slope and Table 3: LD_{50} values). Accordingly, long term ethanol exposure had certain influence on the alcohol tolerance of the pupae as well.

Weight

All three main factors had significant effect on the fresh weights of males (ethanol/E/: $F_{2,139} = 7.15$, $0.05 > P > 0.001$; line/L/: $F_{1,139} = 12.67$, $0.05 > P > 0.001$; strain/S/: $F_{2,139} = 34.57$, $0.05 > P > 0.001$).

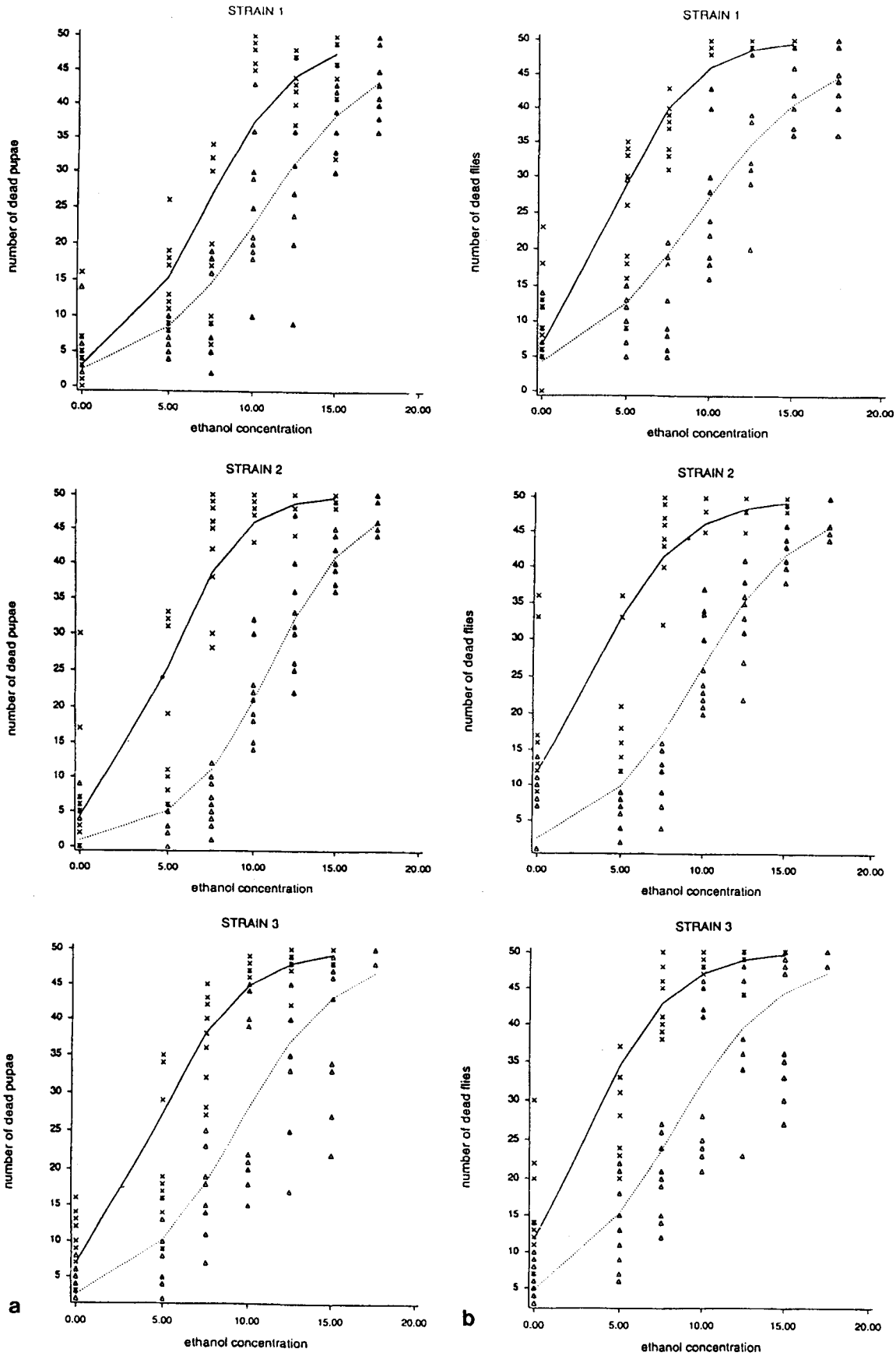

Fig. 1a and b. The observed values and the predicted lines of survival components (× —normal lines; △ —exposure lines). **1a** larva-to-pupa survival. **1b** larva-to-adult survival.

Table 4. *Average male weights together with their standard deviations. NL—normal lines; EL—exposure lines; ethanol concentrations: 0, 5, and 7.5 %*

	Strain 1		Strain 2		Strain 3	
	NL	EL	NL	EL	NL	EL
0 %	7.80 (0.146)	8.30 (0.155)	8.01 (0.160)	8.92 (0.167)	8.67 (0.162)	8.89 (0.164)
5 %	7.75 (0.146)	8.20 (0.154)	8.75 (0.181)	8.39 (0.160)	9.40 (0.187)	8.40 (0.158)
7.5 %	7.09 (0.157)	8.21 (0.157)	7.15 (0.2969)	8.29 (0.154)	8.82 (0.297)	8.53 (0.170)

Specifically, (i) long-term ethanol exposure increased male weights in strains 1 (Odh^S-$Aldox^F$) and 2 (Odh^F-$Aldox^S$), while strain 3 (Odh^S*-$Aldox^S$) did not respond to it (L × S: $F_{2,139} = 12.93$, $0.05 > P > 0.001$); (ii) the three strains had significantly different fresh weights namely, strain 1 (Odh^S-$Aldox^F$) had the lowest male weights; (iii) 5 % ethanol did not affect the weights of the males of the normal lines while 7.5 % ethanol resulted in a considerable decrease in male weights; (iv) neither 5 nor 7.5 % ethanol affected male weights in the exposure lines (E × L: $F_{2,139} = 13.95$, $0.05 > P > 0.001$).

Enzyme activities

The activities of both enzymes differed significantly among the lines and strains (Table 5: L, S, L × S — except for αGPDH); ethanol treatments also had a significant effect on enzyme activities (Table 5: E, E × L). Strain-specific differences were also observed in the influence of environmental ethanol on the activities of the two enzymes (Table 5: E × S or E × L × S). Ethanol treatment is known to induce alcohol dehydrogenase in *D. melanogaster*, resulting in an increase in ADH activity due to enhanced protein synthesis (GEER et al. 1988; KAPOUN et al. 1990). When the larvae of the normal lines were exposed to 5 % ethanol, we observed a considerable increase in ADH activity (approx. 30%) in strains 2 and 3 (Fig. 2: NL). On 7.5 % ethanol supplemented medium, however, larval ADH activity of all three strains was much higher than on normal medium (Fig. 2: NL). In the larvae of all three strains, ADH activity of the exposure lines (kept on 5 % ethanol) was much higher than that of the normal lines measured on 5 % ethanol supplemented medium (Fig. 2:

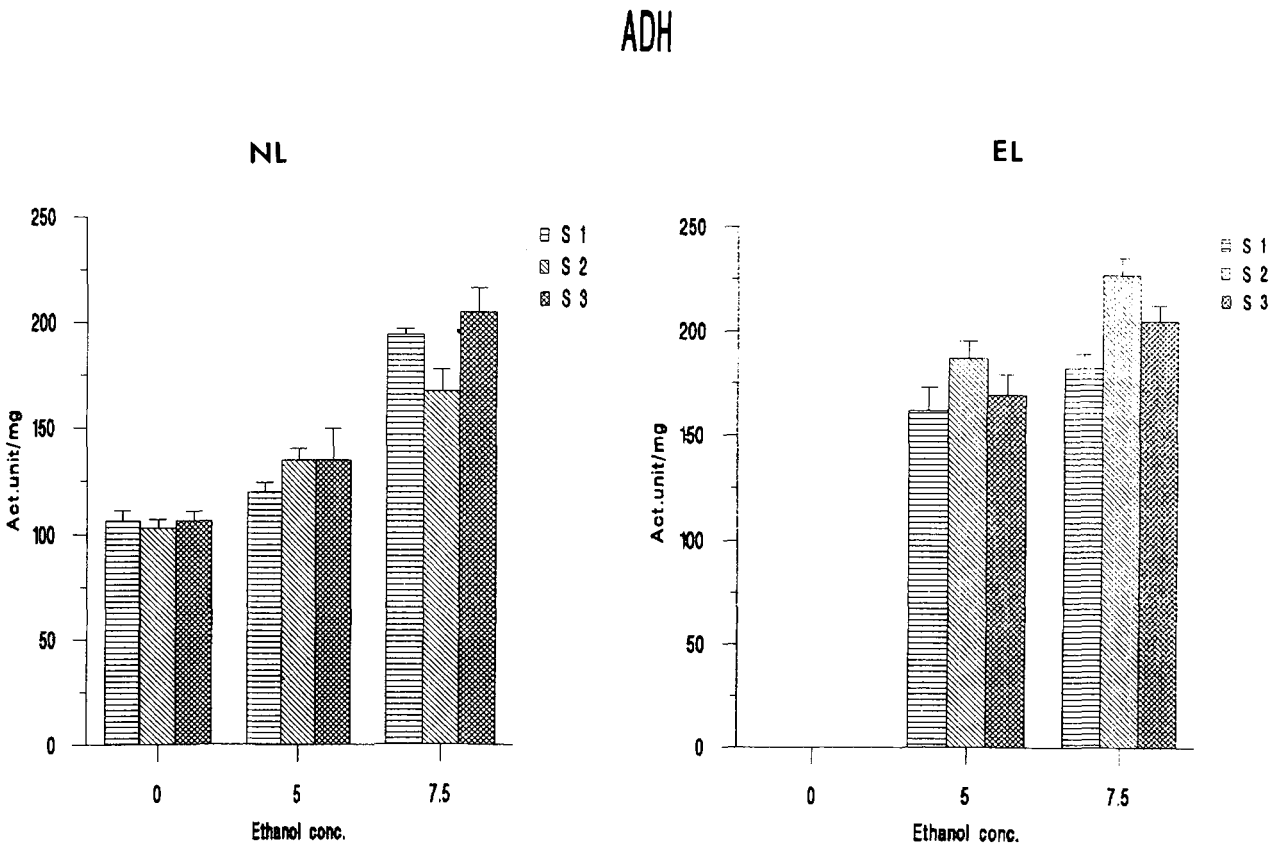

Fig. 2. Larval ADH activities in the normal (NL) and exposure (EL) lines of the three strains obtained at various ethanol concentrations. s 1-strain 1 (Odh^S-$Aldox^F$; s 2-strain 2 (Odh^F-$Aldox^S$); s 3-strain 3 (Odh^S*-$Aldox^S$).

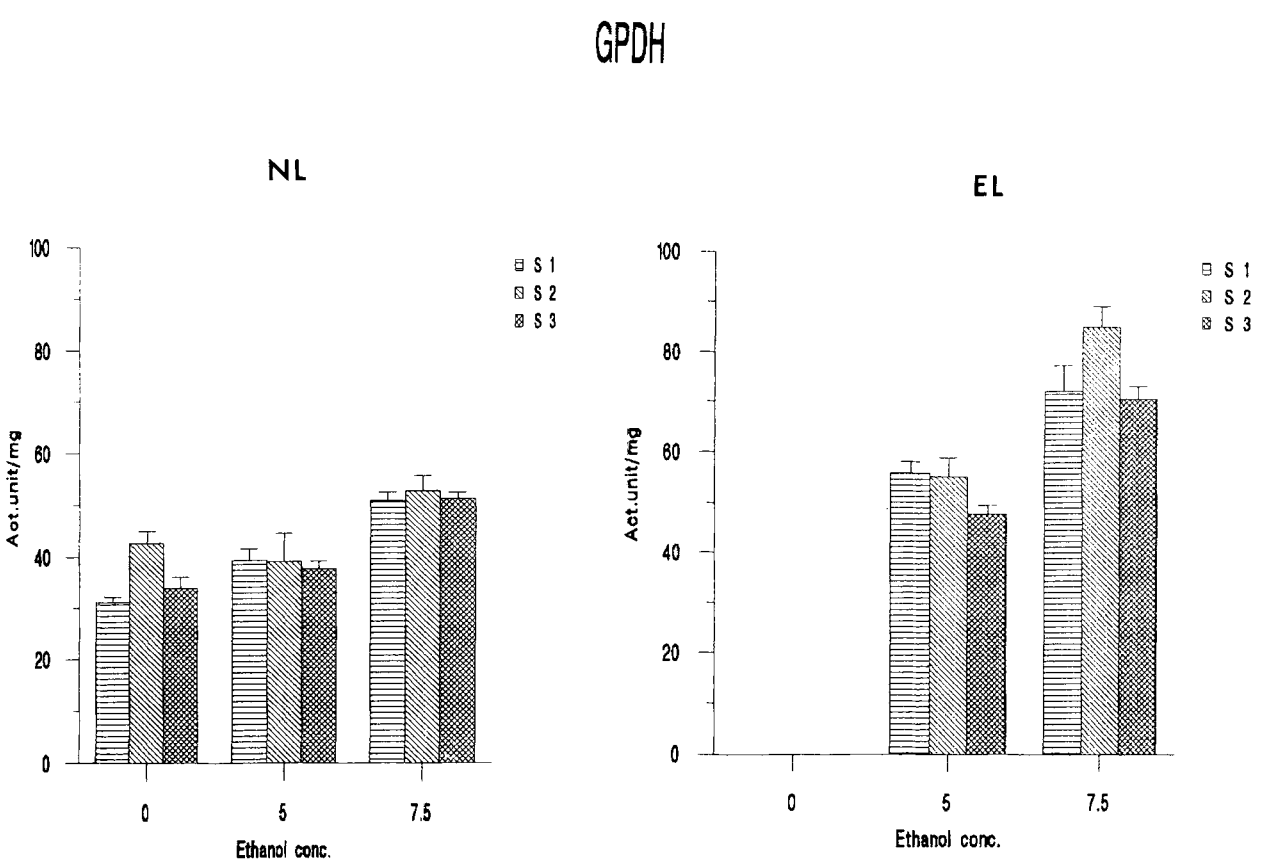

Fig. 3. Larval αGPDH activities in the normal (NL) and exposure (EL) lines of the three strains obtained at various ethanol concentrations. s 1-strain 1 (*Odh^S-Aldox^F*; s 2-strain 2 (*Odh^F-Aldox^S*); s 3-strain 3 (*Odh^{S*}-Aldox^S*).

NL 5 % versus EL 5 %). Nevertheless, the treatment with 7.5 % ethanol resulted in a further increase in the ADH activity of the exposure lines (Fig. 2: EL). When the larvae of strains 1 (*Odh^S-Aldox^F*) and 3 (*Odh^{S*}-Aldox^S*) were treated with 7.5 % ethanol, ADH activities were similar in the normal and exposure lines. The exposure line of strain 2 (*Odh^F-Aldox^S*), however, had much higher ADH activity than the normal line in the presence of 7.5 % ethanol (Fig. 2: NL 7.5 % versus EL 7.5 %).

Long-term ethanol exposure had a significant effect on larval αGPDH activities, exposure lines had much higher activities than the control lines (Fig. 3: NL versus EL). Strain-specific differences were observed in the activities of αGPDH, strain 2 larvae (*Odh^F-Aldox^S*) had slightly but significantly higher activity than the larvae of the other two strains. When the larvae were treated with 7.5 % ethanol, all αGPDH activities increased considerably (Fig. 3).

DISCUSSION

Ethanol tolerance of *D. melanogaster* involves the ability to survive and grow in the presence of ethanol and to utilise it as a source of energy (GEER et al. 1990). Ethanol tolerance has been extensively studied (KAMPING and VAN DELDEN 1978; VAN HERREWEGE and DAVID 1980; OAKESHOTT et al. 1985; GEER et al. 1990). These studies are difficult to evaluate since the experimental conditions have

Table 5. *Analyses of deviance for the enzyme activity data. Factors: E—ethanol concentration; L—lines; S—strains; df—degrees of freedom; MCD—mean change in deviance; Error—residual mean deviance; ns—not significant; all other entries are significant at 0.001 level*

Factor	df	Mean Change in Deviance ADH	GPDH
E	2	2.224	2.827
L	1	0.527	2.148
S	2	0.060	0.200
E × L	1	0.253	0.013 ns
L × S	2	0.092	0.010 ns
E × S	4	0.008 ns	0.211
E × L × S	2	0.038	0.002 ns
Error	73$^+$	0.209	0.297

$^+$: Difference in the degrees of freedom for the error term from those appropriate to a complete model is the consequence of missing values

varied to a great extent. PARSONS (1980), DAVID and VAN HERREWEGE (1983) as well as MERCOT and MASSAAD (1989) exposed adults to ethanol fume while OAKESHOTT et al. (1985) and GIBSON and WILKS (1988) used ethanol supplemented medium. The tolerance has been equated to the concentration of ethanol that killed 50 % of the individuals in a given period of time. MCKECHNIE and MORGAN (1982), DORADO and BARBANCHO (1984), KERVER and VAN DELDEN (1985), as well as MCKECHNIE and GEER (1988) have reported on the changes in different survival components when larvae were exposed to various concentrations of ethanol in the food medium. The sensitivity of *D. melanogaster* to ethanol seems to vary at different life stages; adults are more tolerant to alcohol than larvae (MCKENZIE and PARSONS 1972; KERVER and VAN DELDEN 1985).

Here, larval survival components were studied on regular food medium containing various concentrations of ethanol. Strain-specific differences were found in all survival components. Strain 1 larvae (*Odh^S-Aldox^F*) seemed to be the most tolerant to ethanol while the larvae of strains 2 (*Odh^F-Aldox^S*) and 3 (*Odh^S*-Aldox^S*) appeared to be equally sensitive to it. In contrast, in the presence of ethanol, the lowest pupa-to-adult survival was estimated for strain 1 while the pupae of the other two strains appeared to be more tolerant to alcohol. This result seems to be significant considering the genetic composition of the strains. It is highly probable that the differences in ethanol tolerance among the strains were due to genetic differences on chromosome three. Although we cannot exclude the possibility of other differences in their genetic backgrounds, this seems unlikely to account for the differences we observed. If unknown genetic differences had influenced the responses to ethanol, we would have expected great variation in all responses of the exposure lines (which experienced 20 generations of inbreeding) within each strain. However, the standard deviations for all investigated response variables for each strain were similar in the normal and exposure lines (Tables 2 and 4; Fig. 2 and 3: NL vs. EL). CHAKIR et al. (1996) have recently demonstrated that the genetic basis of both ethanol and acetic acid tolerance is mainly linked to chromosome three. They suggest that the activity differences in acetyl-CoA synthetase carried by chromosome 3 are responsible for the variation in both tolerances. The cytological map position of this locus is on chromosome 3, at 78 C (ASHBURNER pers. commun. 1995), which is fairly close to the *Odh-Aldox* region (cytological map position: 86D1–89A2).

In accordance with other studies (AYALA and MCDONALD 1980; KERVER and VAN DELDEN 1985; SANCHEZ-CANETE et al. 1986; BARBANCHO et al. 1987) we also demonstrated that long-term exposure to ethanol improved all investigated survival components. Furthermore it is important to note that after long-term ethanol exposure strain-specific differences were not detected in ethanol tolerance (Table 1: E × L × S). Assuming that long-term ethanol exposure resulted in adaptation to exogenous ethanol the influence of the *Odh-Aldox* region seems to be limited to the short-term response to alcohol and did not affect this adaptation process.

Table 6. *Changes in fresh male weights, ADH activities and in the three survival components after the normal lines of the three strains were exposed to 5 % ethanol. V_{LP}: larva-to-pupa viability; V_{PA}: pupa-to-adult viability; V_{LA}: larva-to-adult viability*

Strains	Changes in				
	Weight	ADH activity	Survival components		
			V_{LP}	V_{PA}	V_{LA}
1	−0.7 %	+12.7 %	−52.4 %	−35.5 %	−26.2 %
2	+9.2 %	+31.0 %	−53.8 %	−14.3 %	−46.1 %
3	+8.4 %	+26.6 %	−60.8 %	−25.9 %	−47.0 %

Alcohol utilisation and tolerance are two physiological traits which are controlled, at least in part, by different genetic mechanisms in *D. melanogaster* (VAN HERREWEGE and DAVID 1980). Our earlier results have suggested that, if larvae survive the toxic effect of ethanol, it may be utilised as energy source later in the development (PECSENYE et al. 1994b). Ethanol is primarily degraded by the ADH metabolic pathway and is mostly converted into lipids (GEER et al. 1990, 1991). Consequently, the weight of adults developing on ethanol supplemented medium is expected to be higher than those of growing on normal medium. Here we wished to see whether ADH activities were associated to freshly emerged male weights and also to any survival components. Comparing the predicted values of larval ADH activities, fresh male weights and the three survival components (V_{LP}, V_{PA} and V_{LA}) of the normal lines for media containing 0 % and 5 % ethanol, we could detect certain trends in the changes of these variables (Table 6). In strain 1 (*Odh^S-Aldox^F*), ADH was not induced and male weights did not change after the larvae were treated with 5 % ethanol. In contrast, both ADH activities and fresh male weights increased significantly under the same conditions in strains 2 (*Odh^F-Aldox^S*) and 3 (*Odh^S*-Aldox^S*). When the larvae were exposed to 5 % ethanol, pupa-to-adult survival changed in the expected manner in that the largest decrease in this survival component was observed in strain 1. The

decrease in larva-to-pupa survival, however, was much less in strain 1 than in the other two strains under this ethanol treatment. Accordingly, the relationship was only consistent among ADH activity, fresh weight, and pupa-to-adult survival. Larva-to-pupa survival, however, seemed to be independent of both ADH activity and fresh weight. This agrees with a similar observation by GEER et al. (1991). Our results indicate that larva-to-pupa survival mainly depends on the level of resistance to ethanol, which appears to be largely independent of the utilisation of ethanol. When ethanol is efficiently converted to lipids and a considerable amount of fatty acids can accumulate in the larval fat bodies, the sensitivity of pupae to ethanol decreases to a great extent.

The situation, however, was entirely different after long-term exposure to ethanol. The variables did not change in any consistent pattern in the exposure lines of the strains. Larval ADH activities were associated neither with male weights nor with any survival component.

Even though all three strains carried the Adh^S-$\alpha Gpdh^F$ allele combination on their second chromosomes, strain-specific differences were observed in the responses of these enzymes to the different ethanol treatments. It is known that ADH activity increases in third instar larvae during ethanol treatment due to the increased rate of protein synthesis (GEER et al. 1988; KAPOUN et al. 1990). In our study, however, ADH induction was not observed in strain 1 larvae at a low concentration of ethanol (Fig. 2: 5%). After long-term exposure to 5% ethanol all three strains showed a considerable increase in larval ADH activity which was much higher than that measured after short-term exposure to 5% ethanol (Fig. 2: NL 5% vs. EL 5%). It is also remarkable that ADH was still inducible when the larvae of the exposure lines were exposed to 7.5% ethanol. Strains 1 (Odh^S-$Aldox^F$) and 3 (Odh^S*-$Aldox^S$) larvae showed similar ADH activities following the treatment with 7.5% ethanol regardless of the previous culture conditions (Fig. 2: NL 7.5% vs. EL 7.5%). These strains had, accordingly reached the maximum level of larval ADH activity. In contrast, when the larvae of strain 2 (Odh^F-$Aldox^S$) were treated with 7.5% ethanol the exposure line showed much higher ADH activity than the control line. In the presence of 7.5% ethanol, the normal line of this strain did not reach the maximum level of ADH activity yet. Here we give for the first time results on ADH induction in lines exposed to ethanol for several generations. The strains differed in the way they reached the maximum activity of ADH, that is, the dynamics of ADH induction.

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