Genet. Sel. Evol. 30 (1998) 503–516

Original article

Comparative influence of *Odh* and *Adh* loci on alcohol tolerance in *Drosophila melanogaster*

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(Received 28 November 1997; accepted 9 September 1998)

Abstract – The effect of ethanol on larva-to-pupa and larva-to-adult survival was compared in ten laboratory strains of *Drosophila melanogaster*. The strains had five different allele combinations at the *Adh* and *Odh* loci. Two parallel strains of the five two-locus genotypes were isolated from different isofemale lines, and so they had different genetic backgrounds. Second instar larvae of all ten strains were exposed to different ethanol treatments and larva-to-pupa and larva-to-adult survival components were estimated. The strains with different genetic background but identical genotypic combinations at the *Adh* and *Odh* loci had different initial survival rates but they exhibited similar tolerance to ethanol. Ethanol tolerance appeared to depend predominantly on the *Odh* locus. The two *Adh* genotypes did not show significantly different ethanol tolerance. In contrast, the three *Odh* genotypes tolerated exogenous ethanol differently: Odh^F homozygotes had the highest tolerance to ethanol in both the larval and pupal stages. © Inra/Elsevier, Paris

***Drosophila melanogaster* / *Adh* / *Odh* / alcohol tolerance**

Résumé – Le locus *Odh* et la souche génétique ont plus d'influence sur la tolérance à l'alcool que le locus *Adh* chez *Drosophila melanogaster*. L'influence de l'éthanol sur la survie du stade larvaire à la nymphose et de la nymphose à l'état imago a été comparée dans dix souches de *Drosophila melanogaster*. Les souches présentaient cinq combinaisons alléliques aux locus *Adh* et *Odh*. Pour chacun des cinq génotypes, deux souches ont été isolées à partir de lignée isofemelles différentes, c'est-à-dire qu'elles possédaient des fonds génétiques différents. Les larves de second stade des dix souches ont été exposées à différentes concentrations d'éthanol et les survies de la larve à la pupe et de la pupe à l'imago ont été estimées. Les souches ayant une origine génétique différente, mais une même combinaison d'allèles aux locus *Adh* et *Odh* présentent des survies différentes, mais une tolérance similaire à l'éthanol. Le degré de tolérance

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à l'éthanol semble dépendre principalement du locus *Odh*. Les deux génotypes *Adh* ne présentent pas de tolérance significativement différente à l'éthanol. En revanche, les trois génotypes aux locus *Odh* tolèrent des concentrations différentes en éthanol exogène : les homozygotes Odh^F sont les plus tolérants à l'éthanol, aussi bien au stade larvaire qu'au stade pupal.

***Drosophila melanogaster* / *Adh* / *Odh* / tolérance à l'alcool**

1. INTRODUCTION

Alcohol tolerance in *Drosophila melanogaster* is an ideal system for the study of adaptation. The adaptive genetic response can be easily assayed at different levels of the relevant environmental factor. Fruit flies breed in the wild in decaying plant material [8], where different alcohols can accumulate at relatively high concentrations [19, 25]. Environmental ethanol is a significant agent of selection in natural populations of *D. melanogaster*. Both adults and larvae can use a low concentration of external ethanol as an energy source [15, 20, 29], but at higher concentrations, alcohols are toxic [8, 17, 21, 41].

Ethanol tolerance is the ability of the fly to withstand the toxic effect of ethanol [17] and is a quantitative trait, the genetic background of which is poorly understood. Natural populations exhibit considerable genetic variation in the level of ethanol tolerance and both clinal and microgeographic patterns of this variation have been extensively documented [2, 6, 9, 18, 22, 27, 33].

The physiological processes underlying ethanol tolerance are very complex. They involve a series of metabolic pathways, in which ethanol is eliminated and converted to lipids or CO_2 [21, 29]. Furthermore, the mechanisms that stabilize the structure of membranes against the fluidizing effect of ethanol also play important roles in ethanol tolerance [17]. Dietary ethanol has a general effect on the intermediary metabolism, that is the flux from ethanol to lipids and CO_2 increases as a consequence of the changes in the activities of the enzymes involved [14, 16, 20, 24, 26].

Alcohol dehydrogenase (ADH) has been found to play a central role in the metabolic use and detoxification of ethanol [10, 29]. Most natural populations are polymorphic with two common alleles at the genetic locus of this enzyme [30]. A number of experiments have been carried out in order to establish the selective significance of the *Adh* polymorphism in ethanol tolerance ([40] and references therein). There is, however, no consistent evidence from natural or laboratory populations that enhanced ethanol tolerance is the result of exogenous ethanol selecting directly on the genetic variation at the *Adh* locus [11, 19, 32].

D. melanogaster has another enzyme, octanol dehydrogenase (ODH), that uses hydrophobic alcohols as in vitro substrates [39]. The physiological role of the enzyme is barely known [35, 36]. The *Odh* locus is polymorphic for two common alleles in natural populations [31]. When polymorphic laboratory cage populations were grown on ethanol supplemented medium, the Odh^S allele frequency almost doubled in a few generations [34]. This suggests that alcohol stress can cause gene frequency changes at the *Odh* locus. Bokor and Pecsenye [1] and Pecsenye et al. [38] have found that the larvae of different *Odh–Aldox* two-locus genotypes, which had identical Adh^S allele, tolerated environmental

ethanol slightly differently and had different enzymatic responses to ethanol treatments.

The aim of this work was to provide further evidence on the significance of the *Odh* locus in ethanol tolerance and on the interaction between the *Adh* and *Odh* loci in this process. Accordingly, we compared the effect of ethanol on the larval and pupal survival rates of ten *D. melanogaster* strains. The strains were isolated from different isofemale lines collected in a natural population in Hungary and they had five different allele combinations at the *Adh* and *Odh* loci.

2. MATERIALS AND METHODS

2.1. Strains

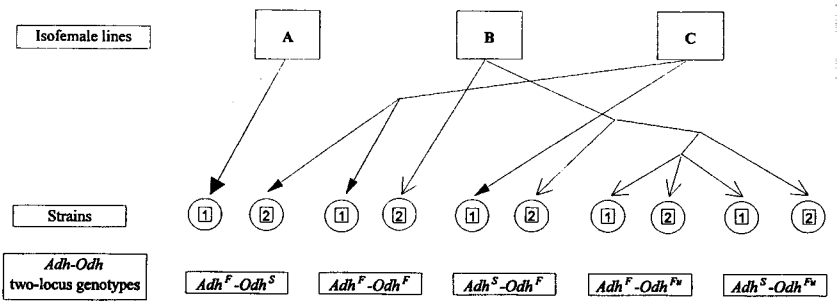
One hundred isofemale lines were established from a *D. melanogaster* population (Sajószentpéter, Hungary, 1993) in order to construct laboratory strains with different *Adh–Odh* two-locus genotypes. Three of these lines were found to be polymorphic at both loci. These three isofemale lines were used to construct the strains surveyed in this study. The strains were monomorphic for five different allele combinations at the *Adh* and *Odh* loci: Adh^F–Odh^F, Adh^F–Odh^S, Adh^F–Odh^{Fu}, Adh^S–Odh^F and Adh^S–Odh^{Fu} (the ODH-Fu allozyme migrates slightly faster than the ODH-F). Except for the four strains with the Odh^{Fu} allele, two parallel strains were isolated from different isofemale lines for the five two-locus genotypes (twin strains), hence their genetic background was expected to be different (*figure 1*). In contrast, all the four strains containing the Odh^{Fu} allele originated from the same isofemale line (*figure 1B*). The isolation of all strains was completed in six generations. Then the strains were kept in separate mass cultures for about two to three generations before the tolerance tests.

2.2. Culture conditions

Prior to all experiments, the strains were kept in mass cultures at 18 °C and approximately 70–80 % relative humidity on standard cornmeal molasses medium. One litre of cornmeal molasses medium contained 72 g maize flour, 10 g agar, 6 g dried 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 after it had been cooled to 50 °C. Ethanol concentrations are given as percentages by volume.

2.3. Alcohol tolerance

Two survival components were studied in both strains of the five different two-locus genotypes: larva-to-pupa and larva-to-adult survival. Adults were allowed to lay eggs on fresh medium for 4 days and then second instar larvae (approximately 4 days old) were collected. Fifty larvae were put into vials containing 5 mL of either normal or ethanol supplemented cornmeal molasses medium. After 10–20 days, pupae and emerging adults were counted. Seven



Adh genotypes	Isofemale lines	*Odh* genotypes	Isofemale lines
Adh^F	A, B, C	Odh^F	B, C
Adh^S	B, C	Odh^S	A, C
		Odh^{Fu}	B

Figure 1. The scheme of the construction of the ten strains with five *Adh–Odh* two-locus genotypes from the three isofemale lines (A, B and C).

ethanol concentrations were used (0, 5, 7.5, 10, 12.5, 15 and 17.5 %) with ten replicates per concentration for each of the strains.

2.4. Statistical procedures

The larva-to-pupa and larva-to-adult data were analysed as proportions of pupae and adults that died out of the original 50. In both cases death rates were analysed using generalized linear model with binomial error and logit link function [13]. Since the two parallel strains of the five *Adh–Odh* two-locus genotypes (twin strains) were isolated from only three isofemale lines they could not be considered as independent samples in the analyses. As a consequence, separate models were used to analyse the effect of the different genetic factors (genetic background, *Adh* and *Odh* loci) on ethanol tolerance. All models were analyses of co-deviance with ethanol concentration as independent variable. The different models contained various factors, the interactions among the main factors and the error terms which were the variations among vials. The terms were included sequentially, i.e. the effect of any term was conditional on all those fitted before. Differences in the degrees of freedom from those appropriate to complete models resulted from missing values. As overdispersion was present in the data, we assumed that the variance was proportional to the binomial variance rather than equal to it. Therefore we calculated a scale parameter by dividing the Pearson χ^2 value by the degrees of freedom and used this estimate to correct the total deviance [7]. Tests of significance were performed by comparing the changes in the corrected deviance with a chi-square distribution. In order to compare the alcohol tolerance of the different strains and genotypes we predicted the slopes and the intercept values of the regression lines using different models (*figure 2A*), and also estimated the initial survival rates in the absence of ethanol (ISR) and the ethanol concentration which killed 50 % of the individuals (*figure 2B*: LD_{50}).

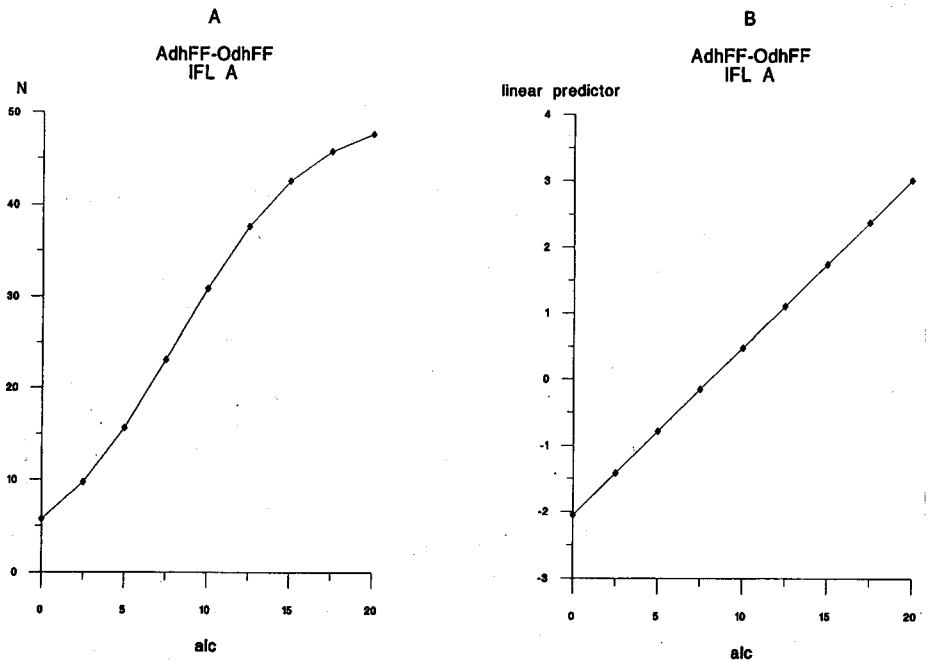


Figure 2. Predicted number of dead pupae (A:N) and values of the linear predictor (B) for the Adh^{FF}–Odh^{FF} strain constructed from IFL A on the basis of the strain model. A) $ISR = (50 - N_0)/50$ where N_0 is the number of dead larvae at 0 % alcohol; LD_{50} is the alcohol concentration at $N = 25$ (50 % of the original number of larvae). B) Linear predictor $\eta = \ln(p/q)$ where p is death rate, q is survival and $p = 1 - q$; $\eta = a + b.x$ where x is alcohol concentration; a is the intercept and b is the slope for the Adh^{FF}–Odh^{FF} strain constructed from IFL A: $\eta = -2.05 + 0.253.x$.

First, we analysed the differences in ethanol tolerance among the ten strains regardless of their genetic background (i.e. isofemale line) or *Adh*–*Odh* two-locus genotypes. As a consequence, the data of the strains were included separately and the co-deviance models contained only strain as main factor (*table I*). Using these models (which we refer to as strain-models) we could calculate the four estimates (slopes, intercepts, LD_{50} and ISR) of ethanol tolerance for all ten strains.

In the second series of the co-deviance analyses, we studied the effect of the *Adh* and *Odh* loci on ethanol tolerance. We therefore pooled the data of the twin strains, i.e. the pairs of strains with identical *Adh*–*Odh* two-locus genotypes. Hence the models (which we refer to as two-locus models) contained *Adh* and *Odh* genotypes as main factors and their interaction (*table I*). Using these models we estimated the four parameters of alcohol tolerance for the five *Adh*–*Odh* two-locus genotypes.

In the third series of the analyses we estimated the relative significance of the three genetic factors (genetic background, *Adh* and *Odh* loci). As a consequence, three types of models were constructed corresponding to these factors. In the

analyses of the genetic background, the data were pooled according to the origin of the strains (i.e. isofemale lines). Hence, in these co-deviance models (which we refer to as IFL-models) isofemale line was the only main factor (*table I*). On the basis of the IFL-models we estimated the measures of alcohol tolerance for the three isofemale lines. Analysing the effect of the *Adh* and *Odh* loci separately, the data were pooled according either to the *Adh* or to the *Odh* genotypes of the strains. These models also contained one main factor: *Adh* genotypes (models will be called *Adh*-models) or *Odh* genotypes (models will be called *Odh*-models). *Adh*-models were used to calculate the four estimates of alcohol tolerance for the two *Adh* genotypes while the four measures of the three *Odh* genotypes were calculated on the basis of the *Odh*-models. All computation was performed using GLIM, release 4 [13].

3. RESULTS

As both pupae and adults were counted we could analyse larva-to-pupa (L–P) and larva-to-adult (L–A) survival in parallel. In all statistical analyses, the greatest change in deviance was attributable to ethanol treatments (*table I*: Alc). The increase in death rates depended significantly on the concentration of ethanol in all experiments: the regressions explained about 72–76 % of the total variation in every model. The variation among the ten strains (all genetic factors) accounted for 7.6 and 8.9 % of the explained deviance in the larva-to-pupa and larva-to-adult stages, respectively (*table I*). The individual effects of the different genetic factors (genetic background, *Adh* and *Odh* loci) contributed about 0–6 % to the explained deviance depending on the models (*table I*).

3.1. Effect of genetic background

When studying the effect of genetic background on the ethanol tolerance of the strains we first used the IFL-models. The results clearly showed that the three isofemale lines differed significantly in their initial survival rates in both life stages (*table I*: IFL). The strains originating from isofemale line A (*figure 1*) had lower survival in the absence of ethanol in both the larva-to-pupa and larva-to-adult stages compared to the others (*table III*: ISR). In contrast, there was no significant difference in the slope of the regression lines of the isofemale lines for either of the two survival components (*table I*: Alc.IFL and *table III*: slope).

We have calculated the four estimates of alcohol tolerance for all ten strains on the basis of the strain-models. The comparison of the slopes and intercepts of the twin strains, i.e. the two strains having identical *Adh–Odh* two-locus genotypes supported the results described above. The intercept values of the twin strains differed significantly for two *Adh–Odh* two-locus genotypes in the larva-to-pupa stage (Adh^F–Odh^S $t_{672} = 5.48$, $P < 0.01$; Adh^S–Odh^{Fu} $t_{672} = 5.29$, $P < 0.01$) and for four *Adh–Odh* allele combinations in the larva-to-adult stage (Adh^F–Odh^F $t_{672} = 2.10$, $P < 0.05$; Adh^F–Odh^S $t_{672} = 3.02$, $P < 0.01$; Adh^F–Odh^{Fu} $t_{672} = 3.68$, $P < 0.01$; Adh^S–Odh^{Fu} $t_{672} = 2.1$, $P < 0.05$). As opposed to the intercept values, the slope of the regression lines were similar in the two strains with identical *Adh–Odh* two-locus genotypes except for the strains with the Adh^S–Odh^{Fu} allele combination (larva-to-pupa stage: $t_{672} = 4.71$, $P < 0.01$; larva-to-adult stage: $t_{672} = 2.40$, $P < 0.05$).

Table I. Results of co-deviance analyses for the larva-to-pupa (L–P) and larva-to-adult (L–A) survival components in all ten strains (strain-models), in the five *Adh–Odh* two-locus genotypes (two-locus models) and in the three isofemale lines (IFL-models).

Strain-models:		L–P	L–A
Factors	df	CD	CD
Alc	1	2 777***	2 100***
Str	9	184.5***	178.8***
Alc.Str	9	42.73***	26.18***
Error	672	613.18	610.64
Two-locus models:			
Factors	df	CD	CD
Alc	1	2 777***	2 100***
Adh	1	0.54	15.15***
Odh	2	83.03***	65.16***
Alc.*Adh*	1	0.01	3.34
Alc.*Odh*	2	12.04***	11.58***
Adh.Odh	1	34.63***	25.62***
Alc.*Adh.Odh*	1	0.01	0.02
Error	682	710.18	694.77
IFL-models:			
Factors	df	CD	CD
Alc	1	2 777***	2 100***
IFL	2	119.7***	66.64***
Alc.IFL	2	0.18	4.2
Error	686	720.55	744.80

CD: change in deviance; df: degrees of freedom; Alc: alcohol concentration (independent variable); Str: strains; *Adh*: *Adh* genotypes; *Odh*: *Odh* genotypes; IFL: isofemale lines; *** significant at 0.001 level.

In general, the differences between the twin strains did not show a consistent pattern with the isofemale lines from which they originated; e.g. the two Adh^F–Odh^{Fu} and Adh^S–Odh^{Fu} strains originated from the same isofemale line (*figure 1*). This indicates that there was a considerable amount of variation even within the isofemale lines.

3.2. Effects of the *Adh* and *Odh* loci

In the second series of the co-deviance analyses, we compared the ethanol tolerance among the five two-locus genotypes. Consequently, we used the two-locus models (i.e. pooled the data of the pairs of the strains with identical *Adh–Odh* two-locus genotypes). The results showed that the *Adh* locus hardly

contributed to the explained deviance, while the effect of the *Odh* locus was considerable (*table I*: *Adh* and Alc.*Adh*, versus *Odh* and Alc.*Odh*). The interaction between the *Adh* and *Odh* loci was also sizable (*table I*: *Adh.Odh*). The intercept values clearly showed the interaction between the two loci: among the Adh^F strains, the Odh^S genotype, and among the Adh^S strains the Odh^F genotype, had considerably lower intercept values than the others in both life stages (*table II*), which implies that these genotypes had the lowest initial survival rates (*table II*). The slopes of the regression lines, however, were consistent with the *Odh* genotypes of the strains. Both in the larva-to-pupa and larva-to-adult stages, the Odh^F genotype combined with either the Adh^S or the Adh^F genotype had the smallest slope (*table I*: Alc.*Odh* and *table II*). Consequently, these two-locus genotypes had the highest ethanol tolerance.

Table II. Parameters of alcohol tolerance predicted for the five two-locus genotypes (on the basis of the two-locus models).

Allele Combinations	L–P				L–A			
	Intercept (SD)	ISR	Slope (SD)	LD_{50}	Intercept (SD)	ISR	Slope (SD)	LD_{50}
Adh^F–Odh^S	−1.622 (0.070)	0.835	0.332 (0.019)	4.9	−0.918 (0.123)	0.715	0.282 (0.016)	3.3
Adh^F–Odh^F	−2.262 (0.482)	0.906	0.276 (0.024)	8.2	−1.464 (0.124)	0.812	0.236 (0.021)	6.2
Adh^F–Odh^{Fu}	−2.064 (0.154)	0.887	0.318 (0.026)	6.5	−1.396 (0.127)	0.802	0.270 (0.022)	5.1
Adh^S–Odh^F	−1.664 (0.141)	0.841	0.286 (0.105)	6.6	−0.550 (0.114)	0.634	0.218 (0.016)	2.5
Adh^S–Odh^{Fu}	−2.274 (0.159)	0.907	0.325 (0.017)	10.1	−1.193 (0.122)	0.767	0.255 (0.026)	4.7

Intercept: intercept of the regression lines; SD: standard deviation; slope: slope of the regression lines; ISR: initial survival rate; LD_{50}: the ethanol concentration which is lethal to 50 % of the individuals.

Similar results were obtained in the third part of the analyses. The regression slopes for the two *Adh* genotypes (SS and FF) estimated on the basis of the *Adh*-models did not differ significantly in any life stage (*table III*: slope). In contrast, when we used the *Odh*-models, the predicted slopes of the strains which were monomorphic for the Odh^F allele were significantly lower than the others, i.e. these strains had higher alcohol tolerance (*table III*).

The degree of alcohol tolerance is generally characterized by the LD_{50} value, that is the alcohol concentration which kills 50 % of the individuals. We also calculated the LD_{50} values on the basis of the regression equations predicted by the two-locus models. In the larva-to-pupa stage, the Adh^S–Odh^{Fu} genotype had the highest LD_{50} value while in the larva-to-adult stage, the Adh^F–Odh^F genotype seemed to be the most tolerant to ethanol (*table II*). Accordingly, when we characterized the alcohol tolerance of the genotypes by their LD_{50} values we did not get a consistent pattern in the two life history stages.

Table III. Parameters of alcohol tolerance estimated for the three isofemale lines on the basis of the IFL-modles, for the two *Adh* genotypes on the basis of the *Adh*-models and for the three *Odh* genotypes, on the basis of the *Odh*-models.

Isofemale lines	L–P			L–A		
	ISR	Slope	LD_{50}	ISR	Slope	LD_{50}
A	0.712	0.313	2.89	0.636	0.296	1.89
B	0.893	0.302	6.95	0.746	0.242	4.45
C	0.888	0.305	6.86	0.766	0.246	4.71
Alleles	L–P			L–A		
	ISR	Slope	LD_{50}	ISR	Slope	LD_{50}
Adh^F	0.872	0.294	6.53	0.774	0.253	4.86
Adh^S	0.876	0.304	6.42	0.703	0.235	3.66
Alleles	L–P			L–A		
	ISR	Slope	LD_{50}	ISR	Slope	LD_{50}
Odh^S	0.835	0.332	4.89	0.715	0.282	3.25
Odh^F	0.872	0.275	7.01	0.726	0.221	4.42
Odh^{Fu}	0.897	0.321	6.75	0.785	0.262	4.93

L–P: larva-to-pupa stage; L–A: larva-to-adult stage; ISR: initial survival rate; slope: slope of the regression lines; LD_{50}: the ethanol concentration which is lethal to 50 % of the individuals.

4. DISCUSSION

Here, we studied the ethanol tolerance of ten strains with five different *Adh–Odh* two-locus genotypes. As our strains were constructed from different isofemale lines, their genetic background was expected to be different. The variation in the level of ethanol tolerance among our strains was the consequence of the differences in their genetic composition, both in their allele combinations at the *Adh* and *Odh* loci and in their genetic background. The size of the change in deviance indicates the contribution of each factor to ethanol tolerance. The differences between the strains with specific *Odh* genotypes accounted for 3.3 and 3.5 % of the explained deviance in the larva-to-pupa and larva-to-adult stages, respectively (*table I*: *Odh* and Alc.*Odh*). The differences in the genetic background contributed 4.1 and 3.3 % to the explained deviance in the larva-to-pupa and larva-to-adult stages, respectively (*table I*: IFL and Alc.IFL). This shows that both the *Odh* locus and the genetic background had a strong effect on ethanol tolerance in our strains. At the same time, the differences between the two *Adh* genotypes did not contribute to the explained deviance in the larva-to-pupa stage while they accounted for 0.8 % of the explained deviance in the larva-to-adult stage (*table I*: *Adh* and Alc.*Adh*). The influence of the *Adh* locus was mostly expressed through the *Adh–Odh* interaction which contributed 1.2 % to the explained deviance both in the larva-to-pupa and larva-to-adult

stages (*table I*: *Adh.Odh*). This indicates that *Adh* genotypes had a considerably weaker effect on ethanol tolerance than *Odh* genotypes and genetic background.

The most remarkable result of our study was that the strains with different *Adh* genotypes did not differ significantly in their larval ethanol tolerance (*table III*: slope). This observation is especially striking as six strains with Adh^F genotype (originating from three different isofemale lines) and four strains with Adh^S genotype (originating from two isofemale lines) were analysed in this study (*figure 1*). McKenzie and Parsons [28] have found that ethanol tolerance and *Adh* genotypes were not correlated in some Australian strains. Chakir et al. [4] have also demonstrated that the large difference in ethanol tolerance between some French and Congolian strains was not entirely due to differences in their allele frequencies at the *Adh* locus. In other studies [12, 23], however, the Adh^F homozygotes had considerably higher ethanol tolerance than the Adh^S homozygotes. One possible explanation of this apparent contradiction between the results reported in the literature lies in the history of the strains used in different tolerance tests. Studying selection in laboratory cage populations Oakshott et al. [32] have proposed that selection at the *Adh* locus in response to exogenous ethanol occurs only in population samples which have been maintained in the laboratory for some time. It is quite possible that the age of the laboratory strains used in the different tolerance tests also influences the correlation between their alcohol tolerance and genotypic composition. In fact, whenever correlation has been detected between the *Adh* genotypes and ethanol tolerance, the strains had been kept in the laboratory for a long time before the experiments started [12, 23]. When McKenzie and Parsons [28] used freshly collected samples in their experiments they found that *Adh* genotypes and ethanol tolerance were independent. Our strains were isolated from fresh population samples, so that eight to nine generations (approximately 24–26 weeks) had elapsed between the collection of the samples and the beginning of the experiments.

Pecsenye et al. [35–37] observed different enzymatic responses in some laboratory strains when larvae were exposed to environmental ethanol. These strains had identical *Adh–Gpdh* two-locus genotypes but different *Odh–Aldox* allele combinations. Bokor and Pecsenye [1] studied the alcohol tolerance of these strains. Even though the outcome of these experiments indicated that the *Odh* locus had a certain influence on ethanol tolerance, the genetic composition of the strains did not allow an unequivocal conclusion. On the one hand, the strains that had been used differed in their *Odh–Aldox* allele combinations, which made it impossible to determine the influence of the *Odh* locus alone. On the other hand, all strains carried the Adh^S allele, which did not allow a study of the interaction between the *Adh* and *Odh* loci. The strains used in the present study satisfy both conditions; they all had the $Aldox^S$ allele and carried one of five different allele combinations at the *Adh* and *Odh* loci. The results presented here clearly show that the influence of the *Odh* locus on ethanol tolerance is considerably higher than that of *Adh* (*table I*). The comparison of the three *Odh* genotypes revealed that the Odh^F homozygotes were the most tolerant to ethanol in both life stages (*table III*). The origin and the genotypic composition of our strains had certain limitations: 1) the Adh^S–Odh^S two-locus genotype was missing because these allele frequencies are very low in nature (unbalanced design); 2) all Odh^{Fu} strains originated from a single isofemale

line (homogeneity in their genetic background). As a consequence, it is not possible to disentangle the effects of the isofemale lines (genetic background), the *Adh* genotypes and *Odh* genotypes exactly. Nevertheless, we believe that our results are suggestive. Six strains monomorphic for either the Odh^S or the Odh^{Fu} alleles and originating from three isofemale lines all showed significantly lower levels of ethanol tolerance (measured by the slope of the regression lines) than the four Odh^F strains which originated from two isofemale lines. Chakir et al. [3, 5] have recently demonstrated that the genetic basis of both ethanol and acetic acid tolerance is mainly linked to chromosome 3. They suggest that activity differences in acetyl-CoA synthetase are responsible for the variation in both tolerances. The cytological map position of the acetyl-CoA synthetase locus is on 3L at 78C (Ashburner, pers. comm. 1995), which is fairly close to the *Odh* locus (cytological map position: 86 D1–D4).

The results of the analyses of the slopes seem to contradict the conclusions drawn from the comparison of the LD_{50} values. The regression slopes showed a consistent pattern throughout the life history stages: the *Adh* genotypes did not differ in their alcohol tolerance, while the *Odh* genotypes showed significantly different tolerance to ethanol. In contrast, different two-locus genotypes proved to be the most tolerant to ethanol in different life history stages on the basis of their LD_{50} values. One explanation of this contradiction emerges from the comparison of the ISR values, regression slopes and LD_{50} values of the five different *Adh–Odh* genotypes (*table II*). In the larva-to-pupa stage, the highest LD_{50} value was observed in the strains having the Adh^S–Odh^{Fu} two-locus genotype. At the same time, the slope of this genotype was close to those of the Adh^F–Odh^S and Adh^F–Odh^{Fu} genotypes which had the lowest LD_{50} values. Comparing the ISR values of these three genotypes it is clear that the initial larva-to-pupa survival rates of the Adh^F–Odh^S and Adh^F–Odh^{Fu} genotypes were lower than that of the Adh^S–Odh^{Fu}. In the larva-to-adult stage, a similar relation was found between the Adh^F–Odh^{Fu} and Adh^F–Odh^S genotypes. Accordingly, the LD_{50} values of these strains were correlated with their ISR values rather than with their slopes. As a consequence, the slopes give more accurate information on the ethanol tolerance of these strains than the LD_{50} values.

The experimental design of our survey allowed us to study the effects on ethanol tolerance of three genetic components (genetic background, *Adh* and *Odh* loci) relative to each other. The results of the co-deviance analyses clearly showed that the influence of the *Adh* locus was marginal, while the other two components had significant effects (*table I*). The *Adh* locus only had a significant effect on larva-to-adult survival and it was mainly expressed in the initial survival rates of the strains (*table III*). The *Odh* locus and the genetic background have similarly strong effect on both survival components (*table I*). Nevertheless, there was a certain difference in the manifestation of their influence. Differences in the genetic background of the strains mostly resulted in variation in their initial survival rates (*table III*) while the ethanol tolerance of the strains (characterized by the slopes of the regression lines) showed a consistent pattern according to their *Odh* genotypes (*table III*).

ACKNOWLEDGEMENTS

We would like to thank Z. Varga, Kossuth Lajos University, Debrecen, Hungary and A. Saura, Umeå University, Umeå, Sweden, for invaluable and stimulating discussions on the manuscript and B. Francis and M. Green, University of Lancaster, UK, for helpful suggestions concerning the statistical models; we are also grateful to Z. Barta, Kossuth Lajos University, Debrecen, Hungary, for helpful discussion on the interpretation of the statistical analyses and to V. Mester for excellent assistance. The project was supported by OTKA 16336.

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