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Differences in the effect of ethanol on fertility and viability components among laboratory strains of *Drosophila melanogaster*

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We studied the effect of ethanol on several fitness components in six *Drosophila melanogaster* strains. Mating success, fecundity, egg-to-larva, egg-to-pupa and egg-to-adult survival and the number of emerging adults were estimated in a single series of experiments. The strains either had different combinations of genetic background and *Adh* genotypes with identical Odh^F genotype or different *Adh-Odh* two-locus genotypes with similar genetic background. Ethanol had the greatest effect on mating success and fecundity, while its influence was lower on survival. When the experimental conditions were contrasted to the natural environment of the flies the most significant results were the ones related to fecundity and larval survival. Ethanol had the highest selective effect on fecundity. The genetic factors contributed substantially to the variation in the fertility and viability components. The *Adh* locus hardly influenced mating success while it had a sizable effect on fecundity and on all survival components. The influence of *Adh* on fecundity greatly depended on the other genetic factors. Genetic background had the largest influence on the different survival components. The influence of the *Odh* locus was mostly observed through the *Adh-Odh* interaction.

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Adaptation to environmental alcohol in *Drosophila melanogaster* is one of the most extensively studied fields in population genetics. Exogenous ethanol has a complex effect on the fruit fly: at low concentrations it is used as an energy source (DAVID et al. 1976; DELTOMBE-LIETAERT et al. 1979; van HERREWEGE and DAVID 1980; MIDDLETON and KACSER 1983; GEER et al. 1985; MCKECHNIE and GEER 1984), while at high concentrations ethanol is toxic (DAVID and BOCQUET 1976; KERVER and VAN DELDEN 1985; GEER et al. 1993). Consequently there must exist a critical concentration where the utilization of ethanol is shifted to the resistance to it. In certain studies this critical ethanol concentration seems to lay between 5 and 7.5 % (SANCHEZ-CANETE et al. 1986).

In nature, fruit flies live, mate and lay eggs in the presence of exogenous ethanol. Furthermore eggs and larvae develop physically immersed in the breeding substrate. Environmental alcohol is, therefore, expected to affect various fitness components.

In many studies, aimed at comparing the fitness of different genotypes at a particular enzyme locus, various fitness components have often been determined in different sets of experiments and the results for the different fitness components were combined to estimate the net fitness of a genotype (DORADO and BARBANCHO 1984; MCKECHNIE and MORGAN 1982; MCKECHNIE and MCKENZIE 1983; OUDMAN et al. 1991). This experimental design has some disadvantages. First, depending on the aim of the study perturbations in the life cycle (e.g. collection of eggs, larvae or virgin females)—occur in different life stages in the different sets of experiments. The different life history stages, however, may vary in their sensitivity to these perturbations (BIJLSMA-MEELES 1979). Second, except for using isogenic lines, it is hard to account for unknown factors in the genetic background, which might be unevenly distributed among the different groups of individuals used in the estimation of the different fitness components.

We studied the effect of ethanol on several fitness components (mating success, fecundity, egg-to-larva, egg-to-pupa and egg-to-adult survival) in six *D. melanogaster* strains. To circumvent the problems mentioned above we investigated all fitness components in a single series of experiments. It started with the observation of the mating success of randomly selected pairs of flies and ended with counting their emerging offspring. Naturally this design also has certain disadvantages: (i) Larva-to-pupa survival is conditional on the number of hatched eggs, and similarly pupa-to-adult survival is conditional on the number of surviving larvae. This is a chain binomial model hence larva-to-pupa and pupa-to-adult survival cannot be analyzed directly. (ii) As the initial number of larvae differed considerably on the different test media, we had to account for the effect of density dependence.

Alcohol dehydrogenase (ADH) initiates the pathway where ethanol is degraded (GEER et al. 1985; HEINSTRA et al. 1987) and has been considered to be the key enzyme both in alcohol utilization and alcohol tolerance (DAVID et al., 1976). Several authors suggest that survival on media containing alcohol is directly related to alcohol dehydrogenase activity (BRISCOE et al. 1975; CAVENER and CLEGG 1978; VAN DELDEN et al., 1978; HICKEY and MCLEAN 1980). This relationship, however, does not seem to be simple and straightforward. In fact, ethanol tolerance has proved to be influenced by many genetic and physiological factors (GEER et al. 1983; MCELFRESH and MCDONALD 1983; MCKECHNIE and GEER 1984; GEER et al. 1990). Nevertheless, the analysis of the molecular data suggests that the polymorphism at the *Adh* locus is maintained by a balancing selection (KREITMAN and HUDSON 1991). Consequently, there must be significant differences among the *Adh* genotypes in some fitness related characters other than tolerance to the toxic effect of high concentration of environmental ethanol (GEER et al. 1993). Certain differences (e.g. in developmental rate, survival at higher temperature) has already been described between strains with different genetic composition at the *Adh* locus (MCKECHNIE and MORGAN 1982; OUDMAN et al. 1991).

We have previously demonstrated that among other genetic factors ethanol tolerance is greatly affected by the octanol dehydrogenase (*Odh*) locus as well (BOKOR and PECSENYE 1997, 1998). Although the physiological role of ODH is poorly understood several lines of evidence indicate that it is associated with alcohol tolerance. When polymorphic populations were grown on ethanol supplemented medium, the Odh^S allele frequency almost doubled in a few generations (PECSENYE and LÕRINCZ 1988). Larvae of different *Odh-Aldox* two-locus genotypes which were homozygous for Adh^S allele tolerated environmental ethanol slightly differently and had different enzymatic responses to ethanol treatments (PECSENYE et al. 1994a,b, 1997).

One goal of the present study was to compare the effect of ethanol on different fitness components in six *D. melanogaster* strains with different genetic compositions. The strains either had different genetic background with identical two-locus genotypes at the *Adh* and *Odh* loci or different allele combinations at these two loci with similar genetic background. Thus, it was possible to determine the influence of the *Adh* and *Odh* loci and the genetic background relative to each other on the investigated fitness components.

MATERIAL AND METHODS

Strains

Six strains of *D. melanogaster* were constructed from two isofemale lines collected in Hungary (BOKOR and PECSENYE 1998). Four strains were isolated from one of the two lines with different allele combinations at the *Adh* and *Odh* loci (Adh^F-Odh^F, Adh^S-Odh^F, Adh^F-Odh^{Fu}, Adh^S-Odh^{Fu}). These four strains had similar genetic background as they originated from the same

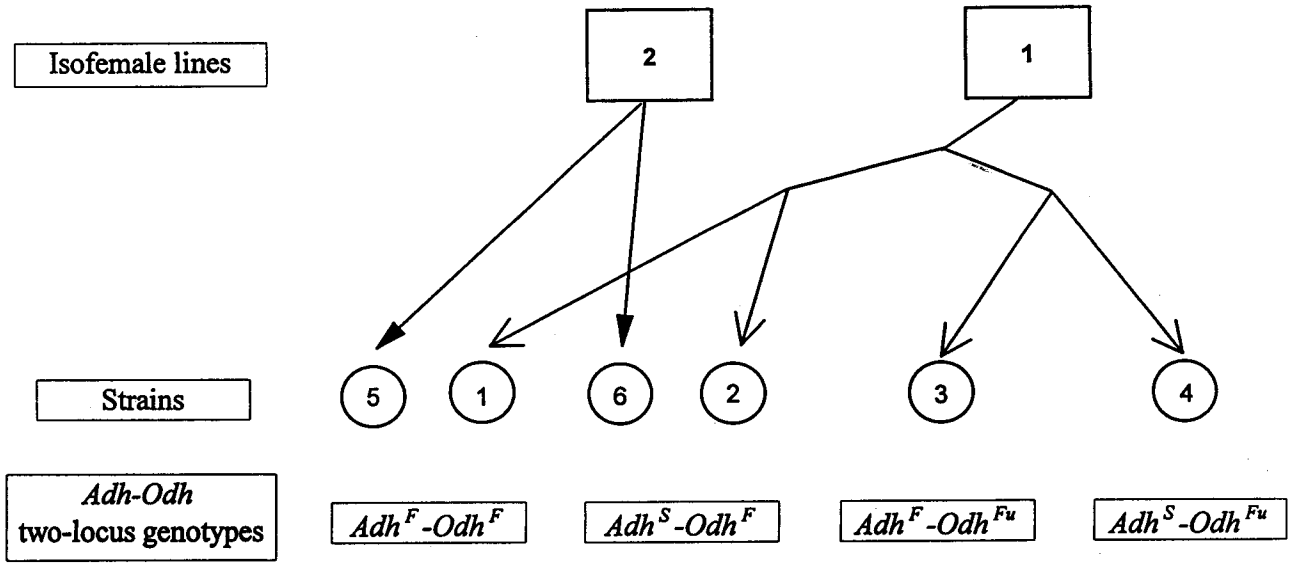

Fig. 1. Genetic composition of the six strains used in this study. S1 and S5 strains originated from line 1 while S2 and S6 strains were isolated from line 2; consequently, they had a different genetic background. At the same time, they had identical allele combinations at the *Adh* and *Odh* loci. S1, S2, S3 and S4 strains all originated from line 1, therefore, they had similar genetic background while they had different allele combination at the *Adh* and *Odh* loci.

isofemale line. Two further strains were isolated from the other isofemale line (Fig. 1). They were monomorphic for the Adh^F-Odh^F and Adh^S-Odh^F allele combinations, respectively. In this way, we had two pairs of strains which carried the same Odh^F allele but originated from different isofemale lines. These two pairs of strains were, therefore, expected to have different genetic background (Fig. 1). Nevertheless all six strains contained the same alleles at the *αGpdh* and *Aldox* loci.

Culture conditions

Before all experiments, the strains were kept in mass cultures on normal cornmeal molasses medium at 18°C and approximately 70–80 % relative humidity. One l of normal cornmeal molasses medium contained 72 g maize flour, 10 g agar, 6 g dry yeast, 60 g sucrose and 4 ml propionic acid. The ethanol supplemented media were prepared by adding the appropriate volume of 96 % ethanol to freshly cooked medium after it had been cooled to 40–50°C. Four ethanol concentrations were used:0, 5, 10 and 15 %. To get equally large surfaces of the egg laying media we poured them diagonally.

Investigation of mating success

Virgin females and males were collected from all six strains and kept on normal medium for at least three days. Then randomly selected pairs were put separately into the vials containing the test media. Ten pairs of flies were used for each combination of ethanol concentrations and strains. The pairs were allowed to mate and two days later the males were removed while the females were put on to fresh egg laying media. Mating was considered successful when the female laid one egg that hatched.

Estimation of fecundity

The females were allowed to lay eggs for four days on all treatment media. Since some of the inseminated females started to oviposit immediately after successful mating the eggs were counted both on the media where mating occurred and also on the fresh media where only the females were transferred. On the fifth day the females were removed and the number of eggs were counted in each vial. Fecundity was characterized by the total number of eggs laid by a female. The number of replicates varied depending on the number of successful mating out of the original ten but it was always larger than 5.

Study of survival components

The number of hatched eggs, pupae and emerging adults were counted in each vial. Thus, the egg-to-larva, egg-to-pupa and egg-to-adult survival of the strains were estimated at all ethanol concentrations.

Statistical analyses

All data were analyzed by generalized linear models (CRAWLEY, 1993; FRANCIS et al., 1994). We had two sets of strains: (i) two pairs of strains with either Adh^S or Adh^F genotype combined with different genetic backgrounds (Fig. 1: S1–S5, S2–S6); (ii) four strains with different allele combinations at the *Adh* and *Odh* loci but with similar genetic background (Fig. 1: S1, S2, S3, S4). We, therefore, carried out all statistical analyses in two series according to the two sets of strains. In the first series, where the first set of strains were included, we studied the effect of the *Adh* locus on the different fitness components relative to the genetic background. Consequently, we called these analyses as "analysis of genetic background". In the second series, the second set of strains were considered. Here, we could investigate the influence of *Adh* relative to the *Odh* locus. Accordingly, we called these analyses as "analyses of the *Odh* locus".

Data obtained in different parts of the experiments were analyzed by different types of models. Mating success was characterized by a dichotomous variable. Consequently, the data were analyzed by using binomial error and logit link function. In both models of the analysis of mating success, ethanol concentration (E) and *Adh* genotypes (Adh) were involved as main factors. In the analysis of the genetic background, line (L) was the third main factor, while in the analysis of the *Odh* locus, the third main factor was the *Odh* genotype (Odh). The models also contained all interaction terms (Appendix 1 and 2). Tests of significance were performed by comparing the changes in deviance with the appropriate chi-square distribution.

In all other models, ethanol concentration was considered as independent variable. Consequently, these analyses were actually co-deviance analyses. Since the number of eggs was very low at 15 % ethanol concentration with a very high variance among the repeats these data were excluded from all further analyses. The structure of the models were similar in all co-deviance analyses. In the first series (analyses of genetic background), all five models contained *Adh* genotype and line (L) as main factors and the interaction terms (Appendix 1). While all models of the "analyses of the *Odh* locus", consisted of *Adh* and *Odh* genotypes as main factors and the interactions among the main factors (Appendix 2). The terms were included sequentially i.e. the effect of any term was conditional on all those fitted above.

Fecundity was characterized by the total number of eggs observed after successful mating. Originally ten pairs of virgins were set for each combination of the investigated factors. As unsuccessful matings were

Table 1. *Percent of explained deviance at specific factor levels in the analysis of genetic background. E: ethanol treatments; Adh: Adh genotypes; L—lines. Bold characters indicate that a large portion of the total deviance is explained by the given factor*

	Mating success	Number of eggs	Survival			Number of adults
			Egg-to-larva	Egg-to-pupa	Egg-to-adult	
E	**67.2**	**49.0**	**13.5**	1.8	4.1	**47.8**
Adh	6.6	**43.8**	**62.8**	**38.2**	**45.7**	**48.0**
L	**25.5**	6.0	**22.5**	**58.9**	**47.5**	4.4
Adh.L	0.7	1.2	1.2	1.1	2.7	0.8

not considered in this part of the analyses; the number of repeats varied for the different ethanol concentrations. The data were analyzed by assuming Poisson error distribution with log link function. All survival components were analyzed as the proportion of larvae, pupae or adults dying out of the original number of eggs by using binomial error and logit link function. The number of emerging adults was also analysed separately by assuming Poisson error distribution with log link function. In all co-deviance analyses, tests of significance were performed by comparing the changes in deviance with the appropriate chi-square distribution.

All arithmetic was performed using GLIM, release 4.

RESULTS

Mating success

In the first part of both sets of analyses, we investigated how the different factors affected mating success in the six strains. Ethanol treatments accounted for a high portion of the explained deviance in both experiments. Even though ethanol had a great influence on mating success in all strains (Table 1 and 2: E) this effects was only expressed at 15 % ethanol concentration, which never occur in natural substrats.

Number of eggs

In the second part of this study, we analyzed the fecundity of the strains. The highest portion of the explained deviance could be attributed to the ethanol treatments in both analyses (Tables 1 and 2); thus, the slopes were highly significant in almost all cases (Table 3). Specifically, the total number of eggs decreased considerably with increasing ethanol concentration in all six strains (Fig. 3). The contribution of the differences between the *Adh* genotypes to the explained deviance was very large in the analysis of the genetic background (Table 1: Adh). In the absence of ethanol, the strains having Adh^F genotype laid more eggs than the Adh^S strains (Fig. 2). The fecundity of the Adh^F genotypes was much more affected by exogenous ethanol than that of the Adh^S genotypes (Table 3 and Fig. 3A). As a consequence, the total number of eggs was similar for the two *Adh* genotypes at higher ethanol concentration (Fig. 3A: 10 %). In the analysis of the *Odh* locus, *Adh* genotypes had a relatively smaller effect on the number of eggs than in the previous analysis (Table 2: Adh). At the same time, the differences between the *Odh* genotypes accounted for a sizable portion of the explained deviance (Table 2: Odh). Nevertheless, the contribution of the *Odh* locus was more pronounced through the *Adh-Odh* interaction (Table 2: Adh.Odh).

Survival components

In the third part of this study, we investigated different survival components. The experimental design allowed us to analyze egg-to-larva, egg-to-pupa and egg-to-adult components directly. From the comparison of the relevant analyses, however, certain inferences could be drawn for the larva-to-pupa and pupa-to-adult components as well.

The total number of eggs varied considerably in the different treatment and strain combinations. As survival components were expected to be affected by the initial number of eggs, we first tested their density dependence. The number of eggs, however, was strongly correlated with ethanol concentration. To circumvent this problem we only used those survival data which were obtained at 0 % ethanol concentration in these analyses. There was a clear difference between the two *Adh* genotypes in the density dependence of their survival components. None of the investigated components of the Adh^S genotypes exhibited density dependent pattern (data not shown). At the same time, the egg-to-pupa ($t_{36} = 2.68$, $P < 0.05$) and egg-to-adult ($t_{36} = 2.27$, $P < 0.05$) survival components of the Adh^F genotypes showed a week but significant density dependence in the first set of experiments (i.e. analyses of the genetic background). Nevertheless, none of the three viability components of the Adh^F genotypes were density dependent in the

analysis of the *Odh* locus. In general, the density dependence of the investigated survival components was week or insignificant and, therefore, we did not include this parameter in any further analysis.

Egg-to-larva survival.—Ethanol had a significant decreasing effect on egg-to-larva survival in both analyses (Appendix 1 and 2: E, Fig. 4). The differences between the two *Adh* genotypes accounted for a very high portion of the explained deviance in both sets of experiments (Tables 1 and 2: Adh). This difference was mostly expressed in the initial survival rates in the absence of ethanol (Appendix 1 and 2: Adh vs. E.Adh). Specifically, the Adh^F genotypes had a slightly higher probability of hatching than the Adh^S genotypes (Fig. 4). A clear difference was, however, observed between the results of the two analyses. While the genetic background and the *Adh* locus had a similarly strong effect on egg-to-larva survival (Table 1: Adh vs. L), the influence of the *Odh* locus was small compared to that of the *Adh* (Table 2: Adh vs. Odh). Nevertheless, the interaction between the *Adh* and *Odh* loci was sizable (Table 2: Adh.Odh).

Egg-to-pupa and egg-to-adult survival.—Ethanol had a very week effect on these survival components in the analysis of the genetic background (Table 1:E). It was due to the fact that ethanol treatments had a strong interaction both with *Adh* genotypes and lines (Appendix 1:E.Adh, E.L) implying that the effect of ethanol was different in the different strains. While ethanol significantly decreased both egg-to-pupa and egg-to-adult survivals in strain 2 (Adh^S-L1), both survival components increased slightly but significantly together with the ethanol concentration in strain 5 (Adh^F-L2) (Table 4, Fig. 5A and 6A). Egg-to-pupa and egg-to-adult survival of the other two strains (strains 1 and 6) were not affected by ethanol (Table 3, Fig. 5A and 6A).

In contrast, the effect of ethanol was relatively strong on both survival components in the analyses of the *Odh* locus (Table 2: E). This effect (i.e. slight decrease with increasing ethanol concentration), however, was only significant in strain 2 which had the Adh^S-Odh^F two-locus genotype.

The influence of the *Adh* locus was also relatively strong on both egg-to-pupa and egg-to-adult survival (Tables 1 and 2: E). In the analyses of the genetic background, the effect of ethanol was different in the two *Adh* genotypes i.e. the slopes differed significantly (Appendix 1: Adh vs. E.Adh, Table 3). In the analysis of the *Odh* locus, however, the initial survival rate was considerably different in the two *Adh* genotypes (Appendix 2: Adh vs. E.Adh). Larvae with the Adh^F allele had significantly higher initial survival in the absence of ethanol than those having Adh^S allele (Fig. 2) In the analyses of the genetic background, the influence of the two genetic components (*Adh* locus and lines) was equally strong on both survival components (Table 1: *Adh*, L). The differences between the two lines with different genetic background were particularly clear at higher ethanol concentration (Fig. 5A and 6A: 10%). As opposed to the influence of the genetic background relative to *Adh* the effect of the *Odh* locus relative to *Adh* proved to be much more limited on both survival components (Table 2: Odh). The influence of the *Odh* locus on egg-to-pupa and egg-to-adult survival was mostly manifested in the *Adh-Odh* interaction (Table 2: Adh.Odh).

Larva-to-pupa survival.—Comparing egg-to-larva and egg-to-pupa survival, we can deduce certain features of the larva-to-pupa survival component. In general, egg-to-larva survival was much higher than egg-to-pupa survival (Fig. 4 and 5). It implies that there are far more risks in survival at the larval stages compared to the egg stage. Ethanol is an important selective factor, which can considerably decrease larval survival especially at higher concentration while it much less affects the probability of hatching (Fig. 4 and 5: 10%). Nevertheless, an interesting feature of the influence of ethanol was detected in these analyses: the six strains exhibited three distinct pattern in their responses to ethanol in the different life stages (Table 3). In strain 1 (Adh^F-Odh^F-L1), egg-to-larva

Table 2. *Percent of explained deviance at specific factor levels in the analysis of the Odh locus. E: ethanol treatments; Adh: Adh genotypes; Odh: Odh genotypes. Bold characters indicate that a large portion of the total deviance is explained by the given factor*

	Mating success	Number of eggs	Survival			Number of adults
			Egg-to-larva	Egg-to-pupa	Egg-to-adult	
E	**52.7**	**58.5**	**29.6**	**29.1**	**26.3**	**56.4**
Adh	6.2	**12.9**	**54.8**	**41.7**	**51.3**	**29.1**
Odh	**38.2**	9.8	5.7	0.3	1.7	9.3
Adh.Odh	2.9	**18.8**	**12.9**	**28.8**	**20.7**	5.2

Table 3. *The slope values together with their standard errors predicted by the co-deviance models for the different genotypes. The upper part of the table shows the predicted values obtained in the analysis of the genetic background while the lower part of the table contains the predicted values from the analysis of the Odh locus. *—significant at 0.05 level; **—significant at 0.01 level; ***—significant at 0.005 level*

Genotypes	Number of eggs	Survival			Number of adults
		Egg-to-larva	Egg-to-pupa	Egg-to-adult	
AdhF-L1	−0.131*** (0.017)	0.113*** (0.037)	0.015 (0.036)	0.022 (0.032)	−0.141 *** (0.020)
AdhF-L2	−0.166*** (0.027)	−0.015 (0.070)	−0.136** (0.059)	−0.129* (0.052)	−0.108*** (0.031)
AdhS-L1	−0.082** (0.029)	0.029 (0.052)	0.175** (0.061)	0.168** (0.056)	−0.170*** (0.045)
AdhS-L2	−0.033 (0.045)	−0.019 (0.094)	0.016 (0.096)	0.061 (0.086)	−0.062 (0.043)
AdhF–OdhF	−0.131*** (0.016)	0.115* (0.046)	0.015 (0.037)	0.022 (0.032)	−0.141*** (0.019)
AdhF–OdhFu	−0.167*** (0.034)	0.074 (0.076)	0.107 (0.064)	0.070 (0.065)	−0.199 *** (0.041)
AdhS–OdhF	−0.082** (0.028)	0.063 (0.063)	0.175** (0.063)	0.168** (0.059)	−0.170** (0.065)
AdhS–OdhFu	−0.102* (0.045)	0.091 (0.101)	0.037 (0.105)	0.032 (0.097)	−0.111** (0.039)

survival was significantly affected by ethanol treatments, while neither egg-to-pupa nor egg-to-adult survivals were. In contrast, egg-to-larva survival was not influenced by ethanol in strains 5 (Adh^F-Odh^F-L2) and 2 (Adh^S-Odh^F-L1) but both egg-to-pupa and egg-to-adult survivals of these two strains were significantly affected by it. As opposed to strains 1, 2 and 5, none of the three survival components was affected by the ethanol treatments in strains 3 (Adh^F-Odh^{Fu}-L1), 4 (Adh^S-Odh^{Fu}-L1) and 6 (Adh^S-Odh^F-L2).

Pupa-to-adult survival.—Egg-to-adult survival was only slightly lower than egg-to-pupa survival indicating that there are not much further risks in survival during the pupal stage. Moreover, comparing egg-to-pupa and egg-to-adult survivals we found similar tendencies suggesting that the effect of the investigated genetic and environmental factors was similar in the larval and pupal stages.

Number of adults

In the fourth part of this study, we analysed the number of emerging adults in both sets of experiments. The distribution of the explained deviance was similar in both analyses (Tables 1 and 2). Ethanol had a strong effect on the number of adults, the slopes were highly significant in all strains except for strain 6 (Table 3: Adh^S-L2). The differences between the two *Adh* genotypes also accounted for a considerable amount of the explained deviance. This difference was mostly expressed in the initial number of adults in the absence of ethanol (Fig. 2); Adh^F genotypes had higher values than the Adh^S genotypes in both analyses. The influence of ethanol, however, was similar in the strains (Appendix 1 and 2: E.Adh, Table 3); the number of adults decreased considerably with increasing ethanol concentration in all strains (Fig. 7). Nevertheless, there was one slight difference between the two analyses. Namely, the line effect was small relative to the influence of the *Adh* locus in the analysis of the genetic background (Table 1). At the same time, both the *Odh* locus alone and the interaction between the *Adh* and *Odh* loci accounted for a sizable amount of the explained deviance relative to the effect of the *Adh* locus (Table 2).

DISCUSSION

The experimental design allowed us to study the effect of ethanol on different fertility and viability components. Assuming that the portion of deviance explained by ethanol treatments indicates the sensitivity of the given fitness component to ethanol we notice that ethanol had a great influence on fecundity characterized by the number of eggs (Tables 1 and 2). Moreover, the greatest change in fecundity was induced by 5% ethanol (Fig. 3) which is about the maximum concentration of alcohols in the natural habitat of the flies (McKenzie and Parsons 1974; Briscoe et al. 1975; Gibson et al. 1981; McKechnie and Morgan 1982). The number of emerging adults was influenced by environmental ethanol in a similar manner as the number of eggs. This suggests that the number of adults which can be considered as the net result of all fitness components was mostly dependent on the number of eggs. Nevertheless, adults are free to disperse in their environment and can choose the most appropriate breeding substrate to lay eggs. Richmond and Gerking (1979) and Hougouto et al. (1982) have shown that females prefer substrates with lower ethanol concentration for oviposition. It implies that the strong selective effect of ethanol on fecundity we observed under experimental conditions is likely to act in nature as well.

The results of the survival analyses indicated that ethanol did not have a consistent selective effect in the different strains and life stages (Table 3 and Figs. 4–6). In our earlier studies, II instar larvae were exposed to exogenous ethanol and their survival decreased dramatically in the usual dose-response manner (BOKOR and PECSENYE 1997, 1998). In the present series of experiments, however, larval survival did not decrease together with increasing ethanol concentration in all strains (Table 3). The disagreement between the results of the two series of experiments with different experimental designs draws attention to the significance of the timing of the treatment; i.e., exposure to ethanol results in different survival rates depending on the life stage in which

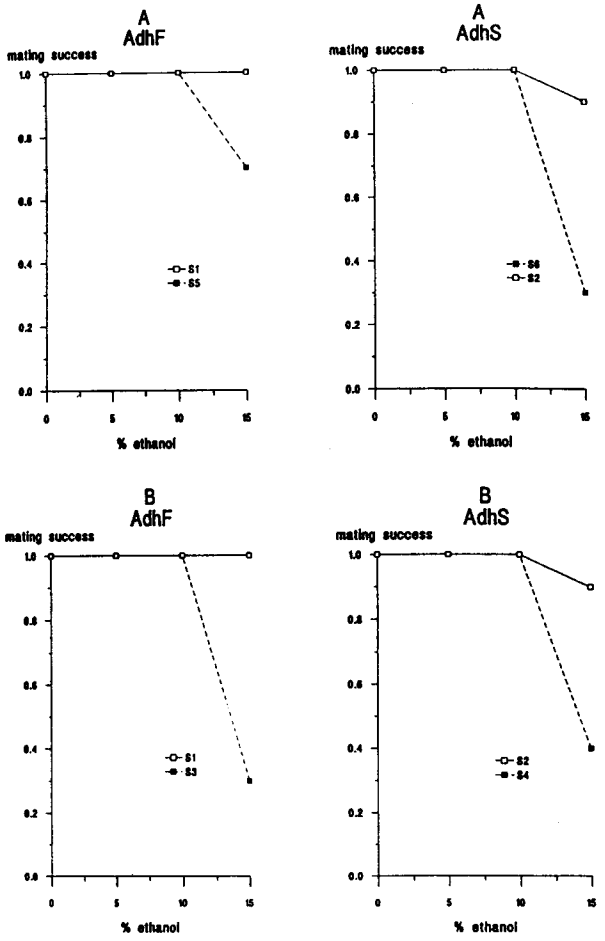

Fig. 2A and B. The effect of ethanol on mating success. The points are predicted values based on the models. **A** The effect of the genetic background. Two of the four strains had different genetic background with identical *Adh-Odh* genotypes: S1 and S5 was monomorphic for the Adh^F and Odh^F alleles while S2 and S6 was monomorphic for the Adh^S and Odh^F alleles. **B** The effect of the *Odh* locus. All four strains had similar genetic background. S1 had Adh^F-Odh^F, S2 had Adh^S-Odh^F, S3 had Adh^F-Odh^{Fu} and S4 had Adh^S-Odh^{Fu} two-locus genotypes.

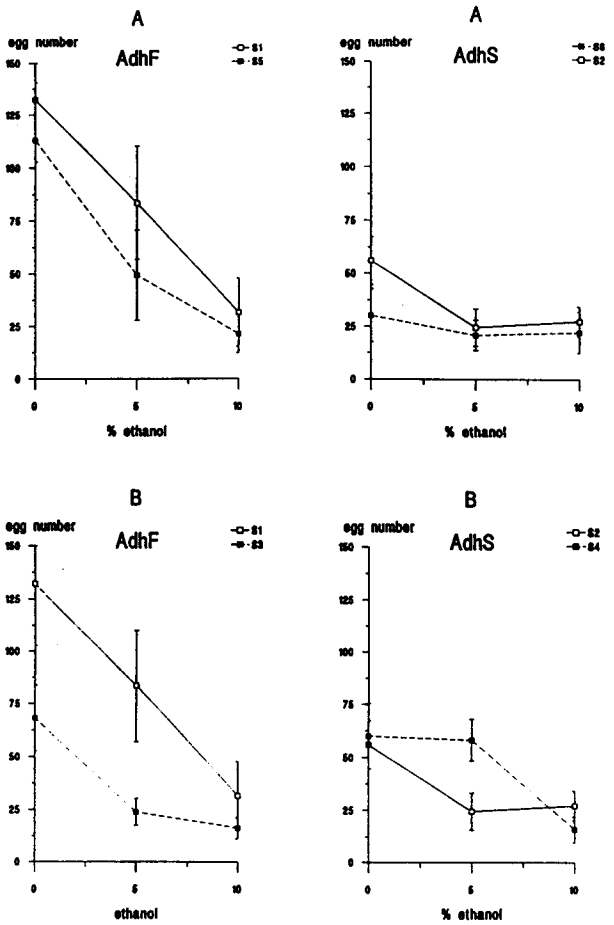

Fig. 3A and B. The effect of ethanol on the total number of eggs. The points are predicted values together with their standard deviations on the basis of the co-deviance models. For further details see the legend of Fig. 2.

flies first cope with the alcohol. In a survey, II instar larvae proved to be the most sensitive to 12 % ethanol (BIJLSMA-MEELES 1979). In nature, eggs are already experiencing environmental ethanol and from the time of hatching larvae continuously live and develop in the presence of it. Moreover, larvae have to cope with ethanol at a constantly increasing concentration due to fermentation in decaying fruits. This implies that the experimental conditions of our study were reasonably similar to the natural environment of the flies.

The genetic components accounted for about 30–85 % of the explained deviance in the different analyses. The significance of the genetic factors (*Adh* and *Odh* loci, genetic background) relative to each other exhibited various patterns for the different fitness components. The *Adh* locus had a considerable effect on all fitness components except for mating success. An interesting result of this study was that the greatest difference between the two *Adh* genotypes was

observed in their fecundities. These differences were most obvious in the absence and at low concentrations of ethanol (up to 5 %). Since the natural breeding substrates of the flies contain alcohols at fairly low concentrations these results are of special interest. As it has been proposed by GEER et al. (1993) the results of this study also suggest that selection may influence the variation present at the *Adh* locus due to differences between the two genotypes in other phenotypic traits than their ethanol tolerance. Nevertheless, the relative significance of the *Adh* locus in relation to fecundity (i.e., the number of eggs) strongly depended on other genetic components. Namely, the two *Adh* genotypes differed to a great extent in their fecundity compared to the differences between the two lines with different genetic background. At the same time, the influence of the *Adh* locus was far weaker on the number of eggs compared to that of the *Odh*.

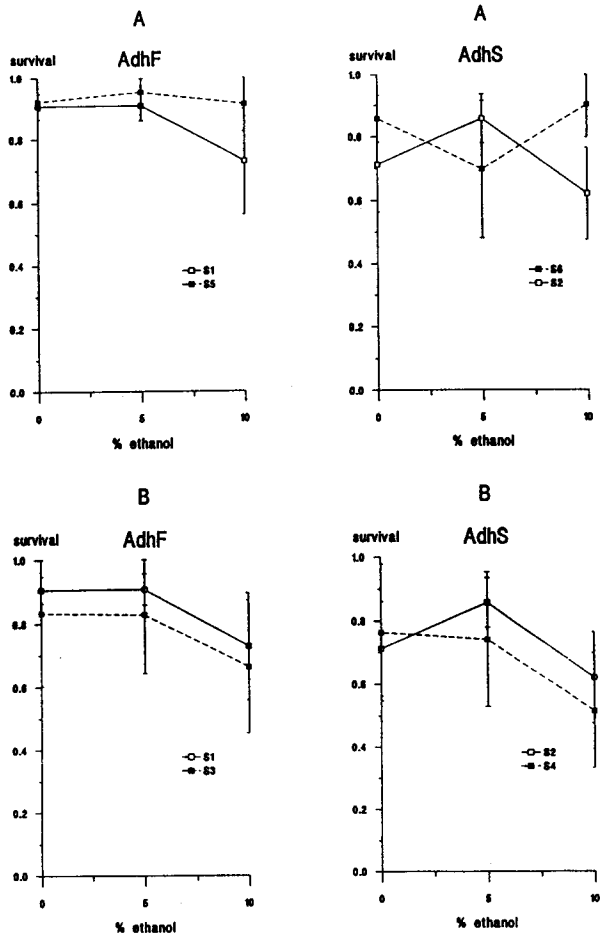

Fig. 4A and B. The effect of ethanol on the egg-to-larva survival. The points are predicted values together with their standard deviations on the basis of the models. For further details see the legend of Fig. 2.

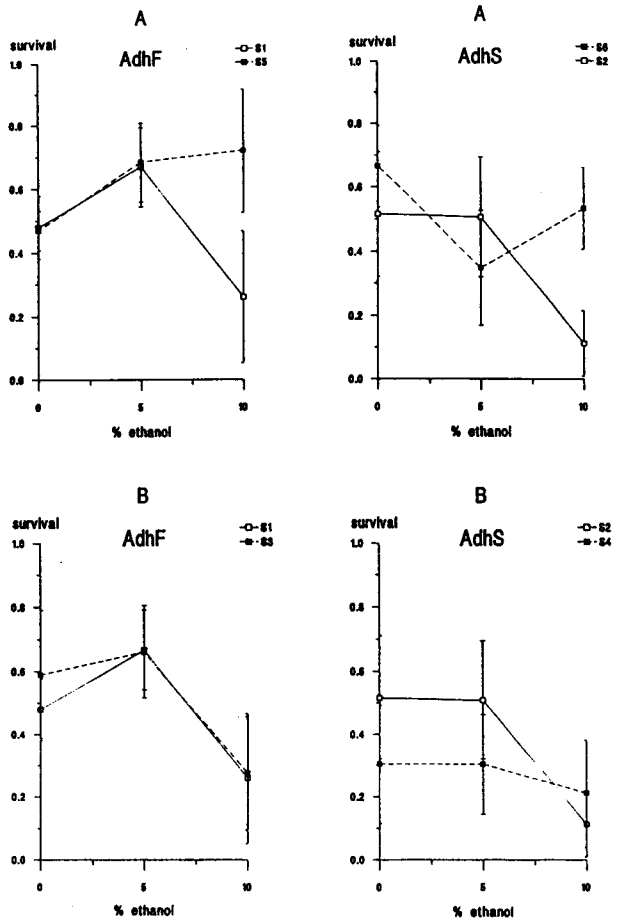

Fig. 5A and B. The effect of ethanol on the egg-to-pupa survival. The points are predicted values together with their standard deviations on the basis of the models. For further details see the legend of Fig. 2.

SANCHEZ-CANETE et al. (1986) have found that the critical ethanol concentration where the utilization of ethanol is shifted to resistance to it lies between 5 and 7.5 %; at levels higher than this concentration, ethanol appears to be toxic rather than nutritive for the larvae. Our results on egg-to-pupa survival also suggest that the critical concentration lies between 5 % and 10 % (Fig. 5). In accordance with the studies of HEINSTRA et al. (1983), GEER et al. (1985), ANDERSON and BARNETT (1991) and BARBANCHO (1992) certain elements of our results also indicate that different genetic factors are responsible for the utilization and detoxification of ethanol in the larvae. In the analysis of the genetic background, we found that at low concentrations, when utilization is assumed to be the crucial process, differences in larval survival were larger between the two *Adh* genotypes than between the two lines (Fig. 5A: 0 and 5 %). This indicates that *Adh* might be an important genetic factor in the utilization of ethanol. At high concentrations, however, when the resistance to the toxic

effect of enthanol becomes the dominating trait, larval survival of the two lines differed to a greater extent compared to that of the two *Adh* genotypes (Fig. 5A: 10 %). It implies that resistance to ethanol mostly depends on unknown factors in the genetic background. This shift in the significance of the genetic factors around the critical ethanol concentration was not observed in the analysis of the *Odh* locus (Fig. 5B). This suggests that the *Odh* locus is neither directly involved in the utilization nor the detoxification of ethanol.

There is no consensus in the literature (reviewed by GEER et al., 1993) on the significance of ADH in alcohol tolerance and on the importance of the polymorphism at the genetic locus of this enzyme in adaptation to environmental ethanol. In certain studies, ethanol tolerance of the Adh^F genotypes was higher than that of the Adh^S genotypes (BRISCOE et al. 1975; HICKEY and MCLEAN 1980). In other surveys, however, ethanol tolerance proved to be independent of the genotypic composition at the *Adh* locus (MCKENZIE and PARSONS 1974; OAKESHOTT et al. 1984; BARBANCHO et al., 1987). At the same time, molecular data indicate that the *Adh* polymorphism is maintained by balancing selection (KREITMAN and HUDSON 1991). It seems, therefore, quite plausible that the *Adh* locus is essential in other physiological processes rather than in tolerance to high ethanol concentration (OUDMAN et al. 1991). Summarizing our results, we can conclude that the differences between the *Adh* genotypes were significant for all investigated fitness components except for mating success (Appendix 1 and 2: Adh). The greatest differences between the two *Adh* genotypes were detected in their fecundities.

The effect of *Odh* and genetic background was analyzed relative to *Adh*. The relative significance of

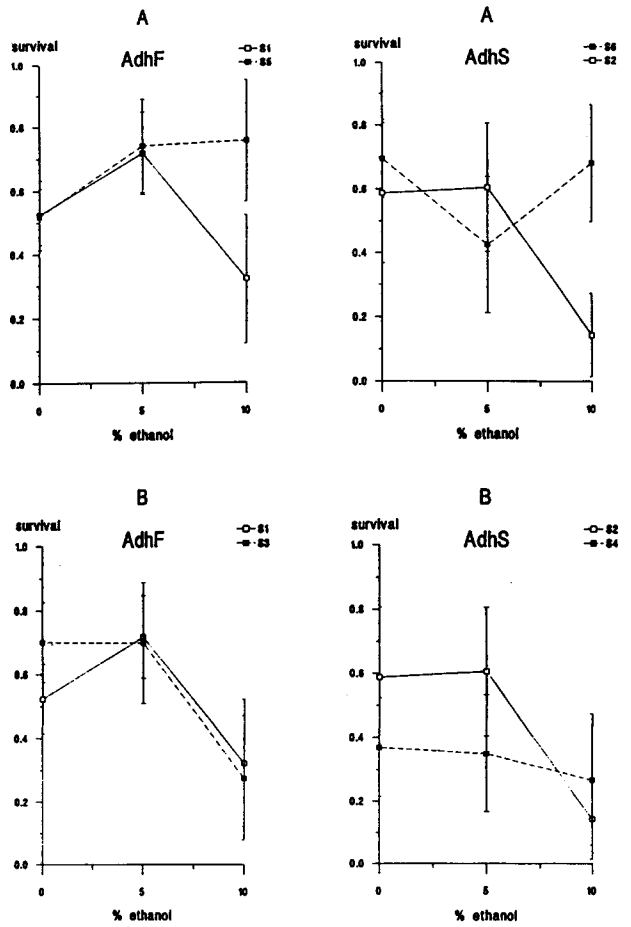

Fig. 6A and B. The effect of ethanol on the egg-to-adult survival. The points are predicted values together with their standard deviations on the basis of the models. For further details see the legend of Fig. 2.

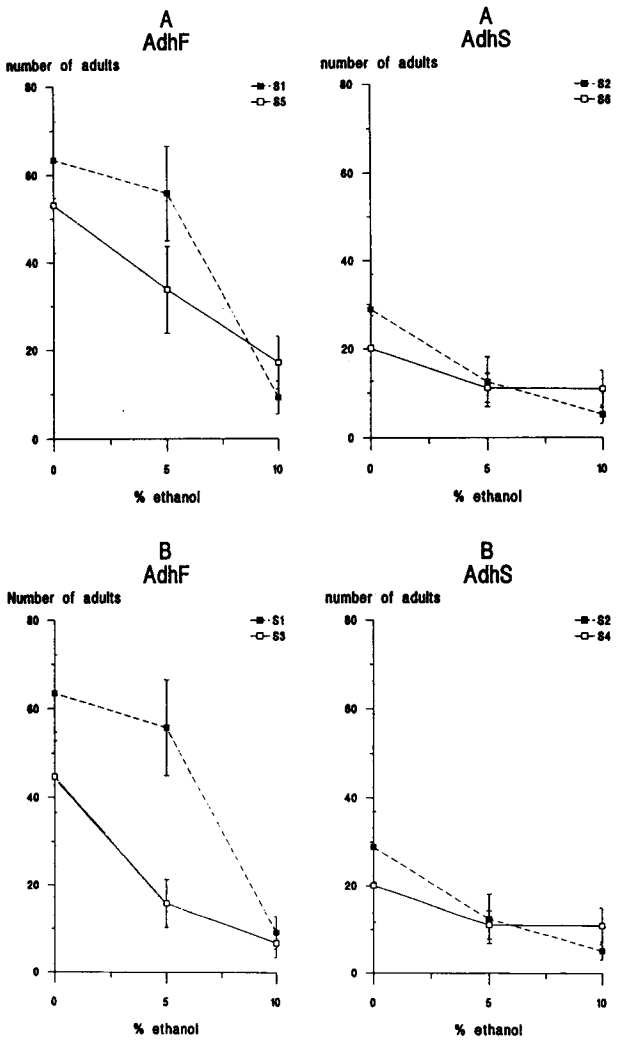

Fig. 7A and B. The effect of ethanol on the number of emerging adults. The points are predicted values together with their standard deviations on the basis of the co-deviance models. For further details see the legend of Fig. 2.

the *Odh* locus and genetic background exhibited different patterns for the different fertility and viability components. In fecundity the significance of the genetic background relative to *Adh* differed from that of the *Odh*. Specifically, the effect of *Odh* locus was higher than that of the genetic background. In addition, the interaction between the *Adh* locus and the genetic background was negligible while that of between the *Adh* and *Odh* loci was sizable. It implies that the effect of the *Adh* locus on fecundity greatly depended on other genetic factors. One of these factors seems to be the *Odh* locus. As the physiological role of the ODH enzyme is poorly understood, we are unable to explain the differences between the two *Odh* genotypes in their fecundities. Nevertheless, our results indicate that ODH might influence egg production.

All survival components exhibited a similar pattern in the significance of the *Odh* locus and genetic background relative to *Adh*. The differences between the two lines and between the two *Adh* genotypes accounted for an equal portion of the explained, while the effect of the *Odh* locus was marginal on the different survival components. Yet, the differences between the two *Odh* genotypes in their egg-to-pupa and egg-to-adult survival were significant (Appendix 2).

The analysis of the number of emerging adults enabled us to study the significance of the investigated fitness components relative to each other. The distribution of explained deviance among the different factors for the number of adults was similar to that for the number of eggs in both sets of experiments (Tables 1 and 2). This indicates that the number of adults which can be considered as the net result of all fitness components mostly depended on fecundity.

Considering the effect of ethanol on different fitness components, however, we can refine this general pattern. In most strains, survival components were not significantly affected by ethanol (Table 3). Thus in these strains (i.e. Adh^F-L1, Adh^S-L2, Adh^F-Odh^{Fu}, Adh^S-Odh^{Fu}), the slope estimated for the number of adults were mostly determined by the slope estimated for the number of eggs. In contrast, the slopes of egg-to-pupa and consequently egg-to-adult survival was highly significant for strains 5 (Adh^F-L2) and 2 (Adh^S-L1) resulting in great difference between the slopes of the number of eggs and adults for both strains.

In summary, the contribution of the genetic factors to the variation in the different fitness components varied greatly. *Adh* had a strong effect on all investigated components except for matting success. The influence of the genetic background was most pronounced on the different survival components. The interaction between the *Adh* locus and the genetic background only accounted for a small portion of the explained deviance. In contrast, the influence of the *Odh* locus was mostly expressed through the *Adh*-*Odh* interaction.

At a lower ethanol concentration—5 % can be considered as the maximum concentration in nature—the fecundity of the Adh^F genotype was only superior to Adh^S when the strain was monomorphic for the Odh^F allele (Fig. 3); all survival components were slightly higher for the Adh^F genotypes compared to the Adh^S regardless of the differences in the genetic background or in the genetic composition at the *Odh* locus (Fig. 4–6).

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APPENDIX A

Deviance analyses of the genetic background for the investigated fertility and viability components. Ethanol treatment was considered as a factor in the analysis of the mating success while in all other analyses (number of eggs, all three viability components and number of adults), it was considered as an independent variable. For test of significance, the change in deviance (CD) was compared to the chi-square distribution for. E—ethanol treatments; Adh—*Adh* genotypes; L—lines; df—degrees of freedom; *—significant at 0.05 level; **—significant at 0.01 level; ***—significant at 0.001 level

Factor	Mating success		Number of eggs		Survival					
						Egg-to-larva	Egg-to-pupa	Egg-to-adult	Number of adults	
	df	CD	df	CD	df	CD	CD	CD	df	CD
E	3	158.7***	1	139.1***	1	7.9***	0.5	1.5	1	94.5***
Adh	1	15.5***	1	110.7***	1	33.9***	1.1	2.7	1	94.4***
E.Adh	3	0	1	13.9***	1	2.8	9.7**	13.3***	1	0.3
L	1	60.2***	1	16.7***	1	9.6**	4.0*	4.4*	1	4.3*
E.L	3	0	1	0.2	1	3.5	12.6***	12.2***	1	4.1*
Adh.L	1	1.6	1	0.2	1	0	0.3	0.7	1	0
E.Adh.L	3	0	1	3.4	1	0.8	0	0.3	1	1.7
Residual	144	148.5	112	105.7	112	111.8	123.2	121.8	106	102.3

APPENDIX B

Deviance analyses of the *Odh* genotypes for the investigated fertility and viability components. Ethanol treatment was considered as a factor in the analysis of the mating success while in all other analysis (number of eggs, all three viability components and number of adults), it was considered as an independent variable. For test of significance, the change in deviance (CD) was compared to the chi-square distribution. E—ethanol treatments; Adh—*Adh* geonotypes; Odh—*Odh* genotypes; df—degrees of freedom; *—significant at 0.05 level; **—significant at 0.01 level; ***—significant at 0.001 level

Factor	Mating success		Number of eggs		Survival					
						Egg-to-larva	Egg-to-pupa	Egg-to-adult	Number of adults	
	df	CD	df	CD	df	CD	CD	CD	df	CD
E	3	84.1***	1	136.1***	1	11.6***	12.5***	11.6***	1	120.7***
Adh	1	0	1	26.4***	1	21.0***	16.4***	20.6***	1	62.1***
E.Adh	3	9.8**	1	3.5	1	0.5	1.6	2.2	1	0.1
Odh	1	60.3***	1	22.8***	1	2.2	0.1	0.5	1	16.9***
E.Odh	3	0.6	1	0.1	1	0.1	0	0.3	1	3.0
Adh.Odh	1	4.7*	1	43.5***	1	2.8	6.8**	5.6*	1	11.2***
E.Adh.Odh	3	0	1	0.1	1	1.1	5.6*	3.6	1	0
Residual	136	127.9	133	41.3	108	105.2	120.7	120.7	97	99.3