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The influence of the *Odh-Aldox* region of the third chromosome on the response of *Drosophila melanogaster* to environmental alcohol

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Second instar larvae of *Drosophila melanogaster* were exposed to exogenous alcohol, which is known to influence the activities of several enzymes. In this study, the activity changes were followed in four enzymes (ADH, ODH, αGPDH and AOX) during ethanol exposure and compared in three inbred lines that had different allelic combinations at the *Odh* and *Aldox* loci. The results indicate that the *Odh-Aldox* region of the third chromosome may alter the general response to ethanol. The activity of ADH increased considerably in two strains in the larval stages in the presence of alcohol; nevertheless, strain 1, with the Odh^S-$Aldox^F$ allelic combination, showed a delay in the ADH induction compared to strain 2, which had the Odh^F-$Aldox^S$ combination. In strain 3 (Odh^{S*}-$Aldox^S$) larvae, ADH induction by environmental ethanol was not detected. Moreover, the activities of αGPDH and AOX in strains 2 and 3 were not affected by ethanol. In contrast, the activities of all four enzymes in strain 1 changed after exposure to ethanol.

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Since many *Drosophila* species feed on fermenting and rotting fruits, low concentrations of ethanol and other short chain alcohols are commonly present in their natural environments (GIBSON et al. 1981; MCKECHNIE and MORGAN 1982). The metabolism of external alcohols preliminarily depends on a single enzyme, alcohol dehydrogenase (ADH) (CLARKE 1975; DAVID et al. 1976). The *Adh* locus (Table 1), which codes for the enzyme, is polymorphic in *Drosophila melanogaster* populations, and has two common codominant alleles: *S* and *F* (GRELL et al. 1965; OAKESHOTT et al. 1982).

Several studies have shown that the alcohol tolerance of the files depends on their *Adh* genotypes; Adh^{FF} homozygotes are the most tolerant (BRISCOE et al. 1975; KAMPING and VAN DELDEN 1978; CAVENER and CLEGG 1978, 1981; VAN DELDEN 1982; VAN HERREWEGE and DAVID 1984). Other evidence, however, indicates that tolerance to ethanol cannot be explained solely by differences in ADH allozymes. Increase in alcohol tolerance is not invariably associated with an increase in the ADH activity of the flies or with an increase in the Adh^F allele frequency in natural populations (OAKESHOTT et al. 1984; BARBANCHO et al. 1987; MERCOT and MASSAAD 1989). Adaptation to ethanol is a very complex process and the induction of ADH, which occurs in the larval life stages (GEER et al. 1993 and references therein), is only one of the mechanisms involved.

In *Drosophila melanogaster*, there is another enzyme, octanol dehydrogenase (ODH), whose in vitro substrates are known to be hydrophobic alcohols (SIEBER et al. 1972). The *Odh* locus (Table 1) is also polymorphic in natural populations, with two common alleles: also called *S* (Slow) and *F* (Fast). Little information about the physiological role of the enzyme exists. MADHAVAN and his coworkers (1973) suggested that the octanol dehydrogenase may be involved in juvenile hormone degradation. DANIELSSON et al. (1994) have lately identified *Drosophila* ODH as a typical class III (medium chain) alcohol dehydrogenase. They suggest that ODH might be involved in glutathion-dependent elimination of formaldehyde.

Table 1. Enzymes assayed in this study

Enzyme	Abbreviation	E.C. number	Genetic locus	Map position
Alcohol dehydrogenase	ADH	1.1.1.1	*Adh*	2–50.1
Aldehyde oxidase	AOX	1.2.3.1	*Aldox*	3–56.6
α-Glycerol-3-phosphate dehydrogenase	αGPDH	1.1.1.8	*αGpdh*	2–20.5
Octanol dehydrogenase	ODH	1.1.1.73	*Odh*	3–49.1

(DICKINSON and SULLIVAN 1975; O'BRIEN and MACINTYRE 1978)

When polymorphic populations were grown on ethanol supplemented medium, the *Odh-*^{S} allele frequency almost doubled in a few generations (PECSENYE and LÖRINCZ 1988), suggesting that alcohol stress can cause gene frequency changes at the *Odh* locus. This finding was especially interesting since ethanol is hardly a substrate for ODH. Moreover, the response appeared to result from an interaction between the effect of selection at the *Adh* and *Odh* loci. When the *Adh* locus was monomorphic, allelic frequencies changed at the *Odh* locus in response to exogenously applied ethanol. However, in populations which were polymorphic at both loci, selective changes could only be detected at the *Adh* locus (PECSENYE, unpublished data).

In *Drosophila* ethanol can be used as an energy source which, via the metabolic pathways, is converted either to carbon dioxide for immediate energy utilisation, or to fatty acids for energy storage (DAVID et al. 1976; DELTOMBE-LIETAERT et al. 1979; MIDDLETON and KACSER 1983; GEER et al. 1985). However, ethanol is also a toxic agent, which appears to inhibit the flies' ability to respire. In this case, the primary role of the metabolic pathways is alcohol detoxification (VAN HERREWEGE and DAVID 1984; HEINSTRA et al 1987; DAVID 1988). Although ADH is a key enzyme in both energy metabolism and alcohol detoxification, it cannot be said that environmental ethanol is a selective force acting simply and directly on the *Adh* genotypes. Evidence is accumulating which shows that alcohol stress influences the entire larval energy metabolism, altering several enzyme activities (GEER et al 1983; MCELFRESH and MCDONALD 1983; MCKECHNIE and GEER 1984).

In this series of experiments, we investigated the effect of the ethanol treatment on the activites of four enzymes (Table 1) in *Drosophila melanogaster* in different developmental stages. We also studied the modifying effect of the *Odh-Aldox* region of the III chromosome on the general response to environmental ethanol. In addition, we tried to establish whether there is evidence for functional metabolic relationships between the alcohol and octanol dehydrogenases during ethanol stress.

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) maintained at the Umeå Drosophila Stock Center. Strain 1 has the *S* allele at the *Odh* locus and the *F* allele at the *Aldox* locus, strain 2 has the *F* allele at the *Odh* locus and the *S* allele at *Aldox*, while strain 3 has a new allele (designated *S**) at the *Odh* locus, and the *S* allele at *Aldox*. These two loci are closely linked, with 7.5 map units between them (Table 1). All three strains carried the *In(2L)t* inversion, which fixed the *Adh*^{S}-*αGpdh*^{F} allele combination on their second chromosomes.

Culture conditions. — Before the experiment, the strains were kept in mass cultures at 18°C and approximately 63 % relative humidity on standard cornmeal molasses medium, in continuous light. 1 l cornmeal molasses medium consisted of 72 g maize flour, 21 g agar, 6 g dead yeast, 75 g sucrose, and 4 ml propionic acid. Flies raised on this regular medium were allowed to lay eggs for four days and then II instar larvae (approximately 4 days old) were collected. These larvae were then subjected to one of two treatments. In treatment 1, 20 larvae were put into vials containing 5 ml of normal potato-mash molasses medium (N). In treatment 2, another 20 larvae were placed on ethanol supple-

mented potato-mash molasses medium (NE). 1 l of this medium contained 40 g potato-mash, 10 g agar, 15 g dead yeast, 75 g sucrose, 1 g ascorbic acid and 2 ml propionic acid. The ethanol supplemented food was prepared from normal medium which had been cooled to 45–50°C, after which ethanol was added to a concentration of 5 %. Five samples were studied in different developmental stages on both media: early III instar (L1), late III instar (L2), early pupa (P1), late pupa (P2), and 7 day old females (o). Three parallel vials were set up for all five samples of all three strains growing on both media.

Enzyme assays. — Four enzymes were followed at all developmental stages (Table 1): alcohol dehydrogenase (ADH), octanol dehydrogenase (ODH), α-glycerophophate dehydrogenase (α-GPDH), and aldehyde oxidase (AOX).

Since there is some overlap in the substrate specificities of ADH and ODH, spectrophotometric assays do not provide independent measurements of ADH and ODH activities. The enzyme activities were therefore measured on starch gels after the separation of the proteins by gel electrophoresis.

The sampling procedure was identical in all cases. 5–10 larvae, pupae, or adults were washed and dried carefully and their weights measured. 10 μl of the following buffer were added to each mg of living material: 0.01 M Tris-HCl (pH = 7.5), 0.001 M EDTA (pH = 8.0), 2 mg/ml Dithiothreitol. After homogenization, the samples were centrifuged (10 min, 10,000 rpm, 4°C) and 10 μl of the supernatant were applied on the gels. In this way the enzyme activities of 1 mg living material were directly compared. Separate homogenates were prepared from the three parallel vials of each strain, and two samples of each supernatant were applied to each gel; i.e., the six estimates of enzyme activity were collected for each strain and life history combination from the same gel. Samples from each of the two different treatments were run on parallel gels on the same day.

The starch gel electrophoresis was always carried out in exactly the same way: electrode buffer: 0.05 M Tris-HCl (pH = 8.6); gel buffer: 1:5 dilution of the electrode buffer. Running conditions were: 15 V/cm, 6–7°C, 6 h. After electrophoresis, the gels were sliced and 3 different slices were systematically stained for the four different enzyme systems. The conditions of the staining procedure were strictly controlled. *ADH and ODH assays*: 0.3 M Tris HCl (pH = 8.5), 0.001 M EDTA, 0.6 mM NAD, 0.25 mM nitro blue tetrazolium (NBT), 0.1 mM phenazine methosulfate (PMS), 1 v % ethanol, 3 v % butanol; 60 min at 34°C in the dark. *αGPDH assay*: 0.1 M Tris-HCl (pH = 8.5), 0.001 M EDTA, 0.6 mM NAD, 0.25 mM NBT, 0.1 mM PMS, 12 mM DL-α-glycerophosphate; 25 min at 34°C in the dark. *AOX assay*: 0.1 M Tris-HCl (pH = 8.5), 0.001 M EDTA, 13.4 mM KCl, 0.6 mM NAD, 0.25 mM NBT, 0.1 mM PMS, 1 v % benzaldehyde; 20 min at 22°C in the dark.

After staining, the gels were immersed in distilled water and photographed immediately. The photographic negatives were scanned with a Beckman DU-8 spectrophotometer at 568 nm. The enzyme activities were measured on the basis of the area of the peaks: one unit area defined one unit of enzyme activity.

Statistical procedures. — The data obtained were analysed with generalised linear models — a class of statistical models that is a natural generalisation of classical linear models; e.g., linear regression, analyses of variance, log-linear models, multinomial response models for counts are special cases of generalised linear models (see MCCULLAGH and NELDER 1989). Since generalised linear models do not yet appear to be in common use for biological data, the reviewers have suggested we provide some details about this method of analysis.

Initially, we performed classical analyses of variance, but for all responses in all analyses, a strong positive relationship was found between the sizes of the residuals and the fitted values. Such positive associations between means and variances are not uncommon for sets of variables whose measured values cannot be negative (here enzyme activities and fresh weights). When a relationship of this sort is found in biological data, the assumption of constant variance (homogeneity of variance), which is required for methods of analysis based on least squares estimations, is violated. Some of the consequences of violations are (a) that some treatments having truly different mean responses may fail to be distinguished, while others truly different may be asserted as such; (b) some standard errors will be too small, others too large, giving spurious appearance of precision or leading to a rejection of the experiment; (c) statistical interactions may not imply biological interactions. It has been common practice to find transformations of the response so as to satisfy the homogeneity assumption. Transformation of a response variable, however, is also

a simultaneous transformation of the systematic and random components of a statistical model and often results in a confused mixture of these components. For example, if the data are log transformed, the predicted values (from treatments) and the random errors are multiplied together. Many biological experiments, including those performed here, were designed to be analysed by additive, not multiplicative, models. In addition, transformation means that the data are analysed in units other than those used for measurement, which makes the inferences even more remote from the observations. Thus, in cases where the assumption of homogeneity of variance is patently false, generalised linear models are more appropriate. Assumption of normality and constancy of variance are no longer required, since the way in which the variance depends on the mean is a function of the error distribution.

As a consequence of finding a strong correlation of the means and variances in the response variables in our data, we therefore chose to analyse the data by generalised linear models under the assumption that a constant coefficient of variation, rather than a constant variance, is true. This can be approximated by specifying a gamma error distribution coupled with an inverse link (MCCULLAGH and NELDER 1989). The model (factor abbreviations and degrees of freedom in parentheses) used to analyse these data (see Table 2), consists of the main effects-life history stages (LHS-4, strains (S-2) and food (F-1); all two and three-way interactions (110) among main effects and replicates, and the error term (41) which is the variation among vials. The terms were included sequentially, i.e., the effect of any term is conditional on all those fitted above it in the table. Differences in the degrees of freedom from those appropriate to a complete model are a consequence of missing values; i.e., in Table 2, a complete model should have 63 df for error, in contrast with the actual value of 41. Tests of significance were performed by comparing the ratio of the mean deviances to the appropriate error term, which is the variation among the vials. In order to exclude any factor levels which were significant but did not explain much of the response deviance, and therefore should not be given much weight in our inferences, we applied the Akaike information (ATKINSON 1981) to each of our analyses. The Akaike infor-

Table 2 Analyses of Deviance for the enzymes ADH, ODH, αGPDH, AOX, and fresh weight (FRWT). The mean deviance ratios are the mean changes in deviance divided by the error terms and are compared with an *F*-distribution. Degrees of freedom (df) are for changes in deviance for each factor of the total model. ns = non significant,* = significant at P = 0.05. All other entries are significant at p = 0.001. LHS = life history stage; S = strain; F = food; × implies an interaction between the main factors. Error consists of replications and interactions among the above terms. Akaike information indices are in parentheses. Factors underlined were not considered meaningful even if they were significant since they did not explain much of the response deviances. (+: The df for the error term of FRWT is 20. For explanation, see sampling procedure in Materials and methods)

Factors	df	Mean change in deviance				
		ADH	ODH	αGPDH	AOX	FRWT
LHS	4	3.080 (3.557)	8.017 (24.430)	13.430 (4.632)	11.110 (11.521)	1.850 (3.530)
S	2	0.229 (3.073)	6.027 (12.310)	0.006ns (4.590)	0.024* (11.423)	0.115 (3.279)
S × LHS	8	0.049 (2.757)	0.774 (6.331)	0.287 (2.637)	0.905 (4.340)	0.143 (2.193)
F	1	0.443 (2.221)	1.030 (5.052)	0.038 (2.172)	0.103 (4.052)	0.159 (1.972)
F × LHS	4	0.187 (1.524)	0.467 (3.288)	0.256 (1.253)	0.234 (3.187)	0.155 (1.371)
S × F	2	0.090 (1.308)	0.021* (3.181)	0.074 (1.078)	0.103 (2.929)	0.059 (1.233)
S × F × LHS	8	0.086 (0.698)	0.155 (2.151)	0.0054 (0.721)	0.208 (1.432)	0.075 (0.682)
Error[a]	41	0.00332	0.00899	0.00323	0.00661	0.00222+

[a] Residual mean deviance

mation is:

$$Q = D + \alpha q \phi$$

where D is the residual deviance, α is a constant, in this case 4 (see MCCULLAGH and NELDER 1989, 90), q is the number of parameters fitted, and ϕ is the residual mean deviance. This information index was applied sequentially to each factor of the model until a minimum had been obtained. Additional factors, significant or otherwise, which caused Q to increase again, were not considered in our inferences. Several figures are presented in this paper which contain predicted mean values and their estimated standard errors. These predicted values are the expected means and standard errors under the model outlined in Table 2 and the standard errors have an extra degree of approximation since the model is non-linear. All arithmetic was performed using Genstat, release 4.04.

Results

Fresh weight

On normal medium, the changes in the fresh weight during most of the investigated period were similar in the three strains (Fig. 1). The late III instar larvae were the heaviest (1.55–1.90 mg/individual). The average fresh weight then decreased until the late pupal stage and remained constant in the 7-day old females. The changes in fresh weight observed over all life history stages differed significantly among the three strains (Table 2: S × LHS): in strains 1 and 3, the weight decreased in the early pupa (P1), while in strain 2 the decrease occurred in the late pupa (P2 in Fig. 1).

Although Table 2 (S × F) shows significant differences in the influence of the alcohol treatment on the fresh weight among the three strains, the Akaike information indices indicate that these differences are not remarkable. In addition, we observed that 5 % ethanol in the medium did not decrease the larva-to-adult survival of either of the strains.

ADH activity

The alcohol dehydrogenase activity had a very characteristic profile during the developmental stages (Fig. 2, Table 2: LHS). It increased in the larval samples and reached a maximum in the late III instar. The activity then decreased considerably until the late pupal stage and increased again in the adults. Our findings are in a good agreement with other results for the developmental profile of ADH (DICKINSON and SULLIVAN 1975; O'BRIEN and MACINTYRE 1978; MARONI and STAMEY 1983). The developmental patterns for the ADH activity in the three strains were rather similar, although the magnitude of the changes was slightly larger in strain 2 than in the other two (Table 2: S × LHS). ADH activity was slightly but significantly higher in strain 2 than in strains 1 and 3 (Fig. 2). This difference among the strains was mostly due to the adult ADH activities: strain 2 had the highest activity, more than 20 % higher than that of strain 1, while the activity in strain 3 was intermediate (Fig. 2).

In the presence of alcohol, the larval activity of ADH increased in strains 1 and 2 compared to the normal conditions. In the alcohol treated larvae, ADH activity was 60 % and 30 % higher than the normal activity in strain 1 (L1) and strain 2(L2), respectively (Fig. 2). This alcohol-induced increase of the ADH activity, however, was not observed in strain 3 larvae (Table 2: S × F). The alcohol treatment did not change the ADH activity in the adults.

ODH activity

The ODH activities were approximately 10 times lower than those measured for ADH. Nevertheless, the pattern of changes in the activities of the two enzymes were similar over all life history stages (Fig. 3). There were considerable differences in the octanol dehydrogenase activities among the three strains (Table 2: S). The activity was more than 70 % higher in strains 1 and 3 than in strain 2 on normal media (Fig. 3).

The influence of alcohol on the ODH activity also differed in the strains. In strain 2, the alcohol treatment did not affect the ODH activity. In strains 1 and 3, however, the enzyme activity was significantly higher in the larval (strain 1: L2, strain 3: L1) and adult stages (Fig. 3).

αGPDH activity

αGPDH is an important enzyme in insect energy metabolism. The α-glycerophosphate shuttle provides the adult flies with ATP during flight. In the larvae, however, it has another function; it is a key enzyme in fatty acid synthesis (GEER et al. 1983; BEENAKKERS et al. 1985).

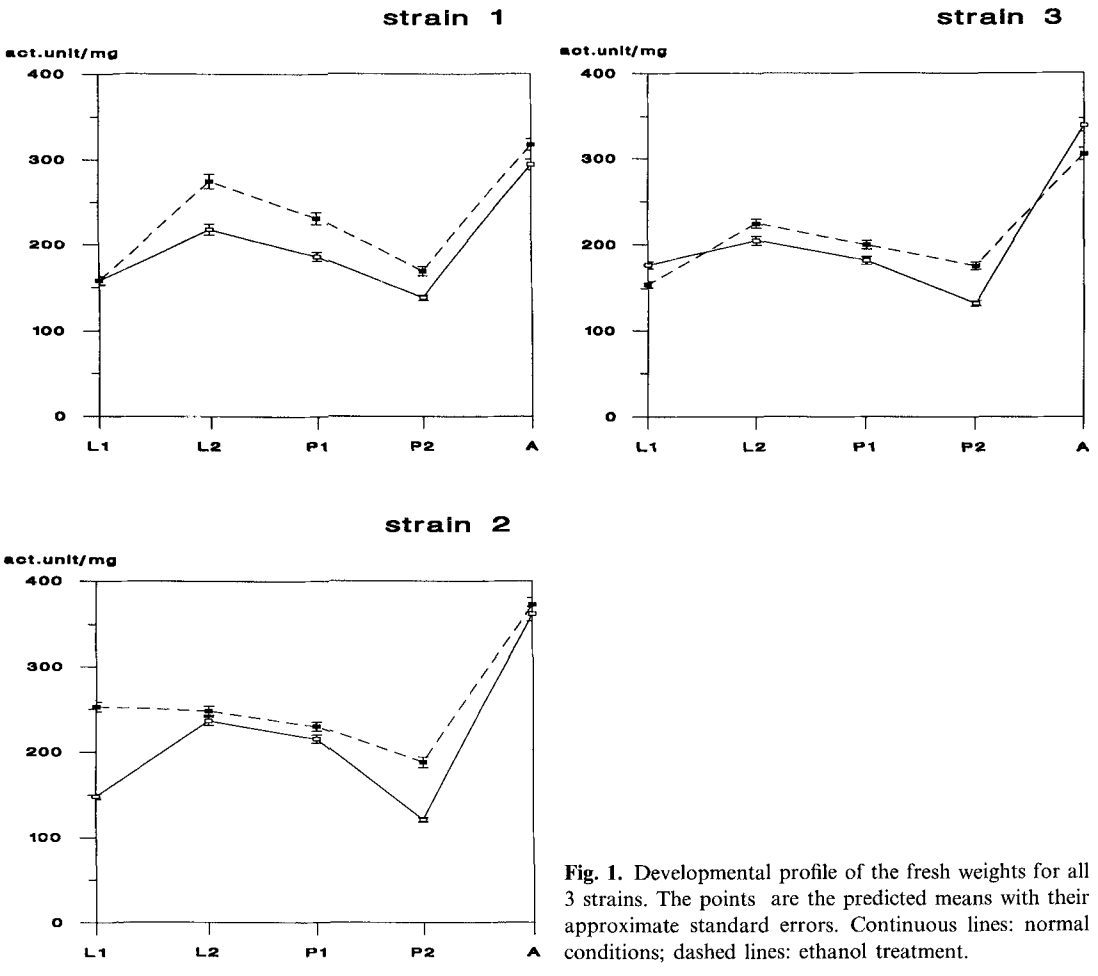

Fig. 1. Developmental profile of the fresh weights for all 3 strains. The points are the predicted means with their approximate standard errors. Continuous lines: normal conditions; dashed lines: ethanol treatment.

The αGPDH had a low activity in the larval stages but its level increased dramatically in the adults (Table 2: LHS, Fig. 4). This result is in a good agreement with other observations (DICKINSON and SULLIVAN 1975; O'BRIEN and MACINTYRE 1978). In normal conditions, there were no significant differences among the activities of the three strains (Table 2: S), although, in the adults the αGPDH activity was approximately 16 % and 25 % higher in strains 2 and 3, respectively, than that of strain 1 (Fig. 4).

In strains 2 and 3, the developmental profile of the αGPDH did not change under ethanol treatment compared to the control (Fig. 4). In strain 1, a slight increase of the activity appeared from the late III instar to the adult stages (Fig. 4).

AOX activity

On normal medium, the developmental profile of AOX was quite similar in the three strains. The enzyme activity increased continuously during the investigated period (Fig. 5). The only remarkable difference among the three strains occurred in the adult stage: the AOX activity in strains 2 and 3 (AOX-S allozyme) was approximately 30 % higher than in strain 1 (AOX-F allozyme).

The influence of ethanol stress on the AOX activity was rather different in the three strains (Fig. 5). The level of activity in strains 2 and 3 did not seem to be affected by the ethanol treatment, while in strain 1, the AOX activity increased considerably in the alcohol treated late III instar larvae compared to the controls (Fig. 5).

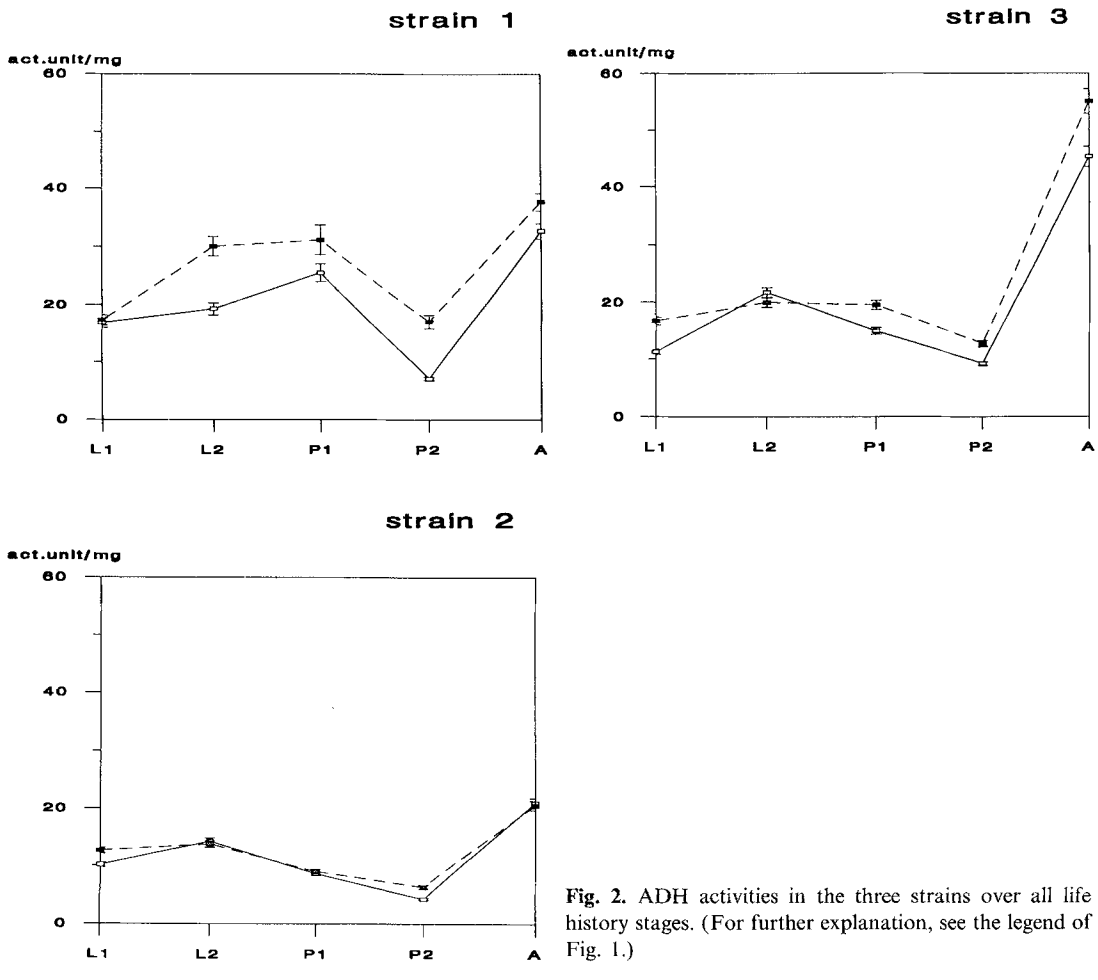

Fig. 2. ADH activities in the three strains over all life history stages. (For further explanation, see the legend of Fig. 1.)

Discussion

Several authors have reported that the ADH activity of the III instar larvae increases during ethanol stress (MCKECHNIE and GEER 1984; KERVER and VAN DELDEN 1985; KAPOUN et al. 1990). It is also known that the increase in the ADH activity is mostly due to an increase in the number of ADH molecules, resulting from increased rates of ADH protein synthesis (GEER et al. 1988). In two of our three strains, the ADH activity was also increased when the larvae were kept on ethanol supplemented medium. It seems, however, that the ADH activity of strain 3 did not change in response to exogenous ethanol (Fig. 2). In addition, our data indicate that there is a difference in the ADH induction between the two inducible strains: in strain 1, it occurs later than in strain 2. The alcohol-induced increase of the ADH activity does not last long. It returns to the control level after 3 days in strain 2 (Fig. 2: L2 sample), while strain 1 levels off to the normal activity 10 days after the induction (Fig. 2: P2 sample). Thus, strain 1 shows a delay not only in the ADH induction but also in the decrease of the ADH activity to the normal level. Certain loci on the third chromosome have been shown to influence the ADH activity of the adult flies (KING and MCDONALD 1987; MERCOT and MASSAAD 1989). However, it is not yet known how the dynamics of ADH induction are affected by other genes. Our data show that the region of *Odh-Aldox* genes on the third chromosome may have an influence on this process.

The ODH enzyme has not been studied in the experiments designed to investigate the physiological and biochemical consequences of ethanol stress.

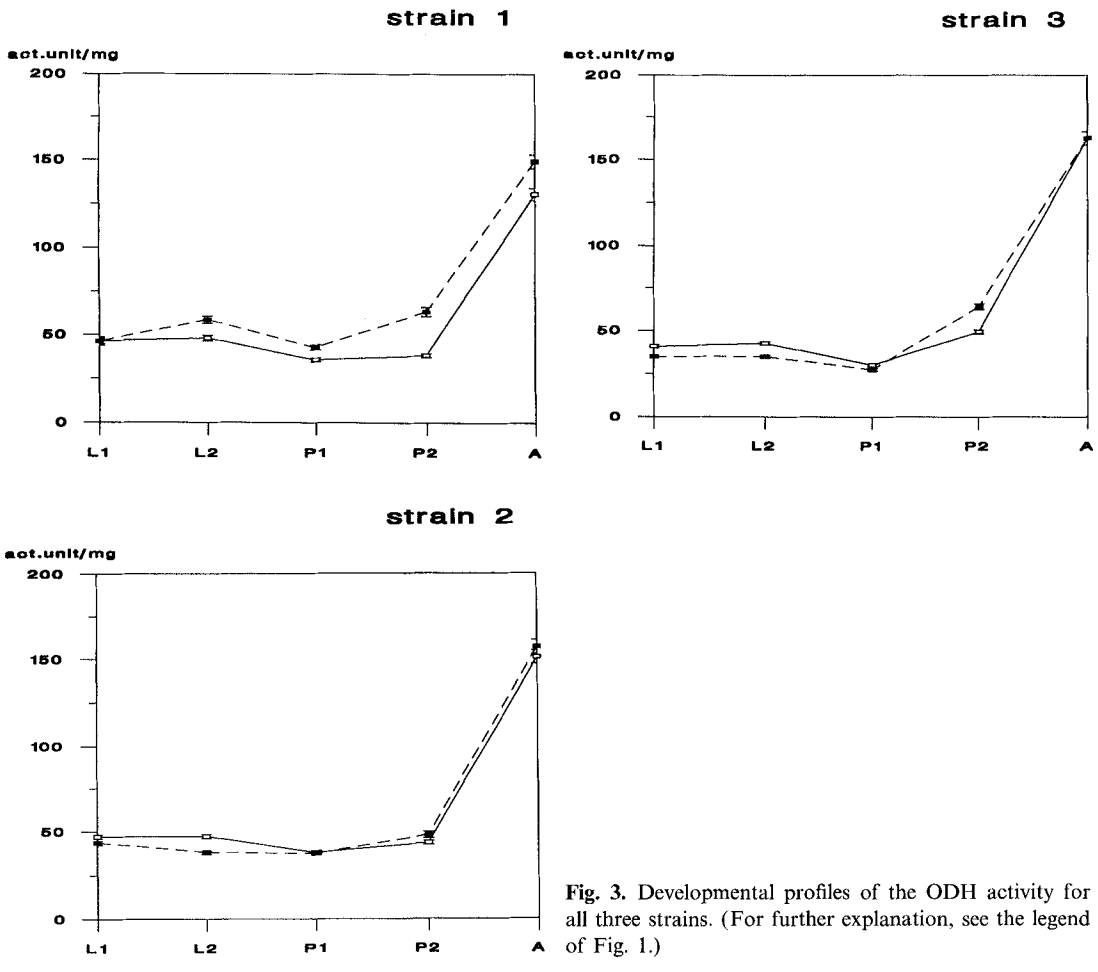

Fig. 3. Developmental profiles of the ODH activity for all three strains. (For further explanation, see the legend of Fig. 1.)

PECSENYE and LÖRINCZ (1988) and PECSENYE (1989) have shown that ethanol may also act selectively on the *Odh* locus. The results of this investigation appear to be consistent with this observation, since the three ODH allozymes were found to be affected in different ways during the ethanol treatment. The activity of the ODH-F allozyme, measured in the presence of alcohol, did not differ from that of the control. The activity of the ODH-S allozyme was, however, slightly increased in all the developmental stages when the medium was supplemented with ethanol. ODH-S* allozyme showed an intermediate response to ethanol: increased activities were mainly observed in the larval and adult stages (Fig. 3). It is not yet clear how the ethanol treatment affects the activity of ODH, since the exact physiological role of this enzyme is not known. Nevertheless, we showed that the ODH activity can be changed by using ethanol in the medium and that the two allozymes have different responses to the alcohol treatment.

When ethanol is present at a low concentration, it is used as an energy source and much of its carbon is incorporated into lipids. The αGPDH activity is associated with lipid synthesis in larvae (O'BRIEN and MACINTYRE 1972). GEER et al. (1983) have shown that the αGPDH is sensitive to ethanol stress in the late III instar larvae. HEINSTRA et al. (1990) have found an interaction between alcohol degradation and the glycerol-3-phosphate cycle. Our results are partly in agreement with these studies. In strain 1 we found a slight but significant increase of the αGPDH activity in the presence of ethanol. This increase appeared 6 days after the treatment had started, i.e., when the ADH was induced in this strain (Fig. 4:

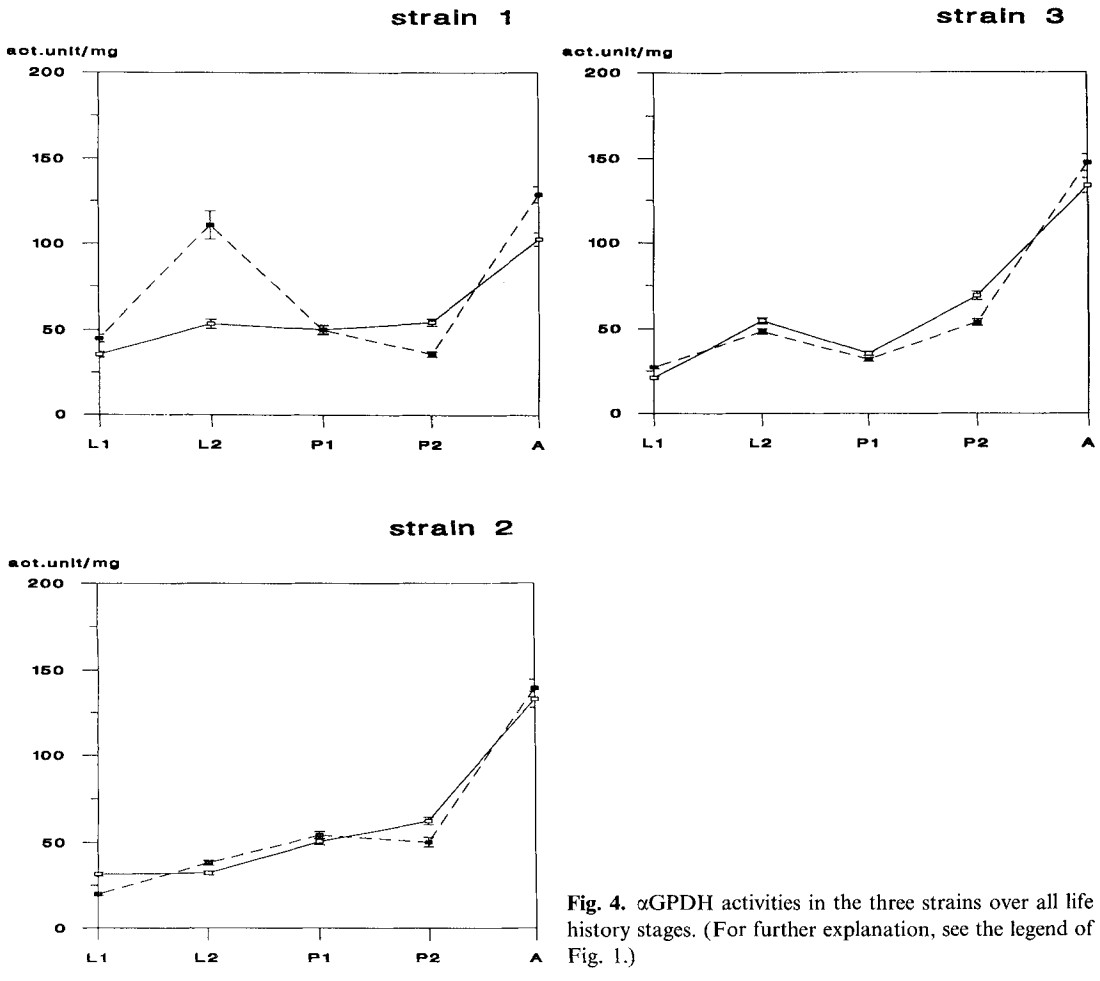

Fig. 4. αGPDH activities in the three strains over all life history stages. (For further explanation, see the legend of Fig. 1.)

L2 sample). In strains 2 and 3, however, the αGPDH activity was not influenced when the larvae were put on ethanol supplemented medium. This between-strain difference in αGPDH activity during the alcohol treatment is remarkable, since all three have the αGPDH-F allozyme.

In vitro aldehyde oxidase can use acetaldehyde as a substrate (DICKINSON and SULLIVAN 1975). Hence, it was believed that this enzyme detoxifies acetaldehyde, the product of ethanol conversion, in vivo as well. This hypothesis was clearly ruled out by the analysis of *Aldox* null mutants, which are able to metabolise both ethanol and acetaldehyde (DAVID et al. 1978). EISSES (1989) and HEINSTRA et al. (1983) have demonstrated that the two subsequent conversions (ethanol → acetaldehyde → acetate) in the first stages of the ethanol metabolism are both catalysed by alcohol dehydrogenase. Thus the in vivo function of the aldehyde oxidase is at present unknown.

MCKECHNIE and GEER (1984) showed that the AOX activity of the III instar larvae decreased slightly during alcohol stress, when the sugar concentration was high in the medium. In our experiment, the AOX activity in strains 2 and 3 did not differ between the alcohol treated and the normal samples. It thus appears that the activity of the AOX-S allozyme is not affected by the ethanol treatment. However, the response to exogenous alcohol is strikingly different in strain 1 (AOX-F allozyme). In the ethanol-treated late III instar larvae, we measured 100 % higher activity than in the controls (Fig. 5: L2 sample). This considerable increase in AOX activity occurred at the same time as the ADH induction (i.e., in L2 sample). As with ADH, the ethanol-induced increase of the AOX

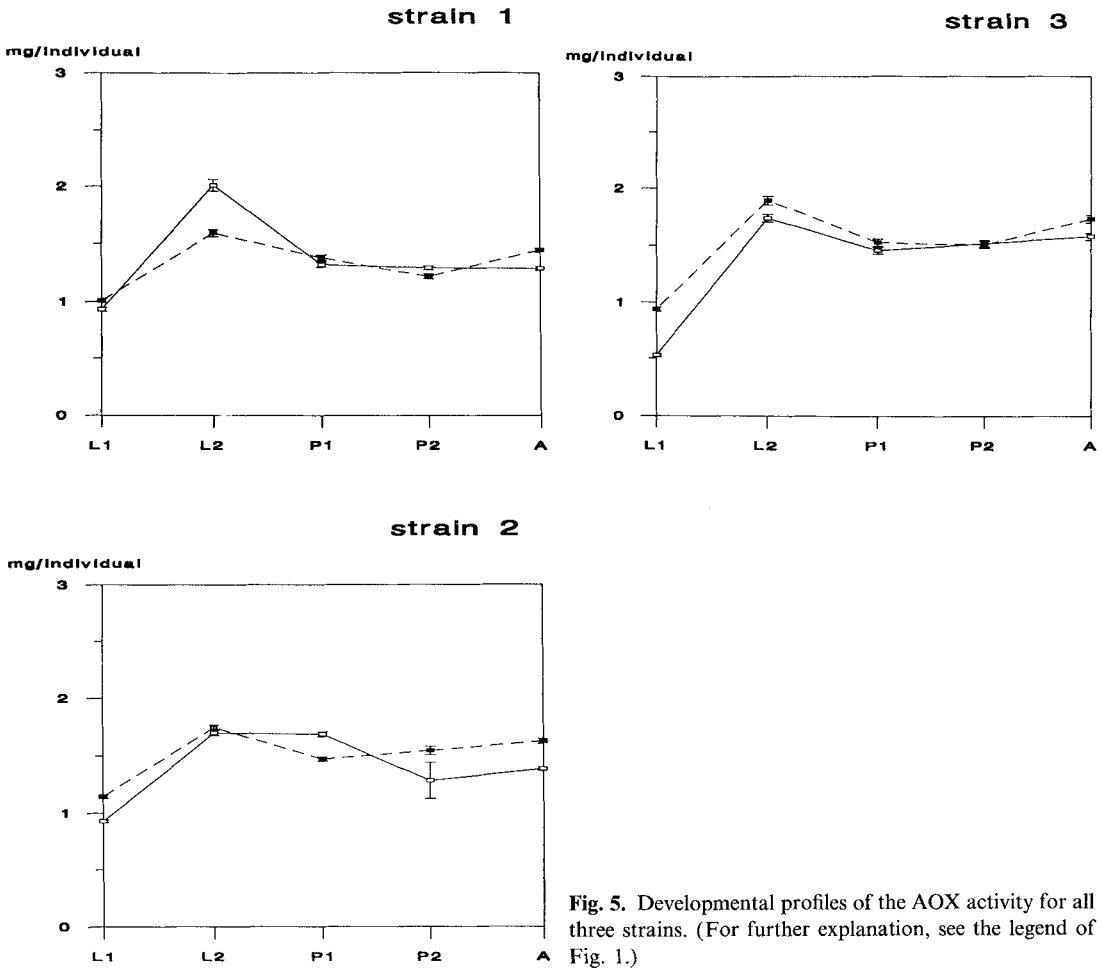

Fig. 5. Developmental profiles of the AOX activity for all three strains. (For further explanation, see the legend of Fig. 1.)

activity also returned to the normal level in samples collected from later life history stages. Both enzymes seem to be affected by alcohol in the larval stages only. While these results do not cast any further light on the physiological role of aldehyde oxidase, it is clear that the response of this enzyme to alcohol is highly allozyme specific.

GEER et al. (1983) and MCKECHNIE and GEER (1984) have shown that the activities of several enzymes are changed during alcohol stress. They have concluded that ethanol stress has a general effect on the metabolism of the larva. This hypothesis is also supported by other evidence (CAVENER and CLEGG 1981; HEINSTRA et al. 1990; GEER et al. 1989). Our results, however, show that the region of the *Odh-Aldox* genes on the third chromosome appear to alter the general response to ethanol. That is, the activities of ODH, αGPDH, and AOX in strain 2, with the Odh^F-$Aldox^S$ allelic combination, were not affected by environmental ethanol. In strain 3 (Odh^{S*}-$Aldox^S$ allelic combination), αGPDH and AOX did not respond to the ethanol treatment. In contrast, strain 1, with the Odh^S-$Aldox^F$ allelic combination, appears to respond generally to ethanol treatment since the activities of all enzymes changed after exposure to exogenous alcohol. Thus the biochemical reactions related to ethanol degradation appear to be complex with the actual response depending on the specific allelic combinations of the loci concerned, suggesting that this region of chromosome III warrants closer investigation.

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References

ATKINSON, A. C. 1981. Likelihood ratios, posterior odds and information criteria. — *J. Econometrics 16*: 15–20

BARBANCHO, M., SANCHEZ-CANETE, F. J. S., DORADO, G and PINEDA, M. 1987. Relation between tolerance to ethanol and alcohol dehydrogenase (ADH) activity in *Drosophila melanogaster*: Selection, genotype and sex effects. — *Heredity 58*: 443–450

BEENAKKERS, A. M. TH., VAN DER HORST, D. J. and VAN MARREWIJK, W. J. A 1985. Biochemical process directed to flight muscle metabolism. — *Comp. Insect Physiol., Biochem. and Pharmacol. 10*: 451–486

BRISCOE, D. A., ROBERTSON, A. and MALPICA, J. 1975. Dominance at *Adh* locus in response of adult *Drosophila melanogaster* to environmental alcohol. — *Nature 255*: 148–149

CAVENER, D. R. and CLEGG, M. T. 1978. Dynamics of correlated genetic systems. IV. Multilocus effects of ethanol stress environment. — *Genetics 90*: 629–644

CAVENER, D. R. and CLEGG, M. T. 1981. Multigenic response to ethanol in *Drosophila melanogaster*. — *Evolution 35*: 1–10

CLARKE, B. 1975. The contribution of ecological genetics to the evolutionary theory: Detecting the direct effects of natural selection on particular polymorphic loci. — *Genetics 79*: 101–113

DANIELSSON, O., ATRIAN, S., LOQUE, T., HJELMQVIST, L., GONZALEZ-DUARTRE, R. and JÖRNVALL, H. 1994. Fundamental molecular differences between alcohol dehydrogenase classes. — *Proc. Natl. Acad. Sci. USA 91*: 4980-4984

DAVID, J. R. 1988. Ethanol adaptation and alcohol dehydrogenase polymorphism in *Drosophila*: From phenotypic functions to genetic structures. — In: *Population Genetics and Evolution* (*ed.* G. DE JONG), *Springer-Verlag, Berlin*, p. 163–172

DAVID, J. R., BOCQUET, C., ARENS, M. and FOUILLET, P. 1976. Biological role of alcohol dehydrogenase in the tolerance of *Drosophila melanogaster* to aliphatic alcohols: Utilization of an ADH-null mutant. — *Biochem. Genet. 14*: 989–997

DAVID, J. R., BOCQUET, C., VAN HERREWEGE, W., FOUILLET, P. and ARENS, M. 1978. Alcohol metabolism in *Drosophila melanogaster*: Uselessness of the most active aldehyde oxidase produced by the *Aldox* locus. — *Biochem. Genet. 16*: 203–211

DELTOMBE-LIETAERT, M. C., DELCOUR, J. D., LENELLE-MONFORT, N. and ELENS, A. 1979. Ethanol metabolism in *Drosophila melanogaster*. — *Experientia 35*: 579–580

DICKINSON, W. J. and SULLIVAN, D. T. 1975. Gene-enzyme systems in *Drosophila*. — *Springer-Verlag, Berlin*

EISSES, K. Th. 1989. On the oxidation of aldehydes by alcohol dehydrogenase of *Drosophila melanogaster*: Evidence for the *gem*-diol as the reacting substrate. — *Bioorg. Chem. 17*: 268–274

GEER, B. W., MCKECHNIE, S. W. and LANGEVIN, M. L. 1983. Regulation of sn-Glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster* larvae by dietary ethanol and sucrose. — *J. Nutr. 113*: 1632–1642

GEER, B. W., LANGEVIN, M. L. and MCKECHNIE, S. W. 1985. Dietary ethanol and lipid synthesis in *Drosophila melanogaster*. — *Biochem. Genet. 23*: 607–622.

GEER, B. W., MCKECHNIE, S. W., BENTLEY, M. M., OAKESHOTT, J. G., QUINN, E. M. and LANGEVIN, M. L. 1988. Induction of alcohol dehydrogenase by ethanol in *Drosophila melanogaster*. — *J. Nutr. 118*: 398–407

GEER, B. W., HEINSTRA, P. W. H., KAPOUN, A. M. and VAN DER ZEL, A. 1989. Alcohol dehydrogenase and alcohol tolerance in *Drosophila melanogaster*. — In: *Ecological and Evolutionary Genetics of Drosophila* (*eds* J. S. F. BARKER *and* W. T. STARMER). *Plenum Press, New York*, p. 231–252

GEER, B. W., HEINSTRA, P. W. H. and MCKECHNIE, S. W. 1993. The biological basis of ethanol tolerance in *Drosophila*. — *Comp. Biochem. Physiol. 105B*: 203–229

GIBSON, J. B., MAY, T. W. and WILKS, A. V. 1981. Genetic variation at the alcohol dehydrogenase locus in *Drosophila melanogaster* in relation to environmental variations: Ethanol levels in breeding sites and allozyme frequencies. — *Oecologia 51*: 191–198

GRELL, E. H., JACOBSON, K. B. and MURPHY, J. B 1965. ADH in *Drosophila melanogaster*: Isozymes and genetic variants. — *Science 149*: 80–82

HEINSTRA, P. H. W., EISSES, K. T., SCHOONEN, W. G. E. J., ABEN, W., DE WINTER, A. J., VAN DER HORST, D. J., VAN MARREWIJK, W. J. A., BEENAKKERS, A. M. T., SCHARLOO, W. and THÖRIG, G. E. W. 1983. A dual function of alcohol dehydrogenase in *Drosophila*. — *Genetica 60*: 129–137

HEINSTRA, P. W. H., SCHARLOO, W. and THÖRIG. E. W. 1987. Physiological significance of the alcohol dehydrogenase polymorphism in larvae of *Drosophila*. — *Genetics 117*: 75–84

HEINSTRA, P. W. H. , SEYKENS, D., FRERIKSEN, A. and GEER, B. W. 1990. Metabolic physiology of alcohol degradation and adaptation in *Drosophila* larvae as studied by means of carbon-13 nuclear magnetic resonance spectroscopy. — *Insect Biochem. 20*: 343–348

KAMPING, A. and VAN DELDEN, W. 1978. Alcohol dehydrogenase polymorphism in populations of *Drosophila melanogaster*. II. Relation between ADH activity and adult mortality. — *Biochem. Genet. 16*: 541–551

KAPOUN, A. M. B., GEER, B. W., HEINSTRA, P. W. H., CORBIN, V. and MCKECHNIE, S. W. 1990. Molecular control of the induction of alcohol dehydrogenase locus in *Drosophila melanogaster* larvae. — Genetics *124*: 881–888

KERVER, J. W. M. and VAN DELDEN, W. 1985 Development of tolerance to ethanol in relation to the alcohol dehydrogenase locus in *Drosophila melanogaster*: I. Adult and egg-to-adult survival in relation to ADH activity. — *Heredity 55*: 355–367

KING, J. J. and MCDONALD, J. F. 1987 Post-translational control of alcohol dehydrogenase levels in *Drosophila melanogaster*. — *Genetics 115*: 693–699

MADHAVAN, K. CONCIENCE-EGLI, M., SIEBER, F. and URSPRUNG, H. 1973. Farnesol metabolism in *Drosophila melanogaster*: Ontogeny and tissue distribution of octanol dehydrogenase and aldehyde oxidase. — *J. Insect Physiol. 19*: 235–241

MARONI, G. and STAMEY, S. C. 1983. Developmental profile and tissue distribution of alcohol dehydrogenase. — *Drosophila Inf. Serv. 59*: 77–79

MCCULLAGH, P. and NELDER, J. A. 1989. Generalised linear models. — *Chapman and Hall, London*

MCELFRESH, K. C. and MCDONALD, J. F. 1983. The effect of alcohol stress on Nicotinamide Adenine Dinucleotide (NAD) levels in *Drosophila*. — *Biochem. Genet. 21*: 365–374

MCKECHNIE, S. W. and GEER, B. W. 1984. Regulation of alcohol dehydrogenase in *Drosophila melanogaster* by dietary alcohol and carbohydrate. — *Insect Biochem. 14*: 231–242

MCKECHNIE, S. W. and MORGAN, P. 1982. Alcohol dehydrogenase polymorphism of *Drosophila melanogaster*; Aspects of alcohol and temperature variation in the larval environment. — *Aust. J. Biol. Sci. 35*: 85–93

MERCOT, H. and MASSAAD, L. 1989. ADH activity and ethanol tolerance in third chromosome substitution lines in *Drosophila melanogaster*. — *Heredity 62*: 35–44

MIDDLETON, R. J. and KACSER, H. 1983. Enzyme variation, metabolic flux and fitness: Alcohol dehydrogenase in *Drosophila melanogaster*. — *Genetics 105*: 633–650

OAKESHOTT, J. G., GIBSON, J. B., ANDERSON, P. R. and KNIBB, W. R. 1982. Alcohol dehydrogenase and glycerol-3-phosphate

dehydrogenase clines in *Drosphila melanogaster* on different continents. — *Evolution 36*: 86–96

OAKESHOTT, J. G., GIBSON, J. B. and WILSON, S. R. 1984. Selective effects of the genetic background and ethanol on the alcohol dehydrogenase polymorphism in *Drosophila melanogaster*. — *Heredity 53*: 51–67

O'BRIEN, S. J. and MACINTYRE, R. J. 1972. The α-glycerophosphate cycle in *Drosophila melanogaster*. I. Biochemical and developmental aspects. — *Biochem. Genet. 7*: 141–161

O'BRIEN, S. J. and MACINTYRE, R. J. 1978. Genetics and biochemistry of enzymes and specific proteins of *Drosophila*. — In: *The Genetics and Biology of Drosophila, Vol. 2a* (*eds* M. ASHBURNER *and* T. R. F. WRIGHT) *Academic Press, London*, p. 395–551

PECSENYE, K. 1989. Fitness components at the octanol dehydrogenase locus in *Drosophila melanogaster*. — *Genetica 79*: 133–138

PECSENYE, K. and LÖRINCZ, G. 1988. Changes in gene frequencies at the octanol dehydrogenase locus of *Drosophila melanogaster* imposed by environmental ethanol. — *Genetica 77*: 171–177

SIEBER, F., FOX, D. J. and URSPRUNG, H. 1972. Properties of octanol dehydrogenase from *Drosophila*. — *FEBS Letters 26*: 274–276

VAN DELDEN, W. 1982. The alcohol dehydrogenase polymorphism in *Drosophila melanogaster*: Selection at an enzyme locus. — *Evol. Biol. 15*: 187–222

VAN HERREWEGE, J. and DAVID, J. R. 1984. Extension of life duration by dietary ethanol in *Drosophila melanogaster*: Response to selection in two strains of different origin. — *Genetica 63*: 61–70