ADH activity and ethanol tolerance in third chromosome substitution lines in *Drosophila melanogaster*

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In vitro ADH activity and ethanol tolerance were studied in males of a series of third chromosome substitution lines in *Drosophila melanogaster*. The lines were divided into those with a random third chromosome from a vineyard population (VO lines) and those with a selected third chromosome from males obtained after an egg-to-adult ethanol survival test on the F4 of the previous population (VE lines). Both ADH activity and ethanol tolerance varied significantly among the lines, but the characters showed no significant correlation. Ethanol tolerance (at the higher ethanol concentrations) was higher in the selected lines (VE lines) but ADH activity was not. In our lines, the *in vitro* ADH activity variability, linked to the regulatory genes (located on the third chromosome) and unrelated to the polymorphism of the *Adh* locus (located on the second chromosome), is not involved in the ethanol tolerance variability. The data suggest that in this population ethanol tolerance was acquired in nature, at least partially, by means other than increasing ADH activity.

INTRODUCTION

Drosophila melanogaster is remarkable for its capacity to tolerate and exploit the presence of alcohols in its habitat (Monclus and Prevosti, 1978–79; David and Van Herrewege, 1983). In this adaptation the enzyme alcohol dehydrogenase (ADH EC 1.1.1.1) plays a key role. Indeed ADH ensures both metabolic use and detoxification of ethanol (Van Herrewege and David, 1980). The presence of an active ADH is necessary to the survival of the flies in ethanol supplemented media (David *et al.*, 1976; Middleton and Kacser, 1983; Freeth *et al.*, 1986).

There are two sources of variation for the *in vitro* ADH activity level:

—Firstly, the polymorphism of the *Adh* structural gene, with different allozyme activities (Chambers *et al.*, 1984). All natural populations contain two widespread alleles, *Adh*^S and *Adh*^F (Oakeshott *et al.*, 1982; David *et al.*, 1986). Generally flies from homozygous *Adh*^{FF} strains possess greater *in vitro* ADH activity than those from homozygous *Adh*^{SS} strains. This difference is due for the most part to the higher number of ADH molecules associated with the *Adh*^{FF} genotype (Lewis and Gibson, 1978; McDonald *et al.*, 1980), but also to the greater activity of the catalytic-centre of the ADH-F allozyme (Winberg *et al.*, 1985).

—Secondly, the existence of polymorphic regulatory genes (Ayala and McDonald, 1980). The role of these regulatory (or modifier) genes is to act on the amount of synthesized enzyme (Maroni *et al.*, 1982), or even on the enzyme stability (King and McDonald, 1987). It has been shown that these modifiers of ADH activity are located on the X, second and third chromosomes (Pipkin and Hewitt, 1972; Ward, 1975; Barnes and Birley, 1978; McDonald and Ayala, 1978; Maroni *et al.*, 1982).

Extensive experiments have been carried out in order to emphasize the selective significance of the alcohol dehydrogenase polymorphism on the ethanol tolerance (see Van Delden, 1982, for review, and Oakeshott *et al.*, 1984, for controversy). However, few experiments have been made on the relationship between the ethanol tolerance and the ADH activity variability which is unrelated to the structural locus. The first result concerned an experiment by David and Bocquet (1977), in which they were able to increase the median lethal dose (LD₅₀) of ethanol by more than 100 per cent in their selected strain. No evidence was found of the existence of structural differences in ADH between selected and control strains. The selected strain, however, had higher ADH activity (McDonald *et al.*, 1977). Other authors have described a positive relationship between ethanol tolerance and ADH activity in strains homozygous for the same allele

of *Adh*. Thompson and Kaiser (1977) found a positive correlation between egg-to-adult survival and ADH activity when two *Adh^{SS}* strains, differing in activity, were tested on ethanol-supplemented food. Nevertheless this result is not unambiguous, because the potato-flake dextrose medium used seems to be, itself, a nutritive selective agent. Kamping and Van Delden (1978) studied seven *Adh^{FF}* homozygous lines and one *Adh-null* line. Although the positive correlation observed between ADH activity and LD₅₀ of ethanol included the *Adh-null* strain, the correlation remained significantly positive considering only the seven *Adh^{FF}* strains. Nevertheless one must point out that this result was due only to one strain which showed an atypically low activity. Furthermore, none of these experiments had used the chromosome substitution lines which are the most appropriate for the study of variations of enzyme activity linked to modifier genes in *D. melanogaster* (Laurie-Ahlberg *et al.*, 1980; 1982). Therefore we investigated the existence of a relationship between the ADH activity variation due to regulatory genes and the ethanol tolerance in chromosome substitution lines.

The present study concerns only the effects of the third chromosome. Since the *Adh* locus is located on the second chromosome (2—50.1), the choice of the third chromosome avoids:

- the variability linked to the different alleles of *Adh*,
- the possibility of linkage disequilibrium between the *Adh* locus and modifier genes of ADH activity,

—the difficulty of distinguishing between *cis*- and *trans*-acting factors. Here, only *trans*-acting factors need to be invoked to explain the observed differences.

MATERIALS AND METHODS

(i) Strains

(a) Balancer stock: the substitution of third chromosomes onto an isogenic background was carried out using a balancer stock, 39A1. The procedure for the constitution of the 39A1 stock is given in fig. 1, the male used originated from the SA-FIV population (Merçot, 1985). Chromosome II of 39A1 (marked II^a in fig. 1) carries the *Adh^S* and *purple* alleles. Therefore 39A1 is symbolised by: Xⁱ/Xⁱ; *pr/pr*; *Ser/Sb* in fig. 2.

(b) third chromosome lines: the procedure for substitution of third chromosomes into an isogenic background is shown in fig. 2. Nevertheless, the substitution lines are different also for the Y chromosome (from the FO males) and the origin of the little fourth chromosome is uncontrolled. But the size of the Y chromosome is insignificant and the fourth is 23 times smaller than the third chromosome. Consequently their possible effect was ignored.

(c) origin of third chromosomes: two sets of third chromosome lines were constituted: (1) the VO lines derived from wild males trapped in Venteuil (Marne, France) in October 1984, (2) the VE lines derived from males obtained from an egg-to-adult survival experiment on 18 per cent of

Figure 1 Mating scheme used to obtain the 39A1 balancer stock.

Figure 2 Mating scheme used to obtain strain homozygous for a third chromosome in the isogenic 39A1 background. In F2, for the second chromosome, a fly with the *pr/pr* genotype is distinguished from a fly with the *II/pr* because the former has the *pr* phenotype whilst the latter has a wild phenotype for eye-color.

ethanol (David *et al.*, 1986); this experiment was carried out, on one single generation, with the F4 of the Venteuil population and caused 74 per cent of mortality.

(ii) ADH activity

(a) Assays: the assay procedure for *in vitro* ADH activity is described by Merçot and Higuët (1987).

(b) rearing conditions and sampling: for each isogenic line 600 eggs were placed in two bottles (300 eggs per bottle) of cornmeal axenic medium with killed yeast (David, 1962). The off-spring were reared at 25°C, collected within 18 h of emergence. Adult males were aged in three groups of 15 individuals for seven, eight and nine days on axenic medium. Live flies were then weighed and their ADH activity measured, twice, on the same day. Thus there were six samples of 15 pooled males (from two bottles and with three different ages) assayed for each line. Thirteen VO lines and 13 VE lines were analysed during March and April 1986. In order to estimate the repeatability of line differences, seven VO lines and seven VE lines were resampled in March 1987.

(c) Statistical analysis: the analysis of the ADH activity variations was carried out with an ANOVA with respect to three hierarchical factors, origin of the third chromosomes (O), chromosome lines (L), bottles (B) and one cross-factor, the age of males (A).

(iii) Ethanol tolerance

(a) Experimental procedures: Experiments were carried out at 25°C on seven VO lines and seven VE lines during June and July 1986. Ethanol tolerance was measured according to the method of David *et al.* (1974). Larvae were grown at low larval density on the axenic killed yeast medium. After emergence, adult males were separated from females, and groups of 20 were kept on nutrient medium for seven days. Each group of males was then transferred into an air-tight plastic vial containing 2 ml of a 3 per cent sucrose solution supplemented with the suitable concentration of ethanol and absorbed by a cellulose paper. For each test, five concentrations of ethanol (5, 7, 9, 11 and 13 per cent) were used, with five replicates per concentration. Dead flies were counted daily for three consecutive days.

(b) Statistical analysis: For each concentration and for each day scored, we carried out the following analysis:

- comparison of the percentage of mortality between the 14 lines by the Kruskal-Wallis test,
- comparison of the mean percentage of mortality between the VO lines and the VE lines by the Mann and Whitney test,
- computation of the correlation coefficient, by the Spearman rank test, between the percentage of ethanol mortality of lines and both their ADH activity and their body weight.

RESULTS

(i) ADH activity

Table 1 gives the mean values, over the three days, of fresh weight, ADH activity per fly and ADH activity per mg of fresh weight for the 26 lines

Table 1 Means of fresh weight (*w*), ADH activity by fly (*A/f*) and ADH activity by mg of fresh weight (*A/mg*) measured in males from the VO lines and VE lines. Roman type: results from 1986; italics: results from 1987

Lines	<i>w</i> *	SE(<i>w</i>)	<i>A</i> †/ <i>f</i>	SE(<i>A/f</i>)	<i>A/mg</i>	SE(<i>A/mg</i>)
VO3A	0.803 <i>0.850</i>	0.006 <i>0.005</i>	2.609 <i>2.641</i>	0.012 <i>0.012</i>	3.254 <i>3.111</i>	0.021 <i>0.016</i>
VO11D	0.802	0.003	2.990	0.014	3.734	0.025
VO13B	0.714	0.003	2.634	0.011	3.687	0.015
VO24C	0.803 <i>0.866</i>	0.004 <i>0.004</i>	3.275 <i>3.310</i>	0.008 <i>0.017</i>	4.081 <i>3.820</i>	0.013 <i>0.018</i>
VO30B	0.787 <i>0.778</i>	0.002 <i>0.002</i>	3.018 <i>3.195</i>	0.014 <i>0.010</i>	3.835 <i>4.107</i>	0.019 <i>0.013</i>
VO36A	0.894 <i>0.895</i>	0.005 <i>0.006</i>	3.068 <i>3.195</i>	0.014 <i>0.013</i>	3.436 <i>3.575</i>	0.021 <i>0.018</i>
VO41D	0.770	0.002	2.899	0.020	3.767	0.026
VO43B	0.769	0.007	2.795	0.017	3.638	0.023
VO47B	0.850 <i>0.887</i>	0.005 <i>0.003</i>	2.007 <i>2.010</i>	0.017 <i>0.009</i>	2.359 <i>2.266</i>	0.017 <i>0.009</i>
VO54A	0.747	0.008	3.084	0.012	4.144	0.026
VO63B	0.883 <i>0.886</i>	0.004 <i>0.004</i>	3.457 <i>3.396</i>	0.013 <i>0.019</i>	3.917 <i>3.832</i>	0.016 <i>0.020</i>
VO64B	0.795	0.005	3.743	0.018	4.716	0.028
VO77A	0.797 <i>0.807</i>	0.003 <i>0.002</i>	3.137 <i>2.885</i>	0.008 <i>0.016</i>	3.936 <i>3.575</i>	0.010 <i>0.021</i>
VO _{mean}	0.801	0.004	2.978	0.033	3.731	0.042
VO _{mean}	<i>0.859</i>	<i>0.006</i>	<i>2.947</i>	<i>0.070</i>	<i>3.469</i>	<i>0.088</i>
VE3D	0.918	0.004	2.723	0.013	2.971	0.019
VE14C	0.794 <i>0.781</i>	0.003 <i>0.006</i>	2.199 <i>2.180</i>	0.015 <i>0.009</i>	2.770 <i>2.796</i>	0.016 <i>0.015</i>
VE35B	0.749 <i>0.751</i>	0.002 <i>0.004</i>	2.432 <i>2.396</i>	0.008 <i>0.012</i>	3.246 <i>3.208</i>	0.011 <i>0.011</i>
VE37F	0.912	0.005	2.999	0.009	3.291	0.012
VE39F	0.925	0.008	2.481	0.011	2.693	0.022
VE41F	0.914	0.002	2.700	0.015	2.954	0.015
VE42A	0.734 <i>0.744</i>	0.007 <i>0.003</i>	2.910 <i>2.813</i>	0.015 <i>0.014</i>	3.974 <i>3.784</i>	0.025 <i>0.019</i>
VE46A	0.806 <i>0.731</i>	0.002 <i>0.004</i>	3.070 <i>2.999</i>	0.010 <i>0.011</i>	3.810 <i>4.110</i>	0.014 <i>0.021</i>
VE46B	0.819	0.003	2.971	0.009	3.629	0.014
VE47A	0.780	0.009	2.925	0.019	3.771	0.039
VE51C	0.768 <i>0.786</i>	0.004 <i>0.003</i>	2.949 <i>3.059</i>	0.013 <i>0.017</i>	3.845 <i>3.897</i>	0.022 <i>0.025</i>
VE51E	0.783 <i>0.797</i>	0.005 <i>0.001</i>	2.828 <i>2.923</i>	0.013 <i>0.019</i>	3.617 <i>3.671</i>	0.024 <i>0.025</i>
VE58B	0.829 <i>0.838</i>	0.003 <i>0.005</i>	3.214 <i>3.002</i>	0.018 <i>0.019</i>	3.876 <i>3.591</i>	0.021 <i>0.029</i>
VE _{mean}	0.825	0.005	2.800	0.022	3.419	0.035
VE _{mean}	<i>0.775</i>	<i>0.005</i>	<i>2.768</i>	<i>0.045</i>	<i>3.580</i>	<i>0.059</i>

* in mg.

† ADH activity is expressed as $\Delta \text{DO} \times 10^3/\text{min}$.
SE() = Standard Error.

analysed in 1986 and for the 14 lines retested in 1987.

It is usual to standardize the raw enzyme activity (units per fly) by dividing by the live weight per fly. We have tested the validity of this practice. For each line (*i.e.*, for the environmental relationship between ADH activity per fly and weight of the six samples) the linear regression is not significant. Over the lines (*i.e.*, for the genetic relationship between ADH activity per fly and weight of lines) the linear regression is also non-significant (1986: $r = -0.028$, 24 df, ns; 1987: $r = 0.155$, 12 df, ns). These findings corroborate the results obtained by Birley and Marson (1981) and by Maroni *et al.* (1982) from their chromosome substitution lines. We have therefore used the ADH activity per fly to characterize the level of ADH activity for each line in the rest of this work.

Table 2 gives the results of the analysis of variance of the ADH activity per fly (data from 1986). The origin of lines (VO lines *vs* VE lines) has no influence, while a highly significant line effect is obvious. A significant effect of the third chromosome on the level of ADH activity per fly is observed in our sample of substitution lines. The age of males (seven, eight or nine days) has an influence on ADH activity. The activity of seven-day-old males ($A/f = 2.845$) is lower than the activity of eight- and nine-day-old males (2.919 and 2.903, respectively). Though Dunn *et al.* (1969), Hewitt *et al.* (1974) and Ward (1975) have shown that ADH activity remains relatively constant in adults aged between 4 and 12 days, our results confirms that the *in vitro* ADH activity shows an increase with time during this period (Kerver and Van Delden, 1985). The bottle effect and the age $\times$ bottle interaction are also significant. They display an environmental effect linked to

Table 2 Analysis of variance of ADH activity per fly

Source	df	Mean square	<i>F</i>	<i>P</i>
Origin (VO <i>vs.</i> VE)	1	2.475	$F_{24}^1 = 1.563$	ns
Line (L)	24	1.584	$F_{26}^{24} = 22.005$	***
Bottle (B)	26	0.072	$F_{156}^{16} = 8.170$	***
Age (A)	2	0.160	$F_{52}^2 = 3.308$	*
O $\times$ A	2	0.034	$F_{48}^2 = 0.872$	ns
L $\times$ A	48	0.039	$F_{52}^{48} = 0.808$	ns
B $\times$ A	52	0.048	$F_{156}^{52} = 5.481$	***
Error	156	0.009		

* $P < 0.05$. *** $P < 0.001$.

ns = non-significant ($P > 0.05$).

bottle culture and extract preparation. Nevertheless this environmental effect has not prevented the detection of an obvious genetic effect linked to the third chromosome. This evidence is strengthened by the high repeatability of the results in the 14 lines sampled about one year apart (table 1). This repeatability of the line differences in the ADH activity per fly is demonstrated by the highly significant value of the correlation coefficient ($r = 0.956$; $P < 0.001$; 12 df) (fig. 3). The same outcome is obvious for the body weight line differences ($r = 0.822$; $P < 0.001$; 12 df).

(ii) Ethanol mortality

It is important to first point out that not all lines were tested for their tolerance to ethanol. In fact, the fecundity, fertility and/or viability of some lines were so low that the test of ethanol tolerance, which required a minimum number of flies, was impossible. Nevertheless the 14 lines tested (seven VO lines and seven VE lines; table 3) reflect satisfactorily the range of the ADH activity values of all 26 lines (table 1); and the mean ADH activity of the seven VO lines tested ($A/f = 2.939$) and this of the seven VE lines tested ($A/f = 2.800$) are very similar to these of the early 13 VO and 13 VE lines (table 1).

Table 3 gives the percentage of male mortality in the 14 lines after one, two and three days of exposure to the five ethanol concentrations used (i.e., 15 observations). Each line was tested using

Figure 3 Repeatability of the ADH activity measurements: correlation between the measurements assayed in 1986 and 1987.

three vials containing the sucrose solution without ethanol, to show an absence of mortality in control conditions.

The difference of death rate between the 14 lines was tested for each of 15 mortality observations. Table 4 gives the results of the Kruskal-Wallis test carried out for this purpose. Except for the least severe condition (one day of exposure to a concentration of five per cent ethanol) and the

Table 3 Percentages of male mortality in the VO and Ve lines after 24 hours (D1), 48 hours (D2) and 72 hours (D3) of exposure to the different ethanol concentrations (Conc.)

Conc. Lines	5%			7%			9%			11%			13%		
	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3
VO3A	0	0	1	13	37	56	2	61	85	17	92	99	51	100	100
VO24C	3	7	11	11	21	30	10	23	65	62	95	100	66	98	100
VO30B	3	3	3	2	2	2	2	23	45	28	81	89	69	90	91
VO36A	2	4	6	0	4	8	6	42	71	9	74	99	22	89	98
VO47B	15	32	47	39	71	91	33	75	88	44	94	100	67	99	100
VO63B	3	5	7	5	17	44	21	22	32	45	96	100	36	93	100
VO77A	5	10	15	20	23	35	32	48	84	51	79	98	57	88	99
VO _{mean}	4	9	13	13	25	38	15	42	67	37	87	98	55	94	98
VE14C	10	20	42	11	36	73	18	46	63	34	65	93	27	77	99
VE35B	0	4	6	0	12	28	8	55	78	15	78	95	44	95	97
VE42A	0	1	1	4	16	41	6	42	88	21	64	88	75	87	100
VE46A	1	1	2	16	16	18	10	12	13	2	19	65	7	69	97
VE51C	2	3	4	3	4	6	21	61	91	38	79	100	42	85	99
VE51E	11	13	16	26	39	66	25	49	73	51	77	86	56	88	100
VE58B	2	14	26	22	37	59	37	96	98	79	100	100	65	100	100
VE _{mean}	4	8	14	12	23	42	18	52	72	34	69	90	45	86	99

Table 4 Comparison of the percentages of male mortality between the lines after each day of exposure to each ethanol concentration (Conc.): Values of *H* from the Kruskal-Wallis test (13 df)

Conc.	Days of exposure		
	D1	D2	D3
5%	19.331	27.631*	35.707***
7%	30.970**	37.222***	45.487***
9%	31.793**	37.806***	41.344***
11%	38.158***	37.218***	27.714**
13%	26.727*	25.079*	4.283

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 5 Comparison of mean percentages of male mortality between VO lines and VE lines after each day of exposure to each ethanol concentration (Conc.): Standardized values of *U* from the Mann-Whitney test

Conc.	Day of exposure		
	D1	D2	D3
5%	0.546	0.229	0.135
7%	0.534	0.070	0.781
9%	0.699	1.386	1.239
11%	0.511	2.807**	2.473*
13%	0.987	2.954***	0.560

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

most severe (three days of exposure to a concentration of 13 per cent), there were always significant differences in the death rate between the 14 lines.

The comparison of the mean percentages of ethanol mortality between the VO and VE lines (table 5) shows a significantly lower mortality of the VE lines after two and three days of exposure to an ethanol concentration of 11 per cent and after two days of exposure to an ethanol concentration of 13 per cent (*i.e.*, to the higher concentrations of ethanol). As the third chromosomes of the VE lines were from males of which the pre-imaginal development was accomplished on a larval medium with 18 per cent of ethanol (which caused a mortality of 74 per cent), these differences may display a selective effect in favour of third chromosomes which allowed a higher ethanol tolerance both in pre-imaginal and adult stages.

(iii) Relationship between ADH activity and ethanol mortality

The preceding results showed both an ADH activity and ethanol tolerance variability linked to

the third chromosome. The final aim of this work was to find out whether there was any correlation between these two variables. Thus we tested, with the Spearman rank test, the correlation between the ADH activity of the 14 lines and their ethanol male mortality in each of the 15 observations. Table 6 gives the value of the 15 correlation coefficients. No significant value was obtained in any case. We did not find any conclusive relationship between *in vitro* ADH activity and ethanol tolerance of males from our third chromosome substitution lines.

Table 6 Relationship between the percentage of male mortality and the ADH activity of lines: values of the Spearman's rank correlation coefficients ($df = 14$), all were not significant ($P > 0.05$)

Conc.	Day of exposure		
	D1	D2	D3
5%	-0.051	-0.085	-0.071
7%	-0.023	-0.231	-0.362
9%	0.160	-0.481	-0.257
11%	0.354	0.358	0.296
13%	-0.064	0.074	0.129

(iv) Relationship between body weight and ethanol tolerance

We have also tested the relationship between ethanol tolerance and body weight of lines (table 7). Again the correlation coefficients were not significant. Van Delden and Kamping (1983) observed, both in *Adh^{FF}* and *Adh^{SS}* strains maintained on hexanol, an increase in ADH activity, ethanol tolerance and body weight. The authors raised the question as to whether there had been a selection for body weight with a concomitant increase in ADH activity (and con-

Table 7 Relationship between the percentage of male mortality and the fresh weight of lines: values of the Spearman's rank correlation coefficients ($df = 14$), all were not significant ($P > 0.05$)

Conc.	Day of exposure		
	D1	D2	D3
5%	0.222	0.254	0.312
7%	0.276	0.257	0.187
9%	0.204	-0.103	-0.240
11%	0.064	0.393	0.480
13%	-0.323	0.381	0.232

sequently in ethanol tolerance) or *vice versa*. For our lines, we can answer that there is no correlation between these three traits.

DISCUSSION

The results reported here confirm the previous observations that a large degree of genetic variability, affecting the *in vitro* ADH activity level, exists in the third chromosomes of *D. melanogaster* (Ward, 1975; Barnes and Birley, 1978; McDonald and Ayala, 1978; Maroni *et al.*, 1982). In spite of appreciable environmental and experimental effects on ADH levels, the direction and magnitude of the differences between our third chromosome substitution lines were highly repeatable. Furthermore, our study shown an ethanol tolerance variability linked to the third chromosome. Although the effects of the genetic background on the ethanol tolerance, independently of the *Adh* polymorphism, were already known (David and Bocquet, 1977; Gibson *et al.*, 1979), this is the first time that the role of any one particular chromosome was demonstrated. Ours is the first study of the relationship between ADH activity and ethanol tolerance in chromosome substitution lines. The results show that *in vitro* ADH activity variability—irrespective of the *Adh* polymorphism—and ethanol tolerance variability are unrelated. Nevertheless only the third chromosome has been surveyed. A study of the X chromosome and even more importantly the second chromosome should be undertaken in order to see whether this lack of correlation can extend to these other chromosomes. Otherwise, the population from which our third chromosomes originate presents two peculiarities. It is both a Palearctic and vineyard population. Precisely these two kinds of population exhibit higher ethanol tolerance than Tropical (David *et al.*, 1986) and non-vineyard (McKenzie and Parsons, 1974; McKenzie and McKechnie, 1978; Hickey and McLean, 1980) populations. Would the same kind of results have been obtained with a different population (*i.e.*, not very tolerant, such as the Afro-tropical populations, or not from a vineyard)? Such a study is in progress. Moreover one must point out that the use of isogenic lines represents an artificial laboratory situation. Inbreeding being a source of homeostatic disturbances, the differences observed could have nothing to do with the detoxification processes which occur in the wild. Nevertheless, during both the aging of males and the experiment in control vials, no natural mortality was observed, suggesting that weak physiological states was not

preponderant in the lines. This observation argues against the between-line mortality variation being due to homeostatic disturbances.

One can also object that the ADH activity which we have measured was on flies which had never been exposed to ethanol. Now, ethanol may have elicited changes on the ADH activity of the flies over the experimental period of the tolerance test. If ethanol has such an effect, it may completely change the correlation of ADH activity on ethanol tolerance. For our part, we have not a response because we have not measured the ADH activity of the survivor flies to the tolerance tests. But two recent reports seem to indicate a lack of such an influence on the adult flies. Indeed, Kerver and Van Delden (1985), from eight tests, have not observed differences in ADH activity between adult flies raised for three days on regular or ethanol supplemented food, except on one occasion where the ethanol food seemed to arrest the age dependent increase in ADH activity. In the same way, Guillen *et al.* (1987) have not observed changes in ADH activity of adults exposed for one day to a solution of ten per cent ethanol. In contrast, the presence of ethanol in the medium induces a significant increase of ADH activity in the larval stages (McKechnie and Geer, 1984; Kerver and Van Delden, 1985). The existence of two promoters for the *Adh* locus, one acting during the pre-imaginal stages, the other during the imaginal stage (Benyajati *et al.*, 1983), may occur in this discordance.

The fact remains that our results deviate from the earlier reports (David and Bocquet, 1976; Thompson and Kaiser, 1977; Kamping and Van Delden, 1977) which argued for a positive correlation between ethanol tolerance and ADH activity unrelated to the *Adh* polymorphism. On the other hand, they agree with the observations of Kerver and Van Delden (1985) and Barbancho *et al.* (1987). Indeed these latter authors demonstrated that after 90 and 60 generations of raising on ethanol supplemented medium an increase in tolerance was not accompanied by an increase in ADH activity in adults with the same *Adh* genotype. But again, another situation seems occur in the juvenile life stages since increasing both in ADH activity and ethanol tolerance was observed by Kerver and Van Delden (1985) in these stages, nevertheless the authors concluded that ADH activity was not the only factor involved in the increased tolerance to ethanol.

Though with a different experimental approach, our results corroborate these more recent observations, and strengthen the hypothesis

that ADH activity and tolerance to ethanol can be, if not in totality, at least in part, dissociated. As for the *Adh* locus, for which the differences in activity of its allozymes can be unrelated with differences in ethanol tolerance (see Oakeshott *et al.*, 1984 for debate), the ADH activity variability, linked to the regulatory genes, can be unrelated to the ethanol tolerance variability.

Ethanol is neurotoxic, thus the study of tolerance leads to the study of the efficiency of the process of resistance to it (David, 1988). Although the main role of ADH in the detoxification of ethanol is well established (David *et al.*, 1976; Middleton and Kacser, 1983; Freeth *et al.*, 1986), the study of *in vitro* ADH activity alone does not take account of the whole problem. The rate of entry of ethanol via the cuticle, spiracles or gut, the role of the hemolymph (Cohan and Graf, 1985), both the permeability and fluidity of cell membranes (Taraschi and Rubin, 1984; Geer *et al.*, 1986) and the tissue specificity of the ADH expression (Ursprung *et al.*, 1970; Maroni and Stamey, 1983; Fisher and Maniatis, 1986) may also be relevant to ethanol tolerance.

Yet, at low concentrations, ethanol, like other primary alcohols, functions as a food component. It has been shown (Van Herreweghe and David, 1974; Deltombe-Lietard *et al.*, 1979; Van Herreweghe *et al.*, 1980; Anderson *et al.*, 1981) that the life span of flies deprived of any other food source is extended when ethanol is provided at low concentrations. Moreover, Van Herreweghe and David (1980; 1984) found that ethanol tolerance (at higher concentrations) and metabolic ethanol utilization (at lower concentrations) may be controlled by different genetic mechanisms. Thus we can agree the hypothesis formulated by Gibson *et al.* (1979) that higher ADH activity may be relevant in providing energy in flies that are tolerant to environmental alcohols by some mechanism unrelated to ADH activity. Further experiments, with isogenic lines are in progress in order to investigate the possible relation between ADH activity and the capacity to use ethanol as a metabolic resource.

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