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DIFFERENTIAL RESPONSES IN *DROSOPHILA MELANOGASTER* TO ENVIRONMENTAL ETHANOL: MODIFICATION OF FITNESS COMPONENTS AT THE *Adh* LOCUS

G. DORADO AND M. BARBANCHO

Departamento de Genética, Facultad de Ciencias, Universidad de Córdoba (Córdoba), Spain

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SUMMARY

Homozygous Adh^F Adh^F and Adh^S Adh^S lines, collected from the same *D. melanogaster* winery polymorphic population, were submitted to stress by environmental ethanol. The responses of each of the FF, FS and SS genotypes, for both control and selected lines were analysed in normal and ethanol supplemented medium, in relation to three fitness components: egg-to-adult viability, developmental time and ethanol utilisation. Ethanol selection has resulted in a higher ethanol tolerance, a shorter developmental time and a better ability to use ethanol as food, showing the Adh^F Adh^F genotype to be that with the highest relative fitness.

1. INTRODUCTION

One of the most common ways to show that selection acts on a determined locus is to grow individuals in a medium supplemented with a specific substrate of the products of the locus under consideration. In this way, most of the studies done with *D. melanogaster* involve the *Adh* locus.

When polymorphic populations are grown in media supplemented with different alcohols, a considerable Adh^F increase has been observed (Gibson, 1970; Bijlsma-Meeles and van Delden, 1974; van Delden *et al.*, 1975, 1978; Cavener and Clegg, 1978, 1981). On the other hand, differential responses of the *Adh* genotypes have been obtained in ethanol supplemented media for ethanol tolerance (Morgan, 1975; Briscoe *et al.*, 1975; Oakeshott, 1976; van Delden *et al.*, 1978; McKechnie and Morgan, 1982), or for the ability to use ethanol as food (Libion-Mannaert *et al.*, 1976; David *et al.*, 1976, 1981; Daly and Clarke, 1981). In general, in such media Adh^F Adh^F genotypes show greater fitness, such superiority being associated with a more active ADH-F isozyme (Gibson and Miklovich, 1971; Libion-Mannaert *et al.*, 1976; Kamping and van Delden, 1978). However, there is evidence that ethanol tolerance and ethanol utilisation both seem to be, at least partially, controlled by different genetic mechanisms (van Herrewege and David, 1980; David *et al.*, 1981). Besides, an increase for the ethanol tolerance is not invariably associated with an increase for Adh^F frequencies or with an ADH activity increase (Gibson *et al.*, 1979; Oakeshott, 1979; Ziolo and Parsons, 1982).

But, apart from the above, there are other fitness components that may be important for the maintenance of the genetic variability at the *Adh* locus. In fact, small differences in the developmental times can be an important component of variation of the fitness in species that, like *D. melanogaster*,

feed constantly (Lewontin, 1974). Differences in the developmental times have been found between *Adh* genotypes both on regular food (van Delden and Kamping, 1979) and on alcohol supplemented media (Oakeshott, 1976, 1977), the Adh^F Adh^F homozygotes having developmental times shorter than the Adh^S Adh^S ones. These differences in the developmental times seem to be linearly related to the differences in the ADH activity (Oakeshott, 1976).

All the above information suggests that *Adh* variants are not neutral on regular laboratory food (see also Wilson *et al.*, 1982) nor on alcoholic supplemented media (see also Clarke, 1975; Thompson and Kaiser, 1977; Cavener and Clegg, 1981). The direction of the selective differences does not always seem to be the same for the different components analysed.

In this paper, the responses of both the Adh^F and the Adh^S homozygous lines (and the hybrid one) to a selection imposed by environmental ethanol are studied with respect to egg-to-adult viability, developmental time and ethanol utilisation as food.

2. Materials and methods

The flies used came from a sample collected in the winery named "Lagar de los Reyes" (LR) in Baena, Córdoba (Spain). At the time that the flies were collected, LR population was polymorphic at the *Adh* locus with frequencies of 0·875 and 0·125 for the Adh^F and Adh^S alleles respectively. From the progeny of this sample, 15 homozygous Adh^F and 15 homozygous Adh^S stocks (each coming from one single and independent homozygote × homozygote cross) were founded. 10 males and 10 females of each homozygous stock (300 flies for each allele) were used to originate the Adh^F and Adh^S homozygous control lines (LRCF and LRCS). These lines were independently grown on normal medium for 10 generations before starting selection.

Selected Adh^F and Adh^S homozygous lines (LRSeF and LRSeS) came from each respective LRC line growing on ethanol-supplemented medium. The number of generations of selection was 16. In order to maintain a high number of flies in the lines, two sub-lines of each were maintained and sib-mated each generation. To obtain heterozygous Adh^F Adh^S (FS) flies, virgin Adh^F Adh^F (FF) females and Adh^S Adh^S (SS) males were mated.

Normal medium consists of 1000 ml water, 100 g sucrose, 100 g fresh dead yeast, 12 g agar 0·5 g salt and 5 ml propionic acid. An ethanol-supplemented medium was obtained by adding 11 ml ethanol (99·5 per cent) to 89 ml of normal medium with vigorous stirring after the temperature was below 50°C.

To study egg-to-adult viability and developmental time, eggs laid by three-day-old females of the appropriate genotype were used; 200 per 1 litre bottle of normal medium and 800 per ethanol-supplemented medium. Adults were removed daily as they emerged.

In order to determine the relative abilities to use ethanol as food, 40 three day old flies (20 males and 20 females) were kept in 270 ml glass vials containing 25 ml of basic medium (12 per cent w/v agar in water) or ethanol-basic medium (11 per cent v/v ethanol in basic medium). The Ld_{50} (day on which 50 per cent of the flies died) was used to evaluate ethanol utilisation.

Both the maintenance of lines and the experiments were carried out at approximately 25°C. In the experiments, two replicates for each genotype, medium and line were made and their mean values were obtained for the study of the different effects.

3. Results

Results for each line, genotype and medium for each of the fitness components studied are summarised in fig. 1. Numerical data for viability (as survival egg-to-adult percentages), developmental times (as days from egg-to-adult) and life span (as lethal days 50) are given in table 2.

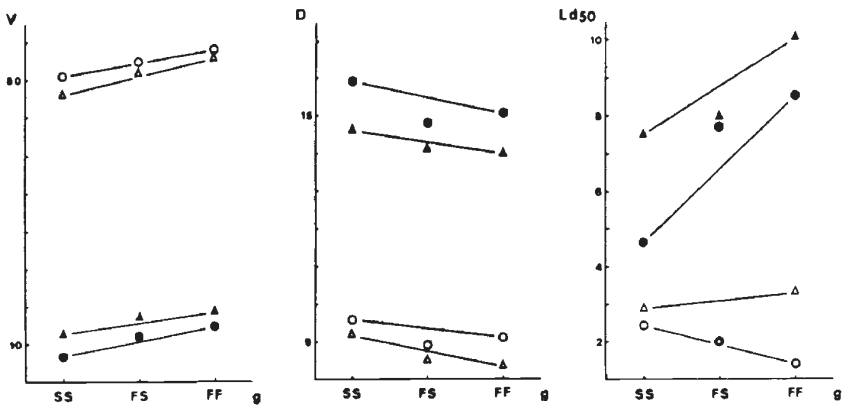

FIG. 1. Percentage survival into adults from eggs (V), mean developmental time, in days (D) and life span as lethal day 50 (Ld50) for each line, genotype and medium. Normal or basic medium: LRC (○), LRSe (△). Supplemented medium: LRC (●), LRSe (▲).

TABLE 1

Factorial analysis of variance of the effects of line, genotype and medium on the egg-to-adult viability, developmental time and life duration

Source of variation	d.f.	Viability		Developmental Time		Life span	
		Mean square	F	Desviance	F	Mean square	F
Lines (L)	1	1·835	0·308 ns	4·002	174·175**	8·158	203·871**
Genotypes (G)	2	116·386	19·569**	1·203	52·376**	4·469	111·666**
Mediums (M)	1	28766·450	4836·892**	198·720	8649·408**	173·887	4345·225**
L×G	2	0·164	0·028 ns	0·125	5·458*	1·550	38·728**
L×M	1	106·851	17·967**	0·459	19·990**	0·986	24·644**
G×M	2	1·850	0·311 ns	0·001	0·064 ns	6·642	165·968**
L×G×M	2	3·717	0·625 ns	0·105	4·554*	1·088	27·184**
Residual	12	5·947		0·023		0·040	
Total	23						

* $P<0·05$, ** $P<0·01$.

(i) *ANOVA*

A factorial ANOVA (summarised in table 1) was used to test the extent of effect upon lines, genotypes and media for each fitness component

TABLE 2

Mean values for each fitness component, genotype, line and medium. Genotype means not underlined by a common line are significantly different, at least at $P < 0{\cdot}05$

Fitness Component	Control lines					
	Regular medium			Supplemented medium		
	SS	FS	FF	SS	FS	FF
Viability	81·00	84·75	88·00	6·69	11·94	14·75
Developmental times	9·60	8·90	9·10	15·89	14·79	15·04
Starvation resistance	2·44	1·99	1·41			
Ethanol utilization				4·64	7·68	8·50
	Selected lines					
Fitness Component	Regular medium			Supplemented medium		
	SS	FS	FF	SS	FS	FF
Viability	75·75	81·50	85·50	12·69	16·94	18·06
Developmental times	9·20	8·50	8·35	14·60	14·13	13·97
Starvation resistance	2·89	1·94	3·30			
Ethanol utilization				7·50	7·97	10·04

studied. Significant differences between lines, for life span and developmental times, indicate that the LRSe line has a higher life span and a shorter developmental time for both media than the LRC. No significant difference was observed for the ethanol tolerance component. However, when an ANOVA for each medium was done, significant differences for the ethanol tolerance in the supplemented medium and for life span and developmental times in both normal and supplemented media were observed.

Significant differences between genotypes were found for all three components, such differences depending on the line and medium (see table 2). These inter-genotypic differences were also found when an ANOVA for each particular medium was done. Significant differences between media were also found for all the components; ethanol produces negative effects on viability and developmental time, and positive ones at life span increases.

L×G interactions were significant for developmental times (interactions only being significant in the supplemented medium) and for life span (probably due to the different pattern of dominance in the supplemented medium).

The significant interactions L×M for all the components are related to a better response of the LRSe line in the supplemented medium (see fig. 1 and table 2).

Significant differences for G×M interactions have only been found for life span components and must be related to the highest resistance to starvation of the homozygous SS and the highest ethanol utilisation of the homozygous FF. As this component really includes two, its effects can be better analysed through an ANOVA for each particular medium. However, when this was done, similar significant differences were observed.

(ii) *Genetic effects*

Intergenotypic differences for each line and medium were tested in table 2 by means of a student's test, by using the error mean squares of the previous analyses of variance. As the differences between genotypes were analysed within lines and within media, the error mean squares used were those of the analyses of variances done for each particular medium.

On the supplemented medium, for both lines, FF and FS individuals show egg-to-adult viabilities higher than that of the SS homozygotes. On the regular medium, though, dominance of the Adh^F allele has not been observed, no significant differences being found among the genotypes of the control lines. On the other hand, small but significant differences can be observed for the developmental times. Apparently there is complete dominance of the Adh^F allele over the Adh^S one in this respect in both lines and media.

In relation to life span, the ability to use the ethanol as food is evaluated by the use of the ethanol-basic medium. Adult FF homozygotes are the best and adult SS homozygotes the worst at using ethanol for metabolic energy, the Adh^S allele showing a dominant effect at the selected lines, though. By using basic medium, the resistance to starvation (basic medium only provides water to the flies) is evaluated, this being very different for the control and selected lines.

(iii) *Ethanol effects*

Ethanol effects for each genotype separately, for both control and selected lines, were quantified by the differences between data from supplemented and normal medium (see table 3). Differences are also tested in table 3 (normal vs. supplemented medium) by means of a student's test by using the error mean squares of the previous analyses of variances.

Highly significant differences ($p < 0{\cdot}001$) are found for both control and selected lines for all three fitness components studied. For both LRC and

TABLE 3

Ethanol and selection effect and their statistical significance for each Adh genotype. Life span on normal medium (implying resistance to starvation) and on supplemented medium (ethanol utilisation). (+): loss of viability, delay in developmental time and life duration increase. (−): the opposite

	Ethanol effect		Selection effect		
Adh	LRC $C^{Su}-C^{N}$	LRSe $Se^{Su}-Se^{N}$	N.M. $Se^{N}-C^{N}$	Su.M. $Se^{Su}-C^{Su}$	
SS	+91·75***	+83·30***	+6·17 ns	−7·42***	
FS	+85·91***	+79·21***	+3·83 ns	−5·90***	Viability
FF	+83·24***	+79·30***	+2·84 ns	−3·76**	
SS	+6·29***	+5·40***	−0·40*	−1·29***	
FS	+5·89***	+5·63***	−0·40*	−0·66**	Developmental Time
FF	+5·94***	+5·62***	−0·75**	−1·07***	
SS	+2·20***	+4·61***	+0·45**	+2·86***	
FS	+5·70***	+6·03***	−0·05 ns	+0·28 ns	Life span
FF	+7·09***	+6·73***	+1·90***	+1·54**	

LRSe lines, the ethanol provokes a great mortality increase (up to 91·75 per cent in LRC-S), a great delay in the developmental times (between 5 and 6 days) and a considerable life span increase when ethanol is the only food (until 7 days for LRC-F). However, negative (egg-to-adult viability and developmental time) effects are slighter and the positive effect (life span) more important for the selected lines with the exception of the Adh^F Adh^F genotype for life span (see also figs. 1, 2 and 3). This smaller life span increase in LRSe-F than in LRC-F may be understood by the exceptional starvation resistance increase provided by selection of the LRC-F.

(iv) *Selection effects*

Differences for significance are tested in table 3 by means of a student's test for each genotype separately for both normal and supplemented medium (LRC vs. LRSe lines). For this, in each respective case, the error mean squares used were that of analyses of variances separately done for each medium. In the absence of ethanol no changes in the egg-adult mortality were noted by the ethanol selection. However, in such normal medium, changes have been found in the developmental times for all three genotypes and in the life span for both homozygous ones. In the presence of ethanol, the same changes were brought about by the ethanol selection but, moreover, the ethanol tolerance of all three genotypes has been improved.

The effects of selection, quantified by the differences between data from selected and control lines, are also shown in table 3. By considering only the significative statistical values for both media, the ethanol selection produces an increase in the egg-to-adult viability (up to a 7·42 per cent of lower mortality), shorter developmental time (to 1·29 days in LRSe in

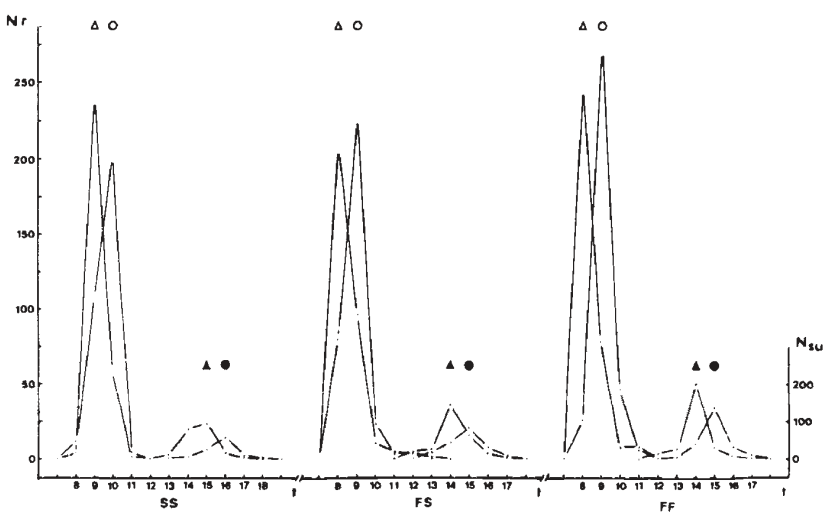

FIG. 2. Egg-to-adult developmental time-viability curves of LRC (○, ●) and LRSe (△, ▲) on regular (○, △) and supplemented (●, ▲) mediums for each *Adh* genotype, as a function of the number of days (t). 400 eggs on regular medium (Nr) and 1600 on the supplemented one (Nsu) were used for each curve.

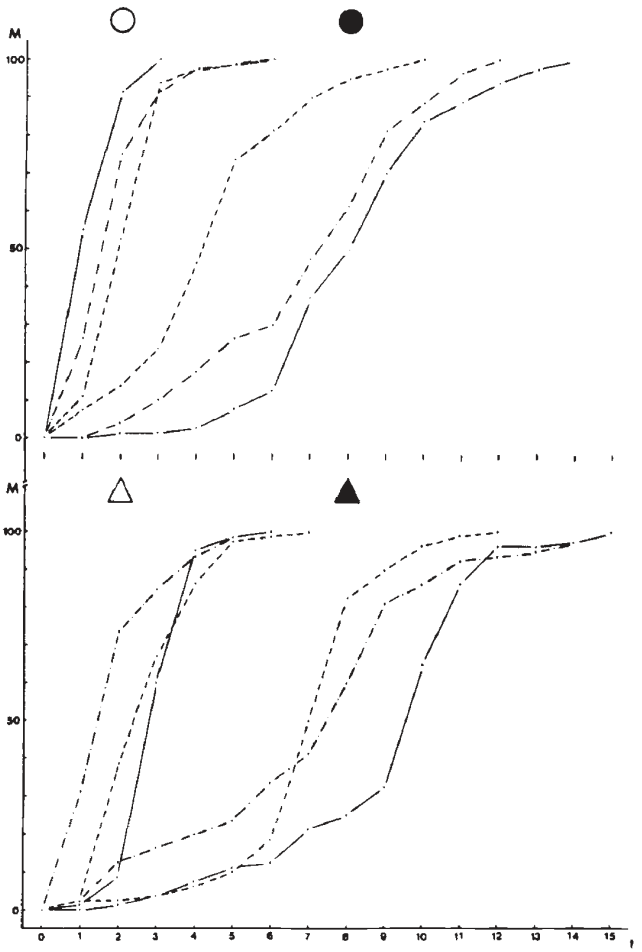

FIG. 3. Adult mortality (M) curves of LRC (○, ●) and LRSe (△, ▲) lines on basic (○, △) or a 11 per cent ethanol basic medium (●, ▲), as a function of the number of days (t). Each curve is based on 80 flies (40♂♂ and 40♀♀). FF: ——; FS: — · —; SS: – – –.

alcoholic medium), and a higher resistance to starvation (to 1·89 days at FF) and a more efficient ethanol utilisation (to an increase of 2·86 days in the life duration for SS) for both homozygotes. For both ethanol tolerance and developmental times, the positive effects of selection are higher in the supplemented medium (see also fig. 2), which can explain the line × medium interaction (see ANOVA in table 4). As life span data from non-alcoholic and alcoholic media have different significance, the effects of the selection do not have to be related. However, both the effects of resistance to starvation and of alcohol utilisation are improved by the selection (see also fig. 3) only in the homozygotes. As the heterozygotes seems not to be improved for this fitness component, different systems (probably involving *Adh* locus) for increasing life span may have been selected at each homozygous line. Further experiments must be done.

TABLE 4

Relative fitness values for each genotype and line in both normal and supplemented medium. 95% confidence intervals in parentheses. Estimates of the relative fitness are based on a ratio of mean values, that with the highest fitness being the "standard" one

Control lines			Selected lines			Fitness Component	Medium
SS	FS	FF	SS	FS	FF		
0·920	0·963	1	0·861	0·926	0·972	Viability	Normal
(1·014, 0·836)	(1·058, 0·876)	(1·097, 0·911)	(0·951, 0·778)	(1·020, 0·841)	(1·067, 0·884)		
0·870	0·938	0·918	0·908	0·982	1	Developmental Time	
(0·907, 0·834)	(0·979, 0·898)	(0·957, 0·879)	(0·947, 0·870)	(1·027, 0·940)	(1·045, 0·956)		
0·739	0·603	0·427	0·876	0·588	1	Starvation Resistance	
(0·800, 0·682)	(0·659, 0·549)	(0·480, 0·377)	(0·941, 0·815)	(0·644, 0·534)	(1·069, 0·935)		
0·370	0·661	0·817	0·703	0·938	1	Viability	
(0·458, 0·288)	(0·761, 0·570)	(0·925, 0·719)	(0·804, 0·610)	(1·053, 0·835)	(1·119, 0·893)		
0·879	0·945	0·929	0·957	0·989	1	Developmental Time	Suppl.
(0·901, 0·857)	(0·969, 0·921)	(0·953, 0·905)	(0·982, 0·932)	(1·015, 0·963)	(1·027, 0·974)		
0·462	0·765	0·847	0·747	0·794	1	Ethanol Utilization	
(0·514, 0·412)	(0·825, 0·708)	(0·909, 0·788)	(0·806, 0·691)	(0·854, 0·736)	(1·067, 0·937)		

(v) *Relative fitnesses*

To summarise the results, relative fitnesses for each genotype, fitness component and medium are shown in table 4. To estimate the relative fitnesses for each component, the ratio of each mean value to the one with the highest fitness within each medium was used. To estimate the error variances of the standard values for each medium, the respective error mean squares of the analyses of variances were used (see Bliss, 1967).

In general, for all three components studied and in both regular and supplemented medium, the FF homozygotes at the selected lines show the highest relative fitness, this superiority being clearer in the alcoholic media than in non-alcoholic ones.

4. Discussion

First of all, we must say that it is unreasonable to attribute all the changes only to selection at the *Adh* locus. Linked unknown genes or even independent genes could be involved. However, our results, in general, are in agreement with others taken from widely separated populations (for example, the Colmar strain, France (David and Bocquet, 1977); Kaduna, Nigeria (Daly and Clarke, 1981); Groningen, The Netherlands (van Delden *et al.*, 1978; van Delden and Kamping, 1979); Puerto Asis, Columbia (van Herewege and David, 1980)).

Van Delden and Kamping (1979), studying the developmental times of Groningen strains for *Adh* locus on regular medium, asked themselves if the shorter developmental time of FF and FS larvae was a general phenomenon. We have found, on regular food, slower developmental times of SS homozygotes and, moreover, the developmental times for all three genotypes have been significantly decreased by the alcohol selection. Colmar selected lines also show a faster developmental time than non-selected ones, though the alcohol selection had eliminated Adh^S alleles (David and Bocquet, 1977). As these differences in developmental times are found in non-alcoholic media, another role must be postulated for the ADH isozymes in addition to the detoxification of alcohols if the *Adh* locus is involved in developmental time. On the other hand, the ethanol in the medium produces a considerable delay in developmental time, though this delay is lower in the selected lines. Oakeshott (1976) linearly relates the differences in developmental time in the ethanol supplemented medium to the differences in the ADH activity. However, doubts about the relationships between the ADH activity alone and developmental time came from the same author (Oakeshott, 1977) on using the pentenol supplemented medium. Selective differences in developmental time were similar in direction for both ethanol and pentenol although Sofer and Hatkoff (1972) suggested that, *in vivo*, ADH oxidizes pentenol into a lethal ketone, ethyl vinyl ketone. Our results increase these doubts, as SS homozygotes seem to have the highest relative reduction in developmental time.

In relation to egg-to-adult viability, in general, no significant intergenotypic differences were observed in normal medium for this fitness component. However, on supplemented medium, significant differences can be noted, homozygous SS being less tolerant, from the first generation of selection (control lines at 11 per cent ethanol medium), which supports the

notion that ADH isozymes must play an important role in ethanol detoxification. Although there are exceptions (Gibson *et al.*, 1979; Oakeshott, 1979; Ziolo and Parsons, 1982), all from Australian winery populations, most of the papers suggest that alcohol treatments modify *Adh* allele frequencies (Gibson, 1970; Bijlsma-Meeles and van Delden, 1974; van Delden *et al.*, 1975, 1978; Cavener and Clegg, 1978, 1981) by favouring the most active one.

Our results show that the selected flies having the Adh^F allele are more tolerant than homozygous SS ones which is in agreement with the above results. This dominance of the Adh^F allele over the Adh^S one was also observed by Briscoe *et al.* (1975), from a Spanish winery population. However, homozygous SS flies are the most improved by selection for ethanol tolerance. Several papers support the idea that ADH plays a significant role in the detoxification of ethanol (David *et al.*, 1976, 1978, 1981; Kamping and van Delden, 1978) and of isopropanol and acetone (David *et al.*, 1981). However, ethanol detoxification and ethanol utilisation as food, both depending on an active ADH, seem to be controlled by different genetic mechanisms (van Herrewege and David, 1980; David *et al.*, 1981). General ethanol detoxification processes involve its conversion into acetate, by acetaldehyde, which is further transformed into metabolic energy in the Krebs cycle (Clarke, 1975; David *et al.*, 1976; Deltombe-Lietaert *et al.*, 1979).

Our results clearly support the view that ethanol can be used as food by *Drosophila melanogaster*. For both control and selected lines and all three genotypes, ethanol considerably increases the survival of adults. In addition, selection has improved ethanol utilisation, although only for FF and SS homozygotes. Again, the homozygous SS line has been the most improved, but FF flies are always the best at using ethanol as food. Similar results were obtained for both, high (Briscoe *et al.*, 1975; Morgan, 1975; Libion-Mannaert *et al.*, 1976) and low ethanol concentrations (Daly and Clarke, 1981). That the better ability to use ethanol for FF flies is not due to a higher toxicity for the SS ones is suggested by a much better life span increase of the SS flies on alcohol than any of the other flies on water alone.

On the other hand, ethanol tolerance and ethanol utilisation do not seem to be related. While selection has decreased the egg-adult mortality for all three genotypes, ethanol utilisation has only been improved for both homozygotes. This fact seems to support the hypothesis of partial independence of these components, proposed by van Herrewege and David (1980).

Finally, we can point out the changes in the resistance to starvation brought about by the ethanol selection. These changes seem not to be related to any known role of the *Adh* locus. However such changes have also been reported in other papers (David and Bocquet, 1977; van Herewege and David, 1980), associated with alcohol selection. Further experiments would be interesting.

In conclusion, the ethanol selection has resulted successfully for all three fitness components studied. After selection a higher ethanol tolerance, a better ability to use ethanol as food and a shorter developmental time have been observed when flies are grown on ethanol supplemented media. Though SS homozygotes have been the most improved, FF homozygotes have the highest relative fitness for all three components. This highest fitness of the FF homozygotes is in agreement with the rapid increase of the Adh^F allele when polymorphic populations for the *Adh* locus are submitted to

an ethanol selection. Without doubt, the high ethanol tolerance of *D. melanogaster* has certainly a great ecological significance (McKenzie and Parsons, 1974) and such a trait must involve a complex genetic system, in which the *Adh* locus plays an important role. When a population with enough genetic variability is submitted to an ethanol stress, any genetic change providing a better and faster adaptive response (a Adh^F allele, for example) can be favourably selected though sometimes a specific physiological change can not be associated with a given genetic one.

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