The Alcohol Dehydrogenase Polymorphism in *Drosophila melanogaster*: Fitness Measurements and Predictions Under Conditions With No Alcohol Stress

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ABSTRACT

The relative fitnesses of the different *Adh* genotypes under normal laboratory conditions and in the absence of alcohol stress were estimated in *Drosophila melanogaster* according to Prout's method. The larval component (viability) did not reveal fitness differences between the genotypes but for the adult component significant differences were observed. The female adult component (fecundity) showed an overdominant pattern: both homozygous genotypes showed a relative fitness significantly lower than the heterozygous genotype. For the male adult component (virility) also differences were observed. The homozygous *SS* genotype showed a lower relative fitness than the other two genotypes. Predictions for gene frequency changes based on the estimated fitness values do show a reasonably good correspondence with frequency changes actually observed in a number of experimental cage populations and indicate a globally stable equilibrium around a frequency of the *F* allele of 0.40–0.70. The relevance of these fitness estimates, obtained under conditions with no alcohol stress, for the explanation of the *Adh* polymorphisms observed in nature is discussed.

THE maintenance of allozyme polymorphisms has been an issue ever since 1966. Still it is not evident whether the majority of these polymorphisms are neutral or maintained by selective forces. However, by applying environmental and physiological situations thought to be relevant to the functioning of the enzymes studied, it has been shown that a number of allozyme polymorphisms in *Drosophila melanogaster* are subject to selection. Especially the *Adh* locus has been studied extensively in relation to environmental alcohols that could serve as a substrate for the enzyme (for a review see VAN DELDEN 1982). In the presence of a deleterious amount of alcohol in the medium there is a highly significant positive correlation between the relative *in vitro* enzyme activity and survival. Experiments of this kind show almost unanimously that the *FF* genotype, with the highest *in vitro* enzyme activity has a significantly better survival than the *SS* genotype which shows a much lower *in vitro* activity. The *FS* genotype, which has an intermediate *in vitro* activity, generally shows a survival rate in between both homozygous genotypes. As these results could be explained by the detoxifying action of ADH it is highly probable that in these cases the selection acts through the *Adh* locus itself and not through other linked fitness genes.

If exposure to alcohol were the only selection pressure, fixation of the *F* allele would eventually occur. However, nearly all natural populations have been observed to be polymorphic (VAN DELDEN 1982), so

there must be either some kind of balancing selection force or else we have to assume that selection by alcohols does not really play an important role in these populations. It has been suggested that the higher temperature stability of the *S* enzyme observed *in vitro*, could create a counter-force favoring the *S* allele at relatively high temperatures. This difference in thermostability has been related to latitudinal clines that have been observed in nature. Experimental populations, however, kept at different temperatures do not confirm this suggestion. Furthermore VAN DELDEN and KAMPING (1980) showed that, although the *S* enzyme has a higher thermostability *in vitro*, this may have no meaning *in vivo*. It has also been suggested that the latitudinal cline is due to an association of the *S* allele with a natural occurring inversion, *In(2L)t* (INOUE *et al.* 1984; VAN DELDEN 1984).

There are also indications, however, of some kind of balancing selection under regular food conditions. BIJLSMA-MEELES and VAN DELDEN (1974) observed a significant convergence of allele frequencies in experimental populations leading to values more or less equal to those in the original population. VAN DELDEN, BOEREMA and KAMPING (1978) argue on the basis of repeated observations of convergence of allele frequencies after many reisolations and the use of high numbers of founding lines that the observed convergences were not due to hitchhiking as was suggested by JONES and YAMAZAKI (1974). Several fitness components have been proposed to influence

the *Adh* polymorphism under regular conditions: overdominance for egg-to-adult survival (VAN DELDEN, BOEREMA and KAMPING 1978), frequency dependent egg-to-adult survival (KOJIMA and TOBARI 1979), differences in developmental time (VAN DELDEN and KAMPING 1979) and differences in mating success (POT, VAN DELDEN and KRUIJT 1980).

So far these data mostly deal with isolated fitness components and it therefore difficult to get a reliable picture of how allele frequencies change from one generation to the next. Therefore the aim of this paper was to measure the relative fitnesses of the different *Adh* genotypes over the whole life cycle under regular laboratory conditions, and to see whether or not the observed fitness values would lead to a stable polymorphism. Out of several procedures that have been proposed to estimate fitness, among others ANDERSON (1969), PROUT (1971a, b) and BUNDGAARD and CHRISTIANSEN (1972), the method described by PROUT (1971a) was chosen (for an extensive review on fitness measurements see HEDRICK and MURRAY, 1983). Furthermore a number of experimental cage populations were established with different initial allele frequencies for the *Adh* locus. The actual behaviour of this polymorphic system will be compared with predictions derived from the fitness estimates.

MATERIALS AND METHODS

Outline of PROUT's Fitness Measurements: The relative fitnesses of the three *Adh* genotypes (homozygotes *SS*, heterozygotes *SF* and homozygotes *FF*) were estimated according to the experimental design formulated by PROUT (1971a). This method allows an estimate of the net fitnesses without studying every detail of the life cycle. For the convenience of the reader the method is basically set forth below in a summarized form, but for a detailed understanding and a much more elaborate description of the theoretical outlines and procedure the reader is referred to the original paper by PROUT.

The net fitness is resolved into two components for each sex: a larval component and an adult component. The larval component is the usual viability and denoted by *L*. The female adult component, although it includes adult survival, principally involves differential fecundity and is denoted by *E* (we have adopted the symbol *E* for egg laying instead of PROUT's *F* for fecundity to avoid confusion with the *Adh*^F allele). The male adult component is denoted by *V* for virility, as differences in male mating success contribute for the greater part to this component. Assuming that the heterozygous genotype has a fitness of unity, the parameters that have to be measured are as shown in Table 1. The larval component can be estimated in a straightforward way from a cross between heterozygotes. Under the assumption of a normal segregation ratio the cross *SF* × *SF* will produce the three genotypes in the expected ratio *SS*:*SF*:*FF* = 1:2:1, so from the offspring proportions *SS* = $\hat{Q}_{SS}$, *SF* = $\hat{Q}_{SF}$ and *FF* = $\hat{Q}_{FF}$, the viability can easily be estimated as follows:

$$L_{SS} = 2\hat{Q}_{SS}/\hat{Q}_{SF} \quad \text{and} \quad L_{FF} = 2\hat{Q}_{FF}/\hat{Q}_{SF}.$$

The estimation of the adult components is much more

TABLE 1
Parameters to be measured

Parameter	Females			Males		
Genotypes	<i>SS</i>	<i>SF</i>	<i>FF</i>	<i>SS</i>	<i>SF</i>	<i>FF</i>
Larval component	L_{SS}	1	L_{FF}	L_{SS}	1	L_{FF}
Adult component	E_{SS}	1	E_{FF}	V_{SS}	1	V_{FF}

complex and is done by mating a mixture of genotypes of one sex to only one genotype of the other sex (the constant parent). For each sex there are three different crosses: with *SS*, *SF* and *FF* as constant parent respectively. A mixture of females is used to estimate *E*, while a mixture of males is used to estimate *V*. PROUT (1971a, Figure 1) shows for a mixture of females mated to heterozygous males the transition from one generation to the next. The same procedure can be performed for all combinations. Table 2, column (a), shows the offspring frequencies, $\hat{Q}_{SS}$, $\hat{Q}_{SF}$ and $\hat{Q}_{FF}$, in the case when a mixture of females with input frequencies Q_{SS} , Q_{SF} and Q_{FF} for *SS*, *SF* and *FF*, respectively, is mated to the three constant males. Note that these equations are similar when the virility is estimated by using females as constant parent, only in that case *E* has to be substituted by *V*. The next column, (b), shows the ratio between the different offspring genotypes for the different constant parents. When the viabilities are known, it is easy to recalculate these ratios and to obtain the ratios at fertilization (column c) by multiplying them by the reciprocal of the viabilities. Next these ratios can be transformed by dividing the numerator and denominator by Q_{SF} and by further substituting $Q_{SS}/Q_{SF} = m$ and $Q_{FF}/Q_{SF} = n$ we get the theoretical expectation of the gene ratio at fertilization, *r*, shown in column d. (A pair of *m* and *n* values represent a particular parental mixture and the actual initial proportions, Q_{SS} , Q_{SF} and Q_{FF} , can be easily recalculated from *m* and *n*). As can be seen from the table the theoretical expectations of *r* are the same for all three constant parents. Given the offspring frequencies of a certain parental mixture (thus *m*, *n* and the resulting *r* value known) there remain two unknown parameters, E_{SS} and E_{FF} or V_{SS} and V_{FF} , to be estimated. Therefore we need two different parental mixtures to provide sufficient independent equations to estimate these two parameters for each of the constant parents:

$$r_1 = \frac{m_1 E_{SS} + 1/2}{n_1 E_{FF} + 1/2} \quad \text{and} \quad r_2 = \frac{m_2 E_{SS} + 1/2}{n_2 E_{FF} + 1/2}$$

in which *r*₁, *m*₁ and *n*₁ represent one mixture and *r*₂, *m*₂ and *n*₂ the other mixture. Now E_{SS} and E_{FF} can be solved leading to:

$$E_{SS} = \frac{r_1 n_1 + r_1 r_2 (n_2 - n_1) - r_2 n_2}{2(r_2 n_2 m_1 - r_1 n_1 m_2)}$$

and

$$E_{FF} = \frac{m_1 - m_2 + m_2 r_1 - m_1 r_2}{2(r_2 n_2 m_1 - r_1 n_1 m_2)}.$$

In our experiments we used the following mixtures:

$$SS:S F:FF = 30:15:5 \quad \text{which gives } m_1 = 2 \text{ and } n_1 = 1/3,$$

and

$$SS:S F:FF = 5:15:30 \quad \text{which gives } m_2 = 1/3 \text{ and } n_2 = 2.$$

TABLE 2

Offspring genotype frequencies for the different constant parents, together with the calculation of the gene ratio at fertilization, r^a

Constant parent	Offspring genotype	Offspring output (a)	Offspring ratio (b)	Ratio at fertilization (c)	Transformed ratio at fertilization (d)
SS:	SS:	$\hat{Q}_{SS} = \frac{1}{EL} (Q_{SS}E_{SS} + \frac{1}{2}Q_{SF})L_{SS}$	$\frac{\hat{Q}_{SS}}{\hat{Q}_{SF}} = \frac{(Q_{SS}E_{SS} + \frac{1}{2}Q_{SF})L_{SS}}{(Q_{FF}E_{FF} + \frac{1}{2}Q_{SF})L_{FF}}$	$\frac{\hat{Q}_{SS}}{\hat{Q}_{SF}} \left(\frac{1}{L_{SS}} \right) =$	$r = \frac{mE_{SS} + \frac{1}{2}}{nE_{FF} + \frac{1}{2}}$
	SF:	$\hat{Q}_{SF} = \frac{1}{EL} (Q_{FF}E_{FF} + \frac{1}{2}Q_{SF})$			
	FF:	$\hat{Q}_{FF} = 0$			
SF:	SS:	$\hat{Q}_{SS} = \frac{1}{2EL} (Q_{SS}E_{SS} + \frac{1}{2}Q_{SF})L_{SS}$	$\frac{\hat{Q}_{SS}}{\hat{Q}_{FF}} = \frac{(Q_{SS}E_{SS} + \frac{1}{2}Q_{SF})L_{SS}}{(Q_{FF}E_{FF} + \frac{1}{2}Q_{SF})L_{FF}}$	$\frac{\hat{Q}_{SS}}{\hat{Q}_{FF}} \left(\frac{L_{FF}}{L_{SS}} \right) =$	$r = \frac{mE_{SS} + \frac{1}{2}}{nE_{FF} + \frac{1}{2}}$
	SF:	$\hat{Q}_{SF} = \frac{1}{2L}$			
	FF:	$\hat{Q}_{FF} = \frac{1}{2EL} (Q_{FF}E_{FF} + \frac{1}{2}Q_{SF})L_{FF}$			
FF:	SS:	$\hat{Q}_{SS} = 0$	$\frac{\hat{Q}_{SF}}{\hat{Q}_{FF}} = \frac{(Q_{SS}E_{SS} + \frac{1}{2}Q_{SF})}{(Q_{FF}E_{FF} + \frac{1}{2}Q_{SF})L_{FF}}$	$\frac{\hat{Q}_{SF}}{\hat{Q}_{FF}} (L_{FF}) =$	$r = \frac{mE_{SS} + \frac{1}{2}}{nE_{FF} + \frac{1}{2}}$
	SF:	$\hat{Q}_{SF} = \frac{1}{EL} (Q_{SS}E_{SS} + \frac{1}{2}Q_{SF})$			
	FF:	$\hat{Q}_{FF} = \frac{1}{EL} (Q_{FF}E_{FF} + \frac{1}{2}Q_{SF})L_{FF}$			

^a Q_{SS} , Q_{SF} and Q_{FF} are the input frequencies of SS, SF and FF, respectively, while $\hat{Q}_{SS}$, $\hat{Q}_{SF}$ and $\hat{Q}_{FF}$ are the respective output frequencies. $\bar{E}$ and $\bar{L}$ are normalizing factors. $m = Q_{SS}/Q_{SF}$ and $n = Q_{FF}/Q_{SF}$.

Substituting these values in the foregoing equations gives:

$$E_{SS} = V_{SS} = \frac{3r_1 + 15r_1r_2 - 18r_2}{72r_2 - 2r_1} \quad (1)$$

$$E_{FF} = V_{FF} = \frac{3r_1 - 18r_2 + 15}{72r_2 - 2r_1} \quad (2)$$

When the constant parent is heterozygous all three offspring genotypes are present (Table 2). This extra information can be used to test whether the heterozygotes form half of the fertilized eggs. Therefore PROUT has derived the following equation:

$$z = \hat{Q}_{SS} \left(\frac{1}{L_{SS}} + 1 \right) + \hat{Q}_{FF} \left(\frac{1}{L_{FF}} + 1 \right).$$

If segregation is Mendelian and the viability estimates are correct, z is expected to be unity.

Lines: The lines used for the experiments were isolated from the Groningen base population. This population is the same as used by BIJLSMA-MEELES and VAN DELDEN (1974) and had been founded with 18 inseminated females collected from a local fruit market in 1967, and kept as a cage population (3,000–5,000 individuals) since then. Four years after the establishment of the cage (a period sufficiently long to ensure decay of initial linkage disequilibria that might have been present at the moment of founding) from this population 16 lines homozygous for the *Adh*^S allele and 16 lines homozygous for the *Adh*^F allele (hereafter called SS and FF lines) were extracted. All lines were independently obtained by collecting the offspring from randomly made single pair crosses, in which both parents were shown afterwards to be of the same homozygous genotype. After isolation these lines (numbered from 1 up to 16 for each genotype) were maintained as separate bottle populations on regular food consisting of 1000 ml water, 19 g agar, 32 g dead yeast, 54 g sucrose and 13 ml nipagin solution (10 g nipagin in 100 ml 96% ethanol). The flies were transferred to new bottles with fresh food every 2 weeks. The culture

conditions were 25° and 50–70% RH. All experiments were performed under these conditions.

Experimental cage populations: One month after the isolation of the homozygous lines, three experimental cage populations were started with different initial frequencies of the *F* allele: 0.10 F, 0.45 F and 0.90 F. Each cage was started with about 1000 fertilized females from the homozygous lines. This was done in such a way that all 16 lines of a given genotype contributed equally to the population. From time to time samples were taken from these cages to determine the allele frequencies. Sampling was performed by inserting two fresh foodcups in each cage and flies were allowed to lay eggs for one day. The emerging adults from these two foodcups were collected for three weeks and mixed together. By means of electrophoresis the genotype of 100 females and 100 males of each sample was determined (during the second half of the experiment sometimes only 75 flies of each sex were assayed).

Estimation of the larval fitness components: Flies for this experiment were obtained by crossing females from the 16 individual SS lines with males from the 16 individual FF lines, and reciprocally; thereafter the female offspring of a particular cross were mated to the male offspring of the reciprocal cross according to the following scheme:

$$\begin{array}{l} P: (SS)_i \times (FF)_{i+1} \delta \quad (FF)_j \times (SS)_{j+1} \delta \\ F_1: \quad \quad \quad \searrow \quad \quad \quad \swarrow \\ \quad \quad \quad S_i F_{i+1} \times F_j S_{j+1} \delta \end{array}$$

($i = 1, \dots, 16$, is line number, note that line 16+1 = line 1). In this way 16 different F_1 crosses were performed involving different line combinations.

Although this procedure might increase the variance between replicates it avoids another important problem. Often the isolated lines homozygous for the same allele are intercrossed to form a single homozygous population (either SS or FF), some time prior to the experiments (BIJLSMA-MEELES 1979; DORADO and BARBANCHO 1984; KOJIMA and TOBARI 1969; MORGAN 1976; VAN DELDEN, BOEREMA and KAMPING 1978). Homozygous flies for either allele then can

be obtained directly from these populations but heterozygous flies can be obtained only by a cross *between* flies of the two populations. Consequently homozygous and heterozygous flies might differ with respect to the amount of variation in the genetic background. The procedure given above avoids this problem. As all 32 lines were independently isolated we may assume that differences in genetic background between lines of the same allele are the same as between lines of different alleles. Hence the genetic background variation is the same for homozygote and heterozygote flies, both in the viability and the fecundity experiment (see below).

From each cross 500 eggs were collected (for the collecting procedure, see BIJLSMA-MEELES 1979) and then transferred to 125 ml glass bottles containing 30 ml of regular food. From each of the 16 bottles all emerging flies were collected, their numbers and sex determined and a sample of 50 females and 50 males was taken for genotype identification by means of electrophoresis.

Estimation of the adult fitness components: To get an estimate of the adult fitness components, mixtures of the genotypes of one sex were mated to just one genotype of the other sex. Both homozygous and heterozygous flies used in the experiments resulted from crosses between different homozygous lines and furthermore the flies of each sex originated also from different line combinations. The replicates within each type of cross involved also different line combinations, as is indicated below:

replicate 1: $S_1S_2 + S_1F_2 + F_1F_2$ ♀♀ mated to S_3S_4 (or S_3F_4 or F_3F_4) ♂♂

replicate 2: $S_2S_3 + S_2F_3 + F_2F_3$ ♀♀ mated to S_4S_5 (or S_4F_5 or F_4F_5) ♂♂

etc.

As mentioned above, this crossing procedure ensures that the different *Adh* genotypes are comparable with respect to the heterozygosity of the genetic background.

For each replicate both mixtures needed to estimate the adult components were established. Fifty flies of each sex were used in each replicate. The flies were collected as virgins and after three days the appropriate mixtures were made so that mating could take place. After 2 more days these flies were, without etherization, transferred to new bottles and were allowed to lay eggs for 24 hr. From these bottles all emerging flies were collected and their numbers and sex determined. From each replicate a random sample of 50 females and 50 males was assayed for genotype identification by means of electrophoresis. A summary of the experiments performed is shown in Table 3.

Electrophoresis: The *Adh* genotype of flies was determined by the electrophoretic method described by BIJLSMA-MEELES and VAN DELDEN (1974). In spite of the fact that agar electrophoresis was used for genotype identification, throughout the paper the polyacrylamide terminology is used for description of the *Adh* alleles.

RESULTS

The larval component: In Table 4A the total offspring numbers over the 16 replicates are shown. The mean number of emerging adults per bottle was 413.3 with a SE of 8.41, so the mean egg-to-adult survival was 82.7%. This indicates that the flies were reared under more or less optimal conditions. Furthermore the table shows the genetic composition of the samples

TABLE 3

Summary of the crosses performed to measure the fecundity and virility of the *Adh* genotypes

Fecundity							
Females			Mated to	Males			No. of replicates
SS	SF	FF		SS	SF	FF	
30	15	5	×	50			13
5	15	30	×	50			12
30	15	5	×		50		13
5	15	30	×		50		12
30	15	5	×			50	12
5	15	30	×			50	14
Virility							
50			×	30	15	5	14
50			×	5	15	30	10
	50		×	30	15	5	14
	50		×	5	15	30	12
		50	×	30	15	5	12
		50	×	5	15	30	12

Fifty flies of each sex were used in each replicate. The numbers of each genotype in the different mixtures are shown.

of 50 females and 50 males summed over the 16 replicates. χ^2 values were calculated to detect heterogeneity among replicates, but the differences were found not to be significant.

Based on the assumption of the occurrence of a normal mendelian segregation the viabilities were estimated for each replicate. This was done for females and males separately. Table 4B shows the mean viabilities together with their standard errors. It is clear that the relative viabilities of the homozygous genotypes differ only very slightly from 1. Student *t*-testing the viabilities against unity shows that these differences are not significant. Within each homozygous genotype the difference in viability between the sexes was tested by means of an analysis of variance of which the results are shown in Table 4C. For both homozygous genotypes the difference between females and males was found to be not significant. In order to get a sex independent viability estimate for each homozygous genotype the data of both sexes were lumped. These estimates, shown at the bottom of Table 4B, are even closer to unity. Therefore the conclusion seems to be straightforward: under the given experimental conditions there are no differences in viability, neither between the different *Adh* genotypes nor between the different sexes.

The adult components: The finding that the viability estimates do not differ from unity simplifies the estimation procedure of the adult fitness components considerably. Firstly the offspring proportions after larval selection become identical with the genotypic proportions at fertilization. This means that corrections for viability differences as shown in Table 2 are unnecessary. Secondly, because there are no sex dif-

TABLE 4
Results of the viability experiment

A	No. of replicates	Total offspring No.	Genotypes in samples			χ^2_{het}	d.f.
			SS	SF	FF		
Females	16	3362	188	415	197	24.5	30
Males	16	3251	209	390	201	30.9	30
Total	16	6613	397	805	398	27.2	30
B		SS	SF		FF		
Viability females		0.956 (0.097)	1		0.987 (0.070)		
Viability males		1.111 (0.085)	1		1.076 (0.096)		
Viability sexes combined		1.015 (0.070)	1		1.011 (0.055)		
C		SS	d.f.	MS	$F_{1,30}$		
Genotype SS:							
Between sexes		0.064	1	0.064	0.570	NS*	
Within sexes		3.380	30	0.113			
Total		3.445	31				
Genotype FF:							
Between sexes		0.191	1	0.191	1.440	NS*	
Within sexes		3.983	30	0.133			
Total		4.174	31				

A. Total offspring numbers and genotypic composition in the samples summed over all replicates together with χ^2 values evaluating differences in genotypic composition among replicates.

B. Viability estimates for the different *Adh* genotypes and different sexes. Standard errors calculated from the variance among replicates in parentheses.

C. Analysis of variance to test the difference in viability estimates between males and females. * NS is not significant at the 5% level.

ferences, males and females can be lumped together for each culture to give one estimate of the fertilization ratio (r) per culture.

The mean number of offspring per bottle was found to be 424.6 with a standard error of 4.89. This number is not significantly different from that observed for the viability experiment. As no indications of crowding were observed, we may assume that the larval densities in the viability and adult experiment were more or less the same.

Table 5 shows the total genotype numbers after electrophoresis for all 12 experiments performed to estimate the adult fitness components. χ^2 values were calculated to detect heterogeneity among replicates within each experiment. The results show in most cases a highly significant heterogeneity, a situation similar to the one observed by PROUT (1971a). This might for the greater part be due to the low number of founding parents, being only 50 pairs in each replicate from which there were only 5 pairs of the less frequent genotype. This will most likely have introduced a considerable component of random drift.

Table 6 shows the calculated mean r values (averaged over all replicates) and the standard errors for each constant parent and mixture. By means of an

analysis of variance we tested whether the r values were independent of the genotype of the constant parent. Table 7 shows the result for each parental mixture for both the fecundity and the virility experiment. It is clear that the F values are small and not significant, indicating that both the female and male adult fitnesses are independent of the genotype of the constant parent. Therefore the data can be pooled to give a combined estimate of the mean r values over the three constant parents. These values are also shown in Table 6.

When the constant parent was heterozygous, Table 6 also shows means and standard errors of z . It is clear that the z values are close to 1 and performing t -tests on the deviation from unity shows that none of these deviations is significant ($t = 0.24$ and $t = 0.54$ for the fecundity experiment and $t = 0.75$ and $t = 1.14$ for the virility experiment). This indicates that there appears to be a 1:1 segregation in the heterozygous constant parent and that the viability estimates used are correct.

The estimates of the adult components now can be easily obtained by substituting the mean r values (pooled over the three constant parents) together with the accompanying m - and n -values in the Equations 1 and 2 derived in the outline of the procedure. The results are shown in Table 8. The data for the fecundity indicate a strong overdominant pattern: both homozygous genotypes show a fitness significantly smaller than unity. For the virility the SS genotype shows a lower fitness than the other two, but the fitness values do not differ significantly from 1. The standard errors in this case were obtained by using Taylor's expansion and the empirical standard errors of the mean r values.

Our experimental setup, however, makes it possible to estimate the standard errors of the adult components in a different way. As has been outlined in MATERIAL AND METHODS each replicate involved a specific set of crosses between the isolated homozygous lines, but of each replicate both mixtures of genotypes were established. This makes it possible to estimate the adult fitnesses for each replicate separately, and in this way means and standard errors of the fitness estimates can be calculated directly. The number of usable replicates, however, is reduced by almost 30% in this case, because data of both mixtures were not always actually available due to escaping flies at the moment of transfer or to considerable mold/bacterial growth in some of the bottles. Moreover, for a few replicates (two for the fecundity and three for the virility experiment) the results of the two mixtures did not fit together for reasons unknown and resulted in unrealistic estimates. For instance one replicate of the virility experiment gave rise to extraordinary negative estimates: $V_{SS} = -24.0$ and $V_{FF} = -17.3$. These

TABLE 5
Genotype numbers in the offspring of the different fecundity and virility experiments

Constant parent	Mixture				No. of replicates	Female offspring					Male offspring					Total offspring		
	SS	SF	FF			SS	SF	FF	χ^2_{het}	d.f.	SS	SF	FF	χ^2_{het}	d.f.	χ^2_{het}	d.f.	
Fecundity																		
SS ♂♂	30	:	15	:	5	13	473	177	...	33.82 ^c	12	471	179	...	32.70 ^c	12	60.40 ^c	12
	5	:	15	:	30	12	195	405	...	12.97	11	213	387	...	19.42	11	22.37 ^a	11
SF ♂♂	30	:	15	:	5	13	235	321	94	34.71	24	213	324	103	36.17	24	42.35 ^a	24
	5	:	15	:	30	12	100	301	199	36.03 ^a	22	83	312	205	44.35 ^b	22	49.76 ^c	22
FF ♂♂	30	:	15	:	5	12	...	446	154	19.25	11	...	448	152	38.13 ^c	11	45.01 ^c	11
	5	:	15	:	30	14	...	210	490	60.19 ^c	13	...	201	499	48.58 ^c	13	98.33 ^c	13
Virility																		
SS ♀♀	30	:	15	:	5	14	490	210	...	74.10 ^c	13	504	196	...	54.96 ^c	13	122.58 ^c	13
	5	:	15	:	30	10	117	383	...	26.79 ^b	9	115	385	...	30.32 ^c	9	47.68 ^c	9
SF ♀♀	30	:	15	:	5	14	249	351	100	32.10	26	238	332	130	31.17	26	49.61 ^b	26
	5	:	15	:	30	12	58	314	228	32.47	22	69	301	230	27.57	22	33.93 ^a	22
FF ♀♀	30	:	15	:	5	12	...	426	174	16.22	11	...	383	217	26.59 ^b	11	28.27 ^b	11
	5	:	15	:	30	12	...	133	467	22.36	11	...	165	435	10.46	11	25.27 ^a	11

^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$.

TABLE 6

Mean gene ratio r after transformation of the data from Table 5, together with the values of z

Fecundity						
Constant parent	Mixture			No. of replicates	r (SE)	z (SE)
	SS	SF	FF			
SS males	30	15	5	13	3.167 (0.488)	
	5	15	30	12	0.529 (0.044)	
SF males	30	15	5	13	2.664 (0.322)	1.008 (0.033)
	5	15	30	12	0.492 (0.063)	0.978 (0.041)
FF males	30	15	5	12	3.229 (0.342)	
	5	15	30	14	0.460 (0.075)	
All males	30	15	5	38	3.037 (0.226)	
	5	15	30	38	0.492 (0.036)	
Virility						
SS females	30	15	5	14	3.378 (0.646)	
	5	15	30	10	0.326 (0.067)	
SF females	30	15	5	14	2.421 (0.262)	1.024 (0.032)
	5	15	30	12	0.291 (0.048)	0.975 (0.022)
FF females	30	15	5	12	2.291 (0.264)	
	5	15	30	12	0.334 (0.031)	
All females	30	15	5	40	2.717 (0.262)	
	5	15	30	34	0.319 (0.028)	

Standard errors in parentheses.

replicates were also excluded from the calculations. Table 8B shows the results for this estimation procedure. The data for the different constant parents were again combined because analyses of variance showed no significant differences between them within each experiment. Comparing the data of the Table 8, A and B, reveals a good agreement between both methods. Standard errors have the same order of magnitude and also the general picture is still the same. For the fecundity there is still a considerable overdomi-

TABLE 7

Analysis of variance of the gene ratio r among the different constant parents for each of the two mixtures of both the fecundity and the virility experiment

	SS	d.f.	MS	
<i>Analysis of mixture 30:15:5, fecundity experiment</i>				
Between constant males	2.847	2	1.423	$F_{2,35} = 0.724$ NS*
Within constant males	68.804	35	1.966	
Total	71.651	37		
<i>Analysis of mixture 5:15:30, fecundity experiment</i>				
Between constant males	0.031	2	0.015	$F_{2,35} = 0.299$ NS*
Within constant males	1.797	35	0.051	
Total	1.828	37		
<i>Analysis of mixture 30:15:5, virility experiment</i>				
Between constant females	9.533	2	4.767	$F_{2,37} = 1.806$ NS*
Within constant females	97.648	37	2.639	
Total	107.181	39		
<i>Analysis of mixture 5:15:30, virility experiment</i>				
Between constant females	0.009	2	0.004	$F_{2,31} = 0.159$ NS*
Within constant females	0.844	31	0.027	
Total	0.853	33		

* NS is not significant at the 5% level.

nance observed, although somewhat less extreme, and for the virility the SS genotype still shows a lower estimate than the other two genotypes but now the deviation from unity is significant.

In conclusion we can say that under the given experimental conditions we observed significant differences in fitness between the different *Adh* geno-

TABLE 8

Estimates of the adult fitness components for both fecundity and virility

	<i>Adh</i> genotype		
	<i>SS</i>	<i>SF</i>	<i>FF</i>
A. Fecundity <i>E</i> (SE)	0.772 (0.089)	1	0.520 (0.075)
Virility <i>V</i> (SE)	0.879 (0.157)	1	0.993 (0.161)
B. Fecundity <i>E</i> (SE)	0.877 (0.118)	1	0.612 (0.097)
Virility <i>V</i> (SE)	0.766 (0.101)	1	1.178 (0.142)

Estimates were calculated both by using the averaged gene ratio values (A) and by using the averaged *E* and *V* values computed for each replicate separately (B). Standard errors (SE) were obtained by using Taylor's expansion in two variables and the use of the empirical variances of *r* (A) or are the empirical standard errors of *E* and *V* (B), respectively.

types but only with respect to the adult components. [This rectifies the premature conclusion that the observed fitness differences were not statistically significant which has been cited by HEDRICK and MURRAY (1983) and VAN DELDEN (1982).]

Predicted and observed frequency changes: Based on the estimated fitness components we can predict the behavior of the *Adh* polymorphism in experimental populations and see whether or not these predictions are in agreement with the observed data. To do so, a number of simulations were performed by using the viability, fecundity and virility estimates as presented in Tables 4B and 8. All estimates were used at face value irrespective of whether they were significantly different from unity or not. For convenience it was assumed that males and females had the same viability (bottom line of Table 4B). Under the assumption that the fecundity estimates are independent of the male partner and the virility estimates independent of the female partner these components can be treated as if they were measures of survival. It is then easy to derive the recurrence equations for the relationships between genotype frequencies of the successive generations. Simulations were carried out for 50 generations with initial allele frequencies of 0.10, 0.45 and 0.90 of the *F* allele with both the estimates from Table 8, A and B. The results are shown in Figure 1A. As might have been expected from the strong overdominant pattern for the fecundity, both sets of simulations lead to a globally stable equilibrium point. By using the estimates of Table 8A the equilibrium point is approximately at *F* = 0.40, while using the estimates of Table 8B this value is somewhat higher, *F* = 0.57. Figure 1B shows the actually observed frequency changes that occurred in the experimental population over more than four years. The figure also shows the changes that occurred in two base populations during the same period. It is clear that the frequencies converge to more or less the same levels as observed for the base populations, around 0.60 *F*. However, the trajectory seems not to

FIGURE 1.—Expected and observed frequency changes for the *Adh* *F* allele. A, Expected changes using the fitness estimates shown in tables 4B and 8A (open circles) or Tables 4B and 8B (closed circles). B, Observed frequency changes in the experimental populations (solid lines) and two duplicate Groningen base populations (broken and dashed lines).

be straightforward. In particular, the population started at 0.45 *F* shows the first two years a significant increase up to 0.80 *F*, a value much higher than the base populations. Thereafter the frequency in the population decreases almost parallel to the one started at 0.90 *F*. A comparison between the Figure 1, A and B, makes it clear that the simulations predict an equilibrium frequency which reasonably fits the observed frequencies, especially when the estimates of Table 7B were used for the simulations. On the other hand the observed trajectories of the changes do not resemble the expected curves so well.

DISCUSSION

Following gene frequency changes in experimental and perturbed populations is a relatively simple and direct method to provide evidence for the occurrence of selection at a particular enzyme locus. One must, however, be aware of the fact that, due to sampling of a restricted number of founder lines, an initial linkage disequilibrium can be created between the enzyme locus under study and other linked fitness genes. The Groningen base population had been founded with 18 wild caught inseminated females, which means with at least 72 independent chromosomes. If it is assumed that about half of the females were inseminated by more than one male (ANDERSON 1974; COBBS 1977) the number of founding chromosomes increases to 90. Even when at establishment of the base population a linkage disequilibrium was introduced, the 4-yr period between establishment and isolation of the homozygous lines should have given

ample time for decay of this linkage disequilibrium. To establish the experimental cages 32 independently isolated lines, equivalent to 128 independent second chromosomes (at which *Adh* is located), were used, which seems enough to avoid problems with the genetic background. In comparison, YAMAZAKI (1971), when measuring the fitness at the esterase-5 locus in *Drosophila pseudoobscura*, used 44 homozygous lines, which in his case amounts to 132 independent *X* chromosomes. They were isolated from a base population founded with only 12 isofemale lines. JONES and YAMAZAKI (1974) have studied the effect of different numbers of founding lines on the behavior of allele frequencies for this *est-5* locus and come to the following conclusion: "But we feel to be absolutely certain of obviating the possibility of genetic interaction overcoming single locus effects, at least forty independent lines (=120 independent chromosomes) should be used to found experimental populations used in estimating fitness differences at such loci." We are therefore as certain as one possibly can be, that the convergence of the allele frequencies observed in the experimental cages (Figure 1B) reflects selection at the *Adh* locus itself and is not due to hitchhiking. This conclusion is furthermore supported by the fact that this finding has now been observed in a number of independent experiments with different sets of isolated lines (BIJLSMA-MEELES and VAN DELDEN 1974; VAN DELDEN, BOEREMA and KAMPING 1978). Moreover, it has been established that the second chromosomes of this population are free of inversions (VAN DELDEN, BOEREMA and KAMPING 1978; A. KAMPING, personal communication).

In this paper, fitness differences between the *Adh* genotypes were also demonstrated by the fitness experiments. Most striking were the differences with respect to the female component. There was a very strong overdominant pattern and compared to the heterozygote, the homozygotes showed a 20–50% reduction in fitness. The applied crossing scheme ensures that the variation in genetic background within replicates is the same for each genotype. The observed overdominance therefore cannot be due to the fact that the heterozygotes are more heterozygous with respect to the genetic background. As on regular food adult mortality during the first days is generally quite low, the observed fitness differences are probably mainly due to differences in fecundity. Therefore the next question is how ADH is related to egg production. At the moment there is no straightforward answer. It becomes, however, increasingly evident that ADH, apart from the conversion of (environmental) ethanol, may have other metabolic functions (EISSES *et al.* 1985; GEER, LANGEVIN and MCKECHNIE 1985). The latter have shown that ADH is involved in the synthesis of lipids from either ethanol or car-

bohydrates. As lipids constitute an important part of the eggs (KING 1970), it is conceivable that ADH in this way can affect egg production. In this respect it is noteworthy that both ADH activity (CLARKE *et al.* 1979) and egg production (DAVID *et al.* 1983) are highly stimulated by addition of extra yeast to the food medium.

For the male adult component also fitness differences were observed. The *SS* genotype showed a lower virility than the other two genotypes. These latter either did not differ in fitness or the *FF* genotype showed a higher (although not significantly) virility than the *FS* genotype. These data are in good agreement with the results of POT, VAN DELDEN and KRUIJT (1980) who investigated the mating success of the different *Adh* genotypes. Their results showed that *FF* males were significantly more successful than *SS* males, while the *FS* males were intermediate but did not differ from either homozygous genotype. Like for the female component, the relation between ADH and virility seems not to be straightforward. However, there is also evidence that the *Adh* genotypes differ in developmental time: the *FF* genotype showing the fastest and the *SS* genotype showing the slowest developmental rate (OAKESHOTT, 1976a, b, 1977; VAN DELDEN and KAMPING 1979). Furthermore, VAN DYKEN (1982), when selecting for locomotor activity, showed that lines selected for high activity became fixed for the *Adh^F* allele while lines selected for lower activity became fixed for the *Adh^S* allele, indicating a possible relation between locomotor activity and the *Adh* locus. If we assume that the differences in virility are due to differences in sexual activity, all three observations possibly might be the manifestation of an underlying difference in "basic activity" that somehow is influenced by the *Adh* locus.

With respect to viability no differences between genotypes were observed. However, viability was estimated under nearly optimal conditions and it might well be that viability is density dependent and that differences would become manifest under more stringent crowding conditions. Testing for density dependence of the fitness estimates would have made the experiment rather impracticable. Evidence for the existence of density dependent selection is still scanty. DEBENEDICTIS (1977), when reviewing the evidence for density dependent selection in *Drosophila*, observed that most evidence was ambiguous. Furthermore, YOSHIMARU and MUKAI (1979) have investigated the larval survival of the different *Adh* genotypes at two density levels and found no effect of the density on the viability estimates. Therefore, it seems unlikely that application of other densities would have changed our viability estimates significantly.

PROUT's estimation procedure seems to be an effective way to estimate fitnesses that predict the behav-

our of a polymorphic system reasonably well, not only with respect to visible mutants (PROUT, 1971a, b) but also, as is shown in this paper, with respect to allozymes. It has to be realized, however, that the fitness estimation experiment and the cage experiment differ distinctly in a number of ways. In the cages there were overlapping generations, which means that re-mating, sperm displacement, longevity, age dependent egg production, age dependent mating success and other age dependent factors may play a significant role in determining the fitnesses and frequency changes. In the fitness experiment all flies were of the same age and all processes, mating, egg laying and larval development, were synchronized. Furthermore, in contrast to the fitness experiment, there were very high densities present in the cages resulting in severe crowding. Although crowding, as already discussed above, may not have influenced the viability estimates, it still could possibly have affected the fecundity and the virility. All these aforementioned differences open ample possibilities for the estimated fitness components to deviate from the actual values. That, in spite of this, predicted and observed equilibrium frequencies and frequency changes fit reasonably, suggests that either these experimental differences do not affect fitness values significantly, or that these effects cancel each other out. The differences may, however, have affected the rate at which the equilibrium is reached, which seems considerably slower in the experimental cages than predicted on the basis of the simulations.

Another aspect concerns frequency dependent selection. CLARKE and ALLENDORF (1979) have pointed out that, for enzymes with external substrates, differences in kinetic properties of the different allozymes can lead to frequency (and density) dependent selection. With respect to the *Adh* locus KOJIMA and TOBARI (1969) reported frequency dependent selection for egg-to-adult survival leading to a stable equilibrium. Also MORGAN (1976) reported frequency dependent viability differences but in his case these did not lead to a stable equilibrium. On the other hand, YOSHIMARU and MUKAI (1979) and WILSON *et al.* (1982) found no evidence for the action of frequency dependent selection, neither did POT, VAN DELDEN and KRUIJT (1980), studying mating success. The occurrence of frequency dependent selection at the *Adh* locus therefore remains uncertain. Increasing the number of different mixtures in PROUT's estimation procedure allows investigation of frequency dependence of the fitness components. But, as already mentioned with respect to density dependence, the amount of experimental work involved would be immense.

Notwithstanding the questions that still await an answer, our experiments show significant selection at the *Adh* locus on regular food, and the observed

selective differences can explain a stable polymorphism at this locus. Indications of some kind of selection at this locus in the absence of alcohol stress, mostly based on studies of isolated fitness components or indirect observations, have also been reported by several other authors (BIJLSMA-MEELES and VAN DELDEN 1974; VAN DELDEN, BOEREMA and KAMPING, 1978; FRANKLIN 1981; WILSON *et al.* 1982; OAKSHOT, GIBSON and WELSON 1984). In most of these cases signs of heterozygous advantage were observed. Because of the biochemical function of ADH, the polymorphism at this locus has been studied mainly in relation to environmental alcohol. One might, however, question the relevance of this relationship under natural conditions. We want to mention two observations in this respect. The first, made by one of us (BIJLSMA-MEELES 1979) might be called the occurrence of "phenotypic adaptation". This observation showed that eggs laid directly on medium containing 12% ethanol showed a significantly better survival than eggs that were not brought into contact with ethanol before they were 14 hours old. It was concluded therefore that the eggs of *Drosophila melanogaster*, when directly brought into contact with environmental ethanol, develop some kind of phenotypic resistance. This does occur in all three genotypes and results in an almost optimal egg-to-adult survival even in the presence of 12% ethanol. This finding has recently been confirmed by KERVER and ROTMAN (1987) and seems also to occur in the presence of other primary alcohols.

The second observation is that flies kept for many generations on ethanol containing food, show a strong genetic adaptation and become highly tolerant to ethanol (KERVER and VAN DELDEN 1985; DORADO and BARBANCHO 1984). This increase in tolerance however is not, or is only partly, accompanied with changes in ADH activity. For instance, KERVER and VAN DELDEN (1985) report that adapted *SS* and *FF* genotypes, in contrast to nonadapted flies, did not differ in viability at 18% and 21% ethanol (their Table 5, page 361) though the genotypes still differ in ADH activity to the same extent as nonadapted flies (their Table 7, page 363). The same table shows that the adapted *SS* genotype still has a significantly lower ADH activity compared to the nonadapted *FF* genotype, while its survival is significantly higher. BARBANCHO *et al.* (1987) come to the same conclusion with respect to the results of DORADO and BARBANCHO (1984).

We believe that phenotypic and genetic adaptation are substantial enough to override differences in enzyme activity between genotypes and are capable of nullifying the selective pressure of environmental alcohol. These processes will protect flies of all three genotypes equally well against concentrations possibly

up to 10%. This concentration is in fact higher than concentrations actually observed in natural habitats (GIBSON, MAY and WILLIS 1981). This does not imply that a high ADH level cannot be of advantage during short term alcohol stresses, but we think that in the long run the behavior of the *Adh* polymorphism in nature is mainly affected by other processes. Therefore, if we want to explain why nearly all natural populations are polymorphic for this locus, it is more realistic to study it in the absence of alcohol stress. Our analysis suggests that, under nonalcohol conditions, the *Adh* polymorphism will be maintained by overdominant selection during the adult part of the life cycle.

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