Genet. Res., Camb. (1976), **26**, *pp.* 265–274
Printed in Great Britain

Selection at the alcohol dehydrogenase locus in *Drosophila melanogaster* imposed by environmental ethanol

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(*Received* 28 *August* 1975)

SUMMARY

This paper describes selective differences imposed by environmental ethanol on six genotypes at the alcohol dehydrogenase (*Adh*) locus in *Drosophila melanogaster*. Probit analyses were used to relate differences between the percentage survival of adults of different *Adh* genotypes to ethanol concentration. Regression analyses were used to relate differences between the pre-adult developmental times of different *Adh* genotypes to ethanol concentration. The directions of differences between some of the genotypes were found to differ in these two components of fitness. The differences in developmental time are linearly related to the differences in the *in vitro* alcohol dehydrogenase activity expressed by these genotypes. Percentage survival differences amongst adults are not linearly related to these differences in enzymic activity. The development of Adh^FAdh^F pre-adults is retarded the least on ethanol impregnated media but Adh^FAdh^S adults are most likely to survive on such media.

1. INTRODUCTION

The alcohol dehydrogenase locus in *Drosophila melanogaster* is one of the few known enzyme loci on which direct effects of natural selection have been demonstrated. Gibson (1970) and Bijlsma-Meeles & Van Delden (1974) showed that the frequency of the Adh^F allele (subsequently denoted F) increased and that of the Adh^S allele (denoted S) decreased in polymorphic populations maintained on ethanol impregnated media. Morgan (1975) provided direct evidence that FF individuals are in fact more tolerant to ethanol than SS individuals. He did not record the tolerance of heterozygotes but subsequently Briscoe, Robertson & Malpica (1975) reported it to be non-significantly different from that of FF homozygotes.

Morgan (1975) and Briscoe *et al.* (1975) confined their studies to genotypes involving the F and S alleles found in natural populations. More importantly they did not investigate the effects of ethanol on components of fitness other than survival. In addition to the three naturally occurring genotypes, the present author studied three genotypes involving the 'null' allele Adh^{n2} (denoted *n2*), which was induced with ethyl-methane sulphonate by Grell, Jacobson & Murphy (1968). *In vitro* alcohol dehydrogenase activities of these genotypes were measured and observations then made of the effects of several ethanol concentrations on

both the pre-adult developmental time and adult survival of each genotype. The relationships between tolerance and enzymic activity were then deduced and differences between the tolerances of various genotypes at the different life cycle stages compared.

2. MATERIALS AND METHODS

(i) *Derivation of strains*

The first population studied was a laboratory stock, LS, polymorphic for the *F*, *S*, and *n2* alleles. From LS three strains were derived, each homozygous for a different *Adh* allele. Each strain contained the progenies of seven pairs of homozygous parents and each of these parents was obtained independently from LS.

The LS stock was chosen because it lacked inversions in the vicinity of the *Adh* locus (2–50·1). Recombination had been studied between the *b* (2–48·5), *pr* (2–54·5) and *vg* (2–67·0) loci on 80 second chromosomes from LS. In all 80, recombination between these loci occurred at the frequencies expected if the regions between these loci lacked inversions.

Supplementary studies were conducted on a population, HV, captured three years previously from the Hunter Valley, New South Wales. HV contained the *F* and *S* alleles and a strain homozygous for each allele was extracted. Each strain contained the progenies of only one pair of homozygous parents and these four parents were sibs. HV had been tested for inversions as above. None of the 25 chromosomes tested contained inversions in the region between the *b* and *vg* loci.

(ii) *Measurement of ethanol tolerance*

The culture medium contained 33 g of agar, 40 g of heat-killed brewer's yeast, 190 g of semolina, 430 ml of treacle, 2130 ml of water and 11 ml of propionic acid. After preparation the medium was cooled to 45 °C and then stirred thoroughly as the required volume of ethanol was added. It was then kept at 4 °C and used 18–24 hours after preparation.

The ethanol tolerance of adults was measured as survival after five days of exposure to ethanol impregnated medium. Flies of the required genotypes were produced from the homozygous strains described above and from the appropriate crosses of these strains. Prior to testing, these flies were maintained in uncrowded conditions on medium lacking ethanol. Sexes were then separated and the flies transferred to the test vials, fifteen flies to each vial. Each vial contained 15 ml of medium of known ethanol concentration. The test vials were kept at 25 °C and after five days the number of flies surviving in each vial was recorded.

In order to measure egg-to-adult developmental times virgins of both sexes were collected from the homozygous strains and then mated to produce the genotypes required for the test. After mating, these flies were maintained for two days on media lacking ethanol and then transferred to test vials, five females and five males to each vial. Each vial contained 15 ml of medium of known ethanol concentration. After 24 h these flies were removed and the vials maintained at 25 °C.

Every 24 h, during the period when the progeny were emerging from pupae, the adult flies in each vial were counted and removed. The developmental time of each culture was taken as the average number of days between removal of the parents and emergence of the progeny as adults.

(iii) *Biochemical techniques*

Samples for electrophoresis were homogenized in 40 ul of distilled water and then centrifuged at 1500 *g* for five minutes. Samples were electrophoresed on starch gels and stained for alcohol dehydrogenase using the methods of Day, Hillier & Clarke (1974).

Methods similar to those of the latter authors were also used during spectrophotometric assays for alcohol dehydrogenase activity. Samples, each of 15 flies, were homogenized in 150 ul of physiological saline, centrifuged at 1500 *g* for 20 min, and kept at 4 °C until assayed (within 8 h of preparation). The assay mixture contained 0·2 ml of 0·003 M NAD^+, 0·2 ml of isopropanol, 0·25 ml of 0·1 M glycine sodium hydroxide buffer, pH 9·3, and 10 ul of sample. The conversion of NAD^+ to NADH was monitored at 340 nm for 3 min, in a Shimadzu QV50 spectrophotometer. Alcohol dehydrogenase activity was expressed as the change in OD_{340} per mg live weight in 3 min.

3. RESULTS

(i) *Enzymic activity*

Table 1 shows the *in vitro* alcohol dehydrogenase activities of each of six *Adh* genotypes from the LS population. There were only trivial differences between the relative activities of different genotypes at different life cycle stages. The relative activities of *FF*, *FS* and *SS* flies were similar to those observed by earlier workers (see Day *et al.* 1974), but there were no reports with which to compare those of *Fn2 and Sn2* flies. The activities of *n2n2* extracts probably represented background rates of conversion of NAD^+ to NADH because Grell *et al.* (1968) showed that *n2n2* flies produce no active alcohol dehydrogenase molecules.

Table 1. *Alcohol dehydrogenase activities of different* Adh *genotypes*

	Life cycle stage		
Genotype	Third instar larvae	Adult ♀♀*	Adult ♂♂*
FF	106 (91, 122)	195 (163, 234)	278 (220, 352)
FS	78 (68, 89)	132 (121, 145)	199 (176, 226)
SS	34 (29, 41)	62 (53, 72)	88 (73, 105)
Fn2	43 (38, 49)	89 (72, 110)	130 (96, 175)
Sn2	20 (16, 25)	44 (33, 59)	57 (51, 65)
n2n2	3 (2, 4)	5 (4, 6)	7 (6, 9)

In each cell is shown the mean activity of about ten samples, with the asymmetrical 95 % confidence limits shown in parentheses.

* Flies in these extracts had aged three days since emergence from pupae.

(ii) *Ethanol tolerance of adults*

Tests of the ethanol tolerance of adults were conducted on flies from the LS population aged 5 days since emergence from pupae. For each sex and genotype the survival percentages of about eight test cultures on each of about eight ethanol concentrations were recorded. Survival percentages of *n2n2* flies of each sex declined erratically with increasing concentrations, but above 7% ethanol they were negligible.

Data for each of the other ten types of culture suggested a sigmoidal relationship between percentage survival and concentration. Finney (1947) showed that such a relationship implied a linear regression of probit transformed percentage survival on logarithmically transformed ethanol concentration. Accordingly a maximum likelihood estimate of the latter function was made for each culture type except those for *n2n2*. The ten regression lines were shown to be parallel ($\chi^2_9 = 11{\cdot}52$, $P > 0{\cdot}10$) and the pooled estimate of the regression coefficient was $+17{\cdot}37 \pm 0{\cdot}14$.

Interpolation into each of the regression lines provided a statistic, median lethal dose (*LD50*), which was the concentration of ethanol required to kill half the population exposed and which, with the regression coefficient, summarized the tolerance of each culture type. These statistics are shown in Table 2.

Table 2. *Median Lethal Doses* (LD50) *of each of two sexes and five* Adh *genotypes*

Genotype	*LD50* of females	*LD50* of males
FF	16·78 (15·86, 17·72)	17·76 (16·76, 19·02)
FS	17·76 (16·74, 19·09)	18·51 (17·49, 20·66)
SS	14·85 (13·20, 16·11)	16·41 (14·07, 18·81)
Fn2	15·62 (14·52, 16·48)	17·65 (16·54, 19·70)
Sn2	12·40 (11·63, 13·29)	13·32 (12·19, 14·46)

Doses are expressed as percentages (by volume) of ethanol in the media. The asymmetrical 95% confidence limits of each *LD50* are shown in parentheses.

For each genotype, males were clearly more tolerant than females, but comparison of tolerances of different genotypes was more difficult. It was necessary to estimate a parameter, mean probit difference (Δ_{12}), defined as the difference between the probits of culture types 1 and 2 for a given concentration of poison (Finney, 1947). For parallel regression lines, the parameter was constant over different concentrations and was estimated as $b\ (log\ LD50_2 - log\ LD50_1)$. For each pair of genotypes two estimates of this parameter were made, one for each sex. A χ^2_1 test was then used to determine the significance of the difference between these two statistics (Finney, 1947, pp. 74–76). Within each pair of genotypes this difference was not significant and an estimate was then made of an average mean probit difference, in which the statistic for each sex was weighted by the reciprocal of its variance (Finney, 1947). Table 3 shows estimates of Δ_{12} averaged over sexes.

Inspection of the 95% confidence limits accompanying the latter showed that the tolerances of several genotypes were significantly different. It was particularly

interesting that *FS* flies were more tolerant than *FF* individuals as this disproved the existence of a simple dependence of ethanol tolerance on alcohol dehydrogenase activity.

Table 3. *Mean Probit Differences* (Δ_{12}), *averaged over sexes, between pairs of* Adh *genotypes*

Pair of genotypes 1	2	Δ_{12}*
FF	*FS*	+0·37 (+0·03, +0·73)
FF	*SS*	−0·76 (−1·14, −0·38)
FF	*Fn2*	−0·32 (−0·65, +0·01)
FF	*Sn2*	−2·23 (−2·79, −1·68)
FS	*SS*	−1·13 (−1·53, −0·73)
FS	*Fn2*	−0·70 (−1·04, −0·35)
FS	*Sn2*	−2·60 (−3·19, −2·01)
SS	*Fn2*	+0·46 (+0·09, +0·84)
SS	*Sn2*	−1·45 (−1·97, −0·94)
Fn2	*Sn2*	−1·91 (−2·43, −1·38)

The 95 % confidence limits of each Δ_{12} statistic are given in parentheses.

* Estimated from the formula $\Delta_{12} = b\,(log\ LD50_2 - log\ LD50_1)$, where $LD50_1$ and $LD50_2$ are the median lethal doses for genotypes 1 and 2 respectively.

(iii) *Ethanol tolerance of adults: possible effects of background genotypes*

Results in section 3(ii) were only taken from strains from LS and might have reflected differences at loci other than *Adh*. These differences might have arisen due to random drift during derivation of strains. Each strain was founded from only a few parents (section 2(i)) and between such small samples gene frequencies at other polymorphic loci would differ just by chance.

In the first of two experiments testing the effects of such random differences in background genotype, comparisons were made between the tolerances of strains from LS and HV. Among strains from the latter, gene frequencies at other loci were even more likely to differ, because fewer parents were used and these parents were sibs. However, sampling variations in these strains should have been uncorrelated with those among strains from LS. If tolerance was significantly affected by random differences at loci other than *Adh*, then the relative tolerance of each LS strain would not necessarily have corresponded with that of the HV strain of the same *Adh* genotype.

Table 4 shows survival percentages on 20 % ethanol of adults of each of two sexes, two ages and three *Adh* genotypes, from strains from both LS and HV. A four-way analysis of variance was conducted on arcsine transformations of these data. This did not show significant effects on percentage survival, of either population differences ($F^1_{172} = 0{\cdot}36$, $P > 0{\cdot}50$) or any of the interaction terms involving population differences (e.g. $F^{11}_{172} = 0{\cdot}93$, $P > 0{\cdot}50$ for the mean square pooled over all interaction terms involving population differences). However, the differences between genotypes were highly significant ($F^2_{172} = 46{\cdot}58$, $P < 0{\cdot}001$) and in the same directions as those observed in section 3(ii). This suggested that results in section 3 (ii) were not due to random differences in gene frequencies at loci other than *Adh*.

The next experiment tested the effects of both random and systematic differences between background genotypes of different *Adh* genotypes. Systematic differences probably resulted from differences in the levels of inbreeding in cultures of different *Adh* genotypes. Strains homozygous for *Adh* alleles were inbred and in flies of

Table 4. *Percentage survival on* 20% *ethanol of* Adh *genotypes from the LS and HV populations*

Population	Genotype	Sex and age: ♀♀ 0–2 days	♀♀ 9–11 days	♂♂ 0–2 days	♂♂ 9–11 days
LS	*FF*	98·3 (90·4, 100·0)	7·8	91·8	4·0
	FS	93·8 (85·6, 98·7)	9·1	98·3	17·8
	SS	73·0 (37·4, 96·8)	1·1	77·4	2·2
HV	*FF*	92·0 (81·8, 98·2)	6·7	90·0	10·7
	FS	94·0 (86·8, 98·5)	9·3	94·0	12·0
	SS	63·3 (45·2, 79·6)	4·7	67·3	8·7

In each cell is shown the mean percentage survival of about eight cultures. Some representative 95% confidence limits are shown in parentheses. Ages are given as days, since emergence, on the first day of exposure.

these strains the levels of heterozygosity at loci other than *Adh* were probably lower than those in *Adh* heterozygotes produced by crossing these strains. Thus the *FS* heterozygote advantage observed in section 3 (ii) might have reflected heterotic effects from modifier loci. This possibility was not adequately tested by the previous experiment because strains from HV and LS were produced by similar breeding systems.

This second experiment used a population, LSA, constructed by mixing *FF* and *SS* strains from LS and hence segregating for *F* and *S*. LSA was not used until fifteen generations after its construction and after this interval all *Adh* genotypes should have had similar genotype frequencies at loci unlinked to *Adh*.

Unfortunately, even in the absence of inversions containing *Adh* alleles, this interval was probably too short to remove all linkage disequilibria between *Adh* alleles and closely linked genes. Thus studies of LSA were adequate tests of heterotic effects from all possible modifier loci except a small minority which were closely linked to *Adh*, still segregating in the inbred strains, and subject to significant random sampling variations between strains.

Studies of LSA were based on samples of 300 like-sexed flies exposed to 10 or 15% ethanol. On the first day of exposure these flies had aged 9–11 days since emergence. Testing procedures were similar to those used previously except that samples were tested in vials containing 70 ml of medium. Table 5 shows frequencies of *Adh* genotypes before exposure and the numbers of each genotype surviving 5 days of exposure. Genotype frequencies changed among both samples of females ($\chi^2_2 = 8{\cdot}57$, $P < 0{\cdot}05$ on 10% and $\chi^2_2 = 8{\cdot}15$, $P < 0{\cdot}05$ on 15%). The changes among males were non-significant ($\chi^2_2 = 2{\cdot}79$, $P > 0{\cdot}10$ on 10% and $\chi^2_2 = 2{\cdot}10$, $P > 0{\cdot}10$ on 15%) but similar in direction to those among females. In all samples *FS* flies were most likely and *SS* flies least likely to survive.

These data were then compared quantitatively with those in section 3(ii). For each sample the percentage survival of each genotype was calculated and transformed to a probit. Within each sample the differences between probits of different genotypes were then obtained. (As a finite probit could not be obtained for *SS* females on 15%, differences involving *SS* could not be calculated from this sample.) These differences were then averaged over different samples. On average the probit for *FS* was 0·34 greater than that for *FF* and 0·72 greater than that for *SS*. Approximate 95% confidence limits of these averages were 0·12 and 0·56, and −0·33 and 1·77 respectively and these results were in satisfactory agreement with the relevant mean probit differences in Table 3.

Table 5. *Frequencies of* Adh *genotypes before, and numbers of each genotype after exposure to* 10 *or* 15% *ethanol*

	Genotype		
	FF	*FS*	*SS*
♀♀ before exposure	47 :	41 :	13
♀♀ after exposure to 10%	24	40	2
♀♀ after exposure to 15%	18	26	0
♂♂ before exposure	35 :	41 :	14
♂♂ after exposure to 10%	26	37	5
♂♂ after exposure to 15%	10	21	4

This confirmed the conclusion from the previous experiment, that results in section 3 (ii) were not due to differences in background genotype caused by random drift. More importantly, it also showed that none of the previous results reflected systematic differences, at loci not closely linked to *Adh*, promoting associative overdominance in *FS* flies.

There remained the possibility of associative overdominance due to modifiers closely linked to *Adh*. However, the likelihood of this was even further reduced by results in Table 4 which, in view of the last results, could have only reflected associative overdominance if modifiers closely linked to *Adh* were also segregating in the HV strains. This was unlikely as each HV strain was founded from two sibs and unless these genes recombined with *Adh* alleles each strain would have included, at each of these loci, only two genes not identical by descent.

(iv) *Ethanol tolerance of pre-adults*

About twenty cultures of each genotype were raised on one of six ethanol concentrations and the developmental time of each culture was recorded. The concentrations ranged from 1·5 to 6·0% for *n2n2* individuals and from 1·5 to 20·0% for all other genotypes.

Data was also collected from cultures raised without ethanol. Under the latter conditions there were no significant differences between the developmental times of different genotypes ($F^5_{15} = 1{\cdot}94$, $P > 0{\cdot}10$).

The data from concentrations greater than or equal to 1·5% suggested that the developmental time of each genotype regressed linearly and positively on ethanol

concentration. Accordingly regression analyses proceeded and the results are shown in Table 6. For each genotype the variance between concentrations explained by regression was highly significant. For *FS* and *Fn2* there were also significant, but apparently unsystematic, deviations from regression. For *n2n2* the deviations from regression were highly significant and suggested a curvilinear regression.

Table 6. *The regression of developmental time on ethanol concentration for each of six* Adh *genotypes*

Genotype	$b_{DT.Eth}$	$F = \frac{MS_{DT.Eth}}{MS_{within}}$†	$F = \frac{MS_{DT}}{MS_{DT.Eth}}$‡
FF	$+0{\cdot}271 \pm 0{\cdot}013$	2·19ns	417·61**
FS	$+0{\cdot}304 \pm 0{\cdot}016$	4·49*	353·29**
SS	$+0{\cdot}336 \pm 0{\cdot}015$	1·68ns	518·99**
Fn2	$+0{\cdot}306 \pm 0{\cdot}017$	4·45*	326·15**
Sn2	$+0{\cdot}343 \pm 0{\cdot}014$	1·98ns	641·68**
n2n2	$+0{\cdot}408 \pm 0{\cdot}082$	5·60**	24·57*

Standard errors are attached to each estimate of $b_{DT.Eth}$.

† Variance ratio for significance of variation unexplained by regression. $MS_{DT.Eth}$ = variance between concentrations unexplained by regression. MS_{within} = variance within concentrations.

‡ Variance ratio for significance of variation explained by regression. MS_{DT} = variance between concentrations explained by regression. ns = not significant, * $P < 0{\cdot}01$, ** $P < 0{\cdot}001$.

An analysis of covariance was used to test the significance of the variation between the regression coefficients ($b_{DT.Eth}$) of different genotypes. Significant heterogeneity was isolated by an analysis in which data from *n2n2* pre-adults were excluded ($F^4_{20} = 2{\cdot}96$, $P < 0{\cdot}05$) but was not isolated when data for this genotype were included ($F^5_{24} = 2{\cdot}38$, $P > 0{\cdot}10$). The latter reflected the much larger error variance from *n2n2*, which inflated the pooled estimate of the variance unexplained by regression.

The differences between the other five $b_{DT.Eth}$ statistics suggested a linear relationship between ethanol tolerance and alcohol dehydrogenase activity. This was tested by an analysis of the regression of estimates of $b_{DT.Eth}$ on the alcohol dehydrogenase activities of the respective genotypes. Data for the latter were taken from Table 1. The analysis was conducted excluding data for *n2n2* pre-adults and it revealed that the differences, between the original $b_{DT.Eth}$ statistics, which were unexplained by regression on enzymic activity, were not significant ($F^3_{20} = 0{\cdot}63$, $P > 0{\cdot}50$), while the differences explained by this regression were significant ($F^1_3 = 15{\cdot}75$, $P < 0{\cdot}05$). A value of $-0{\cdot}00073 \pm 0{\cdot}00018$ was obtained for the regression coefficient, $b_{b.ADH}$, of $b_{DT.Eth}$ on alcohol dehydrogenase activity. Analysis including data from *n2n2* pre-adults was not strictly justified but a similar $b_{b.ADH}$ statistic, $-0{\cdot}00080 \pm 0{\cdot}00020$ was obtained if they were included.

Qualitative comparisons were then made between these results and the relative tolerances obtained from measurement of adult survival. Inspection of Tables 3 and 6 revealed only two genotypes, the relative tolerances of which differed in

direction in the different components of fitness under test. When measured as the percentage survival of adults, the tolerance of *FF* flies was significantly less than that of *FS* flies, but when measured as the change in developmental time of pre-adults, it was greater than that of the latter.

4. DISCUSSION

Probit analysis of data on the ethanol tolerance of adults provided clear evidence of selective differences between several *Adh* genotypes. Of the ten estimates of mean probit differences averaged over sexes, only that between *FF* and *Fn2* was not significant. The tolerance of *n2n2* flies could not be described by the statistics of probit analysis but it was clearly less than those of other genotypes. Results in section 3(iii) provided evidence that the observed differences between *Adh* genotypes were not significantly affected by effects of modifier genes non-randomly associated with *Adh* genotypes.

The pattern of variation in *LD50* and Δ_{12} statistics disproved the existence of any simple relationship between the alcohol dehydrogenase activity and ethanol tolerance of adults. The order of the different homozygotes for tolerance was the same as that for enzymic activity. However *FS* flies showed greatest tolerance but expressed levels of activity approximately intermediate between those expressed by *FF* and *SS* flies. Furthermore, the tolerances of both *Fn2* and *Sn2* heterozygotes were greater than the approximately mid-parental values expected if tolerance was simply proportional to enzymic activity. The existence of this complex relationship between tolerance and enzymic activity implied that selective differences could not be inferred simply from differences in *in vitro* properties of isozymes.

The relative tolerances of *FF*, *FS* and *SS* flies were in the same directions as those inferred by Bijlsma-Meeles & Van Delden (1974) from their study of extinction rates, on 10% ethanol, of various populations polymorphic or monomorphic at the *Adh* locus. However, the results of these workers and the present author both differed in one respect from those of Briscoe *et al.* (1975), who found that the tolerance of *FS* adults was greater than that of *SS* adults but not significantly different from that of *FF* adults. This discrepancy might have reflected differences in experimental techniques as the latter authors used different media and recorded percentage survival after only twenty four hours' exposure. This explanation will be assessed more thoroughly following discussion of tolerance among pre-adults.

The developmental times of all genotypes regressed linearly and positively on the ethanol concentrations tested. For *n2n2*, considerable variation between developmental times on different concentrations remained unexplained by linear regression but among the other five $b_{\mathrm{DT.Eth}}$ statistics significant overall heterogeneity was found. The variation between these five statistics was explained by a linear regression of $b_{\mathrm{DT.Eth}}$ on alcohol dehydrogenase activity.

This linear relationship with enzymic activity contrasted with the complex relationship between adult survival and activity. Whatever its biochemical basis, the contrast indicated that selective differences between ethanol tolerances of

Adh genotypes varied with the conditions of exposure. Possibly the previously described differences in the results of Briscoe *et al.* (1975) also reflected this dependence of relative tolerances on the conditions of exposure.

It is probable that the differences observed between the *FF*, *FS* and *SS* genotypes contribute to selection differentials between these genotypes in wild populations of this species. Many such populations utilise ethanol concentrations as high as 15% (Briscoe *et al.* 1975). The discrepancy with the results of the latter workers suggests that the mean probit differences and $b_{DT.Eth}$ differences between genotypes are not exactly applicable to these wild communities. Nevertheless three general conclusions can be made about selective differences between naturally occurring *Adh* genotypes exposed to ethanol. Firstly, *SS* adults are less likely to survive on ethanol than adults of the other genotypes, and under at least some conditions, *FF* adults are less likely to survive than *FS* adults. Over a range of ethanol concentrations the magnitudes of the percentage survival differences between genotypes vary but their directions, and the magnitudes and directions of mean probit differences, remain constant. Secondly, ethanol retards the development of *SS* pre-adults more than *FS* pre-adults and both these genotypes are retarded more than *FF* pre-adults. The magnitudes of the differences in developmental times increase linearly with increasing ethanol concentrations. Thirdly, the directions of selective differences vary with the component of fitness under test. In particular, selection for the *F* allele on the basis of differences in developmental times tends to counteract the contribution of heterozygote advantage for adult survival, to the maintenance of this polymorphism.

I thank Professor J. H. Bennett, Dr I. R. Franklin and Dr G. M. E. Mayo for valuable discussions and Mrs R. Caston and Mrs D. Golding for technical assistance.

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