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Selection for Ethanol Tolerance in Two Populations of *Drosophila melanogaster* Segregating Alcohol Dehydrogenase Allozymes

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Abstract

Selection for ethanol tolerance was equally successful in two populations of *D. melanogaster* in both of which the frequency of *AdhF* was 0·5 at the start of the experiment.

Increased tolerance to ethanol was not invariably associated with increased frequencies of *AdhF*. In one population alcohol dehydrogenase (ADH) activity was significantly higher in three of the four selected sublines compared with their controls but there was no difference in activity between the selected and control sublines in the second population. The level of ADH activity in the control and selected lines was significantly correlated with the frequency of *AdhF*, but not with ethanol tolerance.

These results show that adaptation to environmental alcohols in populations of *D. melanogaster* can be independent of the ADH system.

Introduction

An experimental approach to the question of how a particular enzyme polymorphism might be maintained is to test the effects, on a polymorphic population, of selection for a character for which the polymorphic alleles differ in their properties (Lewontin 1974; Clarke 1975; Thoday 1975). We describe in this paper the use of this approach in selecting for ethanol tolerance in laboratory populations of *Drosophila melanogaster* segregating for two naturally occurring allozymes of alcohol dehydrogenase (ADH) (EC 1.1.1.1.).

Three sets of observations, each taken at their face value, support the use of this approach in investigating genetic variation in alcohol tolerance. Firstly, natural populations of *D. melanogaster* are commonly polymorphic for genetically determined electrophoretic variants of ADH, *AdhF* and *AdhS*, hereinafter denoted *F* and *S* respectively (Ursprung and Leone 1965; Ward and Hebert 1972; Briscoe *et al.* 1975; Pipkin *et al.* 1976). Secondly, it has generally been shown that strains homozygous for *F* alleles have higher enzyme activity *in vitro* than strains homozygous for *S* alleles (Rasmuson *et al.* 1966; Gibson 1970; Gibson and Miklovich 1971; Vigue and Johnson 1973; Day *et al.* 1974*a*; Ward 1974, 1975) mainly because *FF* flies maintain higher equilibrium levels of ADH molecules than *SS* flies (Gibson 1972; Day *et al.* 1974*b*; Lewis and Gibson 1978). Modifiers of ADH activity are segregating in natural populations of *D. melanogaster* and the level of activity can be changed by directional selection (Ward 1975). Thirdly, there is some evidence that ADH in *D. melanogaster* has a rôle in the breakdown of dietary alcohols (Parsons 1973; McKenzie and Parsons 1974) although the ecological importance of this function for ADH is controversial (Johnson 1974; David *et al.* 1976). McDonald and Avise

(1976) found a significant correlation between ADH activity and survival on media containing propan-2-ol in nine species of *Drosophila*. Thompson and Kaiser (1977) showed that in two strains of *D. melanogaster* homozygous for *S* alleles the strain maintaining the higher number of ADH molecules was consistently associated with the higher relative viability when ethanol or n-butanol were present in the culture media.

If ADH has a part in the breakdown of dietary alcohols, artificial selection for increased ethanol tolerance in a population segregating *F* and *S* alleles might be expected to lead to an increase in the frequency of the *F* allele. Indeed changes in the frequency of the *F* allele in experimental populations have been interpreted using this argument, although direct evidence that ADH activity was involved was not obtained (Gibson 1970; Bijlsma-Meeles and Van Delden 1974; Briscoe *et al.* 1975; see summary in Clarke 1975).

Alternatively, other physiological functions of ADH in *D. melanogaster* have been suggested, for example in lipogenesis through the conversion of glyceraldehyde to glycerol (Johnson 1974). If this were so then selection for increased ethanol tolerance would not necessarily lead to an increase in the frequency of the *F* allele.

We have tested some of the predictions of these alternative hypotheses by selecting for increased ethanol tolerance in two newly established populations of *D. melanogaster*.

Materials and Methods

Base Populations

One of the two populations of *D. melanogaster* used for the selection experiments was derived from fertilized females taken on a waste dump of grape pressings at the Chateau Douglas winery, New South Wales. The second base population was derived from flies trapped with banana bait in an orchard close to the Craigmoor winery, some 140 km to the west of Chateau Douglas. The frequency of the F allele in the trapped flies was 0·66 for Chateau Douglas and 0·67 for Craigmoor. The genotype frequencies were in Hardy–Weinberg proportions (Table 1). Laboratory cultures of Chateau Douglas and Craigmoor were set up with 23 and 18 fertilized females respectively from the trapped flies.

Table 1. *Adh* genotypes in the Chateau Douglas and Craigmoor base populations
(i) Frequencies in the trapped flies; (ii) frequencies after six generations of laboratory culture

		FF	*FS*	*SS*	*F* frequency
Chateau Douglas	(i)	19	24	4	0·66
	(ii)	44	79	38	0·52
			$\chi^2_2 = 6{\cdot}19$, $0{\cdot}05 > P > 0{\cdot}01$		
Craigmoor	(i)	20	18	5	0·67
	(ii)	17	55	14	0·52**
			$\chi^2_2 = 10{\cdot}04$, $0{\cdot}01 > P > 0{\cdot}001$		

** Significant deviation from Hardy–Weinberg proportions, $0{\cdot}01 > P > 0{\cdot}001$.

The selection experiment was started after the Chateau Douglas and Craigmoor stocks had been maintained in the laboratory by mass transfer for six generations on our standard maizemeal and wheat germ culture medium. At this time the frequency of *F* was again scored and in both stocks it was significantly lower than when the stocks were first surveyed (Table 1). In the Craigmoor sample

there was an excess of heterozygotes. It is remarkable that the frequency of *F* should fall from very similar levels to 0·52 in both the Chateau Douglas and Craigmoor cultures. Other stocks derived at the same time from the flies trapped inside the Chateau Douglas and Craigmoor wineries did not show a consistent fall in frequency during laboratory culture. None of the cultures had experienced population bottle-necks during the six generations prior to the start of the selection experiments.

Maintenance of the Selection Lines

From both the Chateau Douglas and Craigmoor base populations four mass cultures were set up. The progenies of each of these eight cultures were used to establish a control culture, with normal food medium, and a series of cultures with ethanol-supplemented medium. Twenty pairs of flies were used as the parents for each culture. The control cultures were maintained separately as eight sublines, four derived from the Chateau Douglas population and four from the Craigmoor population. Similarly, four selected lines from each base population were maintained separately, but in each subline there were a number of cultures with different concentrations of ethanol in the culture medium. All cultures were maintained at 25 ± 1°C with a generation every 3 weeks. In the first few generations the selected cultures had 6, 8 or 9% (v/v) ethanol in the medium. In later generations ethanol concentrations as high as 15% were used.

The flies that emerged successfully in each generation from the ethanol-supplemented cultures were transferred to fresh cultures with a higher concentration of ethanol. For example, flies reared on 8% ethanol in one generation would be used to set up cultures in which there was 9% ethanol in the medium. In every generation each of the selected sublines comprised at least three cultures. One contained a higher concentration of ethanol than a successful culture in the previous generation (the increase was in unit percentage steps, usually each generation). Of the other two cultures, one was used as an insurance culture, and contained a concentration of ethanol that had allowed successful development in the previous generation, and one had an intermediate concentration of ethanol. If less than 20 pairs of flies were available as parents from the culture with the higher ethanol concentration they were supplemented with flies from cultures with the next highest ethanol concentration.

No attempt was made to quantify the response to selection by scoring the number of flies produced at each ethanol concentration. Therefore we are unable to comment on the intensity of selection in the different sublines. The main aim of the selection regime was simply to obtain strains that could be maintained on concentrations of ethanol that would not allow development of flies in the unselected base populations.

Electrophoresis and Enzyme Assays

The frequencies of the *F* and *S* alleles in the base populations and in the control and selected lines were monitored by electrophoresis carried out between 22 and 25°C on cellulose acetate sheets with a continuous buffer system: 35 mM tris, 5 mM boric acid, 3·5 mM EDTA (disodium salt), pH 8·8 (Lewis and Gibson 1978). Activity staining for ADH used nitro blue tetrazolium, 0·5 mM final concentration, and 2-propanol, NAD^+ and phenazine methosulphate at final concentrations of 1%, 0·5 mg/ml and 0·01 mg/ml respectively in 50 mM potassium orthophosphate buffer, pH 7·8.

ADH activity was assayed in crude homogenates of third instar larvae or 6-day-old adults as previously described (Lewis and Gibson 1978). The assay buffer was 50 mM potassium orthophosphate, pH 7·5, containing 0·1 mM EDTA. The final concentrations of substrates in the assay mixture were 0·88 M for ethanol (5% v/v) and 2·7 mM for NAD^+ (2·0 mg/ml). The assay procedure of Vigue and Sofer (1974) was followed and in all cases the endogenous rate of NAD^+ reduction in the absence of ethanol was negligible.

Results

Effects of Selection

Selection for increased ethanol tolerance was successful in both populations (Fig. 1); after 10 generations the four selected sublines derived from each population could be routinely maintained on a culture medium containing 12% ethanol. At the start of the experiment flies from both the Chateau Douglas and Craigmoor

populations would not breed at all on 10% ethanol-supplemented medium. The responses to selection were very similar in all of the eight selected sublines and after eight generations there was no indication that any one of them had a significantly higher tolerance to ethanol than any other selected subline.

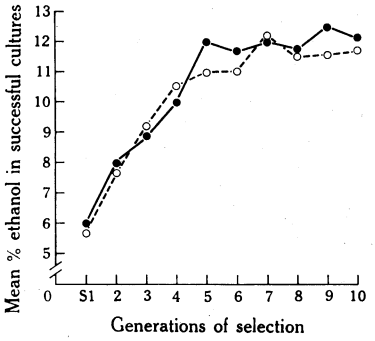

Fig. 1. Response to selection for tolerance to ethanol in the Chateau Douglas population (●) and in the Craigmoor population (○). Tolerance is indicated as the mean concentration of ethanol in the successful cultures each generation. There were differences between the sublines but they were too minor to be included in this figure.

To test whether or not similar increases in ethanol tolerance had occurred in the unselected lines adult flies from the eight control sublines at S_{10} were transferred to culture bottles with 12% ethanol in the medium. None of these cultures produced adult flies, showing that the increased ethanol tolerance in the Chateau Douglas and Craigmoor derived populations had resulted from the selection regime.

Changes in the Frequency of AdhF

At generations 4, 8 and 10 samples of adult flies from the control and selected lines were scored for the frequencies of the *Adh* allozymes. In analysing these data the standard methods of comparing gene frequencies in control and selected lines is inappropriate because it ignores effects of small effective population size (see Appendix). In this experiment the effective population size in each subline was not greater than 20 pairs of flies each generation. The method of analysis we have used, and which is outlined in the Appendix, takes account of the increased variance that would be expected if the only cause of variation in gene frequency was random genetic drift.

These analyses for the Craigmoor derived lines (Table 2) show that the mean frequency of *F* in the pairs of selected and control lines had increased significantly from 0·52 in the two sublines at S_4 and in three sublines at S_8; however at S_{10} none of the four pairs differed significantly in mean *F* frequency from the base population. There was no significant heterogeneity in *F* frequency between the control or selected lines at any of the three generations assayed and the heterogeneity was less at S_{10} than at S_4. The mean *F* frequency in control and selected lines differed from 0·52 at each assay generation except in the selected lines at S_{10}. Of the 12 comparisons of the frequency of *F* in the selected lines with the appropriate controls the only one which was significant was the lower *F* frequency in the third selected subline at S_{10} compared to the corresponding control line.

Similar analyses for the Chateau Douglas derived lines (Table 3) show that the mean *F* frequency in the pairs of selected and control lines had increased significantly from 0·52 in the first and second subline at S_8 and in the first and third subline at

Table 2. Frequency of the *AdhF* allele in the Craigmoor selected and control sublines

Numbers of genes scored are in parentheses. The gene frequency in each selected line was compared with that in the corresponding control line (significance level indicated *above* the gene frequency in the selected line) and the mean gene frequency in each pair of control and selected lines was compared with the initial gene frequency, 0·52*F* (significance level indicated *below* the gene frequency in the selected line). The mean gene frequency in the control or selected sublines was also compared with 0·52*F* after the heterogeneity in gene frequency between the sublines had been taken into account. As so many comparisons are made in the table levels of significance at 0·05 should be considered with caution

Generation		Craigmoor sublines				Heterogeneity between sublines	Divergence of sublines from 0·52
		1	2	3	4		
4	Control	0·74	0·84	0·78	0·72	n.s.	***
		(50)	(50)	(50)	(50)		
	Selected	0·82	0·62	0·58	0·60	n.s.	*
		(50)	(50)	(50)	(50)		
		**	*				
8	Control	0·83	0·83	0·74	0·79	n.s.	***
		(48)	(84)	(100)	(80)		
	Selected	0·87	0·70	0·40	0·70	n.s.	*
		(60)	(160)	(180)	(154)		
		**	*		*		
10	Control	0·72	0·71	0·93	0·73	n.s.	**
		(116)	(112)	(138)	(110)		
	Selected	0·67	0·76	0·34**	0·66	n.s.	n.s.
		(94)	(84)	(70)	(114)		

* $P < 0·05$. ** $P < 0·01$. *** $P < 0·001$. n.s. = $P > 0·05$.

Table 3. Frequency of the *AdhF* allele in the Chateau Douglas selected and control sublines

Numbers of genes scored are in parentheses. The significance of the comparisons made are indicated as in Table 2

Generation		Chateau Douglas sublines				Heterogeneity between sublines	Divergence of sublines from 0·52
		1	2	3	4		
4	Control	0·70	0·58	0·60	0·78	n.s.	*
		(50)	(50)	(48)	(50)		
	Selected	0·64	0·60	0·68	0·52	n.s.	n.s.
		(50)	(40)	(50)	(50)		
8	Control	0·94	0·54	0·52	0·53	n.s.	n.s.
		(48)	(80)	(102)	(90)		
	Selected	0·55*	0·90	0·86	0·53[A]	n.s.	**
		(188)	(160)	(88)	(260)		
		*	*				
10	Control	0·94	0·57	0·34	0·51	*	n.s.
		(106)	(152)	(140)	(128)		
	Selected	0·50*	0·75	1·00**	0·67	*	**
		(108)	(146)	(114)	(132)		
		*		*			

[A] Observed homogeneity is greater than would be expected by chance, $P < 0·05$.
* $P < 0·05$. ** $P < 0·01$. *** $P < 0·001$. n.s. = $P > 0·05$.

S_{10}. Significant heterogeneity in *F* frequency between the control sublines and between the selected sublines was present only at S_{10}. The mean *F* frequency in the control lines at S_4 and in the selected lines at S_8 and S_{10} differed significantly from 0·52.

In the first subline the *F* frequency in the selected line was significantly lower than in the corresponding control line at S_8 and S_{10}; in contrast the third selected line at S_{10} had a significantly higher *F* frequency than the corresponding control. Whereas in all of the Craigmoor control lines the frequency of *F* increased to above the level in the original trapped population (Table 1) there was no such tendency in the Chateau Douglas control lines.

Overall these analyses point to differences in gene frequency changes between the Craigmoor and Chateau Douglas derived lines but in neither set of lines is there evidence for a consistent increase in the frequency of *F* in the selected compared with the control lines.

Differences Between the Lines in Alcohol Dehydrogenase Activity

Although there was no consistent increase in the frequency of *F* in the selected sublines there remains a possibility that ADH activity was consistently higher in these lines than in their respective controls. Genetic modifiers, increasing the amount of ADH protein, are known to be segregating in the base populations (Lewis and Gibson 1978) so that selection for alcohol tolerance might have increased the frequency of such modifiers. These possibilities were tested after S_{10} by assaying ADH activity in samples of third instar larvae from each of the control and selected sublines. (Although the data are not reported here, adults were also assayed and the activities paralleled those obtained in larvae.)

Table 4. Alcohol dehydrogenase activity in selected and control lines

Values are means of triplicate assays in arbitrary units

	Craigmoor sublines				Chateau Douglas sublines			
	1	2	3	4	1	2	3	4
Controls	7·0	8·5	7·6	5·2	9·2	6·2	5·5	7·1
Selected	6·9	7·7	4·9	5·3	6·6	10·1	8·3	9·1

	Analysis of variance				
		Mean squares			
	d.f.	Craigmoor	Chateau Douglas	d.f.	Combined
Selected lines/control lines	1	4·5	13·9**	1	1·3
Craigmoor/Chateau Douglas				1	15·3***
Population × selection				1	17·2***
Sublines	3	8·7	1·9	6	5·3**
Selection within populations × sublines	3	2·3	12·4**	6	7·3***
Within cultures (error)	16	1·2	1·3	32	1·3

** $0{\cdot}01 > P > 0{\cdot}001$. *** $P < 0{\cdot}001$.

These assays (Table 4) show that, overall, the Chateau Douglas derived lines had higher enzyme activity than the Craigmoor lines, though, again overall, there were no significant differences in activity between the selected and control lines. In

the Craigmoor derived lines there was no significant difference in enzyme activity between the selected and control lines (ANOVA, Table 4). However the Chateau Douglas selected lines had higher enzyme activities than the control lines although there was significant heterogeneity between the sublines (ANOVA, Table 4).

Differences in ADH activity between the 16 sublines of both populations were correlated with differences between the lines in the frequency of the *F* allele ($r_{14} = 0{\cdot}61$, $P < 0{\cdot}02$, cf. Tables 2, 3 and 4).

Discussion

These results show that successful selection for increased ethanol tolerance in *D. melanogaster* does not necessarily depend on either an increase in the frequency of the *F* allele or on an increase in the level of ADH activity. The selected lines derived from the Chateau Douglas population produced different results from those derived from the Craigmoor population in terms of both the frequency of *F* and in ADH activity. But these differences were not matched by differences in ethanol tolerance between the two sets of lines; moreover the four Chateau Douglas sublines were significantly heterogeneous. In one Chateau Douglas selected line the frequency of *F* and the level of ADH activity were both lower than in the corresponding control line, yet flies from the selected line bred successfully on 12% ethanol-supplemented medium whilst those from the control line could not be bred on this medium.

Previous laboratory studies have demonstrated increases in the frequency of *F* in populations of *D. melanogaster* maintained on ethanol-supplemented media. One of the present authors (Gibson 1970) described preliminary results of an experiment in which four cultures were set up with a gene frequency of 0·5 *F* and in two of the cultures the medium was supplemented with 6% ethanol. After 18 generations of mass transfer the gene frequency in the two control populations had not changed, whereas the frequency of *F* increased to 0·73 and 0·82 respectively in the ethanol-supplemented cultures. In assays carried out after a further eight generations of mass transfer one of these two ethanol cultures was fixed for *F* and the other maintained a frequency of *F* close to 0·7. The frequency in the control cultures did not deviate significantly from 0·5.

Bijlsma-Meeles and Van Delden (1974) have obtained consonant results. They found that laboratory populations monomorphic for *S* had a higher extinction rate on ethanol-supplemented medium than populations monomorphic for *F* (they obtained their results using a 20% ethanol medium which would not support development of the flies in the selected lines in the experiment described here).

More direct evidence for the greater ethanol tolerance of *FF* adults compared to *SS* adults was obtained by Morgan (1975), Briscoe *et al.* (1975) and Oakeshott (1976), though the last author found that the tolerance of *FF* flies was significantly less than that of *FS* flies. Oakeshott (1976) emphasized that in his experiments the *FS* flies had levels of ADH activity intermediate between those of *FF* and *SS* flies.

McDonald *et al.* (1977) found that a population of *D. melanogaster* selected for increased tolerance to ethanol in adult flies (David and Bouquet 1977) had higher levels of ADH activity and increased amounts of ADH protein compared to unselected controls. Their control and selected lines were fixed for the *F* allele but the frequencies of the *F* and *S* alleles in the initial population were not known (David and Bouquet 1977). Their experiments ran for 100 h during which time they monitored the survival of adults in sealed vials containing filter paper strips impregnated

with a 3% sucrose solution and between 8 and 18% ethanol. Using their technique of assessing tolerance we found that adults from our selected lines had significantly higher survival on 10% ethanol than the controls.

Previous laboratory experiments (Morgan 1975; Oakeshott 1976; Thompson and Kaiser 1977) have shown that stocks differing in ADH activity or in allozyme genotype can differ in fitness when reared on ethanol media. However, our selection experiment shows that adaptation to media supplemented with ethanol can be brought about without changing ADH activity or the frequency of the *F* allele.

In our selection lines all stages in the life cycle were subjected to ethanol, although it is likely that its concentration decreased markedly during the immature stages. However, a culture would only produce adult flies at a particular ethanol concentration if the parents survived to lay eggs on the medium and the larval and pupal stages tolerated the concentration of ethanol that remained in the medium for 7 or more days. Thus successful selection required adaptation of all stages to the ethanol-supplemented medium.

It is clear that a variety of mechanisms could be involved in adapting to the presence of alcohol in the environment. Moreover, in keeping with their different habitats, increased tolerance may arise from different processes in larvae and adults. Nevertheless our results cast doubts on the earlier tentative conclusions, based largely on the results of the laboratory experiments of Gibson (1970), Bijlsma-Meeles and Van Delden (1974) and Clarke (1975), that differences in levels of ADH activity between *F* and *S* in *D. melanogaster* have adaptive significance in natural populations in relation to alcohol tolerance. Indeed our results strongly support the conclusions of McKenzie and Parsons (1974), McKenzie (1975) and McKenzie and McKechnie (1978), based on genetic and ecological studies, that adaptation to the alcohol concentrations in the environment of a winery cellar is independent of the ADH system. They found that the frequency of *F* in samples trapped inside the cellars of the Chateau Tahbilk winery (Victoria, Australia) was the same as in samples taken outside the winery, although the 'inside' samples were more tolerant to ethanol than the 'outside' samples. It is intriguing that these data from an Australian winery (and we have consonant data from the Craigmoor winery to be published elsewhere), differ so markedly from those obtained in wine (sherry) cellers in Spain (Briscoe *et al.* 1975) where the frequency of *F* was significantly higher than in neighbouring non-cellar populations. These conflicting results might reflect differences in the levels of alcohol in the two kinds of winery. In the wine cellars in Spain *D. melanogaster* 'feed on, and breed in, the fermenting and maturing liquor (12–15% ethanol) impregnating floating mats at the surface of wine jars' (Briscoe *et al.* 1975). At Chateau Tahbilk (and at Craigmoor) the flies feed on, and breed in, the red wine seepage from sealed wine casks. J. A. McKechnie (personal communication) found that a dry sample of seepage in which larvae were present from Chateau Tahbilk had 0·22% ethanol; he also found that two samples taken very close to a seepage source gave ethanol levels of 7·0 and 10·5% although larvae were not present in these samples. A further factor distinguishing the population in Spain from the one at Chateau Tahbilk is that the *S* allele is held in an inversion [In(2L)*t*] whereas such linkage disequilibrium has not been found at Chateau Tahbilk (J. G. Oakeshott, personal communication). Inversions will have marked effects on the distribution of the *F* and *S* alleles between the 'inside' and 'outside' of the wine cellar if other factors relevant to alcohol tolerance are included in the inversion.

The following explanations, either singly or in combination, should also be considered in attempts to further reconcile the conflicting evidence implicating ADH activity in alcohol tolerance. Firstly, it is possible that for some unknown reason selection for increased tolerance was unable to exploit the genetic variation for ADH activity present in the Chateau Douglas and Craigmoor base populations (and also in the population of Chateau Tahbilk). Secondly, ADH may have a role in alcohol tolerance only at concentrations above 12% alcohol in the medium. Thirdly, the level of ADH activity may be relevant in determining tolerance to alcohols other than ethanol; there is evidence that, *in vitro*, ADH is more active on secondary alcohols (David *et al.* 1976). Fourthly, it is possible that under some nutritional conditions alcohols will be used as an energy source (Libion-Manneart *et al.* 1976). Higher ADH activity may then be relevant in providing energy in flies that are tolerant to environmental alcohols by some mechanism unrelated to ADH activity. Finally, we need to consider the possibility that the major role of ADH in *D. melanogaster* might be in some other, as yet unidentified, process so that the associations sometimes found in the laboratory between activity and alcohol tolerance are not generally important in natural populations adapted to environmental alcohols.

None of these possible explanations can be fully tested with the data available. Clearly a different experimental approach is now required to explore the adaptive significance of allozymes at the *Adh* locus and the mechanisms of alcohol tolerance in natural populations of *D. melanogaster*.

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Appendix

Suppose we have a set of k observed gene frequencies $\{p_i\}$ based on $\{n_i\}$ individuals. A large number of test statistics are in use to compare the equality of the gene frequencies to an unknown proportion, ρ. These range from Fisher's exact test for $k = 2$ (Kendall and Stuart 1973, p. 572), through variations of the usual chi-squared statistic for $2 \times k$ contingency tables,

$$\sum_{j=1}^{2k} (O_j - E_j)^2 / E_j ,$$

where O_j and E_j are the observed and expected numbers of genes in the $2k$ categories and the deviance,

$$2 \sum_{j=1}^{2k} O_j \ln(O_j / E_j)$$

to

$$2 \sum_{i=1}^{k} n_i (p_i - \bar{p})^2 / \bar{p}(1 - \bar{p})$$

(Cavalli-Sforza and Bodmer 1971, p. 422). This last test statistic is suitable when the n_i are large and the weighted mean gene frequency $\bar{p}$ is neither so small nor so large that the normal approximation to the binomial distribution does not hold. All these tests are equivalent asymptotically (Kendall and Stuart 1973, Chapters 30 and 33), and assume an infinite effective population size.

However, these tests are inappropriate for the analysis of the experiment described in this paper because the effective population size N is not infinite, and so the 'true' gene frequency, p, is not constant, but has a distribution, say $f(p)$. As in an infinite population the expected 'true' gene frequency is ρ, but it has a variance $\rho(1-\rho)(1-e^{-t/2N})$ after t generations of random genetic drift (Kimura 1970, p. 185). Therefore the distribution of the observed gene frequencies is

$$\Pr(p_i - k | 2n_i) = \binom{2n_i}{k} \int_0^1 f(p) p^k (1-p)^{2n_i - k} \, dp .$$

The expected value of p_i is ρ and the variance is

$$\frac{\rho(1-\rho)}{2n_i} + \frac{2n_i - 1}{2n_i} \rho(1-\rho)(1-e^{-t/2N}) .$$

In other words, the variance of the observed proportion consists of two parts—the sampling variance expected when sampling from an infinite population, and that due to random genetic drift in a finite population.

The maximum likelihood method of Nelder and Wedderburn (1972) can be used with either the untransformed or transformed observed gene frequencies when testing their homogeneity with that expected under random genetic drift. The procedure will be illustrated here for the angular transform, $\sin^{-1}\sqrt{p_i}$, modified by the usual convention to be $\sin^{-1}\sqrt{(1/4n_i)}$ for $p_i = 0$ and to be $\sin^{-1}\sqrt{(1-1/4n_i)}$ for $p_i = 1$.

The angular transform was chosen because it stabilizes the variance of the observed gene frequencies to a value approximately independent of the 'true' gene frequencies. This transform is particularly appropriate because it ensures that the variance of the transformed observations are the same under both the null

hypothesis and a wide range of alternative hypotheses. This holds even though the 'true' gene frequencies do appear to be changing not only at random (as assumed by the null hypothesis) but also due to other causes (as postulated by alternative hypotheses). We felt that the weighted least squares method, which may often be more appropriate, could confound the interpretation of results here.

Let $\{u_i\}$ be the set of angularly transformed gene frequencies, with expected 'true' mean μ. The variance of the transformed values, given that only random genetic drift is operating, is

$$\frac{1}{8n_i} + \frac{2n_i - 1}{8n_i}(1 - e^{-t/2N}). \tag{1}$$

The estimate $\hat{\mu}$ of μ is given by

$$\hat{\mu} = \left(\sum_{i=1}^{k} \omega_i u_i\right) \Big/ \left(\sum_{i=1}^{k} \omega_i\right),$$

where the weights ω_i are given by the reciprocal of the variance estimate (1). The estimated mean gene frequency $\hat{p}$ is $(\sin \hat{\mu})^2$. The deviance after estimating μ is calculated as

$$\text{deviance } (\hat{\mu}) = \sum_{i=1}^{k} \omega_i (u_i - \hat{\mu})^2,$$

which is χ^2_{k-1} distributed if the gene frequencies are observed from populations subject only to random genetic drift.

To test the homogeneity of the observed gene frequencies in the control and selected lines in the experiment described above, the deviance was calculated using the within sublines and within generations data. To test the homogeneity within the two experimental groups, this procedure was applied separately to the within generations and across sublines data.

The mean 'true' gene frequency could be taken to be $\hat{p} = \tilde{p}$, the gene frequency at generation 0. To test whether the data are consistent with this hypothesis, the following deviance is appropriate:

$$\text{deviance } (\tilde{\mu}) = \sum_{i=1}^{k} \omega_i (u_i - \tilde{u})^2,$$

where $\tilde{u} = \sin^{-1}\sqrt{\tilde{p}}$, and this deviance is distributed as a χ^2_k distribution if the populations have been subject only to random genetic drift since generation 0. If there is no selection, the test statistic

$$\text{deviance } (\tilde{\mu}) - \text{deviance } (\hat{\mu})$$

is χ^2_1 distributed; high values indicate directional selection while low values are likely if there is stabilizing selection around the gene frequency $\tilde{p}$.

The test statistics for homogeneity of gene frequencies and equality to a predetermined gene frequency can be calculated using the GLIM computer program (GLIM 1975).