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# Selection for ethanol tolerance and *Adh* allozymes in natural populations of *Drosophila melanogaster*

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SUMMARY

Alcohol dehydrogenase (*Adh*) allozyme frequencies and tolerance of adult flies to ethanol were measured in population samples of *D. melanogaster* from a winery in Southern Ontario. Samples were also tested from a number of non-winery sites. $Adh^{fast}$ frequencies decrease as one moves away from the winery, but this drop in allozyme frequency is statistically significant only for those areas that are more than 3 km distant from the winery. Tolerance of adult flies to ethanol also differed between winery and non-winery populations, but these differences were not statistically significant. The data presented here may help to resolve the seeming conflict between the two previous studies of *Adh* allozyme frequencies in natural populations from high- and low-alcohol environments.

## 1. INTRODUCTION

Since the initial demonstration of high levels of allozyme variability in Drosophila populations by Lewontin & Hubby (1966), several attempts have been made to assess the biological significance of allozymic polymorphisms in natural populations. In many studies, the principal aim has been to find a correlation between the frequency of a specific electrophoretic allele and a given environmental variable. Laboratory experiments using *D. melanogaster* have demonstrated predictable responses to changes in dietary carbohydrate for amylase electromorph frequencies (DeJong & Scharloo, 1976; Hickey, 1977). Allozyme frequencies at the alcohol dehydrogenase (*Adh*) locus in *D. melanogaster* have also been shown to respond to changes in dietary treatment: specifically, the $Adh^{fast}$ allele is selected for in the presence of alcohol (Gibson, 1970; van Delden, Boerema & Kaping, 1978; Caverner & Clegg, 1978). This selective response is believed to be due to the greater enzymic activity of the $Adh^{fast}$ electromorph, although much variation between strains exists within a single electrophoretic class for both *in vitro* *Adh* activity (Lewis & Gibson, 1978; MacDonald *et al.* 1977) and for tolerance of high levels of dietary ethanol (Kamping & van Delden, 1978).

Attempts to link the results of these laboratory experiments to surveys of *Adh* frequencies in natural populations of *D. melanogaster* have yielded conflicting results. This species is abundant in wineries all over the world (David, 1978) and has adapted to high concentrations of ethanol, thus allowing the exploitation of

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winery environments (McKenzie & Parsons, 1972). Since laboratory experiments indicate differential susceptibilities of *Adh* phenotypes to environmental ethanol, predictions can be made that flies from winery populations would have greater ethanol tolerance and higher $Adh^{fast}$ frequencies than flies from neighbouring non-winery populations. Both these predictions were borne out by a study comparing a population of *D. melanogaster* from a Spanish wine cellar to a population of flies from a nearby rubbish tip (Briscoe, Robertson & Malpica, 1975). The frequency of the $Adh^{fast}$ electromorph was significantly higher in the wine-cellar population and the tolerance to ethanol of the $Adh^{fast}$ phenotypes was greater than that of the $Adh^{slow}$ phenotypes. In a similar study of an Australian winery population, McKenzie & Parsons (1974) observed differences in ethanol tolerance between the cellar and peripheral sections of the population, but they found no correlation between ethanol tolerance and *Adh* frequencies. A more intensive study of this same population (McKenzie & McKechnie, 1978) showed no relationship between the distributions of ethanol tolerance and of *Adh* phenotypes, although ethanol tolerance could be directly related to the selective effect of environmental alcohol. Thus both studies demonstrate an increased ethanol tolerance in flies from an alcohol-enriched environment, but the Australian study does not show the predicted divergence in *Adh* allozyme frequencies.

The experiments presented here provide data on *Adh* electromorph frequency and tolerance to ethanol of flies from winery and non-winery sites in southern Ontario. The results are compared to those from the Spanish and Australian populations.

## 2. MATERIALS AND METHODS

Adult flies were collected from sites within Barnes Wines, St Catharine's, Ontario and also from neighbouring non-winery sites during the months of September, October and November 1978. Flies for use in the electrophoretic survey were stored at −80 °C. Iso-female lines were established on yeast–agar Drosophila medium and maintained for the ethanol-tolerance experiments. A total of 64 iso-females lines was used.

Electrophoresis was carried out according to the method of Prakash, Lewontin & Hubby (1969). Reference stocks with known *Adh* phenotypes were used as standards.

Ethanol tolerance of adult flies was measured by a procedure similar to that used by Briscoe *et al.* (1975) and by van Delden *et al.* (1978). Control group flies were transferred from normal food to a mixture of 1·5% agar and 7·5% sucrose in 105 ml glass vials. The experimental group was transferred to an identical medium supplemented with 19% ethanol. Two- to four-day-old flies were introduced into these vials. The flies were not etherized as it was observed that etherized flies have reduced alcohol tolerance. Each vial contained between 15 and 40 flies. All vials were kept in an incubator at 25 °C. The number of dead flies in each vial was recorded after 44, 83 ,122, 136 and 162 hours. At the end of the experiment the total number of flies in each vial was recorded and the percentage mortality was calculated. Several replicate vials were used for each iso-female line.

### 3. RESULTS AND DISCUSSION

Natural selection acting at the alcohol dehydrogenase locus in *D. melanogaster* in an ethanol-enriched environment is expected to increase the frequency of the $Adh^{fast}$ electromorph. This expectation is based on the finding of higher enzymic activity for the $Adh^{fast}$ allozyme, compared to $Adh^{slow}$ (Day, Hillier & Clarke, 1974), and also on the response of $Adh^{fast}$ frequencies to alcohol-enriched media in the laboratory (Gibson, 1970; Caverner & Clegg, 1978; van Delden *et al.* 1978). The purpose of this study was to compare $Adh^{fast}$ frequencies in Drosophila populations from a Canadian winery and from surrounding areas. Some sample

Table 1. Adh$^{fast}$ *frequencies among wild-caught* D. melanogaster *adults from three sampling areas*

| Collection areas | Number of files electrophoresed | Frequency of $Adh^{fast} \pm$ S.E. |
|---|---|---|
| Within winery | 125 | $0{\cdot}513 \pm 0{\cdot}032$ |
| Less than 0·5 km from winery | 276 | $0{\cdot}475 \pm 0{\cdot}021$ |
| More than 3 km from winery | 229 | $0{\cdot}410 \pm 0{\cdot}023$ |

Table 2. *Significance levels for pairwise t-test comparisons of* Adh$^{fast}$ *frequencies at different collection sites*

| | Within winery | Less than 0·5 km from winery | More than 3 km from winery |
|---|---|---|---|
| Within winery | — | $0{\cdot}4 > P > 0{\cdot}3$ | $0{\cdot}01 > P > 0{\cdot}001$ |
| Less than 0·5 km from winery | — | — | $0{\cdot}05 > P > 0{\cdot}02$ |
| More than 3 km from winery | — | — | — |

sites were close to the winery (less than 0·5 km away) and therefore are comparable to the non-winery sites of McKenzie & McKechnie (1978). Other sites were considerably further away (more than 3 km) and so these sites are comparable to the non-winery sites studied by Briscoe *et al.* (1975). The data indicates a drop in $Adh^{fast}$ frequencies as one moves away from the winery (Table 1). However, only those differences in gene frequency between the winery population and the more distant non-winery populations are statistically significant (Table 2). These results are consistent with *both* of the previous sets of data, i.e. the non-winery sites for the Australian population were less than 100 m from the cellar area and showed no difference in $Adh^{fast}$ frequency (McKenzie & McKechnie, 1978); the Spanish non-winery population was approximately 1 km away from the cellar area and did have a significantly lower $Adh^{fast}$ frequency (Briscoe *et al.* 1975). Thus, although there may be different adaptive systems operating in the Spanish and Australian populations, as suggested by McKenzie & McKechnie (1978), it is also possible that the differences between the two sets of results may be explained simply on the grounds of different sampling ranges. The results presented here (Tables 1 and 2) support this latter possibility.

The effect of 19% ethanol on the viability of adult flies is shown in Table 3. This level of ethanol in the medium caused an overall mortality level of 45% within 162 h, whereas there was less than 1% mortality among the control flies within the same time period (Table 3). Adult fly mortality in response to 19% ethanol was measured for strains from the winery and from the two non-winery areas. Data are presented in Table 4 for regression coefficients of adult mortality

Table 3. *Effect of* 19% *ethanol on adult mortality in* D. melanogaster

(Control group flies (2–4 days old) were grown on a medium containing 1·5% agar and 7·5% sucrose. Experimental groups were given an identical medium supplemented with 19% = ethanol.)

| | Number of vials | Total number of flies | Number of deaths | Percentage mortality ± S.E. |
|---|---|---|---|---|
| Control | 86 | 932 | 4 | 0·4 ± 0·002 |
| Experimental | 180 | 3753 | 1699 | 45·3 ± 0·008 |

Table 4. *Differences in adult tolerance to ethanol of* D. melanogaster *strains from three different collection areas*

(Data are given for mean regression coefficients (percentage mortality on time (h) and for mean $LT_{10}$ (time (h) to 10% mortality).)

| Collection areas | Number of isofemale lines | Mean regression coefficient ± S.E. | Mean $LT_{10}$ ± S.E. |
|---|---|---|---|
| Within winery | 18 | 0·27 ± 0·076 | 50·83 ± 19·90 |
| Less than 0·5 km from winery | 30 | 0·396 ± 0·100 | 35·92 ± 11·53 |
| More than 3 km from winery | 16 | 0·330 ± 0·073 | 34·83 ± 6·97 |

on time and also for the average $LT_{10}$ (time to 10% mortality) for strains from each of the collection areas. There is a drop in adult tolerance to ethanol as one moves away from the winery. However, the differences between the three areas for adult tolerance are not statistically significant. This is due to the rather limited number of strains used (64 in all) and also to large differences in ethanol tolerance between strains within a collection area. It should be noted that the data presented in Table 4 suggest (but do not demonstrate conclusively) that adult tolerance to ethanol differs between winery and adjacent (< 0·5 km away) non-winery populations. Again, this is consistent with the results of that study which included wine cellar and adjacent sites (McKenzie & McKechnie, 1978) and which showed differences (statistically significant in their results) in ethanol tolerance, but not in $Adh^{fast}$ frequency.

Neither $Adh^{fast}$ frequency nor tolerance to ethanol showed any pattern of overall temporal change during the course of this study (15 September 1978 to 17 November 1978). There was some variation in $Adh^{fast}$ frequency and in ethanol tolerance between sites within a collection area. However, these variations did not correlate with the observed local variations in environmental alcohol level.

The alcohol dehydrogenase enzyme system in Drosophila is one component of the general physiological mechanism whereby the organism can develop a

tolerance to relatively high concentrations of environmental alcohol. Selection for survival in an alcohol-enriched environment within a winery results in selection for all characters which increase tolerance to alcohol. Selection on one component of the tolerance mechanism (*Adh* electromorphs) must necessarily be less intense than the total selection on all components of tolerance. Therefore one expects the cline in ethanol tolerance of adult flies across the winery, non-winery boundary to be more sharply defined than the cline in *Adh* electromorph frequency. This is, indeed, the picture which emerges if we consider the results presented here in combination with the results of previous studies on winery and non-winery populations of *D. melanogaster*.

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