SELECTION ACTING DIRECTLY ON AN ENZYME POLYMORPHISM

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Summary

A biochemical approach is utilised in the study of the maintenance of variation at the *Adh* locus in *Drosophila melanogaster*. There is a direct correlation between biochemical findings and the results of competition experiments. The relevance of these findings to the study of other enzyme polymorphisms is discussed.

1. Introduction

By means of electrophoresis about one-third of proteins have been shown to be polymorphic (Lewontin and Hubby, 1966). How is this protein variation maintained? There are two conflicting views. The neutralist view (Kimura and Ohta, 1971) states that the majority of protein variation is a product of mutation and drift, whilst the selectionist view (Clarke, 1970; Richmond, 1970) stresses the importance of selective forces. The argument can most clearly be resolved by methods which directly measure the selective forces (if any) acting at a particular locus. This paper presents such a method, applied to the alcohol dehydrogenase (ADH) polymorphism in *Drosophila melanogaster*.

Much evidence suggests that selection influences gene-frequencies at this locus (Rasmuson *et al.*, 1966; Kojima and Tobari, 1969; Gibson, 1970; Vigue and Johnson, 1973; Day *et al.*, 1974). Until now, however, there has been no strong evidence that the observed changes in gene-frequency (Kojima and Tobari, 1969; Vigue and Johnson, 1973) are due to selection acting directly on the *Adh* locus, rather than on other loci closely linked to it.

The enzyme produced by the Adh^F allele has a greater specific activity than the enzyme produced by the Adh^S allele when ethanol is used as a substrate (Rasmuson *et al.*, 1966; Gibson, 1970). This difference in activity is also evident when a number of other alcohols are used as substrates (table 1). I have conducted experiments to find out if the difference in enzyme activities are reflected by selective differentials in experimental cultures of *Drosophila*.

2. Materials and methods

Homozygous lines for the *F* and *S* alleles were derived from the Kaduna cage population at the Genetics Department, University of Nottingham. Each line consisted of the progenies of thirty pairs of homozygous parents. Each set of parents was obtained independently from the Kaduna population. The lines had been kept in population cages (as large populations) for approximately 18 months before the experiments were carried out. One hundred *FF* and one hundred *SS* newly emerged larvae (0-3 hours old) were collected for each experiment. They were put in a 10×4 cm *Drosophila* vial containing 10 ml of a medium resembling that of Kalmus (1943),

except that it did not contain sucrose or ethanol; 110 mg of the yeast *Saccharomyces cerevisiae* in 0·5 ml H_2O/10 ml media were added to the control vials. 125 mg of *Saccharomyces cerevisiae* in 0·5 ml H_2O/10 ml media were added to the experimental vials as well as appropriate amounts of alcohol substrates. The amounts of alcohol added were those that gave approximately the same average mortality (regardless of genotype) as the controls. The components of the mixture were shaken until set, in order to disperse evenly the alcohol and the yeast. All emerging flies were electrophoresed, by the method of Day *et al.* (1974), to determine their *Adh* genotype.

TABLE 1

The activities with various alcohols of crude extracts of enzymes produced by the allelic genes Adh^F and Adh^S *in* Drosophila melanogaster (*data of Day et al., 1974*)

Substrate*	Activity† of *F* enzyme	Activity of *S* enzyme	Ratio of activities *F*: *S*
1. Cyclohexanol	0·1022	0·04915	2·08
2. Ethanol	0·03488	0·01921	1·82
3. N-butanol	0·07895	0·05236	1·51
4. Iso-butyl alcohol	0·04511	0·03406	1·31
5. N-propanol	0·03753	0·03089	1·21
6. Iso-propanol	0·1250	0·04952	2·52
7. 1-penten-3-ol‡	0·05216	0·0163	3·2

* Cyclohexanol is completely oxidised *in vivo* (Tecwyn Williams, 1949). Alcohols numbered 2, 3, 4 and 5 are oxidised by alcohol dehydrogenase to aldehydes. Alcohols numbered 6 and 7 are oxidised by alcohol dehydrogenase to ketones.

† Activities were measured in a Pye-Unicam SP 1800 spectrophotometer. Saturated solution (or, in the case of ethanol and N-propanol, molar solutions) of the alcohols were prepared in phosphate buffer (pH 8·0). Equal weights of *FF* and *SS* flies were homogenised and the activities of the crude extracts compared at 25°C, using the different alcohol substrates.

‡ Author's data.

3. RESULTS AND DISCUSSION

The results are given in table 2. If in table 2 we exclude the results for iso-propanol (see below) then a comparison of tables 1 and 2 shows that the degree to which an alcohol selects for *F* in the *Drosophila* vial is clearly related to the ratio of activities of *F* and *S* *in vitro* (regression of β on $F : S$ ratio; $b = -0{\cdot}161$, $t = -5{\cdot}94$, $P < 0{\cdot}01$). This relation is to be expected if the ADH enzyme detoxifies the alcohols, if the products of detoxification are non-toxic, and if the enzymic products of the two alleles act at different rates. The generality of these results has been confirmed by Van Delden and Bijlsma-meeles (personal communication) using *Drosophila* of different geographical origin, and different experimental methods.

The primary alcohols in table 2 are oxidised by ADH to aldehydes and these are presumably further broken down by aldehyde oxidase (Day *et al.*, 1974). Cyclohexanol is completely oxidised *in vivo* (Tecwyn Williams, 1949). Iso-propanol is broken down by ADH into the ketone, acetone. Acetone is toxic, and notoriously difficult to oxidise *in vivo* (Tecwyn Williams, 1949). This fact probably accounts for the anomalous behaviour of iso-propanol in departing from the general association.

If the differences observed are, as they seem to be, due to selection acting directly on the *Adh* locus, then the following prediction can be made. In

mixtures of *FF* and *SS* flies with an alcohol substrate that is metabolized by ADH to give a lethal product, the *SS* flies should be at an advantage to the *FF* flies (because ADH-*F* acts at a faster rate than ADH-*S*). Such an alcohol is 1-penten-3-ol. It is reported to be oxidized by ADH to give a lethal ketone product, ethyl vinyl ketone (Sofer and Hatkoff, 1972). Using the method of Sofer and Hatkoff (1972), I have tested this prediction.

TABLE 2

The relative advantages of FF *flies over* SS *flies when grown on a medium with various alcohols*

Substrate	Quantity added (ml)/10 ml of medium	*FF* input*	*SS* input*	*FF* survived	*SS* survived	β value for *FF*†
Cyclohexanol	0·05	400	400	120	12	0·2556
Ethanol	0·5	400	400	83	17	0·3324
N-butanol	0·1	400	400	80	22	0·3569
Iso-butyl alcohol	0·1	400	400	88	31	0·3719
N-propanol	0·25	400	400	98	57	0·4192
Iso-propanol	0·20	400	400	130	91	0·4315
Control	—	400	400	60	49	0·4745

* Each result is the sum of four pooled replicates (in every case the results of replicates were statistically homogeneous).

† Instead of displaying data as a final gene-frequency I have used the method of Manly (1972), which assigns the relative advantage or disadvantage to a particular morph. In this case the figures describe the relative advantage or disadvantage of the *FF* morph. Values below 0·5 indicate a relative advantage. Values above 0·5 indicate a relative disadvantage.

$$\beta = \frac{\log\left(\frac{FF \text{ put in}}{FF \text{ survived}}\right)}{\log\left(\frac{FF \text{ put in}}{FF \text{ survived}}\right) + \log\left(\frac{SS \text{ put in}}{SS \text{ survived}}\right)}$$

TABLE 3

The effect of exposure to 1-penten-3-ol on the survival of FF *and* SS *flies*

Number of flies exposed	Number of replicates	Sex	ADH genotype	Died	Survived	Percentage survival
430	10	Female	*FF*	277	153	35·6
430		Male	*SS*	194	236	54·8
259	7	Male	*FF*	186	73	28·1
259		Female	*SS*	102	157	60·6

In each experiment, equal numbers of *FF* and *SS* flies (the *FF* flies being of one sex, the *SS* of the other) were placed in a container and exposed to the vapour of a freshly prepared solution of 0·5 per cent 1-penten-3-ol in water for approximately 1¼ minutes. After this time the flies were transferred to a vial containing normal *Drosophila* medium. After a further 12 hours the flies which had died were counted and sexed. The flies used in these experiments were 4 days old (for explanation see Sofer and Hatkoff, 1972). The association between sex and genotype was changed from experiment to experiment.

Of 17 replicate experiments, two gave a non-signficant result and in the other 15 a significantly greater number of *FF* flies died after the treatment

(see table 3). Even without statistically testing the results of individual experiments the total number of experiments going in the expected direction (15/17) is itself clearly significant ($P < 0{\cdot}002$ by the median test).

Using the same experimental design I have exposed flies to the vapour of ethyl vinyl ketone (the ketone produced by the oxidation of 1-penten-3-ol by ADH). There is no significant difference in survival rate between *FF* and *SS* flies. This result means that the differential survival is related to differential activity and not to differential susceptibility to the ketone.

It can be concluded that the variation at the *Adh* locus in *D. melanogaster* is not neutral to applied selection. There is good evidence that the selection acts directly on this locus rather than on other loci linked to it.

The principle of searching for the direct effects of selection, by correlating knowledge of the biochemical properties of enzyme variants with changes of gene-frequencies in experimental populations can be applied to other enzyme polymorphisms. By these means the question " How is protein variation maintained? " could perhaps be finally and conclusively answered.

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4. References

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