ESTIMATION OF FITNESSES AT THE ALCOHOL DEHYDROGENASE AND α-GLYCEROPHOSPHATE DEHYDROGENASE LOCI IN *DROSOPHILA MELANOGASTER*

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Declaration (As required by Regulation 2.4.15)

I declare that this thesis has been composed by myself and that the work contained in it is my own.

ABSTRACT

A two stage, sequential generation sampling method for fitness estimation in random mating *Drosophila* populations is described. Results of its application to the alcohol dehydrogenase and α glycerophosphate dehydrogenase polymorphisms in *D. melanogaster* under different conditions are presented. Populations maintained on medium supplemented with butan-2-ol show a decreased fitness of the S allele at the ADH locus, relative to the F allele. No such effect is observed at the αGPDH locus. Populations maintained on normal medium have been examined for fitness differences in fecundity, mating ability, egg to larval survival and larval to adult survival. No differences have been found between the alternative alleles at either polymorphic locus. These results are discussed and related to the current literature.

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CHAPTER 1

INTRODUCTION

Bryan Clarke has offered an assessment of the contribution that ecological genetics can make to evolutionary theory (Clarke, 1975). At present, the latter is being expanded to assimilate the finding of large amounts of genetic variation in natural populations. Clarke puts forward a methodology to enable the unambiguous demonstration of the action of natural selection at a polymorphic locus in a natural population. He discusses its application to the alcohol dehydrogenase polymorphism in Drosophila melanogaster. The demonstration of the involvement of natural selection in the maintenance of this polymorphism is in itself a major achievement, since there are so few authenticated examples of such involvement (e.g. Lewontin, 1974). On the other hand, even if it were possible to apply the method to, say, several hundred different polymorphisms, the evidence gained would remain anecdotal in the face of the thousands of loci likely to be polymorphic. Further, the method may lead to biased results in that it is applicable only to polymorphisms where genetic variation in protein structure is directly observable. For these reasons, it may be unduly optimistic to hope that when positive results are gained by this approach " ... the arguments between the 'neutralists' and 'selectionists' would finally be resolved" (Clarke, 1975).

It is certain that an improved methodology for the demonstration of natural selection acting in natural populations is necessary; Clarke (op. cit.) points out that no method previously employed can exclude the possibility that selection is acting on alleles closely

linked to, and in linkage disequilibrium with, the locus at which selection is presumed to be acting. The only way to deal with his objection would be to obtain the same results from a number of widely separated populations. Even if this were possible, the objection would only be made less likely to be true; it would not be excluded.

Clarke defines the essentials of a more exact approach as

1) Identification of the selective factor involved.

2) Demonstration of the mechanisms by which this affects the relevant phenotypes.

3) Manipulation of an experimental system based on the results of 1) and 2) to show that predictions can be confirmed.

Applied to an enzyme with electrophoretically detectable variants, such as alcohol dehydrogenase, this expands to four steps, viz.

a) Biochemical and physiological characterisation of the allelic products

b) Inference of possible selective factors from the differences in properties found in a) and from the ecology of the organism.

c) Manipulation of an experimental system to obtain corroborative results.

d) Application of findings to the natural populations, and acquisition of further corroborative evidence.

It can be seen that the success of this strategy depends on a detailed knowledge of the biochemistry, physiology and ecology of the organism. Without this, one might assume that an effect seen <u>in vitro</u> would also occur <u>in vivo</u>, when in fact it did not. In the manipulation

of experimental systems, conditions such as substrate concentrations, must be precisely those found in natural environments, or misleading inferences may be drawn. When lines of individuals homozygous for an allele are used in experimental systems, artefacts due to the linkage disequilibrium set up in such lines may lead to incorrect conclusions (Bijlsma-Meeles pers. comm.).

Clarke's methodology may therefore be suggestive, but alone, it cannot be definitive. Evidence gained by it would be more compelling if it were allied with estimates of relative fitnesses of genotypes in a natural population. This would exclude the possibility that the results obtained were experimental artefacts. Such fitness estimates are rare, and some of the methods used in the past have been shown to be invalid (e.g. Lewontin, 1974). The purpose of the work presented in this thesis is to devise and test an efficient method of fitness estimation in a random mating population, and to apply it to the alcohol dehydrogenase and α-glycerophosphate dehydrogenase polymorphisms in D. melanogaster. This should confirm the results of Clarke and his coworkers, and should vitiate the preceding criticisms.

The ADH locus has been mapped at 2-50.1 (O'Brien & McIntyre, 1972b) and the αGPDH locus at 2-20 (Grell, 1965; Courtright et al., 1966). αGPDH has been implicated in energy metabolism in insect flight muscle (Sacktor, 1970, 1974). The substrate of this enzyme is an internal product of energy metabolism. Because of physiological homeostasis, it can be argued that the "environment" of this enzyme is more uniform than that of ADH, which acts, possibly, on a whole range of alcohols at varying concentrations. It has been suggested

that heterogenous environments support more genetic variation maintained by natural selection than do homogenous environments (e.g. Bryant, 1974). It might be expected, therefore, that the variation in αGPDH is less likely to be maintained by selection than the variation in ADH. For these reasons, αGPDH seems a suitable 'control' locus with which to compare the ADH locus under the conditions of the experiments to be described.

αGPDH is also a more slowly evolving enzyme than ADH, as evidenced by studies on electrophoretic variants in different species of Drosophila (Lakovaara, Saura and Lankinon, 1977). This is compatible with both the possibility that variation, when it exists, is neutral with respect to fitness, and with the possibility that the few polymorphisms in αGPDH are maintained by balancing selection.

The rest of this introduction consists of an outline of the theoretical problems of fitness estimation, summaries of the relevant observations of the ADH and αGPDH polymorphisms, and a discussion of the problems associated with the use of electrophoretic variants.

Problems in fitness estimation

1. Same stage of life cycle transition

It might be thought reasonable to compare genotype frequencies observed at a particular stage of the life cycle with frequencies observed at the same stage, one generation later. However, Prout (1965) has shown that if any selection takes place after the observed stage i.e. if observations are at a partially selected stage, only a component of the total fitness can be estimated by this method. Further, if the actual situation is one of heterozygote advantage, as

it is in Prout's model, then a spurious frequency dependence may be inferred. An extreme example of the first problem is given by Lewontin (1974, p.238), that of the balanced sterile condition. The adult genotypes will be present in a 1:2:1 ratio in each generation, so that comparison of frequencies for two generations would lead to the conclusion that there is no selection. Nevertheless, the fitness of the two homozygotes is actually zero. The second problem is not necessarily dependent on the presence of a fertility component, as Lewontin says, but on a two stage selection process favouring heterozygotes. The first stage may be zygote to adult survival; the second stage could be fertility, fecundity or mating selection. In the extreme case, let

genotypes	A_1A_1	A_1A_2	A_2A_2
survival	near 0	1	1
mating ability	1	1	near 0

have

Then whichever allele is more common, its homozygote will be almost completely selected against, a heterozygote excess will result, and the frequency of the rarer homozygote will increase by segregation from the heterozygotes.

Prout followed this by a more extensive mathematical treatment (Prout, 1969) in which he worked out the minimum number of parent/offspring transitions necessary to estimate fitness for various assumptions about the kind of selection operating. He criticised the experimental design of Tobari & Kojima (1967), who had used this unsatisfactory method to estimate fitnesses of different inversion types in D. ananassae. Prout showed that the data from such population cage selection curves gave excessively large standard errors. (Independently,

and in a different context, Anderson et al (1968) had also reported large standard errors on estimates of fitness derived from population cage selection experiments). Prout showed that as there was no guarantee that further selection was not taking place after sampling, the frequency dependence observed by Tobari & Kojima was not meaningful.

In further papers, (Prout, 1971a,b) he devised a more efficient method of estimating fitnesses. This required the setting up of experimental populations of known parental genotypic proportions, with different ratios between the sexes. Samples of offspring could then be used to estimate fitnesses of the parental genotypes, including mating and fertility components. He applied this system to an experimental population of D. melanogaster using the mutants 'eyeless' (ey^2) and 'shaven-naked' (sv^n). He obtained satisfactory results, and showed that there were large mating and fertility components in selection against these alleles. In spite of Prout's work, other authors continued to use the one-stage transition design. Kojima & Tobari (1969), using the ADH polymorphism in D. melanogaster, estimated larval survival and female fertility by viral experiments. They then compared adult genotype frequencies one generation apart, and observed a frequency dependence of relative fitnesses. They did not exclude the possibility that the genotypes differed in mating ability or male fertility, and therefore the frequency dependence they observed might be a reflection of underlying heterozygote superiority in these components of fitness.

Marinkovic and Ayala (1974), estimated relative fitnesses at the Pgm, Est-5, ODH and MDH-2 loci in D. pseudobscura. They found

effects at all loci for 'hatchability' or fertility, using vial experiments where known proportions of the genotypes were placed in vials as eggs, and the proportion hatching scored. They then did similar experiments for egg to adult survival and development time, but do not seem to appreciate that the effects they observed in these experiments may be a reflection of the differences in 'hatchability' already found. Gromko and Richmond (1978) observed genotype frequencies at the tetrazolium oxidase locus as markers for inversions in D. paulistorum. From a one stage transition they concluded that there was frequency dependent selection maintaining the inversion type. However, from other experiments they presented evidence for heterozygote superiority in some components of fitness. The frequency dependence may therefore be the sort of artefact that Prout describes.

2. Deviation from Hardy Weinberg expectations on one set of observations

In 1958, Wallace quoted numerical examples showing that a simple comparison of observed genotype frequencies with those expected for a Hardy-Weinberg equilibrium (at gene frequencies calculated from the observed genotype frequencies) did not reveal the extent or nature of selection operating on those genotypes. His examples showed that an excess of heterozygotes in such a calculation does not necessarily reflect the superior fitness of the heterozygote, which may in fact be intermediate in fitness between the two homozygotes. One of his examples also shows a spurious frequency dependence, although he did not refer to this. Novitski & Dempster (1958) using a digital computer, were not able to find a unique best fit for fitness values using only observed genotype frequencies. Lewontin and Clark-Cockerham

(1959) and Li (1959) showed that where the fitnesses of three genotypes were related geometrically i.e. where fitnesses $A_1A_1 = W_1$

$A_1A_2 = W_2$

$A_2A_2 = W_3$

and $W_2^2 = W_1W_3$, then there will be no deviation from Hardy Weinberg proportions when the fitnesses do not all equal 1.

If $W_2^2 > W_1W_3$, there will be an apparent excess of heterozygotes. This can include the case of heterozygote advantage, but is not restricted to it. If $W_2^2 < W_1W_3$, there will be an apparent deficiency of heterozygotes. Li refers to the "one generation study of selection" as being "quite fruitless". This seems a reasonable judgement, since neither the apparent presence or absence of selection as given by these comparisons is meaningful.

Nevertheless, the method continues to be used. Rasmuson et al. (1967) observed frequencies at the est-6 locus in population cages of D. melanogaster. At equilibrium, there was an apparent excess of heterozygotes, and the authors claimed this as evidence of heterozygote advantage (although it was not large enough to explain the movement of lines to an equilibrium frequency). Richmond & Powell (1970), measuring frequencies of electrophoretic variants of tetrazolium oxidase in D. paulistorum, reported a similar excess of heterozygotes. They did not refer to Rasmuson et al., and claimed that they had found the first instance of balancing selection maintaining an electrophoretic polymorphism. Vigue and Johnson (1973), reporting ADH frequencies in D. melanogaster in different areas of the U.S.A., say that their data do not deviate from Hardy Weinberg expectations, but they do not discuss

the significance of this. Johnson and Burrows (1976), looking at temporal as well as geographic variation in ADH frequencies, also report no deviation from Hardy Weinberg expectations, and again, do not say what this implies. Mitton and Koehn (1975), in a survey of variation in a marine fish, Fundulus heroclitus, use a test of fit to Hardy Weinberg expectations as a test of selection, without referring to Wallace (1958), Lewontin & Clark-Cockerham (1959) or Li (1959).

3. A more efficient model

Prout's 1971 model is not wholly satisfactory, because it depends on the experimental feasibility of perturbing genotype frequencies. In the case of electrophoretic variants in Drosophila, this can usually only be done by extracting isofemale, homozygous lines. Even if a large number of such lines are extracted and crossed in, effects of artificially created linkage disequilibrium can obscure the fitness differences at the locus studied (Bijlsma-Meeles, pers. comm.).

Bundgaard and Christiansen (1972) devised an experimental procedure which avoids the necessity of perturbation. Instead of measuring fertility, fecundity and mating selection by using different genotypic proportions of the sexes in the parents, they sampled individual female parents and recorded the genotypes of these and their individual offspring. The frequency of transmission of alleles in the male gametes can be estimated from such data, as can fertility differences between female genotypes. The procedure was again applied to a test system in Drosophila melanogaster, using sparkling polient (spa^{pol}) and shaven-naked (sv^{n}). Selection in the experimental population was shown to be almost entirely in mating pattern, and the fitnesses exhibited frequency dependence.

Several authors have referred to the use of parent-offspring combinations (Robertson, 1965; Cooper, 1968; Redfield, 1973) but they were not immediately concerned with overall fitness estimation in natural populations. Christiansen and Frydenberg (1973) expanded the 1972 model and proposed to apply it to a natural population of a live bearing fish, Zoarces viviparus, the eel pout. Christiansen, Frydenberg and Simonsen (1973, 1974, 1976, 1977) have published an extensive series of papers on electrophoretic polymorphisms in Zoarces. In particular, the estIII polymorphism has been studied on samples for five consecutive years, 1969-1974. The polymorphism is found throughout the Kattegat between Denmark and Sweden.

There is a geographic cline running North-South in frequency, with the $estIII^1$ allele at high frequencies going North. The area sampled in the study, Kaloe Cove, is about halfway along the cline, and the $estIII^1$ frequency there is about .36. Throughout the study, a consistent deficiency of heterozygotes compared with Hardy Weinberg expectations was observed. This observation may be due to non-random mating, or to selection such that the square of the fitness of the heterozygote is less than the product of the fitnesses of the two homozygotes. The experimental scheme of Christiansen et al., provided the information that there was selection a) against heterozygotes in the juvenile stage, in all five years; b) possibly male sexual selection favouring the $estIII^1$ allele. This was only seen in the first two years of the study, and was not confirmed in the later data.

Overall, the results do not reveal selection forces acting to balance the polymorphism, and selection against heterozygotes alone will

lead to instability in the polymorphism. However, restricted migration alone can set up a stable, sharp, cline (J. Endler, 1973) and local immigration from the N. Sea may account for the stability of the polymorphism at Kaloe Cove.

Despite the somewhat surprising lack of fitness differences shown, the method is obviously suitable for sampling a natural population where females can be extracted and mother/offspring combinations recorded. Drosophila melanogaster forms such populations, and, like the eel pout, breeds in discrete generations in the British climate. If the method could be shown to be informative in an experimental population of D. melanogaster, it might then be extended to wild populations of native species such as D. subobscura or the fungus feeding D. phalerata and D. cameraria. Accordingly, the work presented in this thesis represents an attempt to apply the method to well known polymorphisms in experimental cage populations of D. melanogaster. Electrophoretic polymorphisms are chosen rather than inversion polymorphisms because the latter are peculiar to dipterans and are less representative of genetic variation in other organisms than the former.

The alcohol dehydrogenase polymorphism in D. melanogaster

The original reports of electrophoretic variation at the ADH locus in D. melanogaster (Johnson & Denniston, 1964; Ursprung & Leone, 1965) were followed by numerous attempts to demonstrate selection operating at the locus. The first suggestion that frequency dependent selection might be involved was made by Kojima & Tobari (1969), but their work involved one stage transitions of the type criticised by Prout and discussed earlier. Dolan and Robertson (1975) examined the effect of

preconditioned medium on larval survival with respect to the ADH locus. Huang, Singh & Kojima (1971) and Kojima & Huang (1972) had reported frequency dependent selection at the est-6 locus for larval survival on preconditioned medium. However, Dolan & Robertson failed to find any effect at the ADH locus. Morgan (1975) set up competition experiments in large vials between larvae from homozygous F and S lines derived from the Kaduna population of D. melanogaster, using three frequencies of the F allele. At two of these frequencies, the relative survivals were almost the same, FF having a higher survival than the SS genotypes. At the highest frequency of F, 0.85, both FF and SS had nearly the same survival values. He claims that this demonstrates frequency dependent selection, but points out that it is not strong enough to maintain the polymorphism. As his experiments do not include heterozygotes, and only larval to adult survival was measured, it would be unsafe to draw inferences about the maintenance of the polymorphism from this work.

Several authors have surveyed natural populations for ADH F and S frequencies, and studied laboratory populations drawn from these surveys. Berger (1971) set up population cages perturbed from the natural equilibrium frequency of the ADH alleles in the USA populations he had studied. All four cages diverged in different directions from the initial frequency. Since a small number of isofemale lines were used to set up the cages, linkage of the ADH alleles to inversions or alleles under strong selection might have been set up, and the divergence might have been due to this. It might also be that there is no selection at the ADH locus in a cage environment. Berger's work

does not make it possible to discriminate between these alternatives, and is therefore not very helpful. Whereas Berger sampled flies from New York State only, Vigue and Johnson (1973) sampled adults of D. melanogaster from 11 states in the U.S.A., ranging from Maine in North to Florida in the South. The standard errors on some of their estimations are quite large, but they show a cline in ADH S frequency from about 0.5 in the North to 0.9 in the South. They measured the temperature dependence of partially purified enzyme activity from all three genotypes, and showed that in vitro at low temperatures, the order of activity was FF $\rangle$ FS $\rangle$ SS whereas at high temperatures (over 40°C) the order reversed; SS $\rangle$ FS $\rangle$ FF. They suggested that this might account for the cline in frequency. Johnson and Burrows (1976) sampled adult flies from several regions in N. Carolina at different seasons of the year for four consecutive years. They found no differences that were statistically significant between frequencies either at different seasons or in different habitats (classified by vegetation). However, the ADH S allele did appear to be at a slightly higher frequency in Summer than in Winter, which is not inconsistent with the suggestion of Vigue & Johnson. Recently, Gionfriddo and Vigue (1978) sampled adult flies from one region in Connecticut, which is in the Northern part of the cline, weekly throughout Summer and Autumn. The average weekly temperature in the collection area was compared with the allele frequencies. Even with assumptions of up to four weeks delay in the manifestation of temperature effects, no correlation was found between temperature and allele frequency. The temperatures observed ranged from 6.5°C to 25.9°C, and the allele

frequencies from 0.66 to 0.34 for the S allele. The standard errors of the allele frequencies are not given, but the authors say that not fewer than 50 flies were used for each measurement. If the estimated frequency were 0.60, this would give a 95% interval of .46 to .74 using exactly 50 individuals. It is therefore possible that sampling errors in the estimation of the ADH allele frequencies may have obscured an effect of temperature. Alternatively, some other explanation of the existence of the cline, such as restricted migration, may be invoked.

None of the studies cited so far report having examined the populations studied for chromosomal polymorphisms. Associations between standard inversion In(2L) and ADH S alleles have been reported for populations of D. melanogaster in Carolina and Texas (Langley et al., 1974; Langley et al., 1977), in Greece, (Alahiotis et al., 1976) and Japan (Watanabe, 1977). Alahiotis also report an association of αGPDH and In(2L). Watanabe reports the same correlation, but in his experiments it was not statistically significant. He also examined cage populations, all initially polymorphic for ADH and αGPDH, but containing no inversions, and samples from the wild containing inversions.

Estimates of viability and productivity were made by extracting chromosomes and comparing them with standard ones for all genotypes at the enzyme loci and for all karyotypes. No effects on fitness were detected at the enzyme loci. However, a significant heterozygote advantage in productivity was found for In(2L)B in flies from wild populations. It is clear from this that reports of heterozygote advantage (and frequency dependent selection if a same stage transition was used to measure genotype frequencies) at loci with electrophoretic

variants must be treated with great caution unless the involvement of inversion polymorphisms has been specifically excluded. Natural populations are more likely to present this problem than cage populations on the evidence so far, since all the cage populations had lost the inversion polymorphisms they had started with. Watanabe & Watanabe suggest that in the laboratory environment, the heterozygote superiority in productivity might be less important than pre-adult viability, in which inversion types are inferior to the normal karyotype. This points up the considerable difference between laboratory conditions and the wild, and emphasises the unsoundness of extrapolating from the former to the latter. With respect to the enzyme loci, their results suggest that certainly in the cage environment, the diverging frequencies of the αGPDH alleles are due to random drift, and that selection is effectively not operating at that locus.

Many authors have demonstrated that ethanol has an effect on ADH frequencies, favouring the F allele (Gibson, 1970; Bijlsma-Meeles & Van Delden, 1974; Morgan, 1975; Oakeshott, 1976; David, 1976; Thompson & Kaiser, 1976; McDonald & Avise, 1976). Further, Briscoe, Robertson and Malpica found, in populations of D. melanogaster caught in Spanish wine cellars, that not only could F be selected for by ethanol, but that frequencies of the S allele were higher the further away from the wine cellars the sample was taken. However, McKenzie & Parsons (1974), Ward (1975) and Barnes & Birley (1978) have demonstrated that ethanol tolerance in D. melanogaster is affected, not only by ADH genotype, but by regions on the third and first chromosome, as well as by a region on chromosome 2 some distance from the ADH

locus. David et al (1978) have shown that lack of activity at the aldox 1 locus on chromosome 3 does not affect ethanol tolerance. This is surprising, since ADH is presumed to transform ethanol to acetaldehyde. The aldox locus produces an aldehyde oxidase which would be expected to catalyse the transformation of acetaldehyde to acetate. Perhaps there are other aldehyde oxidase systems in D. melanogaster which may perform this function.

McKenzie & McKechnie (1978) failed to find any correlation between ADH genotype and ethanol tolerance in D. melanogaster sampled in and around Australian wineries, or any correlation between ADH allele frequencies and distance from the wineries. It may be that the Spanish wine cellars had higher concentrations of ethanol; certainly the laboratory experiments employed concentrations of between 5% and 12% ethanol. 2 native British speciesof Drosophila, D. subobscura and D. phalenata, are known to use decaying rowanberries and fungi respectively as egg laying sites, and no ethanol has been detected in these materials by a method sensitive down to about $\frac{1}{2}$% (B. Shorrocks, pers. comm.). Possibly high concentrations of ethanol, although they have effects at the ADH locus, are irrelevant to the problem of ethanol tolerance in natural populations.

The properties of partially purified extracts of ADH have been investigated and it has been noted that the specific activities, thermal stabilities and temperature dependence of activity of these two allelic products differ (Sofer & Ursprung, 1968; Vigue and Johnson, 1973; Day & Needham, 1974; Day, Hillier & Clarke, 1974; David, 1976;

McDonald & Avise, 1976). The product of the S allele is thought to be more stable at higher temperatures, and the product of the F allele is thought to have a higher activity at lower temperatures than that of the S allele. Three authors give values for the relative activities of extracts of flies of the three ADH genotypes on ethanol, n-butanol, isopropanol and cyclohexanol, and all three disagree (Day, Hillier & Clarke, 1974; Thompson and Kaiser, 1976; Vigue & Johnson, 1973). The most likely explanation of this is that only Vigue and Johnson made any attempt to purify the enzymes, and their preparation was only partial. Consequently, biochemical data from such reports should be treated with caution.

The α glycerophosphate dehydrogenase polymorphism in D. melanogaster

This polymorphism seems a suitable control locus to include in a study of fitness estimation at the ADH locus. Berger (1971) observed an increase in the frequency of the F allele of αGPDH during the Summer in a wild population. He also perturbed cage populations from the average frequency in a natural population, but as he used small numbers of isofemale lines to construct the populations, and did not examine them for inversion polymorphisms, the fact that the lines returned to an equilibrium frequency is not informative. He does not give the standard errors of his frequency estimates, so the reliability of his results is difficult to assess. Miller et al (1975) extracted the enzymes from the three genotypes and measured the thermal stabilities of partially purified preparations. The temperature dependence of activity was such as to favour the F allele at high temperatures and the S allele at lower ones. The thermal stabilities and pH upturned did not differ. McDonald and Avise (1976) showed that interspecific

variation is far less for αGPDH than for ADH. They interpret this to mean that the αGPDH substrate is less variable, having a more 'uniform' function. Bewley and Lucchesi (1977) postulate several functions for αGPDH, including regeneration of NAD^+ in flight muscle, and production of ATP for lipid biosynthesis. No attempts have been made to exert a selection pressure on this locus.

Problems associated with electrophoretic variants

Grell et al (1968) showed that one of the three ADH bands seen for a homozygous individual on electrophoresis, was convertible to another of the bands (ADH_1 to ADH_5) by the addition of NAD. Jacobsen et al (1970) elaborated on this work, and showed that the multiple bands resulted from differential binding of NAD to the enzyme dimer. Dunn et al (1969) showed that there were age dependent changes in ADH activity and in the relative intensity of the minor bands. Schwartz and Sofer (1976) showed that the changes could be induced by feeding NAD in the fly medium.

As well as the standard F and S alleles (4 and 6) Thörig et al (1975) reported an ultrafast allele. The amino acid substitution by which this differs from F and S is now known (Thatcher and Canfield, 1977). Milkman (1976) and Sampsell (1977) reported finding thermostability variants of both F and S, although only 5 in all were found and they were all rare. (The most common was at a frequency of less than 7%). Johnson (1978) has used a gel sieving technique to separate variants on the basis of shape. He found a large variation in shape of non NAD bound ADH in D. melanogaster (approx. 5 times the variation in size of NAD bound enzyme). In an inbred line of D. melanogaster, the variation was much less, about the same as the NAD bound enzyme.

A limited amount of data was given for ADH from an outcrossed *D. melanogaster* population, which seemed to suggest that it had a larger variation in shape than the inbred line. However, post-translational events such as the action of proteases may be responsible for alterations in shape, and in the absence of better data, Johnson is premature in concluding that there is concealed genetic variation in the ADH enzyme leading to variation in conformation.

CHAPTER 2

MATERIALS AND METHODS

1. Flies - All flies used in the experiments were Drosophila melanogaster, 'Malawi' strain, provided by Professor Alan Robertson.

2. Electrophoresis - Both ADH and αGPDH electromorphs (hereafter called genotypes) were classified by horizontal starch gel electrophoresis (Dolan & Robertson, 1975). Whole fly homogenates (in deionised water) were run on 9.1% starch (Connaught Labs) gels, the latter supplemented with 0.045M Tris (Sigma), 0.059M Boric acid (B.D.H.) and 0.00145M EDTA (B.D.H.). Gels were run at 250 volts, 100mA, for 1 hour 20 mins, with a running buffer of 0.5M Tris, 0.65M Boric acid and 0.016M EDTA, pH 8.0. After electrophoresis, gels were sliced with a wire cutter, and staining solutions were applied to the cut surfaces.

The ADH staining solution consisted of 100ml of 0.036M Tris, 0.012M Tris HCl (Sigma) at pH 8.5, to which 50mg NAD (Sigma), 30mg MTT (Sigma), 2mg PMS (Sigma) and 10ml ethanol (Analar, B.D.H.) were added. Deionised water was used to make up all solutions.

The staining solution for αGPDH was the same as for ADH except that the 10ml of ethanol was omitted, and 100mg of α-glycerophosphate (B.D.H.) was added. The αGPDH stain was incubated at 37°C, the ADH at room temperature.

Flies were stored in the deep freeze at -18°C for up to two weeks, before electrophoresis.

3. Fly media

A) Normal medium

20g agar (Davis)

22g dried yeast (Distillers)

150g maize meal

130g Fowler's black treacle

2g Nipagin

1600ml tap water

All ingredients except the nipagin were stirred together and boiled for 2 or 3 minutes. The nipagin powder was then stirred vigorously. The medium was poured into appropriate containers. 3" x 1" vials and 300ml cage food pots were cooled, covered with foil and stored in a cold room at 4°C before use. ½pt bottles were capped with foil and autoclaved.

B) Alcohol supplemented medium

A batch of normal medium lacking 10% of the water content was made as described and allowed to cool to 50°C. An aqueous solution of the necessary volume of alcohol (1 pentyn-3-ol, Aldrich Chemicals or 1 butan-2-ol, B.D.H.) in the remaining 10% of the water was added to the medium, and this was stirred with a mechanical stirrer for 3 minutes. The medium was kept in a water bath at 50°C to prevent it setting during this process. 3" x 1" vials and 300ml food pots were made up immediately before use.

4. Egg collection - Normal medium was diluted by $\frac{1}{3}$ its volume with tap water after being boiled. The mixture was spread, as hot as possible, in a thin layer on plastic petri dishes. When these had cooled and set, they were placed in population cages for periods of between 6 and 18 hours. On removal, the surface of each was divided into sections by scoring with a needle under a binocular microscope, and the number of eggs per section counted. Whole sections were then cut out with a fine scalpel and transferred to the appropriate vials. The sections were so arranged to give an egg number of between 20 & 50.

5. Maintenance of fly cultures - During these experiments, flies were maintained at approximately 25°C, in darkness. Humidity was not controlled. All fly anaesthaesia was done with carbon dioxide. The population cages were approximately 14" cubes, and contained 3 x 300ml food pots, with 200ml food per pot.

6. 'Frydenberg' sampling procedure for random mating, discrete generation population cages

Adult ♂♂ and ♀♀, and larvae, were sampled. Separate samples of individual ♀♀ and offspring from each one were also obtained. Egg counts were done on these ♀♀ to obtain fecundity estimates. The procedure, on a 14 day cycle, is as follows -

Day 1. Adult ♂♂ and ♀♀ sampled from cage.
♂♂ run on gels.
♀♀s (up to 70) placed singly in vials.
3 pots new food placed in cage.

Day 2. ♀♀s in vials tipped into new ones, and eggs counted in previous vials.

Day 3. As Day 2.

Day 4. as Day 2.

Day 5. ♀♀s run on gels; eggs counted in last set of vials. The 3 food pots removed to a new cage.

Day 7. 3rd instar larvae removed from food pots in new cage, and run on gels.

Day 8. 6 larvae sampled from vials of each ♀ that laid more than 6 eggs, and run on gels.

Day 9. As Day 8.

Day 10. As Day 8.

Day 10 (contd.) - single pot of food placed in cage as new generation emerges.

Day 14 = 1 - single pot removed, adults sampled and 3 new pots put in as Day 1.

In the case of the experiment on butanol medium, the life cycle was lengthened by 4 days. The emergence of a new generation did not begin until about day 14. The next adult sampling was on Day 18 = 1, and larvae sampling was on days 9, 10 and 11.

7. Estimates of number of flies in a population cage -

The cage was enclosed in polythene, and CO_2 was run in. The anaesthetised flies were wrapped in preweighed aluminium foil, to keep weight of CO_2 constant, and weighed immediately. The sample to be counted was extracted, resaturated with CO_2, wrapped in foil, and weighed immediately. It was then counted under running CO_2.

CHAPTER 3

Investigation of the effects of 1-pentyn-3-ol and butan-2-ol, agents which might exert a selection pressure at the ADH locus

Before proceeding to attempt to estimate relative fitnesses at the ADH locus by Frydenberg's method, one requires some demonstration of the feasibility of the method in D. melanogaster. Many authors have reported that direct selection may be exerted at the ADH locus by a variety of alcohols. If a suitable agent could be found, the locus could be made to serve as its own control, since relative fitnesses could be compared under direct selection exerted by an alcohol and under ordinary laboratory conditions, where the nature of any selective forces operating is not known.

The results presented in this chapter are of experiments designed to select such an agent, and to determine appropriate concentrations and conditions for its use.

1-pentyn-3-ol has been reported as a toxic substrate of ADH in D. melanogaster (O'Donnell et al., 1975; Robertson, pers. comm. 1974). Butan-2-ol has also been mentioned in this respect (Oakeshott, 1976a; David, 1976; Day et al., 1974; Vigue & Johnson, 1973). ADH has a relatively high affinity for both these substances, so that a greater selective stress may be produced by these agents than by ethanol or other primary alcohols.

1. Tests using pentynol. Egg to Adult viability

Experiment A. To find the range of effective concentrations of pentynol

Eggs were collected by the methods described in Materials and Methods. They were placed in 3" x 1" vials of normal medium containing appropriate concentrations of pentynol. Adults emerging were counted on the 14th day, eggs going in on Day 1.

Conc. of pentynol	Vial no.	No. of eggs added (Day 1)	No. of adults emerged on Day 14	Mean survival for 5 vials
0.32%	1	70	0	0%
	2	43	0	
	3	47	0	
	4	60	0	
	5	54	0	
0.1%	1	67	35	15%
	2	43	4	
	3	52	0	
	4	52	0	
	5	48	0	
0.032%	1	38	28	76%
	2	45	34	
	3	43	36	
	4	41	29	
	5	65	50	
0.01%	1	40	40	89%
	2	34	33	
	3	54	43	
	4	35	27	
	5	37	35	
0 (control)	1	78	73	68%
	2	47	42	
	3	37	24	
	4	47	26	
	5	66	22	

Tests using pentynol. Egg to adult viability

Experiment B. As experiment A

Conc. of pent-ynol	Vial no.	Eggs added (Day 1)	Adults emerged at Day 14	Mean emergence per 10 vials	Conc. of pent-ynol	Vial no.	Eggs added (Day 1)	Adults emerged at Day 14	Mean emergence per 10 vials
0.072 %	1	37	7	29%	0.042 %	1	28	19	72%
	2	44	13			2	21	12	
	3	29	3			3	30	21	
	4	27	2			4	30	17	
	5	31	8			5	31	23	
	6	27	8			6	33	30	
	7	32	6			7	25	24	
	8	28	19			8	25	17	
	9	30	7			9	20	11	
	10	21	17			10	26	21	
0.055 %	1	36	35	57%	0 % (control)	1	32	26	85%
	2	23	14			2	39	32	
	3	21	18			3	26	21	
	4	21	16			4	30	22	
	5	29	20			5	24	24	
	6	28	13			6	28	27	
	7	24	8			7	37	35	
	8	22	12			8	36	33	
	9	31	9			9	46	23	
	10	39	12			10	21	24	

2. Tests using Pentynol. Adult survival on normal medium

Experiment C. To find the range of effective concentrations of pentynol on adult survival and egg production

20 adults (10♂ and 10♀) were placed in vials of normal medium with appropriate concentrations of pentynol. Eggs were counted each day and the flies transferred to another vial. No. of adult survivors was counted after 4 days.

Vial no.	Conc. of pentynol	No. of adults after 4 days	Mean survival for 5 vials	No. of eggs after 4 days	Total eggs per 5 vials	Conc. of pentynol	No. of adults after 4 days	Mean survival for 5 vials	No. of eggs after 4 days	Total eggs per 5 vials
1	0.05%	5	9%	83	274	0.01%	16	68%	71	487
2		1		114			7		66	
3		1		16			17		107	
4		1		49			19		140	
5		1		12			9		103	
1	0.03%	2	16%	41	426	0.005%	16	85%	97	592
2		4		67			16		141	
3		3		40			18		104	
4		3		130			13		144	
5		4		148			18		106	
1	0.02%	6	42%	100	492	0	19	89%	126	650
2		15		137		(control)	18		141	
3		9		41			17		148	
4		5		115			18		105	
5		7		99			17		130	

χ^2 for heterogeneity within vial sets

Concentration	χ^2(4 df)	p
0.03	1.04	> .05
0.02	12.97	<.05; >0.01
0.01	25.55	<.01 (sig)

2. Tests using Pentynol. Adult survival on normal medium

Experiment D. As C, but only adult survival was investigated

Conc. of pentynol	Vial no.	No. of adults after 4 days	Mean survival for 5 vials
0.03%	1	1	28%
	2	19	
	3	0	
	4	7	
	5	1	
0.02%	1	16	34%
	2	2	
	3	3	
	4	4	
	5	9	
0.01%	1	19	66%
	2	18	
	3	14	
	4	7	
	5	8	
0 (control)	1	16	89%
	2	18	
	3	17	
	4	18	
	5	20	

χ^2 for heterogeneity within sets of vials

Concentration	χ^2(4 df)	p
0.03%	63.29	<0.01 (sig)
0.02%	30.0	<0.01 (sig)
0.01%	27.36	<0.01 (sig)

Statistical comparison of total survival on different concentrations of pentynol between experiments C and D

Concentration	Expt. C dead:alive	Expt. D dead:alive	χ^2 (1 df)	p
0.03%	84:16	72:28	4.20	<.05;>.01
0.02%	58:42	66:34	1.36	> .05
0.01%	32:68	34:66	0.09	> .05
0, control	11:89	11:89	0	> .05

3. Tests using Pentynol. Adult survival on different medium containing pentynol

Experiment E. Replicate 1

20 flies (10 of each sex) were placed in 3" x 1" vials. (5 vials of each concentration of pentynol used) and this was done for three different media. Survivors were counted after 3 days.

Conc. of Pentynol	Vial no.	No.of adults after 3 days in			Mean % viability over 5 vials		
		Normal medium	- agar + potato	potato and dextrose	Normal medium	- agar + potato	potato and dextrose
0.04	1	1	2	1	7%	4%	2%
	2	2	0	0			
	3	1	2	0			
	4	1	0	0			
	5	2	0	1			
0.03	1	3	1	0	14%	5%	5%
	2	1	0	0			
	3	2	3	1			
	4	4	1	4			
	5	4	0	0			
0.02	1	6	3	1	24%	12%	8%
	2	8	6	0			
	3	4	0	2			
	4	2	2	3			
	5	4	1	2			
0.01	1	set lost	0	17	-	19%	55%
	2	-	2	13			
	3	-	6	7			
	4	-	1	8			
	5	-	10	10			
0	1	18	2	13	90%	35%	77%
	2	19	6	15			
	3	18	9	15			
	4	18	11	16			
	5	17	7	18			

3. Tests using Pentynol. Adult survival on different media containing pentynol

Experiment E. Replicate 2

20 flies (10 of each sex) were placed in 3" x 1" vials, (5 vials of each, concentration of pentynol used) and this was done for three different media. Adults were counted after 3 days.

Conc. of pentynol	Vial no.	No.of adults after 3 days on			Mean % viability over 5 vials		
		Normal medium	- agar + potato	potato and dextrose	Normal medium	- agar + potato	potato and dextrose
0.04	1	2	2	4	5%	15%	10%
	2	0	3	2			
	3	1	4	1			
	4	0	3	1			
	5	2	3	2			
0.03	1	1	9	3	8%	43%	27%
	2	1	9	7			
	3	1	6	10			
	4	4	10	3			
	5	1	9	4			
0.02	1	5	16	8	30%	69%	61%
	2	7	13	16			
	3	6	10	15			
	4	6	14	15			
	5	6	16	7			
0.01	1	17	18	16	85%	91%	88%
	2	17	19	16			
	3	17	18	18			
	4	18	17	19			
	5	16	19	19			
0 Control	1	20	19	20	97%	96%	90%
	2	19	18	16			
	3	19	20	17			
	4	20	20	18			
	5	19	19	19			

Statistical test of heterogeneity within vials of survival on different concentrations of 1 pentyn-3-ol on different media

	Concentration	Medium		
		Normal	-agar+potato	potato dextrose
	0.02	χ^2=5.7(4 df) $p > .05$	—	-
Expt.E Rep 1	0.01	-	χ^2=22.4(4 df) $p < .01$ (sig)	χ^2=13.3(4 df) $p < .01$ (sig)
	0 (control)	-	χ^2=10.1(4 df) $0.01 < p < .05$	χ^2=3.73(4 df) $p > .05$
Expt.E Rep 2	0.03		χ^2=1.88(4 df) $p > .05$	χ^2=9.44(4 df) $p > .05$
	0.02	χ^2=0.48 $p > .05$	χ^2=5.80(4 df) $p > .05$	χ^2=15.72(4 df) $p < 0.01$ (sig)

Statistical comparisons of survival on normal medium in the experiments of sections 2 and 3

Concentration	Section 2 C+D dead:alive	Section 3		χ^2
		E_1	E_2	
0.03	156:44	86:14	92:8	10.09 (2 df) $p < 0.01$ (sig)
0.02	124:76	76:24	70:30	6.34 (2 df) $0.05 < p < 0.01$
0.01	64:136	-	15:85	9.93 (1 df) $p < 0.01$ (sig)
control	22:178	10:90	3:97	5.60 (2 df) $p > 0.05$

Summary of Results with 1 pentyn-3-ol

The results of Section 1 show that 1 pentyn-3-ol can prevent egg to adult development in D. melanogaster; the concentration in the medium necessary to produce 50% of normal emergence was between 0.055 and 0.072%. At a concentration of 0.32%, no flies emerged at all.

Section 2 shows that 1 pentyn-3-ol is also toxic to the adult fly. This stage of the life cycle seems more sensitive, since only 9% of adults survived for the 4 days on a concentration of 0.05%. The concentration necessary to effect a 50% survival after 4 days was between 0.02%, and 0.01%. The relative survivals on the different 1 pentyn-3-ol concentrations do not differ significantly between experiment A and experiment B, except possibly at a concentration of 0.03%. The survivals were 16% and 28%, and the χ^2 value indicates that this magnitude of difference might be expected by chance between 5% and 1% of the time. A more prominent feature of the data is that in both experiments there is significant heterogeneity within replicate sets of vials. Since the projected experiment in which pentynol might have been used involves the maintenance of ♀ singly in vials, this is important. The results of experiment A also suggest that 1 pentyn-3-ol has no effect on egg laying which is proportional to concentration, at least at the levels used. The overall level of egg production was low, including that of the control, but was apparently proportional to the number of surviving adults.

Section 3 shows the results of repeating the survival experiment of section 2 on different media. The two media without agar gave heterogenous results within sets of vials, whereas the normal medium gave uniform results. This is in contrast to the experiments in

section 2. Further, the % survivals on 0.03% 1 pentyn-3-ol were 14% and 8% for normal medium, lower than previously found in the experiments of section 2. Survival at the 0.02% level was also lower than it had been in the experiments of section 2.

The media without agar tended to become wet and sticky, and this may have contributed to the observed mortalities.

4. Tests using Butan-2-ol. Adult survival

Experiment F. To find the range of effective concentrations of butan-2-ol

20 adult flies (10 of each sex) were placed in appropriate vials and counted after 4 days.

Conc. of butan-2-ol	Vial no.	No. of adults after 4 days	Mean survival per 5 vials
3.2%	1	5	10%
	2	1	
	3	0	
	4	2	
	5	2	
1.0%	1	16	89%
	2	17	
	3	19	
	4	17	
	5	20	
0.32%	1	17	89%
	2	19	
	3	18	
	4	18	
	5	17	
0.01%	1	18	95%
	2	20	
	3	19	
	4	18	
	5	20	
0 (control)	1	18	80%
	2	17	
	3	13	
	4	17	
	5	15	

4. Tests using Butan-2-ol. Adult survival

Experiment G. As F.

Conc. of butan-2-ol	Vial no.	No. of adults after 4 days	Mean survival per 5 vials
3%	1	0	4%
	2	1	
	3	1	
	4	2	
	5	0	
2.5%	1	2	6%
	2	1	
	3	0	
	4	0	
	5	3	
2.0%	1	9	33%
	2	5	
	3	5	
	4	7	
	5	7	
1.5%	1	10	50%
	2	8	
	3	10	
	4	10	
	5	12	
1.0%	1	19	84%
	2	15	
	3	18	
	4	15	
	5	17	
0 (control)	1	19	85%
	2	16	
	3	17	
	4	16	
	5	17	

Statistical tests for heterogeneity between vials in Expts. F and G

	Concentration	χ^2	p
Expt. F	0 (control)	5.00 (4 df)	> .05
Expt. G	2.0%	2.53 (4 df)	> .05
	1.5%	1.60 (4 df)	> .05

Statistical comparison of survival values in Expts. F and G

Concentration	Expt. F dead:alive	Expt. G dead:alive	χ^2	p
1.0%	11:89	16:84	1.07(1 df)	> .05
0 (control)	20:80	15:85	0.87	> .05

5. Preliminary experiment with butanol medium in a population cage.

Experiment H.

Four single pot population cages were set up, each with 200 adult flies. Two cages contained medium supplemented with 2% butanol, and two contained normal medium. A sample from the cage from which the adults were drawn was taken for electrophoresis. After 4 days the survivors in all four cages were removed, counted and samples of them were electrophoresed. Finally, samples of flies eclosing from the experimental pots were also electrophoresed.

Adult survival after 4 days

	0% butanol (control)		2% butanol	
	Rep 1	Rep 2	Rep 1	Rep 2
No. of flies	196	197	65	57
% survival	98	99	33	29

Genotype numbers and frequencies given in the next table

5. Preliminary experiment with butan-2-ol in population cages

Experiment H

Table 1. Genotype frequencies at the ADH locus

Sample	Numbers of genotypes			Genotype frequencies		
	F	H	S	F	H	S
from parent cage	29	53	14	0.30±.043	0.55±.051	0.15±.036
Adults after 4 days on 0%. Rep 1	29	48	19	0.30±.043	0.50±.051	0.20±.040
Rep 2	28	48	20	0.29±.046	0.50±.051	0.21±.042
Adults after 4 days on 2%. Rep 1	19	33	7	0.32±.061	0.56±.065	0.12±.042
Rep 2	16	27	6	0.33±.067	0.55±.071	0.12±.046
Eclosed flies from 0%. Rep 1	30	47	19	0.31±.047	0.49±.051	0.20±.040
Rep 2	34	48	14	0.35±.049	0.50±.051	0.15±.036
Eclosed flies from 2%. Rep 1	36	52	6	0.38±.050	0.54±.051	0.06±.024
Rep 2	29	57	10	0.30±.043	0.60±.050	0.10±.031

Table 2. Gene frequencies at the ADH locus

Sample	$pADH_F$	Sample	$pADH_F$
from parent cage	0.57±.036		
Adults after 4 days on 0%	0.55±.025	Adults after 4 days on 2%	0.61±.033
Eclosed flies from 0%	0.53±.025	Eclosed flies from 2%	0.63±.025

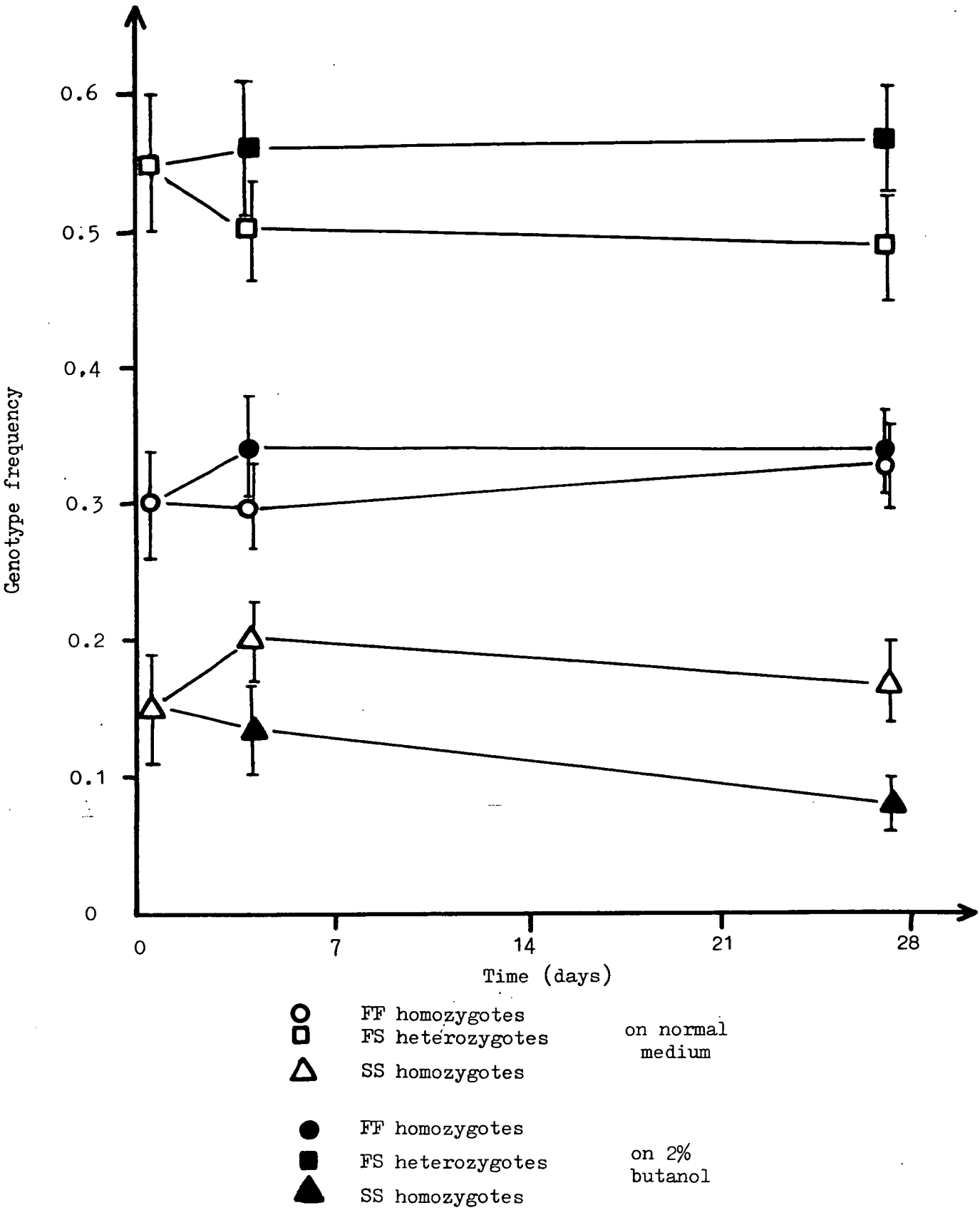


Fig. 3.1 Preliminary experiment on the effect of butan-2-ol on Drosophila melanogaster in single pot population cages (Experiment H): genotype frequencies at the ADH locus.

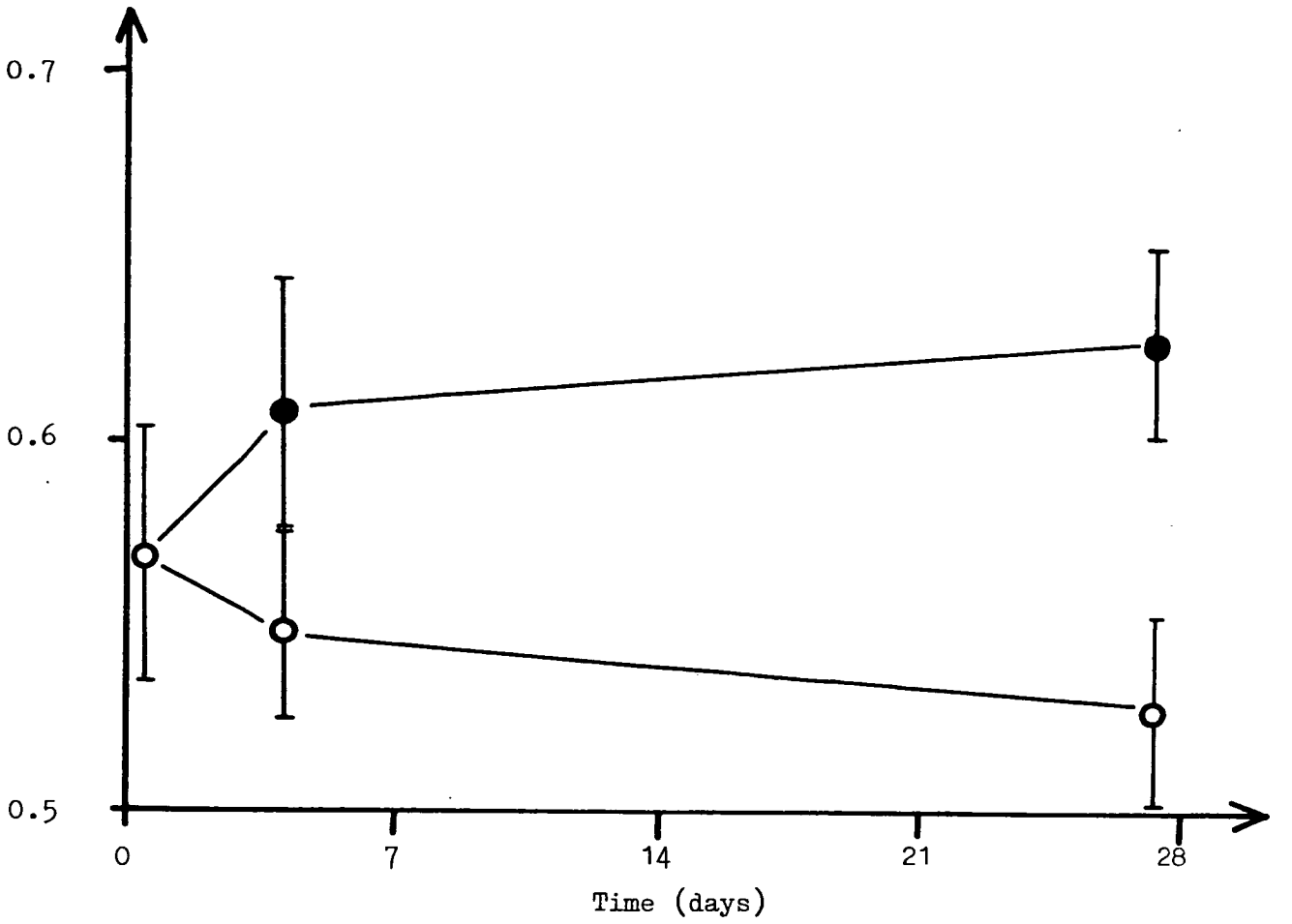


Fig. 3.2 Preliminary experiment on the effect of butan-2-ol on Drosophila melanogaster in single pot population cages (Experiment H): ADH^F gene frequencies.

5. Preliminary experiment with butan-2-ol in cage populations

Experiment H

Statistical comparison of genotype frequencies at the ADH locus

Sample	χ^2	p
1. Parent cage sample with adults after 4 days on 0%	1.48 (2 df)	> .05
2. Parent cage sample with adults after 4 days on 2%	0.33 "	"
3. Adults after 4 days on 0% with the same on 2%	3.31 "	"
4. Eclosed flies on 0% with parent cage sample	0.87 "	"
5. Eclosed flies on 2% with parent cage sample	2.67 "	"
6. Eclosed flies on 2% with eclosed flies on 0%	6.86 "	0.01< p <.05

Statistical comparisons of gene frequencies at the ADH locus

Sample	χ^2	p
1. Parent cage sample with adults after 4 days on 0%	0.43 (1 df)	> .05
2. Parent cage sample with adults after 4 days on 2%	0.36 "	"
3. Adults after 4 days on 0% with the same on 2%	1.89 "	"
4. Eclosed flies on 0% with parent cage sample	0.23 "	"
5. Eclosed flies on 2% with parent cage sample	1.39 "	"
6. Eclosed flies on 2% with eclosed flies on 0%	4.06	0.01<p<0.05

5. Preliminary experiment with butan-2-ol in population cages

Experiment H

Table 3. Genotype frequencies at the αGPDH locus

Sample		Numbers of genotypes			Genotype frequencies		
		F	H	S	F	H	S
from parent cage		42	38	16	0.44±.051	0.40±.050	0.16±.037
Adults after 4 days on 0%.	Rep 1	50	36	10	0.52±.051	0.38±.050	0.10±.031
	Rep 2	52	36	8	0.54±.051	0.38±.050	0.08±.028
Adults after 4 days on 2%.	Rep 1	38	14	7	0.64±.062	0.24±.056	0.12±.042
	Rep 2	30	14	5	0.61±.070	0.29±.065	0.10±.043
Eclosed flies from 0%.	Rep 1	34	37	1	0.47±.059	0.51±.059	0.02±.016
	Rep 2	39	42	15	0.41±.050	0.44±.051	0.15±.036
Eclosed flies from 2%.	Rep 1	50	39	4	0.54±.052	0.42±.051	0.04±.020
	Rep 2	50	39	7	0.52±.051	0.41±.050	0.07±.026

Table 4. Gene frequencies at the αGPDH locus

Sample	$p\alpha GPDH_F$	Sample	$p\alpha GPDH_F$
from parent cage	0.64±.035		
Adults after 4 days on 0%	0.72±.023	Adults after 4 days on 2%	0.76±.029
Eclosed flies from 0%	0.67±.026	Eclosed flies from 2%	0.74±.023

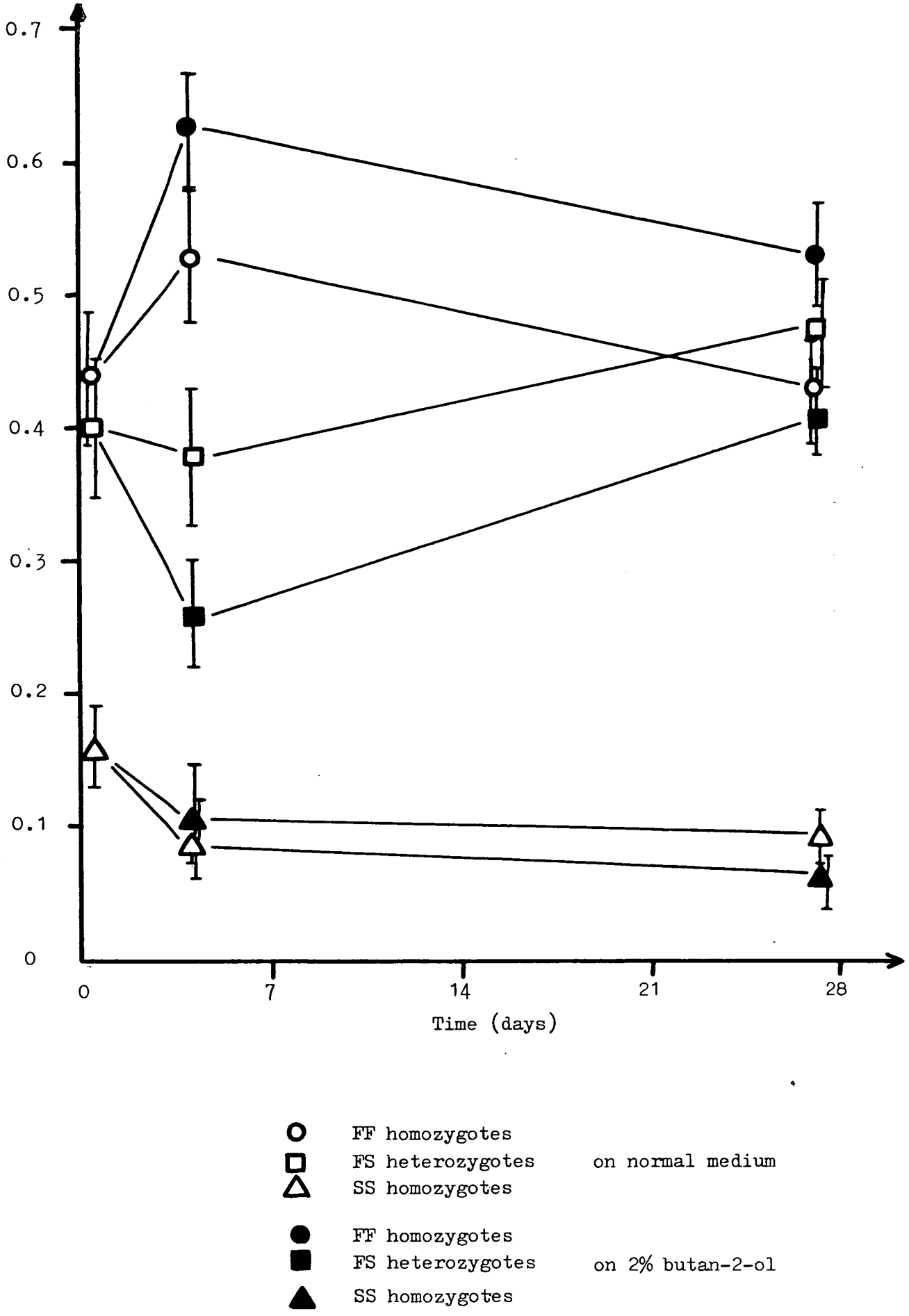


Fig. 3.3 Preliminary experiment on the effect of butan-2-ol on Drosophila melanogaster in single pot population cages (Experiment H): genotype frequencies at the αGPDH locus.

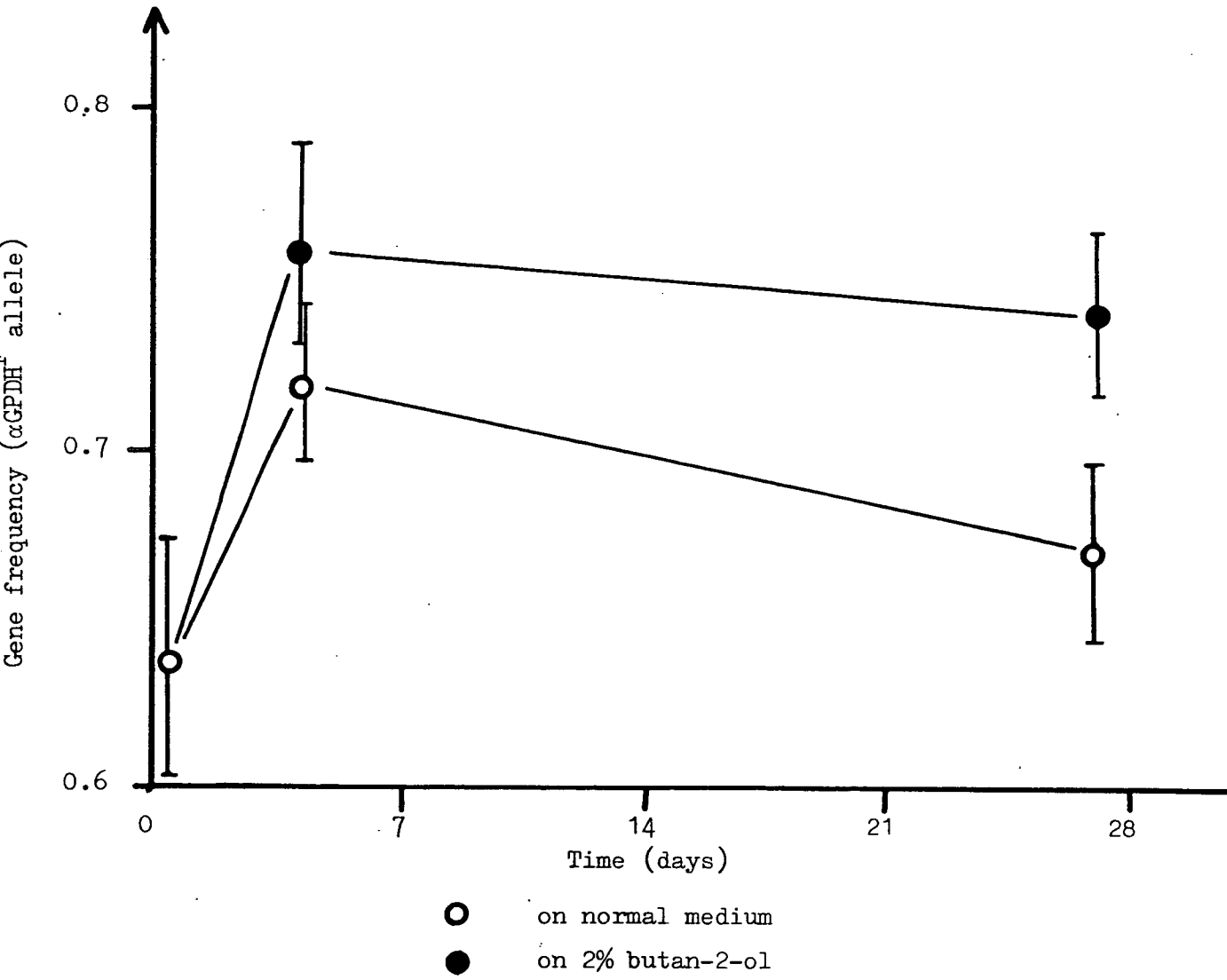


Fig. 3.4 Preliminary experiment on the effect of butan-2-ol on Drosophila melanogaster in single pot population cages (Experiment H): $\alpha GPDH^F$ gene frequencies.

5. Preliminary experiment with butan-2-ol in cage populations

Experiment H

Statistical comparison of genotype frequencies at the αGPDH locus

Sample	χ^2	p
1. Parent cage sample with adults after 4 days on 0%	4.08 (2 df)	>.05
2. Parent cage sample with adults after 4 days on 2%	7.55 "	0.05>p>0.01
3. Adults after 4 days on 0% with same on 2%	4.17 "	>.05
4. Eclosed flies on 0% with parent cage sample	3.34 "	>.05
5. Eclosed flies on 2% with parent cage sample	9.02 "	0.05>p>0.01
6. Eclosed flies on 2% with adults after 4 days on 2%	8.25 "	0.05>p>0.01
7. Eclosed flies on 2% with eclosed flies on 0%	3.92 "	>.05
8. Eclosed flies on 2% with adults after 4 days on 0%	1.93 "	>.05

Statistical comparisons of gene frequency at the αGPDH locus

Sample	χ^2	p
1. Parent cage sample with adults after 4 days on 0%	4.16 (1 df)	0.05>p>0.01
2. Parent cage sample with adults after 4 days on 2%	7.44 "	<.01
3. Adults after 4 days on 0% with same on 2%	1.16 "	>.05
4. Eclosed flies on 0% with parent cage sample	0.64 "	"
5. Eclosed flies on 2% with parent cage sample	6.09 "	0.05>p>0.01
6. Eclosed flies on 2% with adults after 4 days on 2%	0.41 "	>.05
7. Eclosed flies on 2% with eclosed flies on 0%	3.70	>.05
8. Eclosed flies on 2% with adults after 4 days on 0%	0.27	>.05

Summary of preliminary results using Butan-2-ol

Experiments F and G in Section 4 show that butan-2-ol is toxic to the adult fly; the concentration required to effect a 50% survival after 4 days was 1.5%. These experiments did not show any heterogeneity within sets of replicate vials and there is no significant difference between survival rates on given butanol concentrations from one experiment to the next.

The survival rate after 4 days in experiment H on 2% butanol (33%) agrees well with that obtained in Experiment G. This suggests that the butanol concentrations in the medium and air above the medium decline at a rate unaffected by the difference in surface area and volume between vials and the 300ml cage food pots.

The genotype frequencies at the ADH locus show no significant differences between treatments except for the last comparison of the frequencies in flies eclosing from normal medium and medium with butanol, where the deficit of SS homozygotes on the butanol medium approaches significance. The gene frequencies are similarly nonsignificant in their differences, except for the same comparison, where the frequency of the S allele is less on the butanol medium. This suggests that the butanol is effective in selecting at the ADH locus, against SS homozygotes. The sample is not sufficiently large to draw conclusions about the heterozygotes.

The genotype frequencies at the αGPDH locus show three significant differences between the treatments. There are more FF homozygotes after 4 days on butanol medium than there are either at the beginning of the experiment or in the flies eclosing from the butanol medium. However, the flies eclosing from the butanol medium do have significantly more FF homozygotes than the initial sample from the parent cage.

This may represent some effect of the butanol, direct or otherwise. However, it may reflect the small samples used, particularly of the initial parent cage, since the flies eclosing from the butanol medium did not differ significantly in genotype frequency from those eclosing from normal medium, or from the adults surviving after 4 days on normal medium. The gene frequency data make this clearer. The standard errors of the frequency estimates are large; the frequency of the F allele in the adults after 4 days on butanol medium is significantly higher than in the parent population, but does not differ significantly from the frequency in adults after 4 days on normal medium, or from the frequency in flies eclosing from the butanol medium. The frequency of F in the adults after 4 days on normal medium differs from the parent population at a level approaching significance.

CHAPTER 4

Estimation of relative fitnesses at the ADH and αGPDH loci when butan-2-ol is present in the medium

From the results of Chapter 3, it is apparent that butan-2-ol is capable of exerting a selective stress on a single pot cage population of *D. melanogaster*, and that this may involve genotype/gene frequency changes at the ADH locus. The results presented in this chapter are of experiments designed to reveal any fitness differences at the ADH and αGPDH loci, using a modification of Frydenberg's method, as described in Chapter 2, whilst butan-2-ol is present in the medium.

1. Preliminary experiment; sampling of adult and 3rd instar larval stages on 3 generations on butanol medium

Experiment A.

The method used was as described in Chapter 2, except that females tended not to lay eggs easily on the butanol medium, and therefore no adequate samples were obtained of the mother/offspring combinations. The concentration of butanol in the cage food pots was 2%.

Table 1. Genotype frequencies at the ADH locus

Sample		Numbers of genotypes			Frequency		
		F	H	S	F	H	S
Transition 1.	Adult ♂♂	39	37	20	0.41±.050	0.38±.050	0.21±.042
	Adult ♀♀	27	53	15	0.28±.049	0.56±.051	0.16±.038
	larvae from them	25	58	12	0.26±.045	0.61±.050	0.13±.035
Tran. 2	Adult ♂♂	39	48	6	0.42±.051	0.52±.052	0.06±.025
	Adult ♀♀	22	25	1	0.46±.072	0.52±.072	0.02±.020
	larvae from them	35	54	6	0.37±.050	0.57±.051	0.06±.024
Tran. 3	Adult ♂♂	38	52	5	0.40±.050	0.55±.051	0.05±.022
	Adult ♀♀	31	53	10	0.33±.048	0.56±.051	0.11±.032
	larvae from them	52	40	4	0.54±.051	0.42±.050	0.04±.020
Total							
Adult ♂♂		116	137	31	0.41±.029	0.48±.030	0.11±.018
Adult ♀♀		80	131	26	0.34±.031	0.55±.032	0.11±.020
Larvae		112	152	22	0.39±.029	0.53±.030	0.08±.016

Experiment A

Table 2. Gene frequencies at the ADH locus

Sample		$pADH_F$	Sample	$pADH_F$
Transition 1.	Adult ♂♂	0.60±.035	larvae from Tran. 1 parents	0.56±.035
	Adult ♀♀	0.57±.035		
Tran. 2	Adult ♂♂	0.66±.034	larvae from Tran. 2 parents	0.64±.034
	Adult ♀♀	0.72±.046		
Tran. 3	Adult ♂♂	0.66±.034	larvae from Tran. 3 parents	0.75±.031
	Adult ♀♀	0.61±.036		
Totals				
Adult ♂♂		0.65±.020	larvae	0.66±.020
Adult ♀♀		0.61±.022		

Table 3. Summary of egg counts from ♀s in vials.

Because of the poor egg-laying induced by butanol, only 2 generations were counted.

		♀ ADH genotype		
		F	H	S
Tran. 1.	Total eggs	69	72	3
	n	28	25	4
	Mean eggs per ♀	2.46	2.88	0.75
Tran. 2.	Total eggs	89	69	5
	n	14	22	2
	Mean eggs per ♀	6.36	3.14	2.5

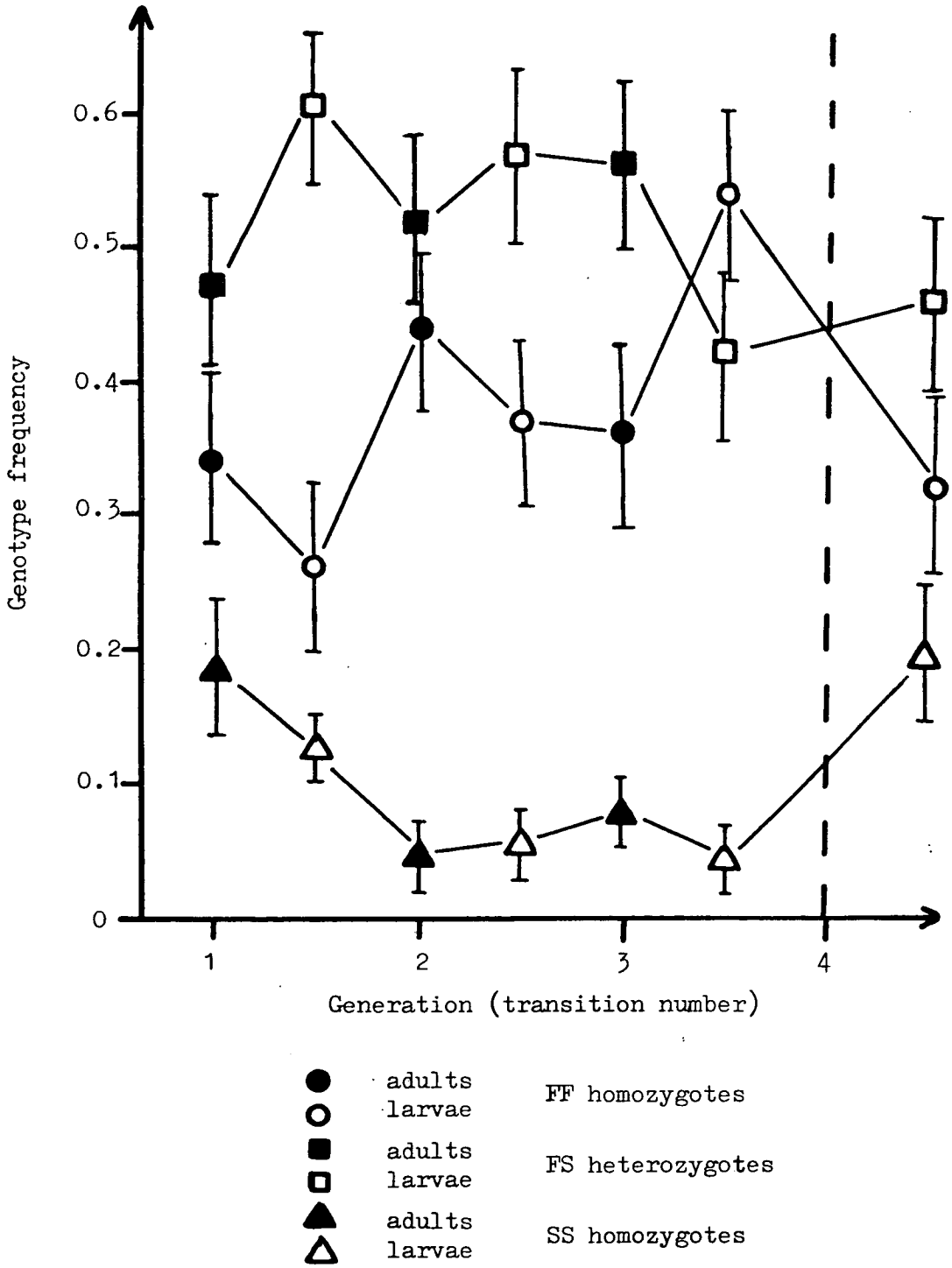


Fig. 4.1 Genotype frequencies at the ADH locus found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on 2% butan-2-ol medium over 3 generations (preliminary experiment A). For the fourth generation, the flies were reared on normal medium.

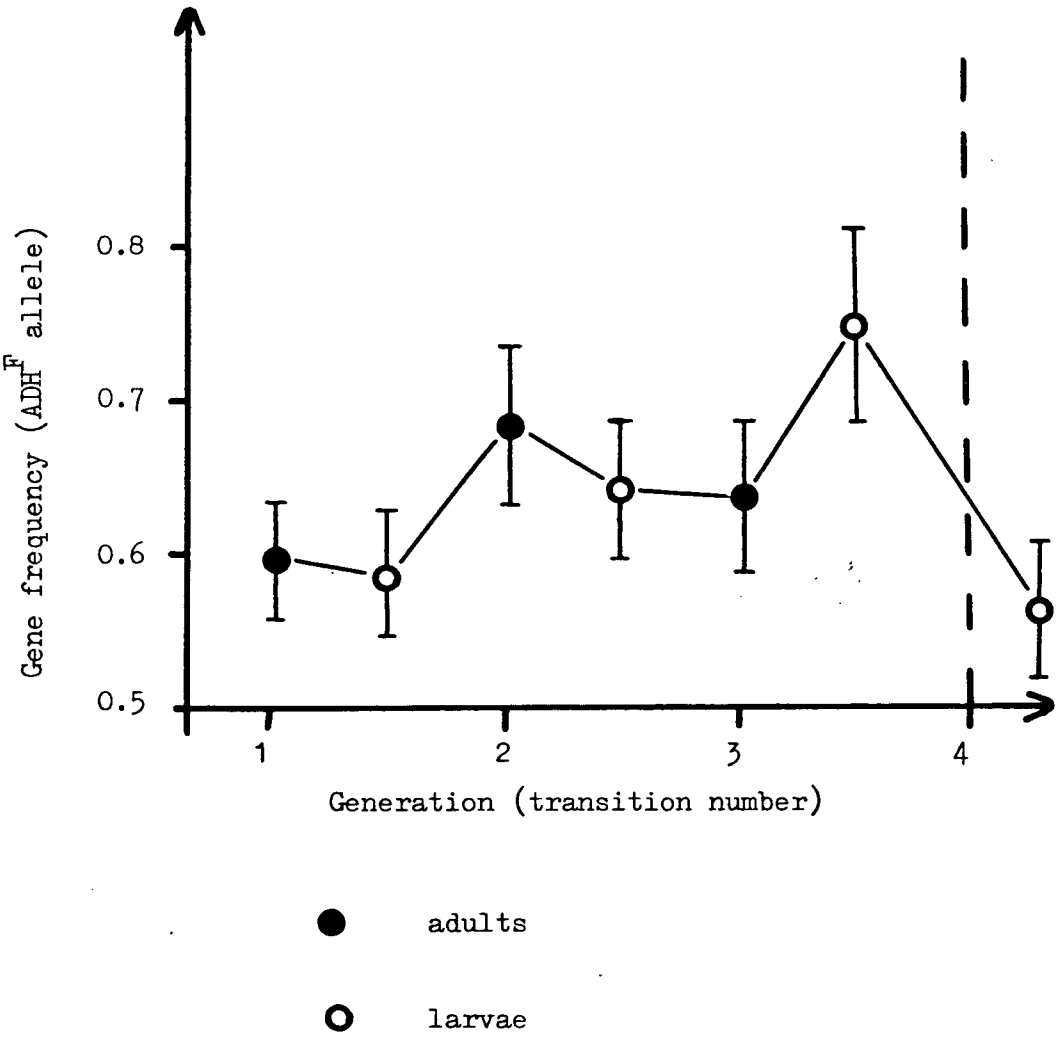


Fig. 4.2 ADH^F gene frequencies found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on 2% butan-2-ol medium over 3 generations (preliminary experiment A). For the fourth generation, the flies were reared on normal medium.

Statistical comparisons of genotype frequences at the ADH locus in Experiment A

Sample	χ^2	p
1. Adult ♂♂ and ♀♀ of Tran.1 (= unselected population)	5.74 (2 df)	> .05
2. Adults of Tran.1 and larvae of Tran.1 (i.e. from these adults)	7.50 "	0.05 > p > 0.01
3. Adult ♂♂ and ♀♀ of Tran.2	1.33 "	> .05
4. Larvae of Tran.1 and adults of Tran.2	9.50 "	< .01 (sig)
5. Adults of Tran.2 and larvae of Tran.2	1.03 "	> .05
6. Adult ♂♂ and ♀♀ of Tran.3	2.38 "	> .05
7. Larvae of Tran.2 with adults of Tran.3	0.25 "	> .05
8. Adults of Tran.3 with larvae of Tran.3	8.45 "	0.05 > p > 0.01
9. Adults of Tran.1 with larvae of Tran.3	20.66 "	< .01 (sig)
10. Total ♂♂ with ♀♀	2.97 "	> .05
11. Total adults with larvae	2.21	> .05

Statistical comparisons of gene frequencies at the ADH locus in Experiment A

Sample	χ^2	p
1. Adult ♂♂ and ♀♀ of Tran.1	0.52 (1 df)	> .05
2. Adult of Tran.1 and larvae of Tran.1	0.17 "	> .05
3. Adult ♂♂ and ♀♀ of Tran.2	0.80 "	> .05
4. Larvae of Tran.1 and adults of Tran.2	7.34 "	< .01 (sig)
5. Adults of Tran.2 and larvae of Tran.2	0.76 "	> .05
6. Adult ♂♂ and ♀♀ of Tran.3	1.89 "	> .05
7. Larvae of Tran.2 with adults of Tran.3	0.09 "	> .05
8. Adults of Tran.3 with larvae of Tran.3	7.97 "	< .01 (sig)
9. Adults of Tran.1 with larvae of Tran.3	15.86 "	< .01 (sig)
10. Total ♂♂ with ♀♀	1.42 "	> .05
11. Total adults with larvae	0.92 "	> .05

Statistical comparisons of egg numbers with respect to ADH genotype

Tran.1. Comparison of means for F and H genotypes; t=0.38, df 51, p > .05

Tran.2. " " " " " " " " ; t=3.23, df 34, p < .01 (sig)

Experiment A

Table 4. Genotype frequencies at the αGPDH locus

Sample	Numbers of genotypes			Frequency		
	F	H	S	F	H	S
Transition 1. Adult ♂♂	28	17	2	0.60±.071	0.36±.070	0.04±.029
Adult ♀♀	63	28	2	0.68±.048	0.30±.048	0.02±.015
larvae from them	58	36	2	0.60±.050	0.38±.050	0.01±.014
Tran. 2 Adult ♂♂	57	31	6	0.61±.050	0.33±.048	0.06±.024
Adult ♀♀	32	13	3	0.67±.068	0.27±.064	0.06±.034
larvae from them	65	28	2	0.68±.048	0.30±.047	0.02±.014
Tran. 3 Adult ♂♂	53	36	7	0.55±.050	0.38±.050	0.07±.026
Adult ♀♀	50	35	10	0.53±.051	0.37±.050	0.10±.031
larvae from them	55	35	6	0.57±.051	0.37±.049	0.06±.024
Total						
Adult ♂♂	138	84	15	0.58±.032	0.36±.031	0.06±.015
Adult ♀♀	145	76	15	0.62±.032	0.32±.030	0.06±.015
Larvae	178	99	10	0.62±.029	0.35±.028	0.03±.010

Experiment A

Table 5. Gene frequencies at the αGPDH locus

Sample		pαGPDH$_F$	Sample	pαGPDH$_F$
Transition 1.	Adult ♂♂	0.78±.043	Larvae from Tran. 1	0.78±.030
	Adult ♀♀	0.83±.027		
Tran. 2	Adult ♂♂	0.77±.031	Larvae from Tran. 2	0.82±.028
	Adult ♀♀	0.80±.041		
Tran. 3	Adult ♂♂	0.74±.032	Larvae from Tran. 3	0.76±.031
	Adult ♀♀	0.71±.033		
Totals				
Adult ♂♂		0.76±.020	Total larvae	0.79±.017
Adult ♀♀		0.78±.019		

Table 6. Summary of egg counts from ♀s in vials

Because of poor egg laying induced by the butanol, only 2 generations were counted.

		♀ genotype for αGPDH		
		F	H	S
Tran. 1.	Total eggs	69	75	0
	n	27	30	0
	Mean eggs per ♀	2.55	2.50	
Tran. 2	Total eggs	108	54	1
	n	21	14	3
	Mean eggs per ♀	5.14	3.86	0.33

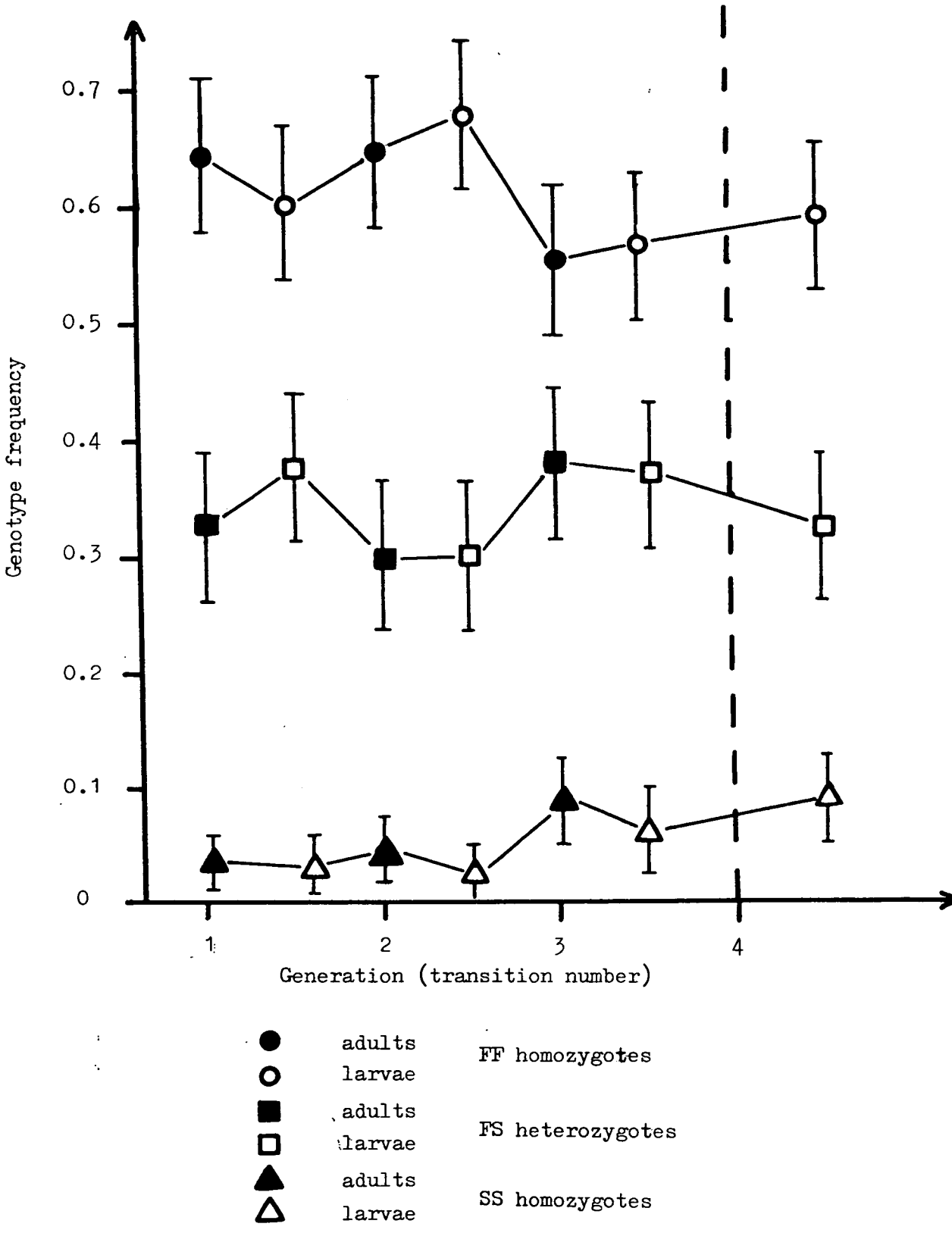


Fig. 4.3 Genotype frequencies at the αGPDH locus found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on 2% butan-2-ol medium over 3 generations (preliminary experiment A). For the fourth generation, the flies were reared on normal medium.

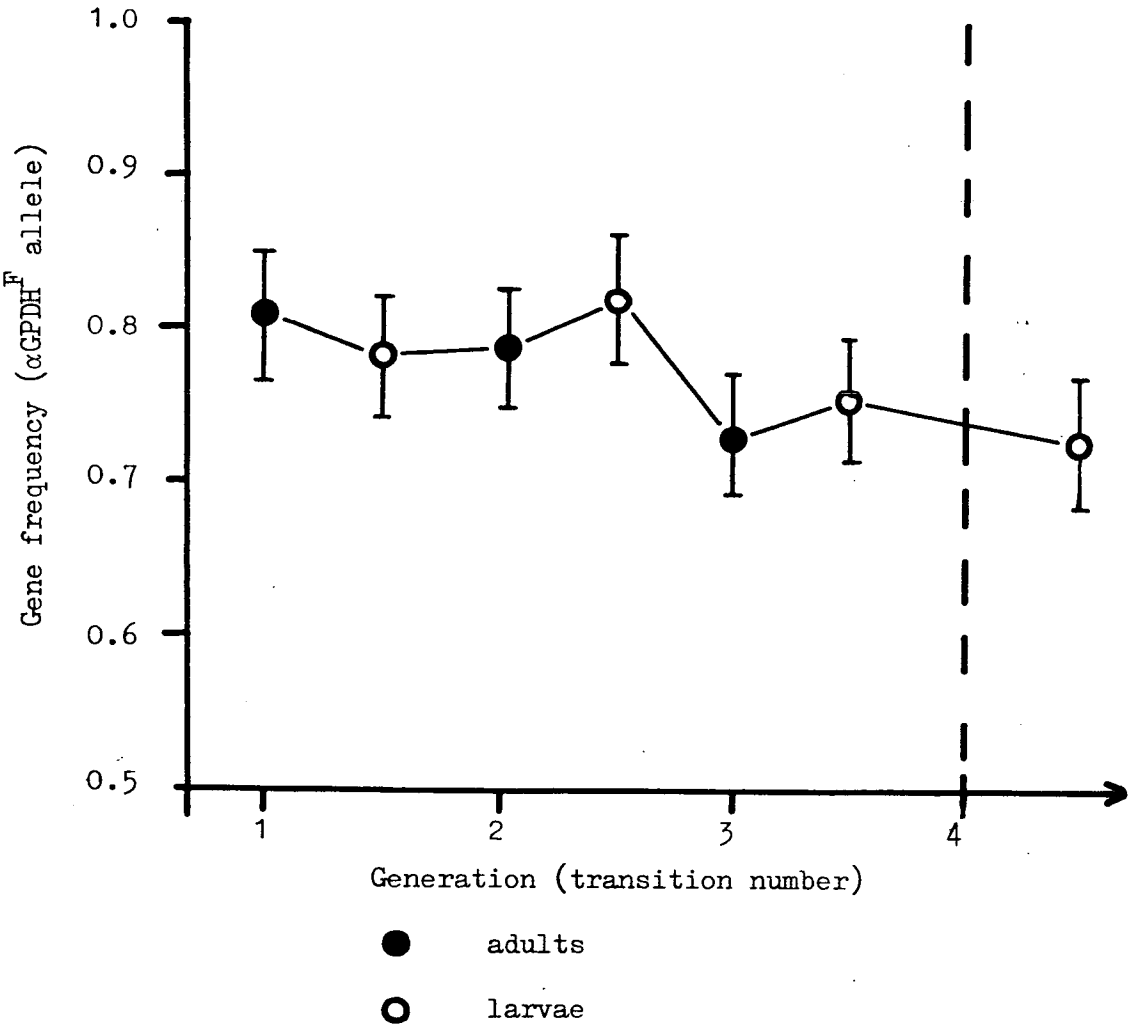


Fig. 4.4 $\alpha GPDH^F$ gene frequencies found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on 2% butan-2-ol medium over 3 generations (preliminary experiment A). For the fourth generation, the flies were reared on normal medium.

Statistical comparisons of genotype frequencies at the αGPDH locus

Experiment A

Sample	χ^2	p
1. Adult ♂♂ and ♀♀ of Tran. 1	1.16 (2 df)	>.05
2. Adults of Tran.1 and larvae from them	0.80	"
3. Adult ♂♂ and ♀♀ of Tran.2	0.54	"
4. Adults of Tran.2 and larvae of Tran.1	3.01	"
5. Adults of Tran.2 and larvae of Tran.2	2.53	"
6. Adult ♂♂ and ♀♀ of Tran.3	0.63	"
7. Adults of Tran.3 with larvae of Tran.2	7.77	0.05>p>.01
8. " " " " " " Tran.3	0.70	>.05
9. Adults of Tran.1 with larvae of Tran.3	2.41	"
10. Total adult ♂♂ with ♀♀	0.57	"
11. Total adults with total larvae	2.94	"

Statistical comparisons of gene frequencies at the αGPDH locus

Experiment A

Sample	χ^2	p
1. Adult ♂♂ and ♀♀ of Tran.1	1.16 (1 df)	>.05
2. Adults of Tran.1 and larvae from them	0.68	"
3. Adult ♂♂ and ♀♀ of Tran.2	0.35	"
4. Adults of Tran.2 and larvae of Tran.1	0.0	"
5. Adults of Tran.2 and larvae of Tran.2	1.21	"
6. Adult ♂♂ and ♀♀ of Tran.3	0.56	"
7. Adults of Tran.3 and larvae of Tran.2	6.98	<.01 (sig)
8. " " " " " " Tran.3	0.70	>.05
9. Adults of Tran.1 with larvae of Tran.3	2.22	"
10. Total adults ♂♂ and ♀♀	0.34	"
11. Total adults with total larvae	1.31	"

Statistical comparison of egg numbers with respect to αGPDH genotype

Gen.1. Comparison of the means of F and H females; t=0.05, df 55, p>.05

Gen.2. " " " " " " " " " t=1.20, df 33, p>.05

Summary of the results of Experiment A

The frequency of the S homozygotes at the ADH locus declined throughout the experiment, so that there were significantly fewer S homozygotes in the larvae from the third transition compared with the adults of the first transition. The adult ♀♀ and larvae of transition 1 show a reduction in the frequency of the FF homozygote which is significantly different from the frequency in the adults of the next generation, and which does not occur at any other stage of the experiment. The frequency of the F homozygotes increases, and the frequency of the heterozygotes decreases, progressively in the larvae. Relative frequencies of F homozygotes and heterozygotes are variable in the adults and exhibit no pattern.

The gene frequency of the F allele at the ADH locus increased through the experiment, and the frequency in the larvae of transition 3 is significantly higher than the frequency in the adults of transition 1. All significant changes are increases in the frequency of the F allele.

The data on egg numbers are so limited that no conclusion can be drawn about the relative performance of the genotypes; the result in generation 2 suggests some superiority of the F homozygote in egg production.

The frequency of the αGPDH genotypes did not change significantly over the experiment, except that the larvae of transition 2 gave rise to adults with fewer F homozygotes, more heterozygotes and more S homozygotes, and the larvae from these adults (Transition 3) retain these proportions, although the differences between them and previous generations are not significant.

The gene frequencies behave similarly; they show no significant differences except that the larvae of transition 2, which have the

next to highest frequency of the F allele in the experiment, give rise to adults in transition 3 with a significantly lower frequency of the F allele.

Again, data on egg numbers is very poor, but, such as it is, shows no significant difference between genotypes.

For neither locus was there any consistent difference in gene or genotype frequency between the sexes or between adults and larvae.

2. Large scale sampling of adults and larvae on butan-2-ol medium for 3 generations.

Experiment B

To obtain unequivocal results, it was decided to repeat experiment A with twice the sample size. The concentration of butan-2-ol used was the same, 2%, and in this experiment no egg counts were made.

Table 1. Genotype frequencies at the ADH locus

Sample		Numbers of genotypes				Frequency		
		F	H	S	N	F	H	S
Transition 1.	Adult ♂♂	54	117	21	192	0.28±.032	0.61±.035	0.11±.023
	Adult ♀♀ butanol medium	67	106	19	192	0.35±.034	0.55±.036	0.10±.021
	larvae from them	71	115	6	192	0.37±.035	0.60±.035	0.03±.012
Tran. 2	Adult ♂♂	83	107	2	192	0.43±.036	0.56±.036	0.01±.007
	Adult ♀♀	92	95	4	191	0.48±.036	0.50±.036	0.02±.010
	larvae from them	86	101	5	192	0.45±.036	0.53±.036	0.01±.010
Tran. 3	Adult ♂♂	86	89	17	192	0.45±.036	0.46±.036	0.09±.021
	Adult ♀♀	66	71	7	144	0.46±.042	0.49±.042	0.05±.018
	larvae from them	102	89	1	192	0.531±.036	0.464±.036	.005±.005
Total								
Adult ♂♂		223	313	40	576	0.39±.020	0.54±.021	0.07±.011
Adult ♀♀		225	272	30	527	0.43±.022	0.52±.022	0.05±.009
Larvae		259	305	12	576	0.45±.021	0.53±.021	0.02±.006

Experiment B

Table 2. Gene frequencies at the ADH locus during Experiment B

Sample		$pADH_F$
Tran. 1	Adult ♂♂	0.59±.025
	Adult ♀♀	0.63±.025
	Larvae	0.67±.024
Tran. 2	Adult ♂♂	0.71±.023
	Adult ♀♀	0.73±.023
	Larvae	0.71±.023
Tran. 3	Adult ♂♂	0.68±.024
	Adult ♀♀	0.72±.026
	Larvae	0.76±.022
Totals	Adult ♂♂	0.65±.014
	Adult ♀♀	0.66±.015
	Larvae	0.71±.013

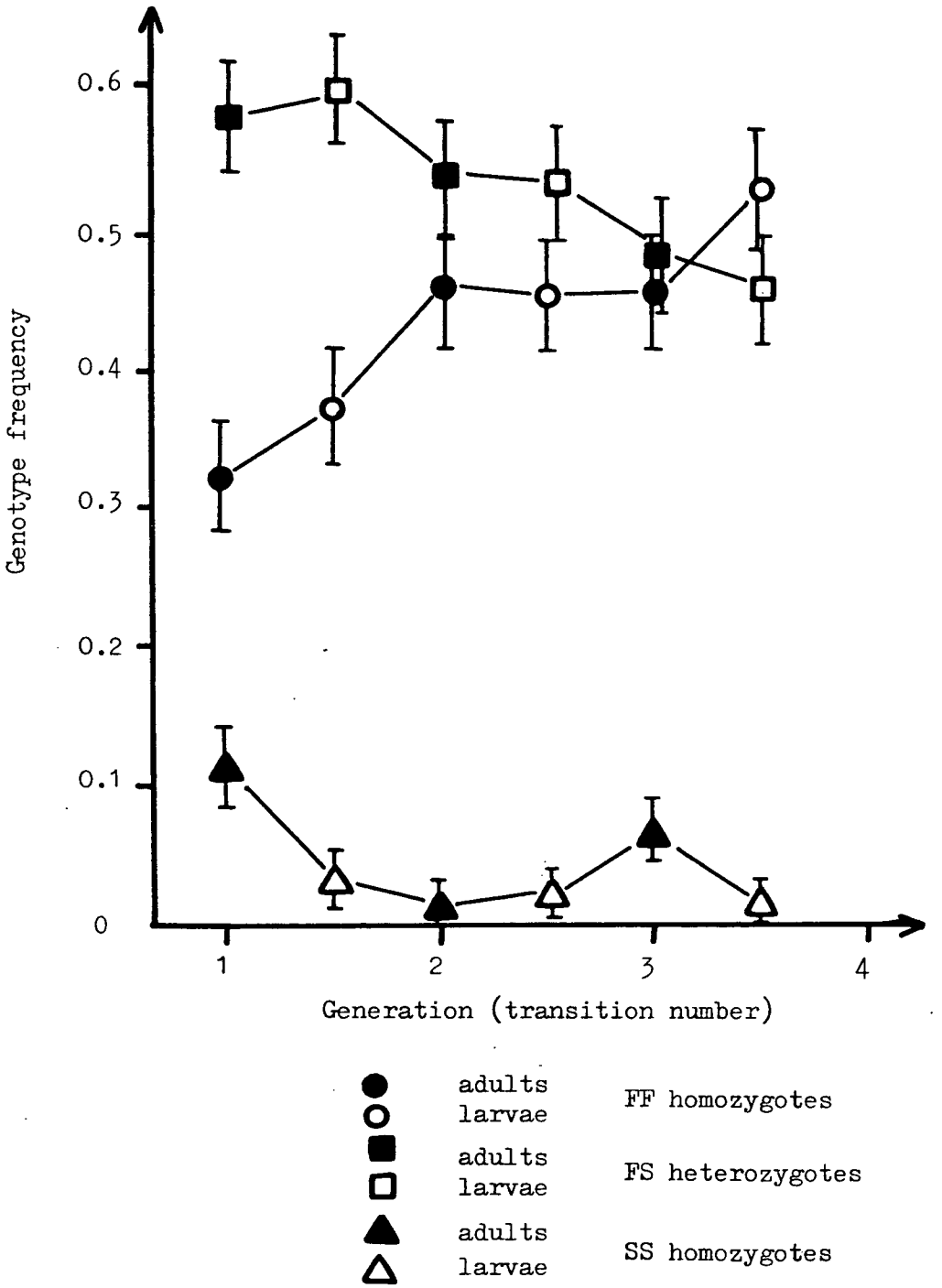


Fig. 4.5 Genotype frequencies at the ADH locus found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on 2% butan-2-ol medium over 3 generations (Experiment B).

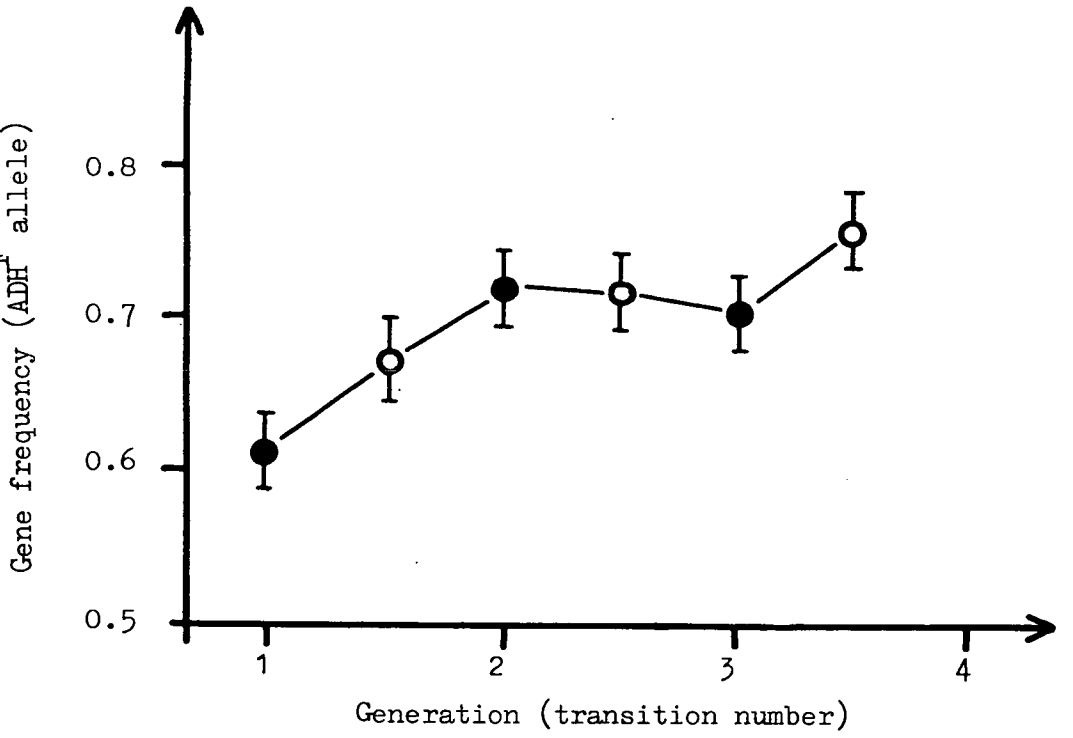


Fig. 4.6 ADH^F gene frequencies found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on 2% butan-2-ol medium over 3 generations (Experiment B).

Statistical comparisons of genotype frequencies for the ADH locus in Experiment B

Sample	χ^2	p
1. Tran.1. Adult ♂♂ and ♀♀	2.04 (2 df)	>.05
2. Adults of Tran.1 and larvae from them	9.74	<.01 (sig)
3. Tran.2. Adult ♂♂ and ♀♀	1.84	>.05
4. Larvae of Tran.1 and adults of Tran.2	4.94	>.05
5. Adults of Tran.2 and larvae of Tran.2	0.74	>.05
6. Tran.3 Adult ♂♂ and ♀♀	2.01	>.05
7. Larvae of Tran.2 and adults of Tran.3	5.20	>.05
8. Adults of Tran.3 and larvae from them	12.94	<.01 (sig)
9. Adults of Tran.1 and larvae of Tran.3	36.30	<.01 (sig)
10. Total ♂♂ with ♀♀	2.14	>.05
11. Total adults with larvae	15.78	<.01 (sig)
12. Larvae of Tran.1 with larvae of Tran.2	2.43	>.05
13. " " Tran.2 with " " Tran.3	4.79	>.05
14. " " Tran.1 with " " " "	12.44	<.01 (sig)

Statistical comparison of gene frequencies at the ADH locus

Sample	χ^2	p
1. Tran.1. Adult ♂♂ and ♀♀	1.23 (1 df)	>.05
2. Adults of Tran.1 and larvae from them	4.45	.05>p>.01
3. Tran.2. Adult ♂♂ and ♀♀	0.36	>.05
4. Larvae of Tran.1 and adults of Tran.2	3.74	>.05
5. Adults of Tran.2 and larvae of Tran.2	0.12	>.05
6. Tran.3. Adult ♂♂ and ♀♀	1.41	>.05
7. Larvae of Tran.2 with adults of Tran.3	0.20	>.05
8. Adults of Tran.3 and larvae of Tran.3	5.16	.05>p>.01
9. Adults of Tran.1 and larvae of Tran.3	28.24	<.01 (sig)
10. Total ♂♂ with ♀♀	1.71	>.05
11. Total adults with larvae	6.52	.05>p>.01
12. Larvae of Tran.1 with larvae of Tran.2	1.56	>.05
13. Larvae of Tran.2 " " " Tran.3	2.69	>.05
14. Larvae of Tran.1 " " " " "	8.30	<.01 (sig)

Table 3. Genotype frequencies at the αGPDH locus in Experiment B

Sample	Numbers of genotypes				Frequency		
	F	H	S	N	F	H	S
Transition 1. Adult ♂♂	135	44	3	182	0.74±.033	0.24±.032	0.02±.010
Adult ♀♀	118	62	7	187	0.63±.034	0.33±.034	0.04±.014
larvae from them	119	66	3	188	0.63±.034	0.35±.035	0.02±.010
Tran. 2 Adult ♂♂	135	52	5	192	0.70±.033	0.27±.032	0.03±.012
Adult ♀♀	125	54	6	185	0.68±.034	0.29±.033	0.03±.012
larvae from them	133	56	3	192	0.69±.033	0.29±.033	0.02±.010
Tran.3 Adult ♂♂	125	38	8	171	0.73±.034	0.22±.032	0.05±.016
Adult ♀♀	121	72	5	198	0.61±.035	0.36±.034	0.03±.012
larvae from them	114	29	1	144	0.79±.034	0.20±.033	0.01±.01
Totals							
Adult ♂♂	395	134	16	545	0.72±.019	0.25±.019	0.03±.007
Adult ♀♀	364	188	18	570	0.64±.020	0.33±.020	0.03±.007
Larvae	366	151	7	524	0.70±.020	0.29±.020	0.01±.004

Experiment B

Table 4. Gene frequencies at the αGPDH locus in Experiment B

Sample		$p\alpha GPDH_F$
Tran. 1	Adult ♂♂	0.86±.018
	Adult ♀♀	0.80±.021
	Larvae	0.81±.020
Tran. 2	Adult ♂♂	0.81±.019
	Adult ♀♀	0.81±.020
	Larvae	0.84±.019
Tran. 3	Adult ♂♂	0.84±.020
	Adult ♀♀	0.79±.020
	Larvae	0.89±.018
Totals	Adult ♂♂	0.84±.011
	Adult ♀♀	0.80±.012
	Larvae	0.84±.011

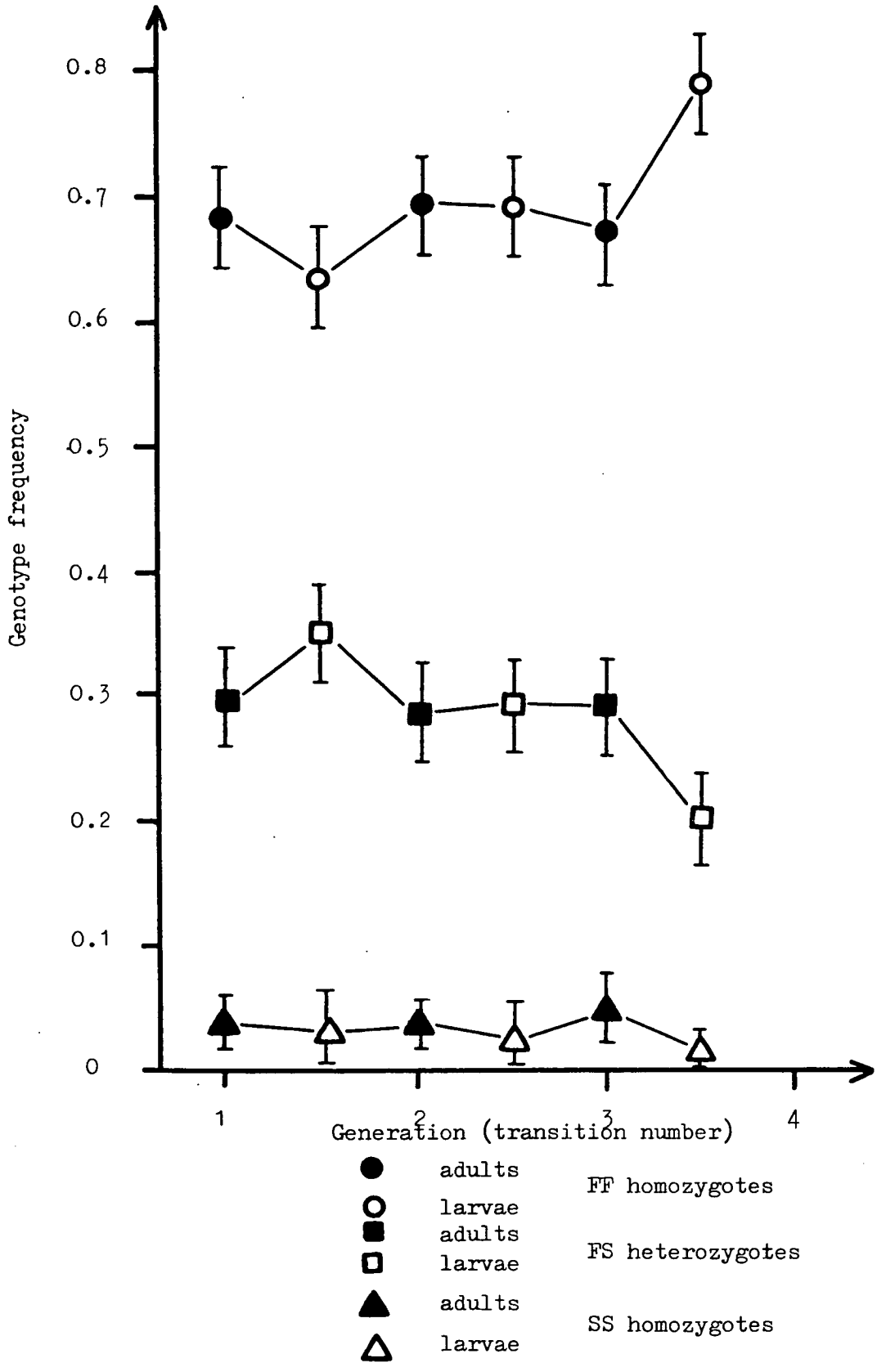


Fig. 4.7 Genotype frequencies at the αGPDH locus found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on 2% butan-2-ol medium over 3 generations (Experiment B).

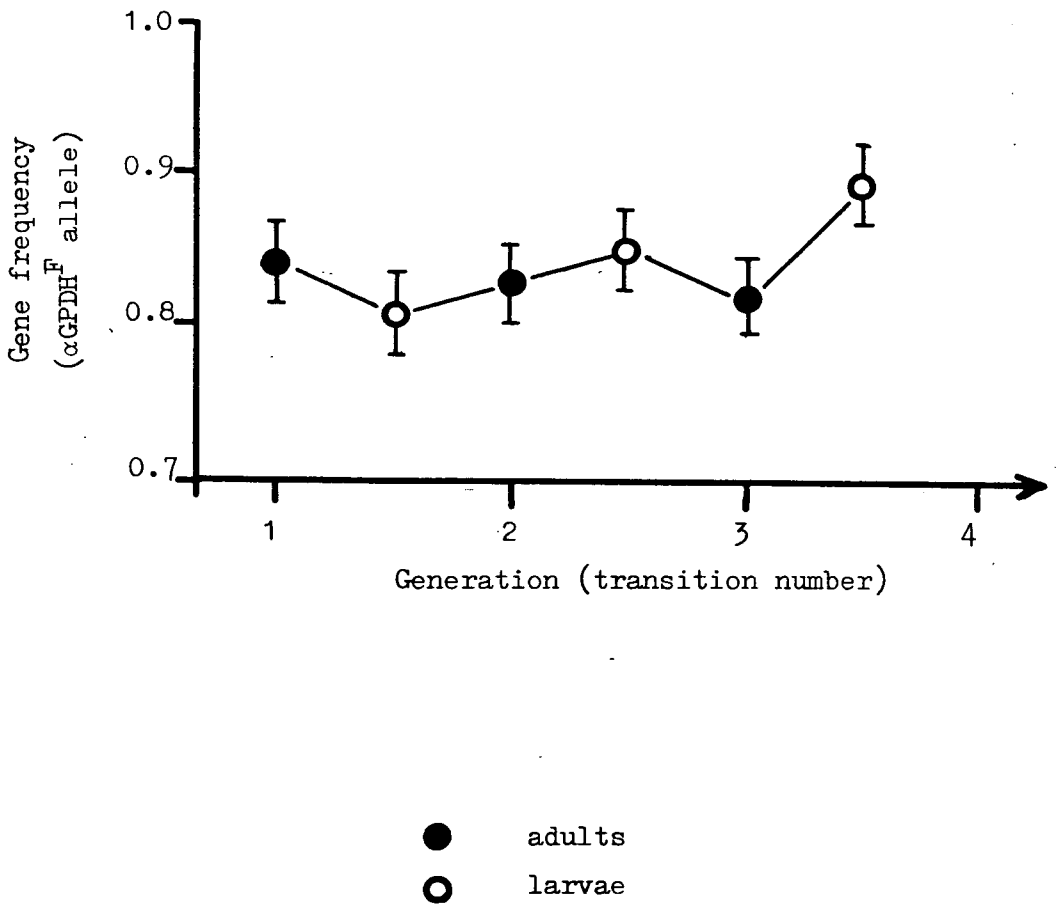


Fig. 4.8 $\alpha GPDH^F$ gene frequencies found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on 2% butan-2-ol medium over 3 generations (Experiment B).

Statistical comparisons of genotype frequencies for the αGPDH locus in Experiment B

Sample	χ^2	p
1. Tran.1. Adult ♂♂ and ♀♀	5.73 (2 df)	>.05
2. Adults of Tran.1 and larvae from them	2.82	>.05
3. Tran.2. Adult ♂♂ and ♀♀	0.38	>.05
4. Larvae of Tran.1 and adults of Tran.2	3.50	>.05
5. Adults of Tran.2 and larvae of Tran.2	1.00	>.05
6. Tran.3. Adult ♂♂ and ♀♀	9.34	<.01 (sig)
7. Larvae of Tran.2 and adult ♂♂ of Tran.3	4.77	>.05
8. " " " " " " ♀♀ " " "	2.98	>.05
9. Adult ♂♂ of Tran.3 and larvae of Tran.3	4.88	>.05
10. " ♀♀ " " " " " " " "	12.98	<.01 (sig)
11. Adults of Tran.1 and larvae of Tran.3	6.49	.05>p>.01
12. Adults of Tran.1 and ♂♂ of Tran.3	3.56	>.05
13. " " " " " ♀♀ " " "	3.50	>.05
14. Total adults, ♂♂ and ♀♀	9.88	<.01 (sig)
15. Total adult ♂♂ and total larvae	5.23	>.05
16. Total adult ♀♀ and total larvae	6.96	.05>p>.01

Statistical comparisons of gene frequencies at the αGPDH locus

Sample	χ^2	p
1. Tran.1. Adult ♂♂ and ♀♀	5.65 (1 df)	.05>p>.01
2. ♂♂ of Tran.1 and larvae of Tran.1	3.94	.05>p>.01
3. ♀♀ " " " " " " " "	0.16	>.05
4. Tran.2. Adult ♂♂ and ♀♀	0.38	>.05
5. Larvae of Tran.1 and adults of Tran.2	0.81	>.05
6. Adults of Tran.2 and larvae of Tran.2	0.13	>.05
7. Tran.3. Adult ♂♂ and ♀♀	2.95	>.05
8. Larvae of Tran.2 and adults of Tran.3	0.91	>.05
9. Adults of Tran.3 and larvae of Tran.3	8.93	>.01 (sig)
10. Adult ♂♂ of Tran.1 and larvae of Tran.3	1.31	>.05
11. Total adults ♂♂ and ♀♀	6.49	.05>p>.01
12. Total ♂♂ and total larvae	0.02	>.05
13. Total ♀♀ and total larvae	5.70	.05>p>.01

Summary of the results of Experiment B

These confirm the results of Experiment A at the ADH locus. There is again a significant difference between genotype frequencies of the adults of Transition 1 at the beginning of the experiment and the larvae from them. There is also a significant difference between the adults of Tran.3 and the larvae from them. These differences are both decreases in the frequency of S homozygotes, with increases in the frequency of F homozygotes. The heterozygote frequencies remain the same. The difference between the adults of Tran.1 at the beginning of the experiment and the larvae of Tran.3 at the end is highly significant and shows a decrease in the frequency of heterozygotes too. Over the whole experiment, the larvae have significantly more F homozygotes and fewer S homozygotes than the adults.

The gene frequency changes repeat the pattern of experiment A. All significant changes are increases in the frequency of the F allele as the experiment progresses. The frequency of the F allele in the summed larval frequencies verges on being significantly higher than in the summed adult frequencies. Rather curiously, the larval gene frequencies again lie in a straight line, and the adult frequencies seem to overshoot and undershoot at the same point as in experiment A.

At the αGPDH locus, there are significant differences in genotype frequencies between the sexes in one generation, and when summed over all generations. The females of Transition 3 have significantly lower frequencies of the FF homozygote than do the larvae of Tran.3. The adults of Tran.1 have lower FF frequencies and higher heterozygote frequencies than the larvae of Tran.3, and this difference borders on significance.

The larvae seem to differ from parents overall in this, having higher FF frequencies and lower heterozygote frequencies when summed overall transitions. This difference again borders on significance.

The gene frequencies show fewer changes. The overall sex difference remains, males having a higher frequency of the F allele, but the difference is barely significant, and the larvae have apparently the same frequency of F as the adult males. The difference between adult ♂♂ in Tran.1 and larvae in Tran.3 is not significant.

Overall, there is a tendency for the gene frequency to increase over the experiment, although this is not statistically significant.

CHAPTER 5

Estimation of relative fitnesses at the ADH and αGPDH loci on normal medium

Both of these loci are polymorphic in the "Malawi" strain of *D. melanogaster* used. Since examination of the genotype frequencies of adults and larvae at the ADH locus shows significant changes when the ADH locus is under selection by butan-2-ol, genotype frequencies have been examined by the same method in a non selected, discrete generation population cage, as described in Chapter 2. Under these conditions, female fecundity is not depressed, and egg numbers were measured. Offspring of individual females were also typed, so that mother-offspring genotype combinations were obtained. Collection of these different data from the same population will be referred to as 'Frydenberg's sampling procedure' since the method is adapted from his work. Details are given in Chapter 2 (Materials and Methods).

Frydenberg's sampling procedure on normal medium.

Experiment A

Table 1. Genotype frequencies at the ADH locus

Sample		Numbers			Frequency		
		F	H	S	F	H	S
Tran. 9.	Adult ♂♂	22	16	10	.46±.072	.33±.068	.21±.059
	Adult ♀♀	32	26	10	.48±.061	.37±.058	.15±.044
	Cage Larvae	33	47	15	.35±.049	.49±.051	.16±.037
Tran. 10.	Adult ♂♂	14	27	7	.29±.066	.56±.071	.15±.052
	Adult ♀♀	25	31	12	.38±.059	.46±.061	.18±.047
	Larvae	41	44	11	.43±.051	.46±.051	.11±.032
Tran. 11.	Adult ♂♂	21	23	4	.44±.071	.48±.072	.08±.039
	Adult ♀♀	26	37	7	.37±.057	.53±.060	.10±.036
	Larvae	47	34	14	.49±.051	.36±.049	.15±.036
Tran. 12.	Adult ♂♂	20	21	7	.42±.071	.44±.071	.14±.050
	Adult ♀♀	28	29	10	.42±.060	.43±.061	.15±.044
	Larvae	13	28	7	0.27±.064	.58±.071	.15±.052
Tran. 13.	Adult ♂♂	14	29	5	.29±.066	.60±.071	.11±.045
	Adult ♀♀	29	21	11	.48±.064	.34±.061	.18±.049
	Larvae	35	42	16	.38±.050	.45±.082	.17±.039
Totals	Adult ♂♂	91	116	33	0.38±.031	0.48±.032	0.14±.022
	Adult ♀♀	140	144	50	0.42±.027	0.43±.027	0.15±.020
	Larvae	169	195	63	0.40±.024	0.46±.024	0.14±.017

Experiment A

Table 2. Gene frequencies at the ADH locus

Sample		$pADH_F$	Sample	$pADH_F$
Tran. 9	Adult ♂♂	0.63±.049	Larvae of Tran.9 adults	0.59±.036
	Adult ♀♀	0.66±.041		
Tran.10	Adult ♀♀	0.57±.051	Larvae of Tran.10 adults	0.66±.034
	Adult ♀♀	0.60±.042		
Tran.11	Adult ♂♂	0.68±.048	Larvae of Tran.11 adults	0.67±.034
	Adult ♀♀	0.64±.041		
Tran.12	Adult ♂♂	0.64±.049	Larvae of Tran.12 adults	0.56±.051
	Adult ♀♀	0.63±.042		
Tran.13	Adult ♂♂	0.59±.050	Larvae of Tran.13 adults	0.60±.036
	Adult ♀♀	0.65±.043		
Total	Adult ♂♂	0.62±.022	Total Larvae	0.63±.017
	Adult ♀♀	0.63±.019		

Table 3. Frequency of transmitted ♂ gametes in offspring of ♀s of different ADH genotypes

Sample	$pADH_F$ in adult ♂♂	P of ADH_F alleles of paternal origin in	
		offspring of FF ♀♀	offspring of SS ♀♀
Tran. 9	0.63±.049	$\frac{43}{66}$ = 0.65±.058	$\frac{8}{18}$ = 0.44±.116
Tran.10	0.57±.051	$\frac{27}{60}$ = 0.45±.064	$\frac{26}{36}$ = 0.72±.075
Tran.11	0.68±.048	no data	no data
Tran.12	0.64±.049	$\frac{80}{120}$ = 0.66±.043	$\frac{40}{60}$ = 0.66±.061
Tran.13	0.59±.050	$\frac{60}{108}$ = 0.55±.048	$\frac{25}{42}$ = 0.59±.075
Total for Tran.9,10,12 & 13	0.61±.025	$\frac{210}{354}$ = 0.59±.026	$\frac{99}{156}$ = 0.63±.039

Experiment A

Table 4. ♀♀ fecundity of different genotypes at the ADH locus

Sample		Total eggs	Number of ♀♀	Mean eggs per ♀
Tran. 9	FF ♀♀	458	32	14.3
	FS ♀♀	402	26	15.46
	SS ♀♀	175	9	19.44
Tran.10	FF ♀♀	289	24	12.04
	FS ♀♀	363	32	11.34
	SS ♀♀	156	12	13.0
Tran.11			NO DATA	
Tran.12	FF ♀♀	725	25	29.00
	FS ♀♀	758	27	28.07
	SS ♀♀	273	9	30.30
Tran.13	FF ♂♂	659	21	31.38
	FS ♀♀	442	14	31.57
	SS ♀♀	279	9	31.00
Total for Tran.9, 10,12 & 13	FF ♀♀	2131	102	20.89
	FS ♀♀	1965	99	19.85
	SS ♀♀	883	39	22.64

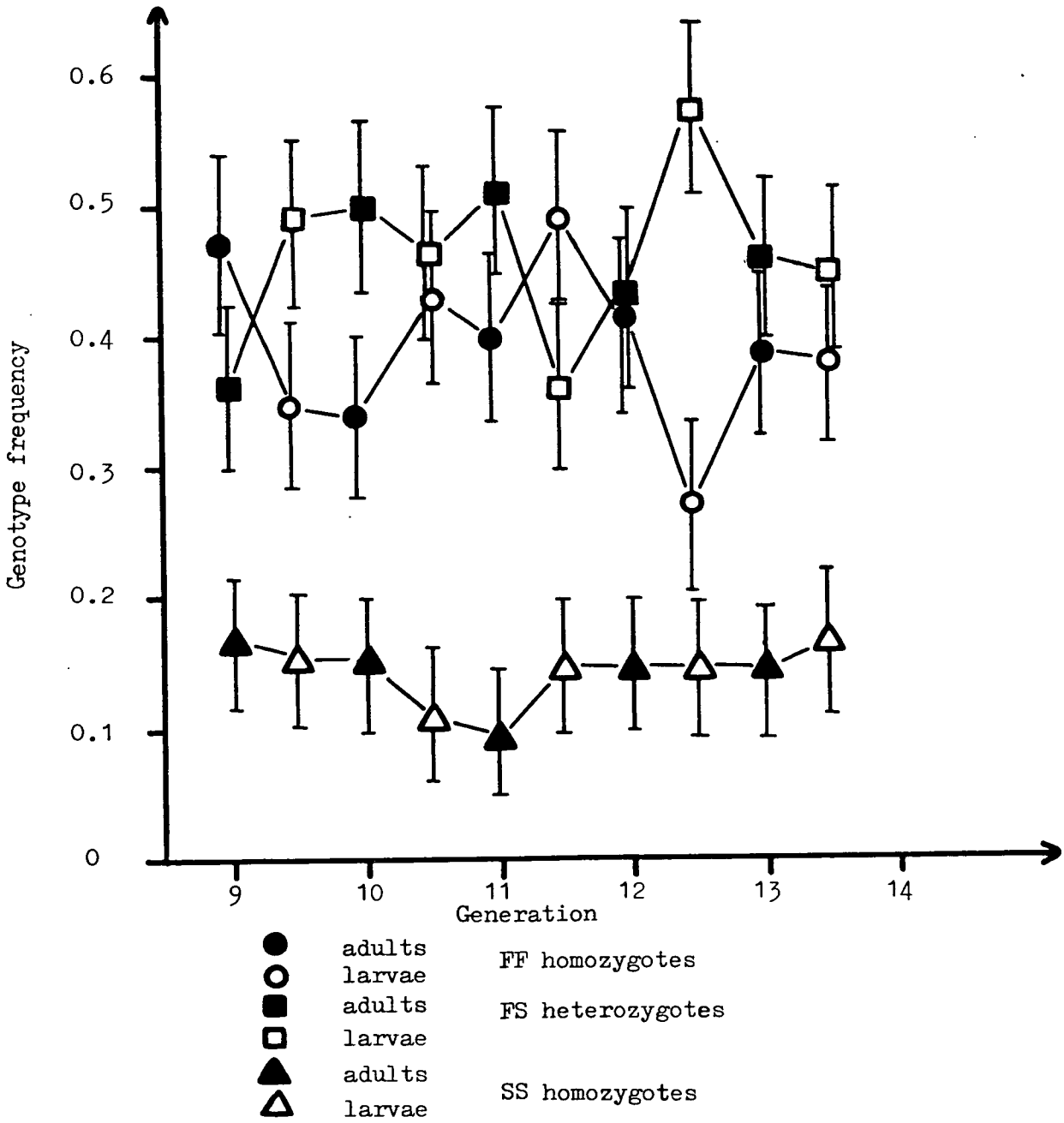


Fig. 5.1 Genotype frequencies at the ADH locus found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on normal medium, over 5 generations (Experiment A).

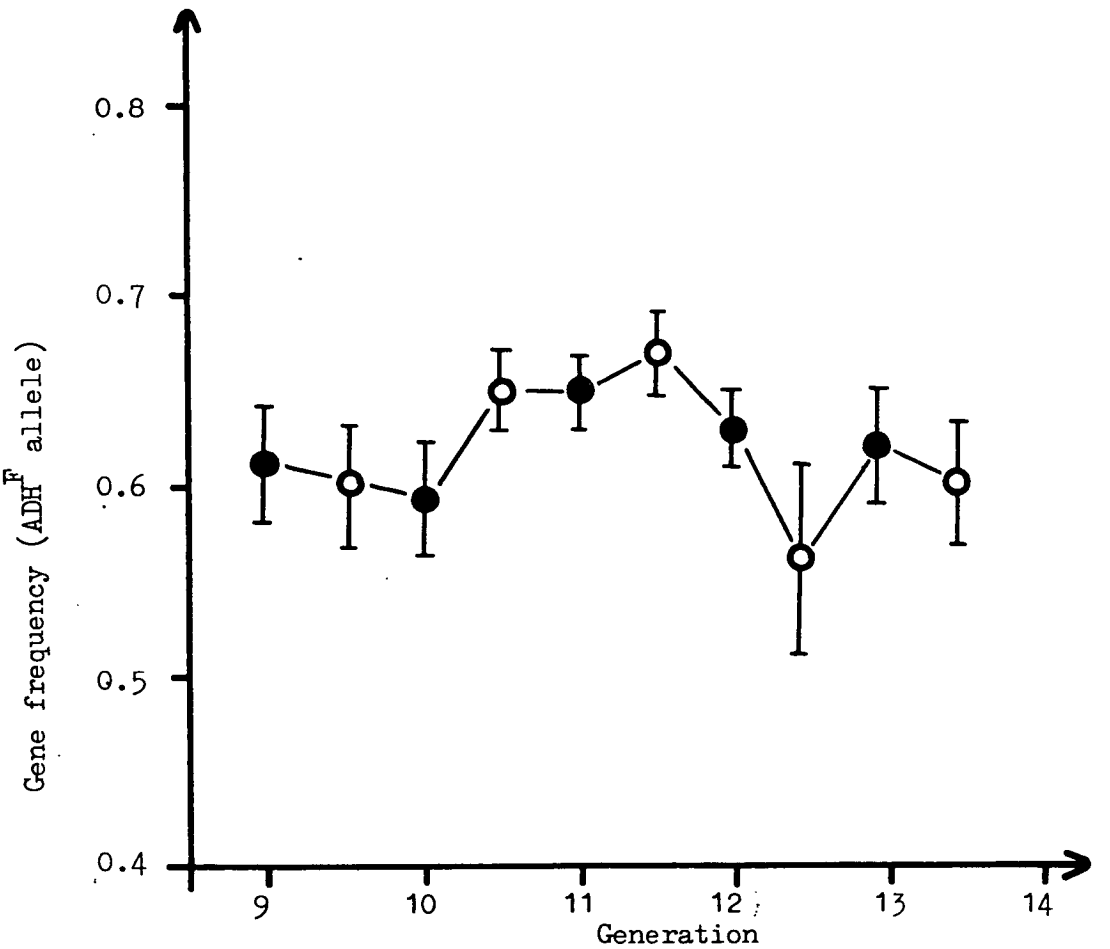


Fig. 5.2 ADH^F gene frequencies found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on normal medium, over 5 generations (Experiment A).

Frydenberg's sampling procedure on normal medium

Experiment A

Table 5. Genotype frequencies at the αGPDH locus

Sample		Numbers			Frequency		
		F	H	S	F	H	S
Tran. 9.	Adult ♂♂	20	25	3	0.42±.071	0.52±.072	0.06±.035
	Adult ♀♀	28	33	7	0.41±.060	0.49±.061	0.10±.036
	Cage Larvae	40	35	15	0.44±0.51	0.39±.051	0.17±.040
Tran. 10.	Adult ♂♂	18	18	3	0.46±.080	0.46±.080	0.08±.040
	Adult ♀♀	37	24	7	0.54±.061	0.35±.057	0.11±.036
	Cage Larvae	40	22	8	0.57±.059	0.31±.056	0.12±.039
Tran. 11.	Adult ♂♂	33	12	3	0.69±.067	0.25±.062	0.06±.035
	Adult ♀♀	30	36	4	0.43±.059	0.51±.060	0.06±.028
	Cage Larvae	53	29	8	0.59±.052	0.32±.049	0.09±.030
Tran. 12.	Adult ♂♂	21	22	4	0.45±.072	0.47±.073	0.08±.040
	Adult ♀♀	31	29	7	0.46±.061	0.43±.061	0.11±.039
	Cage Larvae	29	15	4	0.61±.071	0.31±.067	0.08±.039
Tran. 13.	Adult ♂♂	15	13	3	0.48±.090	0.42±.089	0.10±.054
	Adult ♀♀	34	22	5	0.56±.063	0.36±.062	0.08±.035
	Cage Larvae	43	37	11	0.47±.052	0.41±.052	0.12±.035
Totals	Adult ♂♂	107	90	16	0.50±.034	0.42±.034	0.08±.019
	Adult ♀♀	160	144	30	0.48±.027	0.43±.027	0.09±.016
	Larvae	205	138	46	0.53±.025	0.35±.024	0.12±.016

Experiment A

Table 6. Gene frequencies at the αGPDH locus

Sample		$p\alpha GPDH_F$	Sample	$p\alpha GPDH_F$
Tran. 9	Adult ♂♂	0.68±.048	Larvae of Tran. 9	0.64±.036
	Adult ♀♀	0.65±.041		
Tran.10	Adult ♂♂	0.73±.047	Larvae of Tran.10	0.73±.038
	Adult ♀♀	0.72±.039		
Tran.11	Adult ♂♂	0.81±.040	Larvae of Tran.11	0.75±.032
	Adult ♀♀	0.69±.039		
Tran.12	Adult ♂♂	0.68±.048	Larvae of Tran.12	0.76±.044
	Adult ♀♀	0.68±.040		
Tran.13	Adult ♂♂	0.71±.066	Larvae of Tran.13	0.68±.035
	Adult ♀♀	0.74±.040		
Total	Adult ♂♂	0.72±.022	Larvae	0.70±.016
	Adult ♀♀	0.69±.018		

Table 7. Frequency of transmitted ♂ gametes in offspring of females of different αGPDH genotypes

Sample	$pADH_F$ in adult ♂♂	P of ADH_F in alleles of paternal origin in	
		offspring of FF ♀♀	offspring of SS ♀♀
Tran. 9	0.68±.048	0.89±.045	no data
Tran.10	0.73±.047	0.61±.060	0.83±.118
Tran.11	0.81±.040	no data	no data
Tran.12	0.68±.048	0.69±.042	0.58±.142
Tran.13	0.71±.066	0.83±.037	no data
Total for Tran.9,10,12 & 13	0.70±.025	0.75±.024	-

Experiment A

Table 8. ♀♀ fecundity of different ♀♀ genotypes at the αGPDH locus

Sample		Total eggs	Number of ♀♀	Mean eggs per ♀
Tran. 9	FF ♀♀	420	28	15.00
	FS ♀♀	513	32	16.03
	SS ♀♀	102	7	14.58
Tran.10	FF ♀♀	466	37	12.59
	FS ♀♀	260	25	10.4
	SS ♀♀	82	6	13.67
Tran.11		NO DATA		
Tran.12	FF ♀♀	707	25	28.28
	FS ♀♀	905	31	29.19
	SS ♀♀	144	5	28.8
Tran.13	FF ♀♀	722	24	30.08
	FS ♀♀	519	16	32.44
	SS ♀♀	139	4	34.75
Totals	FF ♀♀	2315	114	20.31
	FS ♀♀	2197	104	21.13
	SS ♀♀	467	22	21.23

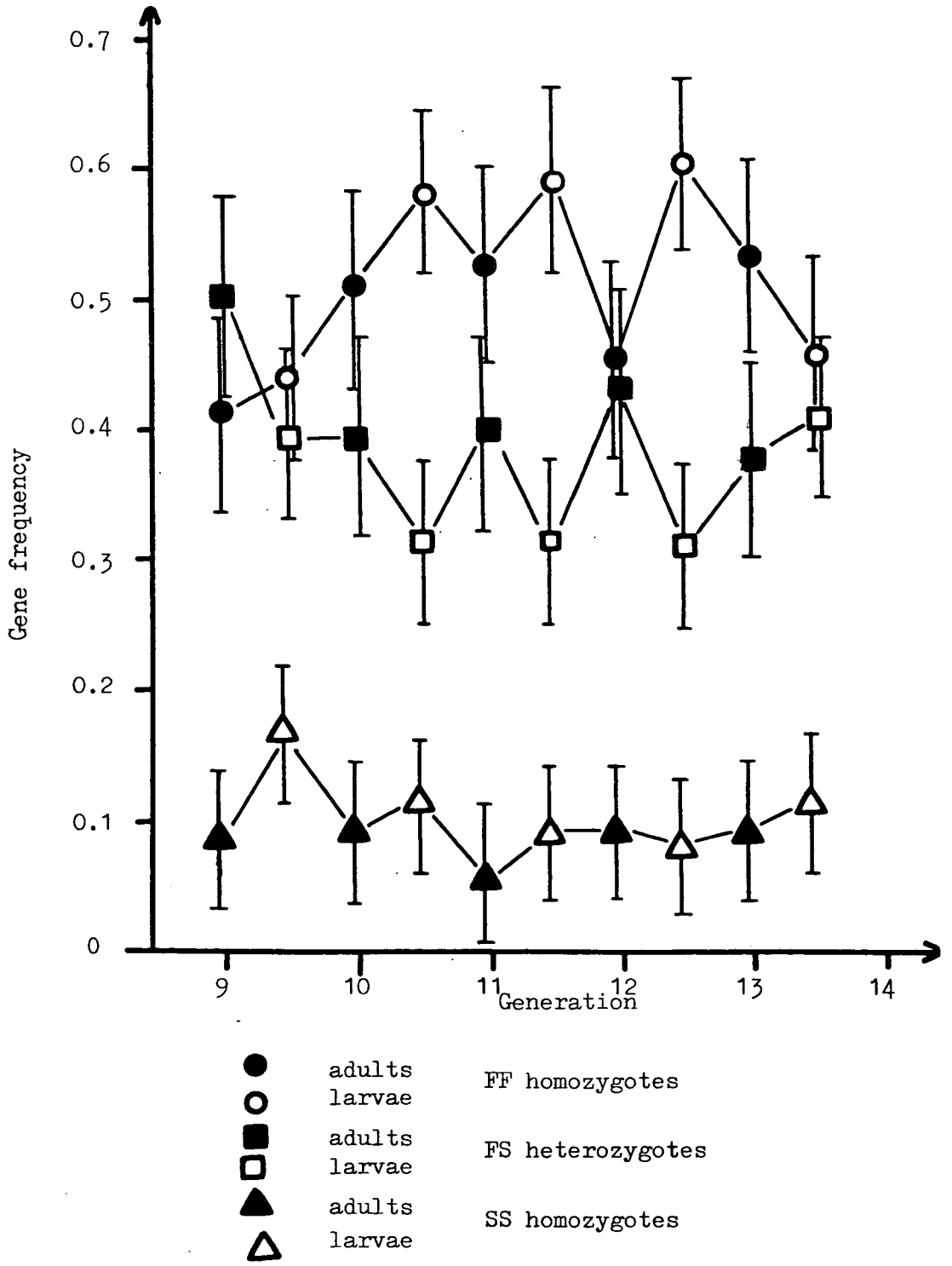


Fig. 5.3 Genotype frequencies at the αGPDH locus found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on

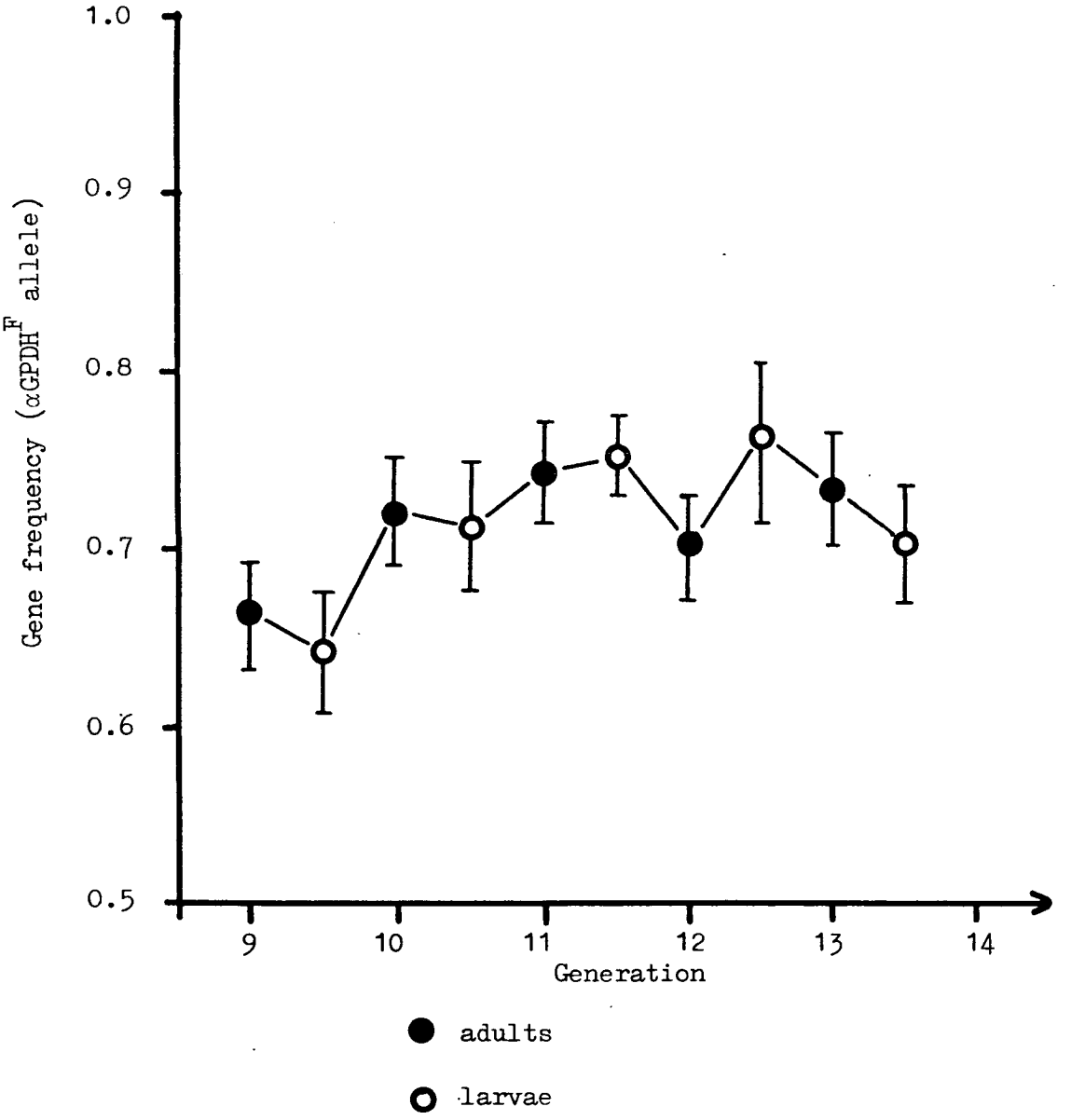


Fig. 5.4 $\alpha GPDH^F$ gene frequencies found by sampling of adult and 3rd instar larval stages of Drosophila melanogaster kept on normal medium, over 5 generations (Experiment A).

Statistical comparisons of ADH genotype frequencies in Expt. A, between adults and larvae. (See Table 1).

Sample	χ^2 (2 df)	p
1. Adults of Tran.9; ♂♂ and ♀♀	0.81	>.05
2. Adults of Tran.9 and larvae from them	4.01	"
3. Adults of Tran.10; ♂♂ and ♀♀	1.28	"
4. Larvae of Tran.9 and adults of Tran.10	0.03	"
5. Adults of Tran.10 and larvae of Tran.10	2.24	"
6. Adults of Tran.11; ♂♂ and ♀♀	0.53	"
7. Adults of Tran.11 and larvae of Tran.10	0.62	"
8. Adults of Tran.11 and larvae of Tran.11	5.13	"
9. Adults of Tran.12; ♂♂ and ♀♀	0.00	"
10. Adults of Tran.12 and larvae of Tran.11	1.46	"
11. Adults of Tran.12 and larvae of Tran.12	3.51	"
12. Adult ♂♂ and ♀♀ of Tran.13	7.32	0.05>p>.01
13. Adult ♂♂ of Tran.13 and larvae of Tran.12	0.39	>.05
14. Adult ♀♀ of Tran.13 and larvae of Tran.12	6.53	0.05>p>0.01
15. Adult ♂♂ of Tran.13 and larvae of Tran.13	3.10	>.05
16. Adult ♀♀ of Tran.13 and larvae of Tran.13	1.92	"
17. Adults of Tran.9 and ♂♂ of Tran.13	8.11	0.05>p>0.01
18. Adults of Tran.9 and ♀♀ of Tran.13	0.06	>.05
19. Larvae of Tran.9 and Tran.13	0.35	"
20. Total ♂♂ and ♀♀	1.54	"
21. Total adults to larvae	0.05	"

Statistical comparisons of ADH gene frequencies in Expt. A, between adults and larvae. (See Table 2)

Sample	χ^2 (1 df)	p
1. Tran.9; adult ♂♂ and ♀♀	0.33	>.05
2. Tran.9; adults with larvae of Tran.9	1.19	>.05
3. Tran.10 adults; ♂♂ and ♀♀	0.12	>.05
4. Tran.10 adults with larvae of Tran.9	0.03	>.05
5. Tran.10 adults with larvae of Tran.10	2.18	"

Statistical comparisons of the gene frequencies listed in Table 2 continued

Sample	χ^2 (1 df)	p
6. Tran.11; adult ♂♂ and ♀♀	0.03	>.05
7. Tran.11 adults with larvae of Tran.10	2.18	"
8. Tran.11 adults with larvae of Tran.11	0.43	"
9. Tran.12; adult ♂♂ and ♀♀	0.01	"
10. Tran.12 adults and larvae of Tran.11	0.21	"
11. Tran.12 adults and larvae of Tran.12	0.00	"
12. Tran.13; adult ♂♂ and ♀♀	0.69	"
13. Adults of Tran.13 and larvae of Tran.12	1.36	"
14. Adults of Tran.13 and larvae of Tran.13	0.66	"
15. Adults of Tran.9 and Tran.13	1.05	"
16. Larvae of Tran.9 and Tran.13	0.20	"
17. Total adults; ♂♂ and ♀♀	0.25	"
18. Total adults and total larvae	0.02	"

Statistical comparisons of the frequencies of transmitted ♂ gametes in Table 3

Sample	χ^2(1 df)	p
1. Comparison of frequency in offspring of FF and SS mothers, Tran.9	0.59	>.05
2. Comparison of frequency in offspring of FF and SS mothers, Tran.10	6.74	<.01 (sig)
3. Comparison of frequency in offspring of FF and SS mothers, Tran.12	0.00	>.05
4. Comparison of frequency in offspring of FF and SS mothers, Tran.13	0.19	"
5. Comparison of frequency in offspring of FF and SS mothers, Total	0.78	"
6. Frequency in adult ♂♂ with frequency of paternal alleles in offspring, Tran.9	0.06	"
7. Frequency in adult ♂♂ with frequency of of paternal alleles in offspring of FF♀, Tran.10	1.50	"

Statistical comparisons of the frequencies of transmitted ♂ gametes in Table 3 (continued)

Sample	χ^2 (1 df)	p
8. Frequency in adult ♂♂ with frequency of paternal alleles in offspring of SS♀, Tran.10	3.26	>.05
9. Frequency in adult ♂♂ with frequency of paternal alleles in offspring, Tran.12	0.27	>.05
10. Frequency in adult ♂♂ with frequency of paternal alleles in offspring, Tran.13	0.18	>.05
11. Frequency in adult ♂♂ with frequency of paternal alleles, Total offspring	0.00	>.05

Statistical comparisons of genotype frequencies at the αGPDH locus (Table 5)

Sample	χ^2 (2 df)	p
1. Adults of Tran.9; ♂♂ and ♀♀	0.61	> .05
2. Adults of Tran.9 and larvae of Tran.9	4.20	"
3. Adults of Tran.10; ♂♂ and ♀♀	1.25	"
4. Adults of Tran.10 and larvae of Tran.9	2.56	"
5. Adults of Tran.10 and larvae of Tran.10	1.16	"
6. Adults of Tran.11; ♂♂ and ♀♀	8.48	.05>p>.01
7. Adult ♂♂ of Tran.11 and larvae of Tran.10	1.85	>.05
8. Adult ♀♀ of Tran.11 and larvae of Tran.10	6.14	.05>p>.01
9. Adult ♂♂ of Tran.11 and larvae of Tran.11	1.31	>.05
10. Adult ♀♀ of Tran.11 and larvae of Tran.11	6.06	.05>p>.01
11. Adults of Tran.12; ♂♂ and ♀♀	0.20	>.05
12. Adults of Tran.12 and larvae of Tran.11	3.76	"
13. Adults of Tran.12 and larvae of Tran.12	3.05	"
14. Adults of Tran.13, ♂♂ and ♀♀	0.45	"
15. Adults of Tran.13 and larvae of Tran.12	0.70	"
16. Adults of Tran.13 and larvae of Tran.13	0.92	"
17. Larvae of Tran.9 and Tran.13	0.77	"
18. Adults of Tran.9 and Tran.13	3.19	"
19. Total ♂♂ to ♀♀	0.50	"
20. Total adults to larvae	6.43	.05>p>.01

Statistical analysis of the data on female fecundity in Table 4

This was done using a NAG package, GLIM. Results and details are given in the Appendix. No effect was found of either ADH genotype or αGPDH genotype, or of any interaction between them.

Statistical comparisons of gene frequencies at the αGPDH locus (Table 6)

Sample	χ^2 (1 df)	p
1. Tran. 9; adult ♂♂ and ♀♀	0.13	>.05
2. Tran.9 adults and larvae of Tran. 9	0.28	"
3. Tran.10 adults; ♂♂ and ♂♂	0.01	"
4. Tran.10 adults and larvae of Tran. 9	3.29	"
5. Tran.10 adults and larvae of Tran.10	0.01	"
6. Tran.11; adult ♂♂ and ♀♀	4.73	0.05>p>.01
7. Tran.11 adult ♂♂ with larvae of preceding Tran.10	2.22	>.05
8. Tran.11 adult ♂♂ with larvae of Tran.11	1.39	"
9. Tran.12; adult ♂♂ and ♀♀	0.00	"
10. Tran.12 adults and larvae of Tran.11	2.41	"
11. Tran.12 adults and larvae of Tran.12	2.10	"
12. Tran.13; adult ♂♂ and ♀♀	0.15	"
13. Tran.13 adults and larvae of Tran.12	0.31	"
14. Tran.13 adults and larvae of Tran.13	1.21	"
15. Larvae of Tran.9 and Tran.13	0.55	"
16. Adults of Tran.9 and Tran.13	1.98	"
17. Total adults; ♂♂ and ♀♀	0.99	"
18. Total adults vs. total larvae	0.00	"

Statistical comparisons of frequency of transmitted ♂ gametes and frequency in adult ♂♂ (Table 7)

Sample	χ^2(1 df)	p
1. Frequency of paternal alleles in offspring of FF and SS ♀♀, Tran.10	1.40	>.05
2. Frequency of paternal alleles in offspring of FF and SS ♀♀, Tran.12	0.59	"
3. Frequency of paternal alleles in offspring of FF ♀♀ only, Totals		
4. Frequency in adult ♂♂ and frequency of paternal alleles in offspring of FF ♀♀, Tran.9	8.17	<.01 (sig)
5. Frequency in adult ♂♂ and frequency of paternal alleles in offspring of FF and SS ♀♀ of Tran.10	1.72	>.05

Statistical comparisons of frequency of transmitted ♂ gametes and frequency in adult ♂♂ (Table 7) (continued)

Sample	χ^2 (1 df)	p
6. Frequency in adult ♂♂ and frequency of paternal alleles in offspring of FF ♀♀, Tran.12	0.00	> .05
7. Frequency in adult ♂♂ and frequency of paternal alleles in offspring of FF ♀♀ only, Tran.13	3.11	"
8. Frequency in all ♂♂ and frequency of paternal allele in all offspring of FF females	2.12	"

Statistical analysis of the data of female fecundity (Table 8)

This was done using GLIM, a NAG package; details are given in the Appendix. No significant effect of αGPDH genotype was found, neither was there any interaction with ADH genotype.

Summary of the results of Experiment A

The polymorphism at the ADH locus remained stable over the experiment, and there were no significant differences between genotype or gene frequencies at the beginning and end of the five generations of sampling. There was only one significant difference between the sexes; genotype frequencies in ♂ and ♀ differed in Tran. 13. However, this is not reflected in the gene frequencies, which are not significantly different. There were no differences between adult and larval, genotype or gene frequencies.

In one transition 10, there appears to be a significant deficit in paternal F alleles in the offspring of FF and SS mothers, but this is not repeated, and the frequency of F alleles in the total adult males does not differ from the frequency of F in paternal alleles of offspring of both FF and SS mothers.

There is no significant difference between genotypes with respect to female fecundity.

The polymorphism at the αGPDH locus is also stable over the experiment, and neither genotype nor gene frequencies differ between the beginning and end of the sampling. In Tran.11, the frequency of the FF homozygote is significantly larger in the male than in the females, and this is reflected in the gene frequencies. However, this difference is not repeated in any other transition and the total ♂ and ♀ genotype frequencies do not differ significantly. There is no pattern of differences between adults and larvae except that the total adults have more FS genotypes and fewer SS genotypes than the total larvae. The difference is not reflected in gene frequencies.

In one transition, 9, there is a significant increase in the frequency of F allele transmitted as ♂ gamete, as compared to the frequency in adult males. However, this is not repeated significantly in other generations, and the total frequencies in adult males and transmitted ♂ gametes do not differ significantly, although the frequency of the F allele is higher in the transmitted male gametes.

There is no significant difference between genotypes in ♀ fecundity.

Data on linkage disequilibrium from Experiment A

Table A2. No. of each genotype (ADH locus first, αGPDH locus second).

	FF FF	FF FS	FF SS	FS FF	FS FS	FS SS	SS FF	SS FS	SS SS
Gen. 9	26	25	4	15	23	4	7	11	2
Gen.10	18	17	0	29	18	7	8	9	2
Gen.11	24	20	3	34	22	4	5	6	0
Gen.12	26	31	3	21	22	7	8	8	1
Gen.13	22	12	2	18	13	5	7	6	0
All Gen.	116	105	12	117	98	27	35	40	5

This data was analysed using DIPCHROM (Hill, 1975) to test for significant associations between alleles at the two loci.

The departure from the associations predicted by Hardy Weinberg appears to be just significant for each generation and for the total. For the latter, the programme gives 2 log LR = 9.2229, DF = 5. This is distributed as χ^2, so $0.01 > p > 0.05$.

Estimation of effective population size

Table A3

	No. of families	$\bar{k}$	σ_k^2	$\frac{\sigma_k^2}{\bar{k}}$	N_e
Gen. 9	67	15.45	131.56	8.515	1452
10	69	11.91	112.15	9.42	1326
11	70	5.06	31.47	6.22	1913
12	62	28.92	206.78	7.15	1695
13	45	30.71	303.46	9.88	1269
Total		18.41	157.08	8.53	1449

N_e is given by $\dfrac{N\bar{k}}{1 + \dfrac{\sigma_k^2}{\bar{k}}}$ (Crow & Kimura, 1970)

where i) $\bar{k}$ is assumed to be finally 2 for replacement in a a population of constant size.

ii) the ratio $\sigma_k^2 : \bar{k}$ is calculated from observed data as above.

iii) N is the number of individuals calculated from the observed number of males, M, and females, F, as follows,

$$N = \frac{4MF}{M+F}$$ (Crow & Kimura, 1970)

M and F were calculated by weighing (see Materials and Methods) in Gen. 13.

Total cage weight 5.700g

Sample weight 0.610g

Sample numbers 382 ♂ and 358 ♀

M = 3,569 and F = 3,345

Multiple matings

The number of multiple matings by females homozygous at both ADH and αGPDH loci was determined by the method of Milkman & Zeitler (1974)

Table A4.

Gen.	No. of females examined	Double matings
9	9	0
10	9	5
12	15	2
13	13	1
Total	46	8

Summary of results A2, 3 and 4

The amount of linkage disequilibrium between the αGPDH and ADH loci shown in the analysis of Table A2 is bordering on significance. If a larger sample had been taken, the result might have been more clear cut. Associations between these two loci have been reported in other populations (Watanabe and Watanabe, 1977; Langley, Ito & Voelker, 1977; Alahiotis, Pelecanos and Zacharopoulon, 1976; Cavener & Clegg, 1978) but these have always involved inversions in natural populations, and the associations have been slight.

Table A3 shows an effective population size of between $\frac{1}{4}$ and $\frac{1}{5}$ of the observed number of individuals. The $\sigma_k^2 : \bar{k}$ ratio is low (around 8.5) and reasonably constant from generation to generation (6.2 to 9.8). Begon (1977) found a ratio of 14.8 for wild D. subobscura maintained on laboratory medium under semi wild conditions.

Table A4 shows the proportion of females detected which have mated with 2 or more males. This is about $\frac{1}{6}$ (8/46), which would suggest that the phenomen is not uncommon in a laboratory cage population.

CHAPTER 6

DISCUSSION

The experiments of Chapters 3, 4 and 5 were designed to add to the evidence which is generally thought to implicate some kind of balancing selection in the maintenance of the alcohol dehydrogenase polymorphism.

The methodology put forward by Clarke (1975) (and discussed in Chapter 1) was felt to be too subject to artefacts of experimental systems; the evidence it provides does not quite stand on its own. Consequently these experiments were suggested as a way of measuring effects of selection directly on a quasi-natural population.

The results of Experiment A in Chapter 5 are therefore disappointing, unfortunate or interesting, depending on the standpoint of the commentator. No evidence of differences in fitness between genotypes at the ADH locus has been found under standard conditions in the laboratory. The components of fitness examined were fecundity, mating ability, zygote to larval viability, and larval to adult viability. No evidence of fitness differences in the preceding components has been found for the αGPDH locus.

However, it has been demonstrated that butan-2-ol is capable of exerting a clearly detectable selective force at the ADH locus (Chapter 4, Experiments A & B).

In this chapter, the possible weaknesses of the findings in Chapter 5 will be discussed, and the results related to those of other authors. The results of Chapter 4 will be similarly dealt with. However, the body of literature on relative survivals, perturbation

and biochemical characteristics of the ADH genotypes when exposed to various alcohols is so large that a review of this literature has been relegated to Appendix 3.

The most obvious reason for failing to detect any balancing selection in the experiments of Chapter 5 (apart from there not being any) is the small size of the samples. The standard errors of the gene frequencies are consequently large, of the order of 0.035 to 0.05 for a gene frequency of around 0.6. If we assume a selection coefficient of 0.1 (i.e. 10%) against the recessive S allele in the zygote to larval stage, then the expected difference in gene frequency between adults and larvae is about 0.01 if the adult frequency of the S allele is 0.4 (Falconer, 1967). 1,000 each of adults and larvae would have to be typed for this to approach significance on a χ^2. Over this experiment, only 500 or so of each stage were typed, and the magnitude of the selection coefficient detectable is more like 20%. However, other authors have followed gene frequencies in the adult stage only at both ADH and αGPDH loci, in natural and cage populations, and their sample sizes have been comparable to, or smaller than those given here. Berger (1971) perturbed cage populations using lines derived from wild populations, and reported apparent selection returning the ADH frequency to an equilibrium value. Bijlsma-Meeles and Van Delden (1974) perturbed populations in vials and cages, and reported selection returning the ADH frequency to an equilibrium value.

There appears to be linkage disequilibrium between the ADH and αGPDH loci at a level just bordering on significance (Chapter 5, Results A2). This has been reported in other populations, e.g. the Weymouth, U.S.A., populations described by Cavener and Clegg (1978). If any selection

had been found at either locus, it would have been a complicating factor, and would have required further investigation.

The effective population size, as given by the variance in family size, is between $\frac{1}{4}$ and $\frac{1}{5}$ of the observed number of individuals (Chapter 5, Results A3). This is relatively large compared to the value found by Begon (1977) for wild D.subobscura; between $\frac{1}{9}$ and $\frac{1}{10}$ of the observed number. This accords with predictions, since the population cage represents the least stringent selective conditions of experimental systems (Spiers, 1974; Kinross, 1969). and Begon measured the variance in family size on females in vials under semi wild conditions. These would be more stringent, and therefore give a larger variance and smaller N_e. The actual value of N_e in Experiment A is around 1400 (the accuracy of this would have been improved by estimating the total individuals in each generation) which is large enough for drift to permit the continued existence of the polymorphism in the absence of balancing selection.

Multiple matings do not appear to have been very frequent; (Chapter 5, Results A4); out of 46 doubly homozygous females examined, only 8 gave evidence of having mated with more than one male. The effect of frequent female multiple mating, assuming males to have a limited mating ability, would be to reduce the contribution of females to the next generation, since more would remain unmated. In extreme circumstances, this could lead to sex differences in gene frequency which were the reverse of, but analogous to, those expected in cattle under artificial insemination schemes (Robertson, 1965).

However, no consistent significant differences in gene frequency were observed between the sexes for either the ADH locus or the αGPDH locus.

With the preceding points in mind, it is possible to consider previous reports of differences in selection values at the ADH locus. Kojima and Tobari (1969), Morgan (1975) and Van Delden, Boerema & Kamping (1978) have all reported finding such differences. All three papers involve the extraction of isofemale lines to provide individuals of the required genotypes; all FS individuals are F_1 individuals between populations derived from such lines. These experiments are therefore open to two possible sources of error immediately; firstly, that the process of extracting isofemale lines sets up large amounts of disequilibrium between loci, and that therefore any effects seen may not be due to the genotypes directly observed, but to genotypes at other, unknown, linked loci (or to associated inversions if they have not been shown to be absent); secondly, that populations derived from isofemale lines will be less heterozygous than the base population, and crossing them may give F_1 individuals exhibiting heterosis or hybrid vigour. This will lead to apparent heterozygote advantage in the experiments to be described.

Kojima and Tobari (1969) used premated females to compare the viability of egg to 3rd instar larvae in crowded and uncrowded conditions. They found no differences between genotypes at the ADH locus. However, they noted that there was a large variance in size of the 3rd instar larvae, and they only typed middle sized ones; if smaller (slow growing) on larger (fast growing) larvae had been typed, they suggest that there might have been differences. They found no fecundity differences. In an egg to adult viability experiment, using the same premated females as for the egg to 3rd instar larvae comparison, they found significant differences between the genotypes such that at two low input frequencies of the F allele, more FF adults emerged, and at a high input frequency of

the F allele, more SS adults emerged. This frequency dependent effect was quite strong; selection coefficients are around 20% for the high and lowest values of F allele frequency. All the experiments were performed in ½pt milk bottles, with 100 ♀ parents per bottle. The premated females were of the type SS♀ x SS♂; FF♀ x FF♂ and SS♀ x FF♂ and vice versa. Hence heterosis could exist, and give rise to spurious frequency dependence, as discussed in the Introduction, but whatever the explanation, strong selection in relatively uncrowded conditions was reported by these authors.

Morgan (1976) compared the viability of FF and SS lines only, in order to avoid artefacts due to heterosis. He placed newly emerged larvae (0 to 3hrs old) into 3" x 1" vials in batches of 200. He used five different frequencies of the F allele; at the two lowest, more FF adults emerged than expected; at the intermediate frequency ($p_F = 0.4$) more SS individuals emerged than expected, at $p_F = 0.5$ the FF individuals were again at an advantage, and at $p_F = 0.85$ there were no differences between genotypes. The total emergences were low (around 35 to 50% of the input) as would be expected, since 200 larvae per vial is relatively crowded. The process of transfer may also have increased mortality. The selective forces demonstrated are very large; at a frequency of 0.5, the S allele has a selection coefficient of 36%, whereas at a frequency of 0.6, it is at an advantage, with a selection coefficient of 108%. Such strong frequency dependent selection would have produced a clearly observable pattern of genotype frequency differences if it had been taking place in the experiments described in Chapter 5, but it has to be admitted that the crowded conditions may have increased the magnitude of the effect. More curiously,

Morgan's results suggest three possible equilibria for the ADH polymorphism in his population; one at $p_F = 0.85$, and one on either side of $p_F = 0.4$. He doesn't give the equilibrium value of p_F for the population from which the lines were extracted.

Van Delden et al (1974) produced two populations homozygous for the F and S alleles at the ADH locus, by the extracted line method. They then self mated the F_1 from a cross of these two populations, and looked for deviations from the expected 1:2:1 ratio of genotypes. They varied the conditions of crowding by allowing females to lay eggs in the ½pt culture bottles for varying lengths of time, up to 5½ days. In the first few days of emergence, more FF genotypes were found. By day 14 the ratio was as expected or, in some cases, reversed so that there were more SS types. The more crowded the conditions, the more marked this effect became. In further experiments, they showed that FF larvae reach a critical weight for pupation before SS larvae. They also set up bottle cultures in which adults were transferred to new bottles on a time scale of 2 weeks or 3 weeks. In the 2 week bottles, a decline in the frequency of the S allele was observed, which did not occur in the 3 week bottles. The selection coefficient of the S allele was around 20%. In the experiments presented in this thesis, a 2 week cycle has always been used, but the ADH polymorphism is quite stable. There is no evidence of an increased number of FF individuals in the larval sample as opposed to the adults. A selective difference of the size found by Van Delden & Kamping would have been detected by these experiments. It may be noted, as it will be discussed in more detail Appendix 3, that the standard food medium used by Van Delden & Kamping contains 1% ethanol final concentration, and ethanol has been

shown to prolong developmental time (Oakeshott, 1976a). This may have some bearing on their results.

Overall, the experiments described in this thesis were capable of detecting effects of the magnitude of those described in these papers, and their failure to confirm this work, which is in part self contradictory, probably lies in other authors' use of extracted lines.

The results of the experiments with butan-2-ol (Experiments A & B, Chapter 4) are more in line with findings generally reported (Van Delden et al., 1975; Van Delden, Boerema & Kamping, 1978). 2% butan-2-ol is clearly capable of perturbing the ADH locus; the frequency of the F allele changing from 0.61 $\pm$ .018 to 0.67 $\pm$.018 over 2 generations (Experiment B). This corresponds to a selection coefficient against the S allele of around 30%. The literature on survivals, gene frequency changes and biochemical characteristics of the ADH locus on different alcohols is profuse, diffuse and confused. A critical review of it, and the relationship of the results of Chapter 4 to those of other workers, will be found in Appendix 3. Cavener and Clegg (1978) report a correlated response at the αGPDH locus to selection at the ADH locus with ethanol supplemented medium. In view of the limited amount of disequilibrium found in Experiment A of Chapter 5, such a response might have been expected. However, it is not reflected in the gene frequencies of the adults in the experiments of Chapter 4.

APPENDIX 1

Mother offspring combinations. (6 offspring per female)

Gen. 9 ♀ parent / offspring

ADH

	F	H	S
F	43	24	-
H	23	25	8
S	-	11	10

αGPDH

	F	H	S
F	43	27	-
H	5	27	0
S	-	12	0

Gen.10

	F	H	S
F	27	13	-
H	33	43	26
S	-	16	10

	F	H	S
F	40	15	-
H	26	16	10
S	-	11	2

Gen.11

	F	H	S
F	6	3	-
H	0	13	0
S	-	2	0

NO DATA

Gen.12

	F	H	S
F	80	46	-
H	40	57	40
S	-	23	20

	F	H	S
F	83	49	-
H	37	67	7
S	-	11	5

Gen.13

	F	H	S
F	60	21	-
H	48	37	25
S	-	14	17

	F	H	S
F	85	45	-
H	17	33	0
S	-	12	6

APPENDIX 2

GLIM (Generalised Linear Interactive Modelling) is a package produced by NAG (Numerical Algorithms Group). Full details are given in Nelder and Wedderburn, "Generalised Linear Models", Journal of Royal Statistical Society, A, 135, p.370-384, (1972).

The program uses a maximum likelihood iterative process to fit possible relationships within the data. No significant relationships were found between any combination of genotype, egg number and generation number.

APPENDIX 3

Table 1. Relative Survivals

Author	Alcohols	Exposure	Findings
McKenzie & Parsons, 1974	Ethanol, 9%	"Newly hatched" larvae transferred	Survival order; inside cellar (40%) outside cellar (30%) far away (20%)
Briscoe et al 1974	Ethanol, 12.5%	Adults (all ages) onto medium for 24 hours	SS 45% survival FS 70% " FF 70-75% "
Morgan, 1975	Ethanol 5%; butanol 1%; isobutyl alcohol 1%; propanol 2.5% isopropanol 2%; cyclohexanol 0.5%	1st instar larvae	FF survival > SS survival in all cases, but all FF survivals also higher than control, and 2 SS survivals similar
David, 1976	First eight primary alcohols; 2 propanol, 2 butanol, 2 pentanol 2 hexanol	Adults exposed to vapour to find LC_{50} at 2 days	FF individuals only tested; LC_{50} for ethanol 16%; for 2 propanol 1.5%
McDonald & Avise, 1976	2 propanol; 1%, 2.5%, 5% and 10%	Adults exposed to medium for 2 days	Electrophoretic status of flies not given; LD_{50} 2 days 5%.
Thompson & Kaiser, 1976	Ethanol, 1%; Butanol, 0.5%	Eggs (up to 20hrs) transferred	FF (92% of control) SS (65% of control) for ethanol; FF, (67% of control) SS (37% of control) for butanol.
Oakeshott, 1976a	Ethanol	Adults transferred to medium	LD_{50} for FF individuals at 5 days 17%; for SS, 15%.
Van Delden et al 1978	Ethanol	Adults transferred to medium	LD_{50} at 5 days; FF ♂ 34% ♀ 27% FS ♂ 28% ♀ 24% SS ♂ 19% ♀ 20%
David, 1978	Ethanol, acetaldehyde	As 1976 paper	LD_{50} ethanol for FF; ♂ 13% ♀ 18% acetaldehyde for FF; ♂ 0.56% ♀ 0.52%

APPENDIX 3

Table 2. Gene frequency changes

Gibson (1970)	ethanol 6%	p_o = 0.5 for F allele after 18 gens, p = 0.78
Bijlsma-Meeles & Van Delden (1974)	ethanol 10% and 20%	On 10% ethanol, $p_o F$ = 0.2 & 0.8 after 3 gens p_F = 0.42 & 0.62 On 20% ethanol, after 5 gens, p_F = 0.65 & 0.87

Van Delden & Kamping (1975)	ethanol 15%; methanol 2.5%; butanol 2.5%; propanol & iso-propanol 2.5%; glycerol 15%; hexanol 0.5%	$p_o F$ = 0.5. After 10 gens
		hexanol p = 0.97; butanol p = 0.75;
		methanol p = .72; propanol p = 0.63;
		isopropanol p = 0.60;
		glycerol p = .42; ethanol p = 0.70;
		control, p = 0.50
		for each frequency, n = 150 individuals

Van Delden et al (1978)	ethanol 15%; other alcohols as previous paper.	$p_o F$ = 0.5. After 19 gens
		control p = 0.52; methanol p = 0.63;
		ethanol p = 0.25; propanol p = 0.59;
		isopropanol p = 0.56; butanol p = 0.
		hexanol p = 1.00; glycerol p = 0.47
		for each frequency, n = 150 individuals.

APPENDIX 3

Table 3. Biochemical characteristics

Sofer & Ursprung (1968)	k_m (no units) ethanol > cyclohexanol > isobutyl alcohol > 2 butanol > 2 propanol. used partially purified extract of enzyme from FF strain.
Vigue & Johnson (1973)	k_m for ethanol; SS 5.7mM; FS 5.0mM; FF 7.7mM. Specific activities for FF enzyme: 2 butanol > 2 propanol > 1 butanol > 1 propanol > ethanol. Specific activities for SS enzyme: 2 butanol > 2 propanol > 1 butanol > 1 propanol > ethanol.
Day, Hillier & Clarke (1974)	Specific activities for FF enzyme: 2 propanol > cyclohexanol > 1 butanol > 2 butanol > 1 propanol > ethanol. For the SS enzyme: 1 butanol ≥ 2 propanol ≥ cyclohexanol > 2 butanol > 1 propanol > ethanol.
Day & Needham (1974)	k_m for ethanol (NAD saturated) for SS_5 3.35 ± 1.65mM SS_3 13.7 ± 1.60mM
Oakeshott (1976b)	Specific activities of FF enzyme: 2 butanol > 1 pentone-3-ol > propan-2-ol > pentanol ≥ butanol ≥ hexanol ≥ propanol > 3 methyl 1 butanol ≥ methyl 2 butanol ≥ 2 methyl propanol > ethanol > methanol. Specific activities of SS enzyme: 1 pentone-3-ol > propan-2-ol ≥ 2 butanol > pentanol > hexanol ≥ butanol > propanol > 3 methyl butanol > methyl 2 butanol ≥ ethanol ≥ 2 methyl propanol > methanol.
Thatcher (1977)	k_{ms} for UF enzyme; ethanol 17.0mM; butanol 15.0mM; propanol 9.0mM; pentanol 6.0mM; 2 propanol 4.0mM; 2 butanol 3.0mM. Order of V_{max} is 2 propanol > 2 butanol > butanol > pentanol > propanol > ethanol.
Thompson & Kaiser (1976)	Activities of SS relative to FF; on ethanol 44%; butanol 55%; isopropanol 43%; cyclohexanol 65%.

APPENDIX 3

Review of the literature on relative survivals, frequency changes and biochemical characteristics of the ADH genotypes when exposed to different alcohols

A) The effect of ethanol alone

i) Relative survivals

Table 1 summarises the publications on relative survivals of the three ADH genotypes on alcohol supplemented medium (in the case of David's work, in ethanol vapour from an ethanol/sucrose solution in a closed container). Whatever concentration is used, the FF genotype is always shown to be at an advantage compared to the SS genotype in terms of survival in the presence of ethanol (Briscoe, 1974; Morgan, 1975; Thompson & Kaiser, 1976; Oakeshott, 1976a; Van Delden, Boerema & Kamping, 1978). The relative survival of the FS heterozygotes is different in each of the three studies in which it was examined, viz.

Briscoe (1974) found FF = FS > SS (Spanish population)

Oakeshott (1976a) found FS > FF > SS (Australian population)

Van Delden *et al* (1978) found FF > FS > SS (Dutch population)

Oakeshott (1976a) showed that young flies (0 to 2 days after eclosion) were far less sensitive to 20% ethanol in the medium (giving around 90% survival after 5 days) than were older flies (9-11 days after eclosion) which gave around 10% survival after 5 days. Dunn *et al* (1969) found peaks in ADH production at 4 and 9 days after eclosion. Hewitt (1974) found a peak at 3 days and Ward (1975) at 8 days. Oakeshott (1976b) found the highest adult activity at 34 days post. eclosion. The activity profile of ADH does not seem particularly consonant with the difference in survival at different ages found by

Oakeshott.

Further, Bijlsma-Meeles (1979) has shown that ADH activity is apparently induced in eggs placed on ethanol containing medium, but that irrespective of this, younger eggs give a much higher survival to adults than older eggs or larvae.

Of the authors listed in Table 2, Briscoe et al (1974) and McDonald & Avise (1976) used adults of unspecified age; Oakeshott (1976a), Van Delden et al (1978) and David (1976, 1978) used adults aged between 3 and 5 days; the others used eggs or larvae of varying ages (not always specified) placed on the ethanol supplemented medium.

Only Van Delden et al (1978) and Oakeshott (1976a) used the same method of exposure, time period for survival and age of flies. All the other authors differ in respect of 2 of these variables if not all three. Comparison of results is therefore very difficult. However, it is clear that the reported range of sensitivities to ethanol is very large. Considering only experiments where adult flies were placed in containers with ethanol supplemented medium, (Briscoe et al., 1974; Oakeshott, 1976a; Van Delden et al., 1978) or ethanol vapour (David, 1976, 1978) then for FF flies the order of sensitivity is

Briscoe,	12.5%	ethanol	1	day	exposure	75%	survival
David, 1976	16%	"	2	day	exposure	50%	"
Oakeshott, 1976a	17%	"	5	"	"	50%	"
Van Delden, 1978	30%	"	5	"	"	50%	"

If one uses the product of ethanol concentration, number of days exposure and percentage survival as being some indicator of sensitivity, then the Spanish "Mancha" population tested by Briscoe et al., is ten times more sensitive than the Dutch "Groningen" population used by Van Delden et al. Both have a history of exposure to

environmental ethanol, since the "Mancha" flies were collected around Spanish vineyards, and the "Groningen" population is maintained on a medium containing 1% ethanol (it is added as a solvent for 'nipagin', an antifungal agent). Several authors have suggested that ethanol resistance is affected by other loci in addition to the ADH locus (e.g. McKenzie & Parsons, 1974; Ward, 1975; Barnes & Birley, 1978; McDonald & Ayala, 1978). It is possible that the Groningen population has been selected for particular alleles at these other loci, making it more resistant to ethanol.

In view of Bijlsma Meeles' recent (1979) demonstration that egg age appears to affect survival on ethanol medium, it is not surprising that the experiments using eggs or larvae of known genotype placed on ethanol supplemented medium are inconsistent and difficult to compare. Thompson and Kaiser (1976) put eggs of mixed ages up to 20hrs onto medium containing 1% ethanol. They used a potato/dextrose medium rather than agar/cornmeal because it is easier to mix alcohol into the former. The control viability of FF and SS genotypes were 45% and 43%; on ethanol supplemented medium viabilities were 43% and 33% i.e. 92% and 65% of the controls respectively. The result was significant. The temperature at which the experiment was performed is not given. The eclosing flies counted were all those that had emerged by the 17th day. 800 eggs per genotype per treatment were initiated.

Morgan (1975) placed larvae which had newly emerged (0 to 3hrs from hatching) onto standard cornmeal/agar medium containing 5% ethanol. Whereas Thompson & Kaiser put 50 eggs of one genotype into a single vial, Morgan put 100 each of FF and SS genotypes into a single vial. The control viabilities under these more crowded conditions were 15% for FF and 12% for SS genotypes; on ethanol supplemented medium they became 20.8% and 4.3% i.e. 138% and 35% of the control values

respectively. The conditions of the experiment, being more crowded, were such as to induce competition, and D. melanogaster can apparently utilise ethanol as an energy source (David, 1978). This may have contributed to the anomalous relationship of the FF survival values on control and ethanol supplemented medium.

Bijlsma Meeles (1979) transferred eggs aged 2, 6, 10, 14 and 18 hours to medium containing 12% ethanol at 25°C. 50 eggs of a single genotype were placed in each vial. As the age of the eggs increased, significantly fewer of the SS genotypes emerged and the viability of all genotypes became lower (average viability of 2hrs old eggs - 60%; of 18hrs old eggs, 9%). Viability of larvae transferred to ethanol medium was even lower (78hr old larvae; average viability 7 to 8%). If eggs were laid directly onto ethanol medium, average viability was around 45%. When the ADH activity of the eggs was measured, an increase in activity in eggs which had been exposed to ethanol was found. However, exposed SS eggs, despite this increase, still had a lower ADH activity than unexposed FF eggs, and yet survived much better in the ethanol medium. Bijlsma Meeles concludes that while the presence of environmental ethanol affects relative fitnesses at the ADH locus, care must be taken in interpreting results of this type of experiment, since ethanol resistance in Drosophila eggs and larvae is not dependent solely on ADH activity.

This supports the conclusions of McKenzie & Parsons (1974), who transferred "newly emerged" larvae onto medium containing 9% ethanol; they used 25 larvae per vial, and maintained them at 20°C. They compared three strains; one from inside a wine cellar; one from just outside it and one from a considerable distance away from any winerys. The average emergence from 200 larvae from each of these three strains

were 40% : 30% : 16%, showing that some selection for ethanol resistance has occurred in and around the wine cellar. However, the three strains were not found to differ significantly in allele frequencies at the ADH locus.

The overall conclusions that can be drawn from experiments of this type are rather limited because there is extensive use of inbred lines, and experimental conditions are invariably not comparable. It is clear that the addition of ethanol to the Drosophila environment usually reduces viability at all stages of the life cycle; under such conditions the SS genotype is usually at a disadvantage. No firm conclusions can be drawn as to the dominance relationships between the genotypes with respect to fitness on ethanol; they appear to be different in different stocks. It is also apparent that ADH activity is not the only factor determining ethanol tolerance in D. melanogaster.

ii) Changes in gene frequency at the ADH locus after exposure to ethanol

Table 2 summarises the published experiments on perturbation of the ADH locus with alcohols. The work on ethanol shows less heterogeneity than the survival experiments just described. Gibson (1970), Van Delden et al (1975) and Van Delden et al (1978) used ½pt milk bottles; Cavener and Clegg (1978) used a vial type population cage and Bijlsma Meeles and Van Delden (1974) used vials. In all of Van Delden's work, the relative humidity was controlled at 60%, whereas other authors have not reported any humidity control.

Gibson (1970) used medium supplemented with 6% ethanol. After 18 generations, the frequency of the F allele had risen from 0.5 to 0.78. He does not give the standard errors on these frequencies, nor had he any control.

Cavener & Clegg (1978) used a slightly higher concentration, 10%, of ethanol in the medium. The frequency of the ADH F allele was 0.33 ± .032 initially, and after 18 generations it reached 0.62 ± .044.

Bijlsma-Meeles & Van Delden (1974) used 10% and 20% ethanol concentrations. They used extracted lines to establish vials with either a frequency of ADH F of 0.2 or 0.8. After 3 generations on 10% ethanol medium, the frequencies were 0.42 ±.054 and 0.62 ±.062 respectively. On 20% ethanol, the direction of movement was different; after 5 generations, the vials with initial frequency of 0.2 were not much different from those on 10% ethanol, with a frequency 0.42 ±.031, but the 0.8 had risen to 0.87 ±.022. Further, the frequencies in the control populations also changed, from 0.2 and 0.8 to 0.42 ±.031 and 0.65 ±.031. It is likely that the effects of ethanol in these experiments were overlayed by selection at loci other than the ADH locus, the former having been brought into disequilibrium with the latter in the process of extracting the original lines.

Van Delden *et al* (1975), again using extracted lines, established bottle populations with a frequency of ADH F of 0.5. After 10 generations on 15% ethanol supplemented medium, the frequency had risen to 0.7 ± .037, while the control remained at 0.5 ± .041. The difference is highly sig.

Van Delden et al (1978) repeated experiments under the same conditions; after 20 generations on 15% ethanol supplemented medium the frequency of the F allele had risen from 0.5 to 0.93 ± .021, whereas the control had risen to 0.61 ± 0.040. In another experiment, after 19 generations the rise was to 0.75 ±.035, while the control moved to 0.52 ± .041. In both these cases, the difference between

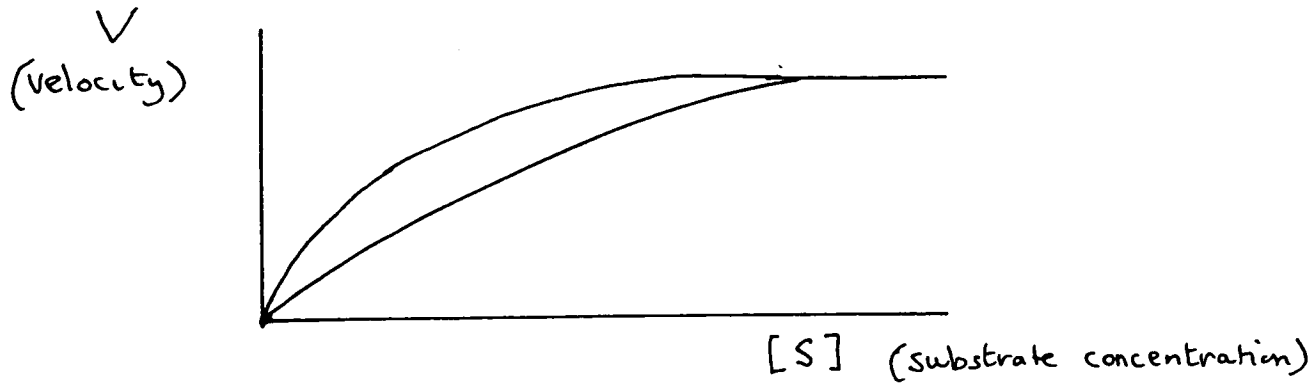
the ethanol supplemented bottles and the control bottles is highly significant. However, there appears to be a considerable discrepancy in the response of the ADH locus in the two experiments.

These five papers indicate clearly that allele frequencies at the ADH locus can be altered by the introduction of ethanol, which causes an increase in the frequency of the F allele. None of the experiments record fixation of the F allele under such conditions. The magnitude of the increase and the behaviour of the controls are variable for the Groningen population used by Van Delden (which is the only population studied in any detail). In the experiments of Bijlsma-Meeles & Van Delden (1974), the population used was constructed from only 10 original isofemale lines (5 SS and 5 FF), which would involve the setting up of a large amount of linkage disequilibrium. In view of the evidence for the involvement of other loci in ethanol tolerance (McKenzie & Parsons, 1974; Ward, 1975; Barnes & Birley, 1978; McDonald and Ayala, 1978; Bijlsma Meeles, 1979) it will be necessary to examine the behaviour of the ADH alleles on ethanol medium when they are in a genetic background defined with respect to these other loci. Until this is possible, more precise information on the magnitude of frequency changes at the ADH locus induced by environmental ethanol will not become available.

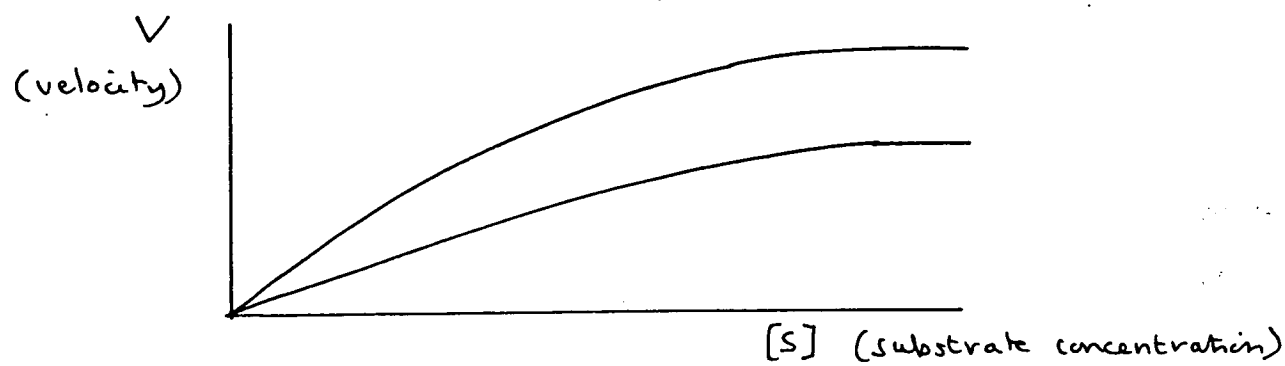
iii) Biochemical characteristics of the enzymes produced by the different ADH alleles when ethanol is the substrate

Table 3 summarises publications on the biochemical parameters of the three enzymes. Only 4 authors have measured K_m values at all. The others rely on "specific activity". Depending on the concentrations involved, a difference in *in vitro* specific activity may imply a difference in K_m, or V_{max}, or both.

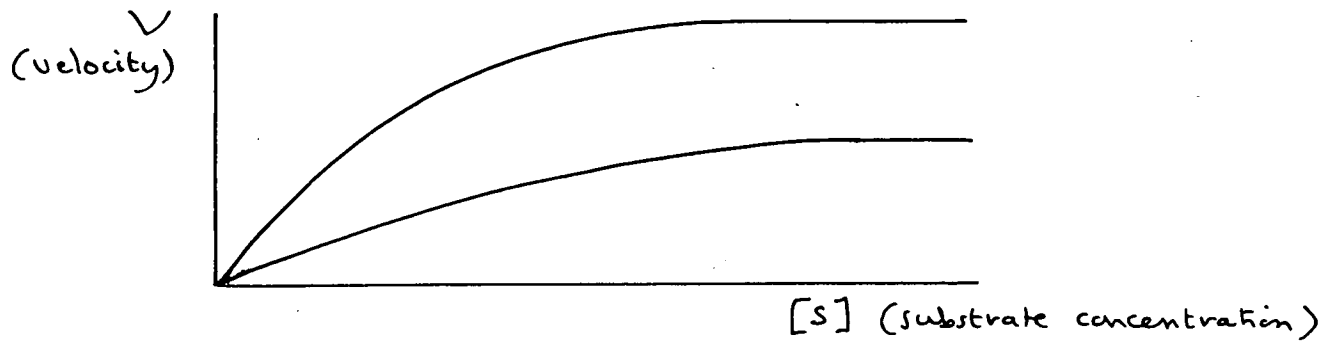
e.g. 1. Different K_{ms}; same V_{max}



2. Different V_{max}; same K_m



3. Different V_{max} and different K_m



Whether or not the enzymes will differ in their behaviour <u>in vivo</u> depends on the concentrations at which the specific activity was measured; a difference in K_m may not be effective if the substrate is always at saturated concentrations and the V_{max} is the same.

However, most of the studies to be described used high concentrations of substrate, so that differences reported are likely to represent differences in V_{max} rather than K_m.

Gibson (1970) gives no details of his assay; he gives a ratio of activities of 8.5 : 5.4 : 2.9 for enzyme from FF, FS and SS individuals respectively.

Thompson & Kaiser (1976) used a crude homogenate of whole flies; they used an assay mixture of total volume 2.53ml of which 0.03ml was ethanol. He does not give the NAD concentration. Under these conditions, SS flies had 44% of the activity of FF flies.

Oakeshott (1976b) used partially deyeasted flies, and used the supernatant from a spun down crude homogenate as his source of enzyme. He used a concentration of 0.015M ethanol, and 0.009M NAD. He gives a ratio of activities of 23 : 15 : 10 for FF, FS and SS extracts respectively.

Day, Hillier and Clarke (1974a) used deyeasted flies, and a partially purified enzyme preparation, which was adjusted 'so that ADH activity was in the range in which it is known to be proportional to enzyme concentration'. The meaning of this is not clear. The concentration of ethanol was 0.013M, and of NAD 0.00013M (nearly a hundredth of the concentration Oakeshott used). At 25°C, the ratio of activities was 0.09 : 0.07 : 0.03 for FF, FS and SS extracts respectively.

Day & Needham (1974) measured the K_{ms} for ethanol of two of the conformers of the enzyme from SS individuals. (Differential binding of NAD gives rise to 5 different conformers of enzyme from each of the two kinds of homozygotes; these can be seen as three bands under electrophoresis (Jacobsen et al., 1972). Crude extracts of enzyme

will contain a mixture of all 5).

They prepared their extracts by isoelectric focussing. In the presence of saturated NAD (> .00001M) the K_m, for ethanol were 3.35 ± 1.65mM ethanol for the S_5 enzyme, and 13.7 ± 1.60mM ethanol for the S_3 enzyme.

Vigue & Johnson (1973) purified enzyme from FF, FS and SS individuals on DEAE cellulose and hydroxyapatite columns, but they did not use isoelectric focussing. With an NAD concentration of 0.005M, the K_{ms} for ethanol were 7.7mM for FF extract; 5.0mM for FS extract and 5.7mM for SS extract. The K_m value measures the affinity of the enzyme for its substrate; it appears from these results that the FF enzyme has a lower affinity for ethanol than the FS or SS enzyme. This is confirmed by Clarke (1975). The differences in specific activity could then be due to differences in V_{max}, since they are in the opposite direction to the differences in K_m.

Sofer & Ursprung (1968) obtained Lineweaver Burke plots for enzyme partially purified from FF individuals. However, they do not report the NAD concentration used, and there are no units on their graphs, making it impossible to estimate the K_m or V_{max} for ethanol. If one assumes the units to be molarities, K_m ethanol is around 5mM, which is the same order of magnitude as Vigne & Johnson's finding. No errors are given.

Thatcher (1977) measured the K_m ethanol for enzyme from 'ultra fast' homozygotes from the Mancha population. (UF differs from the usual ADH F by the substitution of aspartine for alanine in one tryptic peptide (Thatcher & Canfield, 1977)). He used 0.001M NAD and gives a K_m ethanol of 17.0mM, with no standard error. He notes the extreme susceptibility of the F extract to protease activity, and his

purification procedure was designed to eliminate proteinase contamination early on.

Other authors have not taken this precaution, and if the F and S alleles are also proteinase sensitive, the differences in specific activity observed by them may be due not only to different V_{max}'s, but also to different sensitivities to proteinases, or to genetic variation in the proteinases themselves (since most authors have not used isogenic lines for biochemical work, but extracted lines).

Overall, it would appear that the use of 'specific activities' of crude preparations is not particularly informative, and it may be misleading. In spite of the apparent plethora of work on the subject, only one paper has compared the K_{ms} of enzymes from the three standard ADH genotypes, (Vigue & Johnson, 1973) and the authors do not quote standard errors. The only thorough purification, with appropriate checks, appears to have been done on a relatively rare allele, UF, (Thatcher, 1977). A curious relationship exists between the order of specific activities and the order of the K_m values for enzymes from the three genotypes, which may be due to i) the enzymes having large differences in V_{max}, ii) the specific activities differing because of differential sensitivity to proteinases of the three enzymes iii) the specific activities differing because the extracted, homozygous lines used have the ADH locus in disequilibrium with proteinase loci. It is not possible to say whether the enzymes differ <u>in vivo</u>, since no measurements have been made of the alcohol concentrations or free NAD concentrations in Drosophila on ethanol medium. If, for arguments sake, they are very low, at rate limiting values, then the order of activity would be the order of the K_m values, the reverse of the order of specific activities. Since V_{max} values are not known, and since the proteinases may be having effects, further conjectures about the

in vivo relationships of the enzymes are not feasible.

It would be over-optimistic to claim that the biochemical underpinnings of the ADH polymorphism have been exposed.

B) The effects of alcohols other than ethanol (including pentyn-3-ol and butan-2-ol as described in this thesis)

i) Relative survivals

Two authors have examined differential survival of ADH genotypes on alcohols other than ethanol. Morgan (1975) used 1st instar larvae from extracted lines, and transferred them to vials supplemented with one of each of the following; butanol, propanol, isopropanol, cyclohexanol and isobutyl alcohol (2 methyl 1 propanol in UIPAC system) at the following concentrations; 1% butanol, 2.5% propanol, 2.0% isopropanol, 0.5% cyclohexanol, 1% isobutyl alcohol. In his paper he attempts to correlate the relative survivals found with the relative specific activity of the enzymes as measured on these alcohols by Day, Hillier and Clarke (1974). He quotes Day *et al.* as having used isobutyl alochol, but Day's paper does not list it. Isobutanol was used (2 butanol in IUPAC system) by Day *et al.*, so that in this case, Morgan's comparison is not valid. For each alcohol, Morgan found that FF individuals survived better than SS individuals. However, for four alcohols (cyclohexanol, isobutyl alcohol, n-propanol and isopropanol) he reported total survivals higher than control survivals on medium not supplemented with any alcohols. The explanation for this could be similar to that considered for his results on survival on ethanol, or it could be due to some other unknown factor.

Thompson & Kaiser (1976) transferred eggs of up to 20hrs to medium containing 0.5% butanol; more FF individuals survived than SS individuals (68% and 37% of control values respectively. This was less than the survival they found on ethanol at 1%; 92% and 65%

of control values respectively).

In Chapter 3 of this thesis, results of survival experiments on pentyn-3-ol and butan-2-ol are given. Eggs up to 20hrs old were transferred to vials containing varying concentrations of pentyn-3-ol. The concentration necessary to produce 50% of control emergence was between 0.055 and 0.072%, but there was considerable heterogeneity between vials. Use of different media did not improve this. The adult stage seems more sensitive, only 9% of adults of mixed ages surviving for 4 days after transfer to 0.05% pentyn-3-ol supplemented medium. Because of the heterogenous survival values, relative survivals of FF, FS and SS types on this alcohol were not examined. Morgan (Ph.D. thesis) has reported similar problems.

Adult survival was tested on butan-2-ol, and 50% of adult flies survived 4 days on 1.5% butan-2-ol supplemented medium. There was none of the heterogeneity seen with the pentyn-3-ol, and the butan-2-ol was then tested in experiments to perturb the gene frequency at the ADH locus.

It can be seen that relatively little work has been done on examining the survival of Drosophila on exotic alcohols, and no work has been published which could be directly compared with the results of Chapter 3. The latter seems to bear out the results of Thompson & Kaiser in that alcohols higher than ethanol appear to be toxic to Drosophila melanogaster; Morgan's results are equivocal in this respect.

ii) Gene frequency changes induced by alcohols other than ethanol

Van Delden & Kamping (1975) examined gene frequency changes in bottle populations maintained on supplemented media for 10 generations (see Table 2). All populations were started at a frequency of 0.5 for ADH F allele, using extracted lines. After 10 generations, the population on 0.5% hexanol was nearly fixed for ADH F; all the others were still segregating. The control frequency was 0.5 ± .028, with no change. The frequency on methanol had risen to 0.72 ± .026, which is interesting, since ADH is said to be relatively inactive on methanol (Day, Hillier & Clarke, 1974; David, 1978). Both propanol and iso-propanol bottles gave similar increases in frequency, to 0.63 ± .028 and 0.60 ± .028 respectively. This is also interesting, because ADH is said to have a much higher affinity for secondary than for primary alcohols (Sofer & Ursprung, 1968), but this is apparently not reflected in Van Delden's results.

Van Delden et al (1978) performed similar experiments, using lines extracted from the same Groningen base population. They used the same alcohols and concentrations, and obtained similar results, except that after 19 generations the hexanol population became fixed for ADH F, and the methanol population frequency had only risen to 0.63 ± .028, which did not differ significantly from the control frequency of 0.52 ± .028. In both these sets of experiments, butanol (2.5%) caused larger frequency rises than ethanol (15%) although the differences between them are not significant.

The results of Chapter 4 show that butan-2-ol is capable of exerting a strong selective force at the ADH locus, stronger than any of the effects obtained by Van Delden apart from that of hexanol. This may be in part because Van Delden used extracted lines, and may have set up linkage disequilibria between the ADH locus and other loci

affecting resistance to alcohols, such that the effect on the ADH allele frequencies was moderated by their linkage to these other alleles. Further, since the Groningen population has been maintained on 1% alcohol for many generations, it may have become fixed for alleles affecting resistance to alcohols in general, not just ethanol. Under the conditions of the experiments in Chapter 4, and in the Malawi population, use of butan-2-ol appears to be an effective way to perturb the ADH locus.

iii) Biochemical characteristics of the different ADH enzymes with alcohols other than ethanol

Table 3 contains a summary of the measurements of ADH characteristics on different substrates. Sofar & Ursprung (1968) give Lineweaver Burke plots without units, from which it can only be concluded that the secondary alcohols butan-2-ol, propan-2-ol and cyclohexanol have lower K_{ms} and higher V_{max}'s than ethanol or isobutyl alcohol when a partially purified extract from FF individuals is used. The secondary alcohols also appeared to exhibit substrate activation at high substrate concentrations. Thatcher (1977) used enzyme extracted from individuals homozygous for the UF allele. The order of K_{ms} was ethanol > 1 butanol > 1 propanol > 1 pentanol > propan-2-ol > butan-2-ol. The order of V_{max} values was propan-2-ol > butan-2-ol > butan-2-ol > 1 pentanol > 1 propanol > ethanol. It appears that for the UF enzyme too, activity is greatest on secondary alcohols.

Vigue & Johnson (1973), Day et al (1974) and Oakeshott (1976b) have compared specific activities of different ADH enzymes (mostly from FF and SS individuals) on different alcohols. Their findings do not differ greatly from those of Thatcher (1977) and Sofer & Ursprung (1968) and in view of the unsatisfactory nature of specific activity as a measurement, detailed discussion of their results is unlikely to add

very much. A note may be made of the work by Thompson & Kaiser (1976), who compare the activity of SS enzymes on different alochols relative to the FF enzyme. The figures they quote do not represent specific activities, since they added a fixed volume of each alcohol, not a fixed concentration, to their reaction mixtures. The relationships given are therefore for different concentrations of each of the alcohols concerned.

It can be clearly seen that there is even less information on the behaviour of the ADH enzymes with alcohols other than ethanol. Since higher alcohols might be found in the fermenting vegetable matter supporting the yeasts on which Drosophila feed, and since they are more toxic than ethanol, this seems regrettable.

In view of the large number of publications reviewed in this appendix (over 20), the general paucity of incontrovertible results seems to reflect adversely on the biochemical methodology adopted by many population geneticists.

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