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Polymorphism at the G6PD and 6PGD loci in *Drosophila melanogaster*

I. Evidence for selection in experimental populations

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SUMMARY

Gene frequency changes were followed in 17 experimental cage populations of *Drosophila melanogaster* polymorphic for the two loci G6PD and 6PGD. From these cages it was observed that selection was favouring the *F* alleles of both loci when the cages were started with low frequencies of these alleles (0·20*F* and 0·50*F*). A viability experiment and an extinction experiment also provided evidence that the *F* alleles of both loci were favoured. It is argued that the observed selection is not due to selective differences in genetic background of the different alleles, but is acting on the isozyme loci themselves.

1. INTRODUCTION

One of the major questions in population genetics at present concerns the maintenance of the great amount of genetic variation at the molecular level found in populations of most species studied (review by Lewontin, 1974). Some authors (Kimura, 1968; Kimura & Ohta, 1971; King & Jukes, 1969) have argued that this variation is for the greater part a product of mutation, drift and migration of neutral variants. This view is extensively criticized by others (Clark, 1970; Ayala, 1972) who stress the importance of selection affecting allozyme variation. Most evidence in favour of the action of selection is coming from studies on geographical variation (Ayala, 1972; Prakash, Lewontin & Hubby, 1969). Direct experimental data, however, are scarce. Yamazaki (1971) in his accurate experiments found no selective differences between the esterase-5 variants of *D. pseudoobscura*.

On the other hand for the Adh-locus in *D. melanogaster* selective differences between the genotypes were found when ethanol, a substrate of this enzyme, was added to the food (Bijlsma-Meeles & Van Delden, 1974), and also when the food was supplemented with other alcohols (Van Delden, Kamping & Van Dijk, 1975). This relation between selection and the substrate of the enzyme, which renders the possibility that the selection is acting by means of linked loci highly improbable, was also found in the case of the amylase-locus in *D. melanogaster* (De Jong *et al.* 1972; De Jong & Scharloo, 1976).

The present study deals with the polymorphic loci coding for the enzymes

glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44). Both enzymes are biochemically linked in the same biochemical pathway, the pentose phosphate pathway, which is an alternate pathway for the degradation of hexoses. Its major function is probably the production of NADPH necessary for fatty acid synthesis (Newsholme & Start, 1973).

In *D. melanogaster* both enzymes are X-linked and the genes are located at 63 for G6PD and 0·9 for 6PGD (Young *et al.* 1964; Young, 1966). For both loci a fast (*F*) and a slow (*S*) allele are known. For a detailed description of the genetical and biochemical aspects of the two enzymes see Dickinson & Sullivan (1975).

This paper presents the results of an investigation into the behaviour of the polymorphism at these two loci. The pattern of variation at the two loci was studied by determining the gene frequencies in a number of cage populations. Furthermore a number of experiments were done to establish whether these two loci are subject to selection or not. This was done by following gene frequency changes in nineteen experimental cage populations. In addition to this experiment, viability tests and extinction experiments were done in a variety of different environments. In order to relate the results of these experiments to the biochemical action of the two enzymes also different food media were used. Because the pentose phosphate pathway is biochemically linked with glycolysis different kinds of sugars were added to the food, and because of its link with the fatty acid cycle a fatty acid (sodium octanoate) was also added to the food.

MATERIALS AND METHODS

Culture method

Flies were usually raised in half-pint bottles at 25 °C and 50–70 % R.H. on standard food consisting of 1000 ml water, 19 g agar, 32 g dead yeast, 54 g sucrose and 13 ml nipagine solution (10 g nipagine in 100 ml ethanol 96 %).

Stocks

The experiments were started with two strains; the Tuscaloosa strain being homozygous *S* for G6PD and homozygous *F* for 6PGD and the Oregon-R strain being homozygous *F* for G6PD and homozygous *S* for 6PGD, respectively. The strains were kindly supplied by Dr J. W. Young.

Notation

Throughout the paper the following notation is used for the genotypes. The first two letters indicate the alleles of the G6PD-locus and the following two letters refer to the 6PGD-locus. So $\frac{FS}{FF}$ is homozygous *F* for G6PD and heterozygous for 6PGD.

Experimental populations

The cage populations are named after their frequencies of the *F* alleles at both loci, starting with the G6PD-locus; thus a cage population with initial frequencies

of $0{\cdot}20F$ and $0{\cdot}80F$ for G6PD and 6PGD is named 20×80. (For description and construction of the cages see Beardmore *et al.* 1963.)

With the two strains three cage populations were started with 500 females and 500 males each: 20×80 I, 50×50 I and 80×20 I. Six months after the establishment of the 50×50 I cage population, new homozygous lines were re-isolated from this cage by means of single-pair mating, each line thus containing three independent X-chromosomes. Ten lines of each possible homozygous genotype ($\frac{FF}{FF}$, $\frac{FS}{FS}$, $\frac{SF}{SF}$ and $\frac{SS}{SS}$) were isolated in this way. With these lines a second set of cage populations was established; 20×80 II, 50×50 II, 80×20 II, 50×100 II, 50×0 II, 100×50 II and 0×50 II. These cages, and also the cages of the third set (see below), were started in linkage equilibrium with 500 females and 500 males each, and equal numbers of each isolated line were used of the homozygous genotype necessary to establish the initial gene frequency.

Two years after the establishment of the 50×50 I cage population new homozygous lines were again re-isolated from this cage. This time 15 lines of each homozygous genotype were isolated. This was done in such a way that each line contained one single X-chromosome of the 50×50 I cage population. With these new lines a third set of cage populations was established; 80×80 III, 80×20 III, 50×50 III, 20×80 III, 20×20 III, 50×100 III, 50×0 III, 100×50 III and 0×50 III. From these cages samples were taken to determine the gene frequencies. Sampling was done by inserting two fresh food cups in each cage, and flies were then allowed to lay eggs for one day. The emerging adults from these two cups were collected for three weeks and mixed together.

All the following experiments were done with the 40 lines of the first re-isolation.

Egg-to-adult survival

In order to measure egg-to-adult survival eggs were collected from each of the four homozygous genotypes. All ten lines of a given genotype were mixed together. One hundred eggs of a given genotype were put into each vial. This was done in eight different environments with six replicates of each genotype in each environment. At the end of the experiment the total number of emerged adults was counted. The environments were as follows.

(1) Control: normal food, temperature 25 °C and 50–70 % R.H.

(2) Glucose 1 %: as (1) but the 5·4 % sucrose of the normal food is replaced by 1 % glucose.

(3) Maltose 1 %: as (1) but the sucrose is replaced by 1 % maltose.

(4) Lactose 1 %: as (1) but the sucrose is replaced by 1 % lactose.

(5) Lactose 0·5 %: as (1) but the sucrose is replaced by 0·5 % lactose.

(6) No sugar: as (1) but the sucrose is omitted from the food.

(7) Yeast 1·6 %: as (1) but with half the normal amount of yeast.

(8) 29·8 °C: as (1) but temperature 29·8 °C instead of 25 °C.

In all the environments the flies were reared under crowding conditions (100 eggs on 1 ml food).

F_2-experiment

Eggs from the cross $\frac{FS}{SF} \times \frac{FS}{\ }$ were collected and transferred to half-pint bottles with 25 ml food; 250 eggs in each bottle to prevent crowding. The total male offspring from each bottle was collected and the genotypes in a sample of 70 males were determined. This was done in eight different environments, as follows.

(1) Control: normal food, temperature 25 °C and 50–70 % R.H.

(2) 17 °C: as (1) but temperature 17 °C instead of 25 °C.

(3) 29·5 °C: as (1) but temperature 29·5 °C instead of 25 °C.

(4) 13° ~ 29·5 °C: as (1) but a daily fluctuating temperature (8 h 13 °C and 16 h 29·5 °C) instead of a constant temperature of 25 °C.

(5) 35 % R.H.: as (1) but 35 % R.H.

(6) 90 % R.H.: as (1) but 90 % R.H.

(7) Lactose 1 %: as (1) but the sucrose is replaced by 1 % lactose.

(8) Maltose 1 %: as (1) but the sucrose is replaced by 1 % maltose.

Extinction experiment

In this experiment nine different population types were established. Four of them were started with flies homozygous for both loci ($\frac{FF}{FF}$, $\frac{FS}{FS}$, $\frac{SF}{SF}$ and $\frac{SS}{SS}$), four with flies homozygous for one locus and heterozygous for the other ($\frac{FF}{SF}$, $\frac{FS}{SS}$, $\frac{FF}{FS}$ and $\frac{FS}{SS}$) and one with flies heterozygous for both loci (a mixture of $\frac{FF}{SS}$ and $\frac{FS}{SF}$). The polymorphic populations were started with an initial gene frequency of 0·50 at both loci.

Fifty vial populations were established of each population type. The parents were discarded after seven days and after fourteen days the offspring was transferred to new vials without mixing between the vials. This was done for twenty generations, and each generation the number of vials giving no adult offspring at the time of transfer was counted. This was done for three environments.

(1) Control: normal food, 25 °C and 50–70 % R.H.

(2) Lactose 1 %: food in which the 5·4 % sucrose is replaced by 1 % lactose.

(3) Sodium octanoate: normal food supplemented with 0·08 % Na-octanoate.

Electrophoresis

Both enzymes were run in horizontal acrylamide gels. The tank buffer was 0·15 M-Tris/citric acid, pH 7·5 supplemented with 0·005 M-EDTA. The gel contained 5 % acrylamide in ten-times-diluted tank buffer. Per 100 ml gel solution 2 mg NADP was added. Electrophoresis was carried out for 2½ h (200 V and 40 mA). The gels were stained in a 0·1 M-Tris/HCl buffer, pH 7·5, containing 0·01 M-$MgCl_2$. To 100 ml staining buffer 15 mg NADP, 15 mg MTT, 15 mg substrate (G6P or 6PG) and 2 mg PMS were added. The genotype of each fly for both loci was determined.

RESULTS

Laboratory populations

To gain an insight into the pattern of variation for the two loci under study the gene frequencies were determined for eleven randomly chosen cage populations. All these populations were started with flies caught in the wild and maintained

Table 1. *Gene frequency of the F allele of G6PD and 6PGD in eleven cage populations (these data were obtained in* 1972)

Population	Origin of the populations	Kept as cage population since	G6PD *F* allele	6PGD *F* allele
Bogota	Colombia	1965	0·32	0·95
Curaçao	Netherlands Antilles	1971	0·22	0·88
Kaduna	Nigeria	1949	0·49	1·00
Groningen 67	Netherlands	1967	0·95	1·00
Groningen 69	Netherlands	1969	0·88	0·81
Groningen κ 71	Netherlands	1971	0·81	1·00
Haren	Netherlands	1971	0·90	1·00
Loenen	Netherlands	1971	0·86	0·97
Israel	Israel	1970	0·96	1·00
Pacific	USA	1956	0·72	1·00
Evanston	USA	1969	0·69	0·00

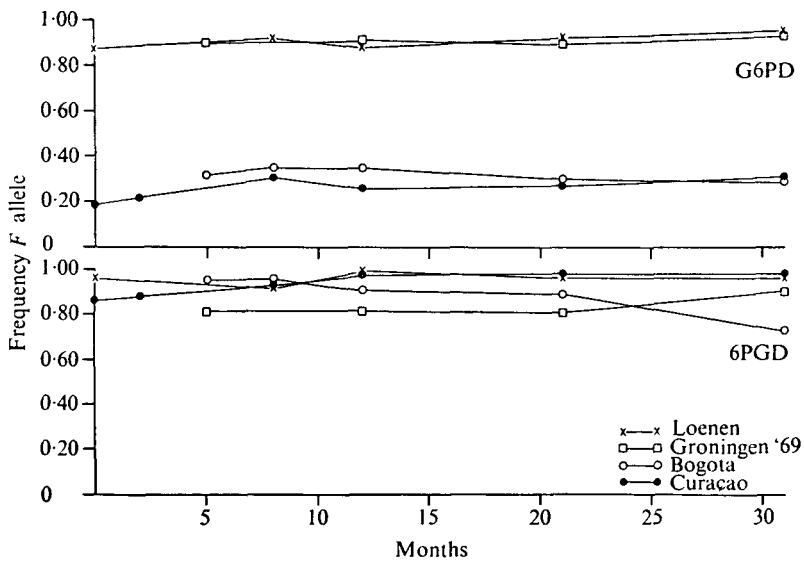

Fig. 1. Changes in *F* frequency in the four laboratory populations polymorphic for both loci (top figure G6PD, bottom figure 6PGD).

in the laboratory as cage populations for a variable period. Table 1 shows the gene frequencies of the *F* alleles found in the populations. As can be seen, all populations are polymorphic for G6PD, with allele frequencies ranging from 0·96*F* to 0·22*F*.

There is an indication of geographical differences. The northern populations

from Holland have a high F frequency whereas the most southern populations (Kaduna, Curaçao and Bogota) have a lower frequency. The degree of polymorphism for 6PGD is totally different from G6PD. Most populations are fixed for the F allele, or segregating with high frequencies of the F allele. The only exception is the Evanston population which is fixed for the S allele. In Fig. 1 it can be seen that the gene frequencies in the four populations segregating for both enzymes are fairly stable over the period tested.

Experimental populations

Figs 2–4 show the changes in gene frequency for both loci in the experimental populations originating from the two original strains. For clarity, confidence intervals are not shown in the figures. With a few exceptions the sample sizes were

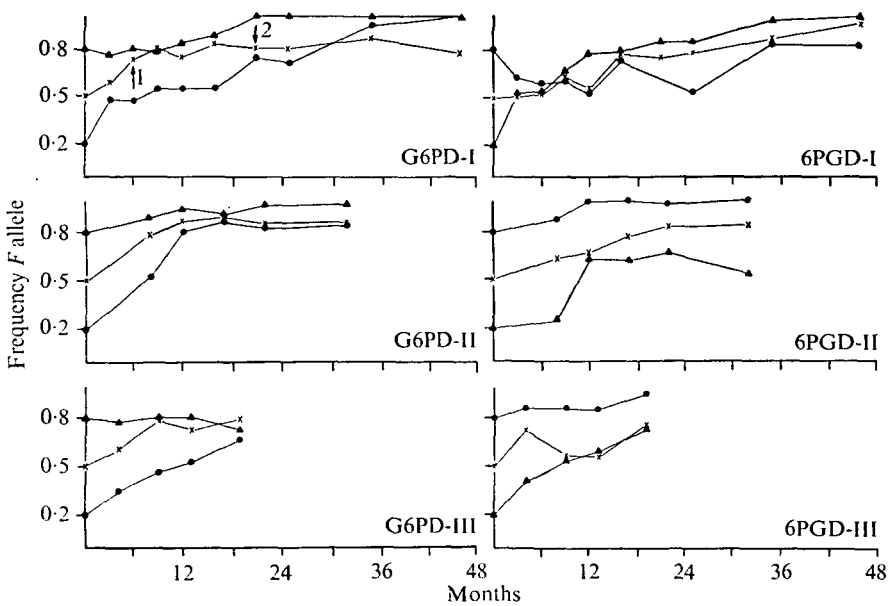

Fig. 2. Changes in F frequency at both loci in the experimental cage populations present in all three sets. The two arrows indicate the points of the first (1) and second (2) re-isolation of homozygous lines. ▲—▲, 80×20; ×—×, 50×50; ●—●, 20×80.

200 genes, so the confidence interval did not exceed $\pm 0{\cdot}07$. Fig. 2 shows the changes in gene frequency of the cages present in all three sets: 80×20, 50×50 and 20×80. It is quite clear that there is an increase in the frequency of the F allele of both loci in the cages founded with the two original strains (G6PD-I and 6PGD-I). It is also clear that the changes are qualitatively the same in the second and third set of cages (G6PD-II and 6PGD-II; G6PD-III and 6PGD-III). This indicates that randomizing of the background does not much alter the changes in gene frequency. In all cages there is an increase in the F frequency to

0·80–0·90 for both loci. Figs 3 and 4 show how the change in gene frequency at one locus depends on the initial gene frequency at the other locus. In Fig. 3 all cages shown were started with an initial gene frequency of 0·50, while Fig. 4 shows the cages with an initial frequency of 0·20 and 0·80. It appears that some

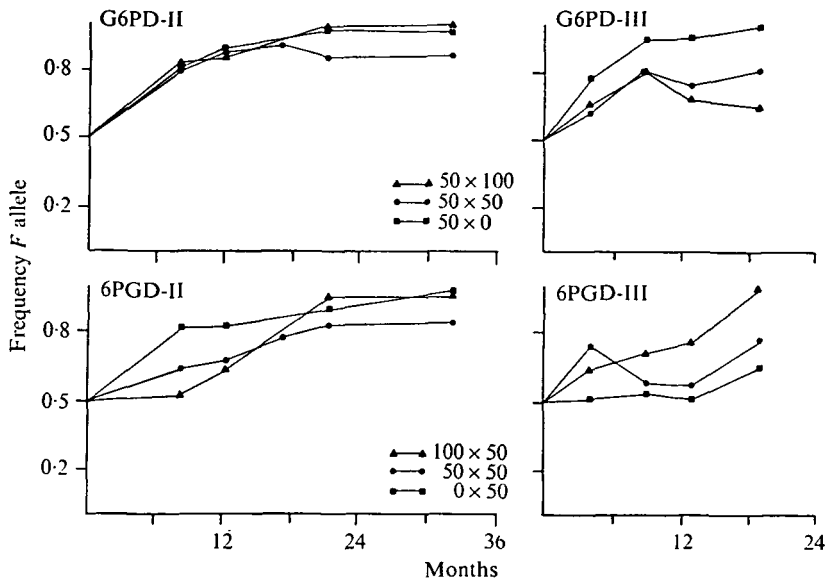

Fig. 3. Changes in F frequency at both loci in the second and third set experimental cage populations (top figures G6PD, bottom figures 6PGD) started at 0·50 F, with different initial frequencies (1; 0·50; 0) at the other locus.

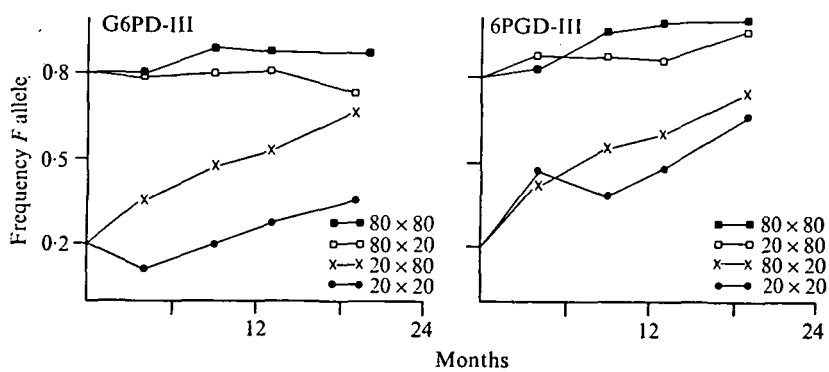

Fig. 4. Changes in F frequency at both loci in the experimental cage populations (third set) started at 0·20 F and 0·80 F with varying genetic background with respect to the other locus.

differences between the cages exist, but these differences are not consistent. In the 50 × 0 III cage, with zero frequency of 6PGD-F, there is a rapid increase compared to the other cages started at 0·50F, but the 20 × 20 III cage (Fig. 4) shows a slower increase as compared to the 20 × 80 III cage. It is obvious that

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there is a rapid increase of the F frequency at both loci when started at lower frequencies, by looking over the three sets of cages. This is also clearly shown in Table 2, which gives the mean change in gene frequency ($\overline{\Delta F}$) after the first nineteen months averaged over all cages started at a particular gene frequency. Started at $0{\cdot}20F$ and $0{\cdot}50F$ respectively, the change, in the case of G6PD, is 0·40 with both initial frequencies, while it is 0·50 and 0·33 in the case of 6PGD. This indicates that there must have been selection favouring the F alleles of both loci.

Table 2. *Mean change in gene frequency* ($\overline{\Delta F}$) *over all cages and the standard error* ($S_{\overline{\Delta F}}$) *of this change*

	G6PD			6PGD		
Started at	0·20	0·50	0·80	0·20	0·50	0·80
Number of cages	4	7	4	4	7	4
$\overline{\Delta F}$	0·39	0·38	0·05	0·50	0·33	0·12
$S_{\overline{\Delta F}}$	0·11	0·05	0·04	0·04	0·05	0·06

The first set of cages was established with a high level of linkage disequilibrium between the two loci. A significant linkage disequilibrium was still found in the first sample (after three months) from two of the three cages. In none of the further samples from these cages was a significant linkage disequilibrium found. The cages of the second and third set were established in linkage equilibrium and in all samples from these cages no significant linkage disequilibrium was found. Also, in nearly all samples from all cages neither a significant departure from Hardy–Weinberg equilibrium was found nor a significant difference in frequency of the alleles between females and males.

Egg-to-adult survival

Table 3 shows the mean survival of the four homozygous genotypes. It is clear that there are marked differences between the environments and that, except for the environments 'control' and 'yeast 1·6%', the $\frac{SS}{SS}$ genotype has the lowest survival. The bottom part of Table 3 gives the summarized results of an analysis of variance of each environment separately. It can be seen that, when there is a significant effect, the S allele of both loci has a lower survival than the F allele. Table 4 gives the results of an analysis of variance of all data. All three main factors (environment, G6PD and 6PGD) have a significant effect on the survival rate. There are also significant interactions between the two loci and between 6PGD and environment. The significant interaction between the two loci is mainly due to the low survival rate of the $\frac{SS}{SS}$ genotype. The mean survival of this genotype differs significantly from the other genotypes when computed over all environments, while the other three do not differ from each other.

Table 3. *Mean survival percentage ($\bar{x}$, in angles) together with the standard deviation (s) of the four homozygous genotypes in the viability experiment (top), and the results of an analysis of variance of the data of each environment separately (bottom). All indicated significant effects are significant at the 5 % level*

		Control	Glucose 1 %	Maltose 1 %	Lactose 0·5 %	Lactose 1 %	No sugar	Yeast 1·6 %	29·8 °C	Total
$\frac{FF}{FF}$	$\bar{x}$	47·8	31·6	35·2	29·9	35·3	29·1	24·8	35·7	33·6
	s	2·9	2·7	5·2	2·7	2·5	2·3	3·0	4·5	7·2
$\frac{FS}{FS}$	$\bar{x}$	43·9	35·4	33·0	32·2	35·5	29·2	27·9	34·3	33·9
	s	6·4	3·9	3·0	3·0	4·7	2·4	3·9	3·3	5·9
$\frac{SF}{SF}$	$\bar{x}$	50·5	32·5	35·8	28·7	36·7	30·2	24·9	38·3	34·7
	s	3·6	3·3	3·9	2·5	2·8	1·6	3·9	2·9	7·9
$\frac{SS}{SS}$	$\bar{x}$	46·8	28·5	31·2	27·5	32·0	25·5	24·9	31·9	30·8
	s	1·4	3·5	3·2	2·2	3·4	3·2	3·9	6·5	7·4
Source										
G6PD-effect		n.s.	$s < f$	n.s.	$s < f$	n.s.	n.s.	n.s.	n.s.	
6PGD-effect		$s < f$	n.s.	$s < f$	n.s.	n.s.	$s < f$	n.s.	$s < f$	
G6PD × 6PGD		n.s.	$\frac{SS}{SS} <$	n.s.	n.s.	n.s.	$\frac{SS}{SS} <$	n.s.	n.s.	

Table 4. *Analysis of variance of all data (in angles) of the viability test* (* $P < 0{\cdot}05$; ** $P < 0{\cdot}001$)

Source	*SS*	d.f.	*MS*	*F*
Environments	7138·05	7	1019·72	81·77**
G6PD	49·52	1	49·52	3·97*
6PGD	158·25	1	158·25	12·69**
Environment × G6PD	146·63	7	20·95	1·68
Environment × 6PGD	212·46	7	30·35	2·43*
G6PD × 6PGD	207·98	1	207·98	16·68**
Environment × G6PD × 6PGD	54·04	7	7·72	0·62
Error	1992·11	160	12·47	
Total	9962·11	191		

F_2-experiment

This experiment was done to see if the observed differences in viability found in the previous experiment also could be observed when the different genotypes were combined in one bottle. Table 5 shows the results of this experiment. There is only one environment which gives a significant deviation from the expected 1:1:1:1 ratio, namely lactose. However, this significant deviation is due to only one of the three bottles. The other two did not show a significant deviation, while the χ^2 for heterogeneity is significant ($\chi^2_6 = 12{\cdot}88$, $P = 0{\cdot}045$). So under these experimental conditions differences in viability do not exist or are too small to detect.

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Extinction experiment

Fig. 5 shows the cumulative extinction percentages from generation to generation of the different population types in the different environments. For this figure the populations were combined for each locus separately. It is clear that the extinction

Table 5. *F_2-experiment, number of males of each genotype (over three bottles) from the cross* $\frac{FS}{SF} \times \frac{FS}{\rightarrow}$ *with* χ^2 *test for deviations from the expected ratio* 1:1:1:1 (* $P < 0{\cdot}05$)

	Control	17 °C	29·5 °C	13 ° ~29·5 °C	35% R.H.	95% R.H.	Lactose	Maltose
$\frac{FF}{\rightarrow}$	54	52	51	51	53	53	34	45
$\frac{FS}{\rightarrow}$	50	50	47	66	57	53	53	62
$\frac{SF}{\rightarrow}$	63	52	61	49	56	49	63	54
$\frac{SS}{\rightarrow}$	43	56	51	44	44	55	60	49
χ^2	3·98	0·36	2·04	5·12	2·00	0·36	9·70*	3·07

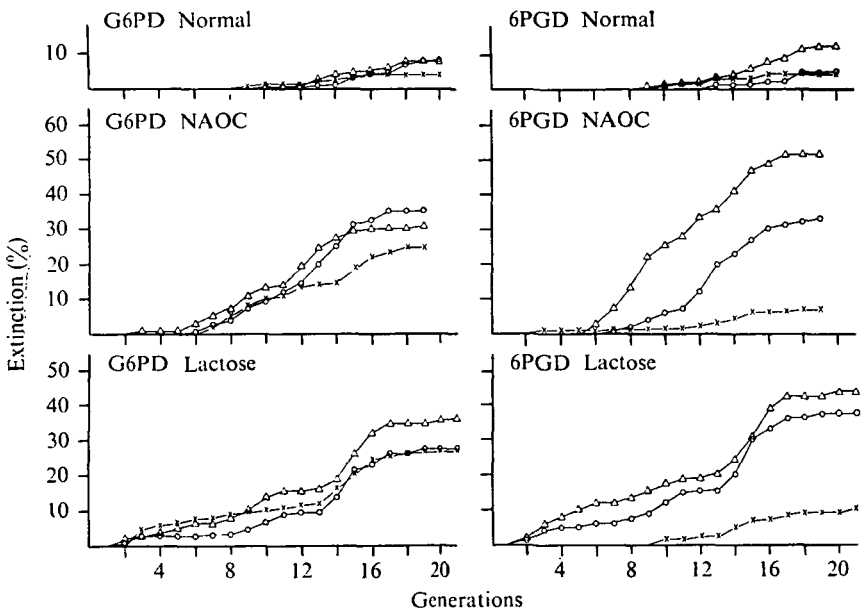

Fig. 5. The cumulative extinction percentages in all three environments of the three different population types for each locus separately. ×—×, monomorphic *F*; ○—○, polymorphic; △—△, monomorphic *S*.

on normal food is much lower than in the other two environments. For G6PD only slight differences are found between the population types. For 6PGD, in all three environments, the homozygous *S* populations seems to have higher extinction than the homozygous *F* populations. These differences are clearly shown in Table 6, in which the total number of extinct populations at the end of the experiment is shown (for normal after 20 generations; for Na-octanoate after 19 generations and for lactose after 21 generations). A χ^2 test of association showed no significant results in each of the three environments, so both loci are, with

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Table 6. *Total number of extinct populations at the end of the experiment with* χ^2 *of independence for each environment and the* χ^2 *of the dead–alive contingency table on the totals of each locus separately* (** $P < 0{\cdot}01$)

		Normal G6PD			
		F/F	*F/S*	*S/S*	Total
6PGD	*F/F*	1	2	3	6
	F/S	1	5	1	7
	S/S	4	6	8	18
Total		6	13	12	

χ^2 independence = 3·42
χ^2 G6PD = 2·98
χ^2 6PGD = 9·22**

		Na-octanoate G6PD			
		F/F	*F/S*	*S/S*	Total
6PGD	*F/F*	5	2	3	10
	F/S	10	20	19	49
	S/S	22	31	24	77
Total		37	53	46	

χ^2 independence = 4·36
χ^2 G6PD = 4·07
χ^2 6PGD = 71·59**

		Lactose G6PD			
		F/F	*F/S*	*S/S*	Total
6PGD	*F/F*	4	5	6	15
	F/S	18	18	20	56
	S/S	19	18	28	65
Total		41	41	54	

χ^2 independence = 0·82
χ^2 G6PD = 3·56
χ^2 6PGD = 44·91**

respect to extinction, independent of each other. Therefore the difference in extinction between population types can be tested, in a 2×3 dead–alive contingency table, on the totals for each locus separately. For G6PD the differences in extinction between the population types are not significant in the three environments. For 6PGD the differences are significant in all cases. The homozygous *S* populations have a significantly higher extinction than the homozygous *F* populations. The extinction of the polymorphic populations differs from environment to environment, with a relatively low extinction on normal food and a relatively high one on lactose.

Table 7. *Extinction experiment, mean gene frequency in the polymorphic populations with its standard error*

Normal

	G6PD-frequency				6PGD-frequency		
		P_F	S.E.			P_F	S.E.
	F/F	0·538	0·082		*F/F*	0·556	0·110
6PGD-background	*F/S*	0·600	0·082	G6PD-background	*F/S*	0·491	0·121
	S/S	0·649	0·107		*S/S*	0·644	0·050
Total		0·595	0·051	Total		0·564	0·056

Lactose

	G6PD-frequency				6PGD-frequency		
		P_F	S.E.			P_F	S.E.
	F/F	0·616	0·122		*F/F*	0·509	0·132
6PGD-background	*F/S*	0·380	0·138	G6PD-background	*F/S*	0·660	0·101
	S/S	0·402	0·117		*S/S*	0·712	0·111
Total		0·466	0·073	Total		0·627	0·066

Na-octanoate

	G6PD-frequency				6PGD-frequency		
		P_F	S.E.			P_F	S.E.
	F/F	0·423	0·067		*F/F*	0·520	0·100
6PGD-background	*F/S*	0·458	0·096	G6PD-background	*F/S*	0·584	0·088
	S/S	0·411	0·091		*S/S*	0·603	0·076
Total		0·431	0·048	Total		0·569	0·050

An important question is whether these differences between the population types are also reflected in changes in gene frequency of both loci. For this reason the gene frequency was determined at the end of the experiment in ten remaining vials of each polymorphic population type. The results are summarized in Table 7. The only significant change found is a decrease in the frequency of the *F* allele of G6PD on sodium octanoate compared to the frequency on normal food ($0{\cdot}431 \pm 0{\cdot}073$ against $0{\cdot}595 \pm 0{\cdot}051$).

DISCUSSION

Natural populations of *D. melanogaster*, and of other organisms, have abundant stores of genetic variation at the molecular level. Both loci involved in this study

are also polymorphic, as can be seen from Table 1. But the gene frequency distribution of the two is different. In the eleven populations surveyed, G6PD is always polymorphic with a wide range of gene frequencies. For 6PGD the gene frequencies are more constant in all populations, except one. This is in contrast with the data reported by O'Brien & MacIntyre (1969) and Berger (1970), who reported considerable variation in gene frequencies from population to population for both G6PD and 6PGD. The question whether these polymorphisms are subject to natural selection remains to be answered.

From the experimental cage populations the conclusion is clear. There is selection favouring the F alleles of both loci. All cages, started with the same initial gene frequency, showed similar changes. The F frequency of both loci increased rapidly to values between 0·80 and 0·90F when started at low values (0·20F and 0·50F). When started at 0·80F of each locus, there is only an appreciable increase for 6PGD, whereas the frequency of G6PD seems to be constant (Table 2). This suggests that the polymorphism on the G6PD locus is perhaps balanced in some way. The rates of change of the F frequency of both loci in these cages are in agreement with the changes found for the Adh-locus by Bijlsma-Meeles & Van Delden (1974), but in contrast with the results of Yamazaki (1971) for esterase-5 of *D. pseudoobscura*. From Figs 2–4 selection coefficients are estimated, for both loci separately, by means of curve fitting by assuming selection against the homozygous S/S female and the hemizygous $\frac{S}{}$ male. These estimated values range from 0·08 to 0·15 for both loci with a mean value of 0·10. These selection coefficients are much higher than the values estimated by MacIntyre (1972) for esterase-6, acid-phosphatase-1 and leucine aminopeptidase-D.

From this it is clear that selection is changing the gene frequencies at both loci. But an important question now is, whether the apparent selective response is due to selective differences at the allozyme loci themselves or to selection at linked fitness genes. This problem was recently discussed by Jones & Yamazaki (1974). Because of the combining of two different strains the first set of cages were probably initiated with a high level of linkage disequilibrium between the loci under study and between these loci and other genes. But for the second and third set the situation is changed. In the 50×50 I cage population the linkage disequilibrium is reduced by recombination in course of time. Using the formula $(1-c)^t = \frac{1}{2}$ (Crow & Kimura, 1970) one can, given the number of generations (t), calculate the minimum recombination frequency (c) necessary to reduce the initial linkage disequilibrium to half of its original value. The first re-isolation was done after approximately thirteen generations, which gives a value of $c = 0{\cdot}052$, and the second re-isolation after 52 generations, which gives a value of $c = 0{\cdot}013$. This indicates that at the time the new lines were isolated, the loci under study probably were in linkage disequilibrium with only a small part of the genome (one to five map units around the locus).

From this reduction in course of time and the high number of founder lines (40 and 60, respectively) one would expect that, if the rapid change in F frequency of both loci was due only to selection at associated loci, this increase should be

much slower in the second and third set of cage populations. It is clear that this is not found. There is a good agreement for all populations started at a particular gene frequency independent of their previous history. This indicates that selection acts at the isozyme loci themselves and not by means of linked fitness genes, unless these genes are very closely linked to the loci under study.

The evidence suggesting selection in favour of the *F* allele of G6PD seems to contradict the constancy in the case of the Bogota and Curaçao populations at approximately 0·35 *F*. However, because both populations were established with quite a number of wild-caught inseminated females and were kept for some time (see Table 1) as cage populations in the laboratory, it is not unlikely that both populations had attained stable allele frequencies at the time the survey of these populations started. The question then arises why the equilibrium allele frequencies of these populations differs from that of the experimental populations. This can be explained in two ways. Firstly it is possible that there are genetic variants within each electrophoretic class as found for instance by Singh, Hubby & Lewontin (1974) for octanol dehydrogenase-I of *D. pseudoobscura* and that the experimental populations possess other *S* and (or) *F* alleles than the Bogota and Curaçao populations. When the different alleles have different selective values this gives rise to differences in equilibrium allele frequencies. Secondly the selective value of a given variant can depend on the total genetic background in which it is embedded. It is unlikely that the experimental populations possess the same genetic background as the Bogota and Curaçao populations and this may have caused the difference in equilibrium allele frequencies. Both possibilities are supported by the finding of the population survey that the northern populations differ in gene frequency from the more southern populations (e.g. Bogota and Curaçao).

The conclusion that selection is favouring the *F* allele of both loci is supported by the egg-to-adult survival experiment. This experiment shows clearly that, when significant differences in survival are found, always the *S* allele has a lower survival than the *F* allele. When all environments are combined the survival of the $\frac{ss}{ss}$ genotype is also significantly lower than the other three genotypes (Table 3). But there are also indications that the relative viability of the genotypes can be modified by the environments, in particular for the 6PGD locus, which gives a significant interaction with the environments (Table 4). In contrast with this experiment the F_2-experiment fails to give additional information. This probably suggests that the differences in viability are measurable only under crowding conditions, but it is not ruled out that the differences in survival become reduced by combining the different genotypes in one bottle.

The extinction experiment also provides evidence, though at a different level, for differences in fitness between the different genotypes. For 6PGD the monomorphic *S* populations have a much higher extinction rate than the monomorphic *F* populations. But the extinction rate of the polymorphic population seems to differ from environment to environment. So differences in genetic composition of a population concerning the 6PGD-locus influence the chance of population survival. This result corresponds to the conclusion with respect to the Adh-locus

(Bijlsma-Meeles & Van Delden, 1974). For G6PD no differences in fitness between the population types are observed, although there is an indication that, compared to the control, the *S* allele is favoured in the environment 'Na-octanoate' (Table 7). This seems to be in contrast with the results of the preceding experiments. One must be aware, however, of the fact that this kind of experiment estimates 'population fitness'.

Although this population fitness is based on the differences in fitness of the different genotypes the relation is not straightforward and can be biased by several causes. The most important bias is that the population fitness of the polymorphic populations is not a constant but changes with the genotypic composition of these populations. On the other hand, in the monomorphic populations all possible interactions between different genotypes, e.g. mating preference and frequency-dependent selection, are not involved.

There is some evidence that the selection found in the extinction experiment on Na-octanoate is selection at the isozyme loci themselves. It is known that larvae of *D. melanogaster* fed on a diet with long-chain fatty acids (12 and more C-atoms) have repressed levels of G6PD and 6PGD, while dietary carbohydrates induce high levels of these enzymes (Geer *et al.* 1976). Experiments in progress now give evidence that also Na-octanoate in the diet causes changes in the enzyme levels. Furthermore, it was found that in rat liver Na-octanoate has an inhibitory effect on both enzymes, when a supernatant of a homogenate is preincubated with Na-octanoate (Weber *et al.* 1967).

Although it is not entirely excluded that the selection is acting on closely linked fitness genes, the results of the preceding experiments strongly point to the occurrence of selection at the polymorphic loci G6PD and 6PGD.

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