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# Adaptive value of PGM polymorphism in laboratory populations of *Drosophila melanogaster*

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SUMMARY

Experiments have been performed to show that PGM polymorphism for the two common electrophoretic allozymes, $PGM^A$ and $PGM^B$, in *Drosophila melanogaster* has adaptive value. Firstly, the allele frequencies converge to the same equilibrium value in six experimental populations. Secondly, density-dependent selection operates. Thirdly, the relative fitness of the three genotypes varies in modified culture media. PGM polymorphism is maintained by frequency-dependent selection and heterotic selection: the first mechanism operates to reach equilibrium frequency, the second cooperates to maintain it. The experiments performed with modified culture media favour the view that the two allozymes have different affinities for two components which are present in the nutritional environment. These components may be either substrates or other factors involved in the reaction catalyzed by PGM.

## 1. INTRODUCTION

The mechanism by which protein variation, particularly enzyme polymorphism, is maintained in natural and experimental populations has been investigated by several authors. However, the biochemical properties responsible for the selective differences between allozyme variants and the corresponding selective environmental factors have been identified only in a few studies. From this point of view the best study in *Drosophila melanogaster* (*D. m.*) has been that of ADH polymorphism. A correlation has been demonstrated between selective differences of the alleles at this locus and both the activities of allozymes on alcohol media (Gibson, 1970; Oakeshott, 1975; Van Delden, Kamping & Van Dijk, 1975; Thompson & Kaiser, 1977) and their thermostability (Vigue & Johnson, 1973; Pipkin, Rhodes & Williams, 1973). For the amylase locus in *D. m.* a close connexion has been demonstrated between selective differences of alleles and the food composition by adding specific substrate (De Jong & Scharloo, 1976; Hickey, 1977). More recently Bijlsma (1978) has found variations of relative fitness among different genotypes at loci G6PD and 6PGD on culture medium supplemented with sodium octanoate which specifically modifies the activities of these two enzymes.

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However, the first clear connexion between a polymorphism and the properties of allozymes has been demonstrated by Koehn (1969) at the locus Esterase-I in the fresh-water fish *Catostomus clarkii*. Currently it is widely accepted that many enzymic polymorphisms are maintained by an heterogeneous environment. Powell (1971) and McDonald & Ayala (1974) have demonstrated that the greater the environmental variation the greater the amount of allozyme polymorphism observed.

Some studies on PGM polymorphism in many species of completely different groups have given evidence in favour of the adaptive value of this polymorphism. Particularly, support of this view has been provided in man by the deviation from the Hardy-Weinberg equilibrium shown by Anantakrishnan, Beck & Walter (1973). The opposite conclusion has been reached by Beckman & Beckman (1975) who had analysed a possible role of PGM in prenatal selection. Marinković & Ayala (1975) have studied the effects of allozyme variants coded by the alleles at the loci Pgm-1 and Me-2 on a variety of fitness components in *Drosophila pseudo-obscura*. Particularly the heterozygote at Pgm-1 locus showed greater egg-to-adult survival than the two homozygotes under conditions of high larval competition. The authors concluded that a balancing selection operates at this locus. This is in agreement with the conclusions of Dobzhansky & Ayala (1973) who have observed cyclic oscillations of alleles frequencies at the two loci over the season.

Trippa, Santolamazza & Scozzari (1970) and Hjorth (1970) have described the PGM polymorphism in *D. m.* and Trippa *et al.* (1974) have investigated its significance in natural populations of *D. m.*, measuring the allele frequencies in two successive years. These were found substantially unchanged despite the strong genetic drift or the so-called 'bottleneck effect'.

We report the results of a study concerning polymorphism at the Pgm locus in laboratory populations of *D. m.*

## 2. MATERIALS AND METHODS

### (i) *Stock*

The Pgm allele frequency was first evaluated in six laboratory populations started 18 years ago from an Oregon-R strain of *D. m.* From these populations were derived two homozygous stocks ($Pgm^A/Pgm^A$ and $Pgm^B/Pgm^B$) which have provided the material for all the later experimental observations.

### (ii) *Experimental populations*

From the two homozygous stocks described above six experimental populations were started, each with 100 females and 100 males. Each population was composed of about 2000 flies at the maximum crowding equilibrium. The initial frequencies of the Pgm alleles were controlled by the proportions of $Pgm^A$ homozygous and heterozygous founders of the populations. Two populations were started with $Pgm^B$ frequency 0·98, two with 0·50 and two with 0·02. Variation

in allele frequencies has been followed, at irregular intervals of time, on samples from each population.

### (iii) *Culture method*

Flies were raised in half-litre bottles at 25 ± 1 °C. A standard culture medium was used consisting of 1000 ml water, 27·5 g commercial Torula yeast, 125 g finely-ground yellow corn meal, 75 g D(+)glucose, 7·5 g agar-agar and nipagine to control mould (about 0·66 g % of final culture medium concentration in 95 % ethanol). In experiments with a modified food composition, D(+)glucose or yellow corn meal were omitted.

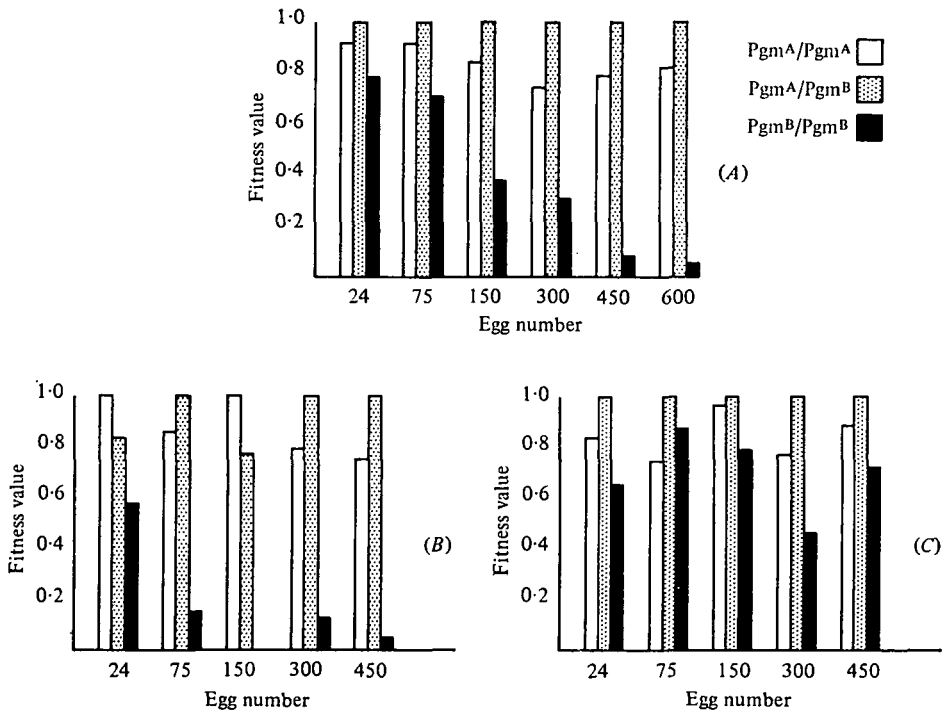


Fig. 1. Relative fitness of the three Pgm genotypes measured by the egg-to-adult survival at different larval densities: on complete culture medium (*A*), on medium without corn meal (*B*) or without D(+)glucose (*C*).

### (iv) *Electrophoresis*

Electrophoresis was carried out on horizontal starch gels according to Spencer, Hopkinson & Harris (1964). The PGM phenotype of each adult fly was determined in all experiments performed on genotype competition. At least 100 flies were electrophoretically analysed to evaluate the variations in the allele frequencies in successive generations of the experimental populations.

### (v) *Fitness parameters*

Fecundity was evaluated in females raised under optimal conditions by counting the eggs laid every day by each female in the age interval of 1–11 days. When egg-to-adult survival and length of development were studied under optimal conditions, 25 eggs were put into a small vial containing 8 ml of culture medium. Different levels of larval crowding were obtained by varying the number of eggs in the presence of a constant amount of food, 4 ml. The rate of hatching was evaluated by counting the eggs hatched every hour for 2 days. The length of total development was evaluated by counting the adult flies at intervals of 4 h beginning from the tenth day of culture life.

### (vi) *Frequency-dependent selection*

In each culture vial containing a constant amount (2 ml) of standard medium and a constant total egg number (450), the ratio of eggs of the three genotypes was varied. Five frequencies of the $Pgm^B$ allele (0·10–0·25–0·50–0·75–0·90) were chosen and for each one three replications were performed. The ratio of the eggs of the three genotypes was chosen, at each frequency, assuming Hardy-Weinberg equilibrium. The electrophoretic PGM phenotype was determined for each adult fly.

## 3. RESULTS

### (i) *Laboratory populations*

As a first approach the frequencies of the PGM electrophoretic variants in the six laboratory populations have been evaluated. In all six populations the only variants detected have been $PGM^A$, always at high frequency, and $PGM^B$. Table 1 gives the frequencies of the three genotypes and of the $Pgm^B$ allele in each population. The frequency of the $Pgm^B$ allele ranged from 0·1 to 0·15 and it was exactly

Table 1. *Pgm genotypes and $Pgm^B$ allele frequencies in six laboratory populations*

(The expected value in H.W. equilibrium is given in parentheses.)

| Population | No of flies examined | Genotypes | | | % $Pgm^B$ allele | P for H.W. equilibrium |
|---|---|---|---|---|---|---|
| | | $Pgm^A/Pgm^A$ | $Pgm^A/Pgm^B$ | $Pgm^B/Pgm^B$ | | |
| 1 | 395 | 315 | 80 | – | 0·10 ± 0·01 | > 0·05 |
| | | (320) | (71) | (4) | | |
| 2 | 381 | 281 | 90 | 10 | 0·14 ± 0·01 | > 0·30 |
| | | (282) | (92) | (7) | | |
| 3 | 391 | 285 | 101 | 5 | 0·14 ± 0·01 | > 0·20 |
| | | (289) | (94) | (8) | | |
| 4 | 407 | 301 | 98 | 8 | 0·14 ± 0·01 | > 0·90 |
| | | (301) | (98) | (8) | | |
| 5 | 361 | 255 | 100 | 6 | 0·15 ± 0·01 | > 0·20 |
| | | (261) | (92) | (8) | | |
| 6 | 355 | 268 | 85 | 2 | 0·12 ± 0·01 | > 0·05 |
| | | (275) | (75) | (5) | | |

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the same (0·14) in three out of the six populations. The differences in $Pgm^B$ frequency between the six populations were not statistically significant.

### (ii) *Fitness parameters*

Means and standard errors of some fitness components have been analysed on flies homozygous for $Pgm^A$ and $Pgm^B$ and on the heterozygotes from the two reciprocal crosses. Table 2 gives the results. One-way analysis of variance has been performed to test the statistical significance of the effect of genotypes on each of the fitness components. The differences among genotypes were highly significant in all parameters. The statistically significant difference in female fecundity was due only to the lower fecundity of $Pgm^B$ homozygous females. The differences

Table 2. *Means and standard errors for five fitness parameters, studied under optimal conditions, in three Pgm genotypes of* Drosophila melanogaster

(The number of replications is given in parentheses.)

| | Genotypes | | | | Degrees of freedom | $F$ |
|---|---|---|---|---|---|---|
| | $Pgm^A/Pgm^A$ | $Pgm^A/Pgm^B$† | $Pgm^B/Pgm^A$‡ | $Pgm^B/Pgm^B$ | | |
| Female fecundity | 196 ± 8 (31) | 192 ± 7 (38) | 271 ± 9 (33) | 163 ± 9 (28) | 3, 126 | 6·92*** |
| Hatchability§ | 23·3 ± 0·1 (180) | 23·5 ± 0·1 (197) | 22·5 ± 0·2 (174) | 22·1 ± 0·2 (197) | 3, 744 | 17·68*** |
| Total egg-to-adult survival§ | 21·4 ± 0·2 (180) | 21·2 ± 0·2 (197) | 20·1 ± 0·2 (174) | 18·9 ± 0·2 (197) | 3, 744 | 38·09*** |
| Rate of hatching (in hours)§ | 21·3 ± 0·3 (18) | 21·7 ± 0·3 (20) | 23·7 ± 0·3 (19) | 22·0 ± 0·2 (19) | 3, 72 | 16·28*** |
| Length of total development (in days)§ | 11·2 ± 0·1 (39) | 11·4 ± 0·1 (38) | 11·4 ± 0·1 (40) | 11·7 ± 0·1 (39) | 3, 152 | 5·27** |

† Heterozygotes from the cross ♀♀$Pgm^A/Pgm^A$ × ♂♂$Pgm^B/Pgm^B$.
‡ Heterozygotes from the reciprocal cross.
§ 25 eggs each replication.
** Statistically significant, $P < 0·01$; *** $P < 0·001$.

between the $Pgm^A$ homozygotes and the heterozygotes, from both reciprocal crosses, were not significant ($P > 0·05$).

The hatchability of $Pgm^A$ homozygous eggs and of the heterozygous eggs from $Pgm^A/Pgm^A$ mothers was significantly higher ($P < 0·001$) than that of reciprocal heterozygotes and $Pgm^B$ homozygotes; there was no significant difference between the first two ($P > 0·20$) or the second two ($P > 0·10$). This was a clear indication of a maternal or cytoplasmic effect on hatchability depending on the preformed allozyme in the egg. The $Pgm^B$ homozygotes had the lowest egg-to-adult survival; the significant difference ($P < 0·001$) in the total egg-to-adult survival between the heterozygotes of the reciprocal crosses was only due to the difference already observed in hatchability. This means that the maternal genotype had no effect on post-hatching survival. The difference in rate of hatching and in length of total

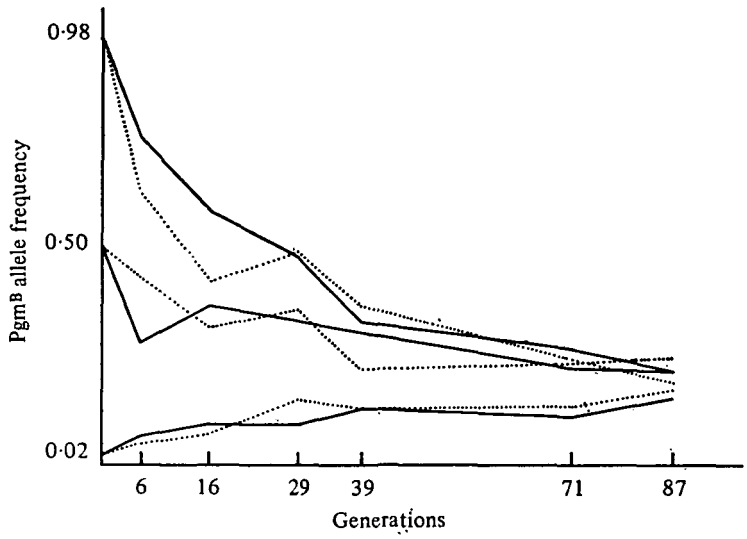


Fig. 2. Variations of $Pgm^B$ allele frequency in the six experimental populations.

development were in the same direction as those in hatchability and in total egg-to-adult survival, respectively.

The general picture of the effect of genotype on the fitness components, tested under optimal conditions, was as follows: (1) the $Pgm^B$ homozygotes had the lowest values for all the components; (2) the heterozygotes from $Pgm^A/Pgm^A$ mothers were better than the heterozygotes from $Pgm^B/Pgm^B$ mothers in the embryonic stage as a consequence of a slight, but significant, maternal effect; (3) the heterozygotes and the $Pgm^A$ homozygotes had substantially the same fitness.

Because the fitness parameter values obtained under optimal conditions were not relevant to the maintenance of the $Pgm^B$ allele at equilibrium in the populations, the assay of egg-to-adult survival was repeated varying the conditions of competition and crowding. The heterozygotes in all the following experiments were obtained from $Pgm^A$ females. The results are reported in Table 3. The effect of geno-

Table 3. *Means and standard errors of egg-to-adult survival under competitive conditions between genotypes at increasing levels of density with constant amount of complete culture medium*

| No. of eggs (one third were of each genotype) | No. of replications | Genotypes: % survival | | | Degrees of freedom | $F$ |
|---|---|---|---|---|---|---|
| | | $Pgm^A/Pgm^A$ | $Pgm^A/Pgm^B$ | $Pgm^B/Pgm^B$ | | |
| 24 | 34 | 77·5 ± 2·5 | 83·7 ± 2·5 | 66·2 ± 2·5 | 2, 99 | 9·91*** |
| 75 | 5 | 86·4 ± 2·1 | 93·6 ± 4·4 | 66·4 ± 2·0 | 2, 12 | 17·90*** |
| 150 | 5 | 78·4 ± 1·6 | 92·0 ± 2·6 | 35·2 ± 7·0 | 2, 12 | 43·68*** |
| 300 | 5 | 53·4 ± 5·4 | 71·2 ± 5·0 | 22·4 ± 3·5 | 2, 12 | 27·36*** |
| 450 | 3 | 38·5 ± 7·9 | 48·7 ± 4·1 | 4·2 ± 1·7 | 2, 6 | 19·99** |
| 600 | 4 | 18·3 ± 3·4 | 22·1 ± 1·8 | 1·3 ± 0·4 | 2, 9 | 23·91*** |

** Statistically significant, $P < 0·01$; *** $P < 0·001$.

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type was statistically significant at each level of crowding. The disadvantageous genotype was always $Pgm^B/Pgm^B$, whose relative fitness was most reduced by crowding. Furthermore, the best genotype in competition was the heterozygote, which survived better than the $Pgm^A$ homozygote. In this connexion the hypothesis was that the heterozygote advantage under conditions of competition was due to the environmental heterogeneity, and for this purpose the assay of egg-to-adult survival was repeated varying the composition of the culture medium. Because in this medium the glucose and yellow corn meal were the main sources of carbohydrates, experiments were performed omitting the corn meal (Table 4)

Table 4. *Means and standard errors of egg-to-adult survival under competitive conditions between genotypes at increasing levels of density with constant amount of culture medium without yellow corn meal*

| No. of eggs (one third were of each genotype) | No. of replications | Genotypes: % survival $Pgm^A/Pgm^A$ | $Pgm^A/Pgm^B$ | $Pgm^B/Pgm^B$ | Degrees of freedom | $F$ |
|---|---|---|---|---|---|---|
| 24 | 30 | 72·5 ± 3·7 | 61·2 ± 5·0 | 41·2 ± 5·0 | 2, 87 | 10·91*** |
| 75 | 26 | 34·4 ± 4·8 | 42·0 ± 4·0 | 6·4 ± 2·8 | 2, 75 | 22·13*** |
| 150 | 14 | 17·0 ± 3·4 | 13·2 ± 3·2 | — | 2, 39 | 10·96* |
| 300 | 4 | 17·7 ± 9·4 | 22·2 ± 7·8 | 2·7 ± 1·2 | 2, 9 | 4·46* |
| 450 | 4 | 10·5 ± 2·1 | 14·0 ± 3·5 | 0·6 ± 0·6 | 2, 9 | 8·25** |

* Statistically significant, $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Table 5. *Means and standard errors of egg-to-adult survival under competitive conditions between genotypes at increasing levels of density with constant amount of culture medium without* D(+)*glucose*

| No. of eggs (one third were of each genotype) | No. of replications | Genotypes: % survival $Pgm^A/Pgm^A$ | $Pgm^A/Pgm^B$ | $Pgm^B/Pgm^B$ | Degrees of freedom | $F$ |
|---|---|---|---|---|---|---|
| 24 | 30 | 66·2 ± 5·0 | 78·4 ± 3·7 | 51·2 ± 5·0 | 2, 87 | 8·01*** |
| 75 | 21 | 46·8 ± 4·4 | 62·4 ± 6·0 | 55·2 ± 4·0 | 2, 60 | 4·30* |
| 150 | 13 | 47·4 ± 7·0 | 48·0 ± 6·6 | 38·2 ± 6·4 | 2, 36 | 0·73 |
| 300 | 4 | 31·2 ± 3·2 | 40·2 ± 2·1 | 18·7 ± 1·0 | 2, 9 | 19·77*** |
| 450 | 4 | 22·6 ± 7·4 | 25·1 ± 6·4 | 18·3 ± 6·8 | 2, 9 | 0·25 |

* Statistically significant, $P < 0{\cdot}05$; *** $P < 0{\cdot}001$.

and the glucose (Table 5) respectively. The egg-to-adult survival of the three genotypes was reduced on medium without corn meal and the $Pgm^B$ homozygote was particularly damaged. Moreover, the survival of the heterozygote was relatively more reduced than that of the $Pgm^A$ homozygote. Essentially flies with the $PGM^B$ allozyme (homozygous or heterozygous) were more damaged by the omission of yellow corn meal.

The omission of D(+)glucose showed opposite results. The egg-to-adult survival of the three genotypes was again reduced, but now the $Pgm^B$ homozygote was the less damaged and consequently its relative fitness increased. The relative

fitness value of the three genotypes at various crowding levels and in the various food media are shown in Fig. 1.

### (iii) *Experimental populations*

The six experimental populations were followed for 87 generations, and all showed convergence of the $Pgm^B$ allele frequency to values close to 0·21 (Fig. 2). This value is not far away from those found in the six laboratory populations (Table 1). The discrepancy may be attributed to the very different lifetime of the two sets of populations.

### (iv) *Frequency-dependent selection*

The results of the experiments performed to evaluate the role of frequency-dependent selection in PGM polymorphism are shown in Table 6 and plotted in

Table 6. *Percentage egg-to-adult survival of the three Pgm genotypes with varying initial $Pgm^B$ allele frequency*

| Initial $Pgm^B$ allele frequency | Egg-to-adult survival % | | |
|---|---|---|---|
| | $Pgm^A/Pgm^A$ | $Pgm^A/Pgm^B$ | $Pgm^B/Pgm^B$ |
| 0·10 | 9·1† | 14·8 | 20·0 |
| 0·25 | 12·4 | 17·9 | 8·3 |
| 0·50 | 15·8 | 15·0 | 5·7 |
| 0·75 | 20·2 | 14·0 | 7·8 |
| 0·90 | 73·0 | 26·7 | 8·5 |

† Each value is based on three replicate experiments of 450 eggs divided between the three genotypes in H.W. equilibrium.

Fig. 3; they indicate the presence of frequency-dependent selection at the Pgm locus. In fact, increasing the $Pgm^B$ allele frequency in the egg stage caused the relative fitness of the $Pgm^B$ homozygote to be lowered, whereas that of the $Pgm^A$ homozygote was raised. Opposite results were obtained by increasing the $Pgm^A$ allele frequency.

The regression coefficient ($b$) for the $Pgm^B$ allele frequency after selection (in the adults) on before selection (in the eggs) was 0·739 and its standard error was 0·037. The deviation of $b$ from 1 (value indicating absence of selection) was highly significant ($P < 0·001$). The theoretical line in the absence of selection and the experimental line were coincident at the $Pgm^B$ allele frequency of 0·25, a frequency, again, not very different from that of equilibrium in the six experimental populations.

## 4. DISCUSSION

The same $Pgm^B$ allele frequency in the six laboratory populations and its convergence to the same equilibrium value in the six experimental ones suggest that the PGM polymorphism in *Drosophila melanogaster* is balanced. The variation of the relative fitness of the three genotypes with both density (Table 3) and culture

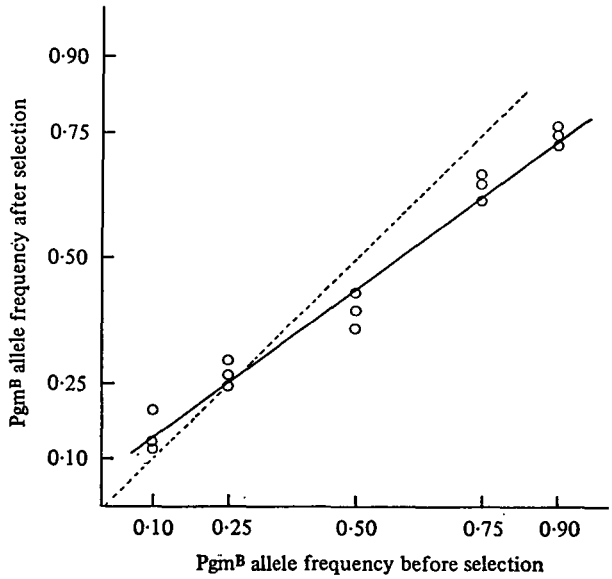


Fig. 3. $Pgm^B$ allele frequency before (eggs) and after (adults) selection in a frequency-dependent selection experiment. ——, experimental line; – – –, theoretical line in absence of selection.

medium composition (Tables 4 and 5) prove the adaptive value of this polymorphism.

The results of this work lead us to believe that some component of the culture medium is the factor responsible for the maintenance of the $Pgm^B$ allele at frequency equilibrium. In our case this is likely to be one of the corn meal components. Its presence seems to produce selective effects directly on the Pgm locus. On the other hand one might postulate that quantitative and qualitative variations of carbohydrate content in the culture medium might have selective effects on a locus like Pgm which is concerned with glucose metabolism. Nevertheless we cannot exclude the possibility of a linkage disequilibrium for a block of genes containing the Pgm locus. Linkage disequilibrium for Pgm and Esterase-6 loci has been found in *D. m.* in previous studies (Charlesworth & Charlesworth, 1973; Langley, Ito & Vorlker, 1974; Costa *et al.* 1979).

Under the assumption that selection acts directly on the Pgm locus, the problem arises of identifying the mechanism of allozyme competition. The conversion of glucose-1-phosphate to glucose-6-phosphate requires the presence of the cofactor-substrate glucose-1,6-diphosphate. In a comparative study of allozyme kinetics (Fucci *et al.* 1979) we have shown that *in vitro* at pH 6·0 the $K_m$ value for G-1, 6-$P_2$ of $PGM^B$ is higher than that of $PGM^A$. This kinetic difference alone might not explain the competitive mechanism of the two allozymes. Our results suggest that the substances involved in the competition must be at least two, one of which is probably the G-1,6-$P_2$. The frequency-dependent selection might therefore be explained by different affinities (in opposite directions) of the allozymes for two

different substrates. This hypothesis postulates a functional relationship between the Pgm allozymes in *D. m.* similar to that shown by Quick, Fisher & Harris (1974) for the PGM isozymes in man. In this species the $PGM_2$ isozyme possesses a lower affinity than the $PGM_1$ isozyme for the glucose-1-phosphate but a higher one for the ribose-1-phosphate. However, such an interpretation of the PGM allozymes competition contrasts with the functional classification of the enzymes according to Gillespie & Kojima (1968) and Kojima, Gillespie & Tobari (1970). In this classification the enzymes of Group I, to which PGM belongs, are characterized by a singular physiological substrate generated and utilized intracellularly and they show little polymorphism. On the contrary the enzymes of Group II, which are more polymorphic, have multiple physiological substrates which reflect environmental diversity. It is already known that PGM is an unusual Group I enzyme, since it is highly and almost universally polymorphic; furthermore, our results indicate that the PGM polymorphism reflects environmental diversity.

The data in Table 6 show that frequency-dependent selection is one of the mechanisms which maintain the PGM polymorphism in *D. m.* The role of this selective mechanism has been already demonstrated in the same species for other polymorphisms (Morgan, 1976). Furthermore, in conditions of strong competition the heterozygote is superior to both the homozygotes and its superiority is greatest at the allele frequencies corresponding to the equilibrium values in populations. It is possible to conclude that frequency-dependent selection is the main force which drives the Pgm allele frequencies to their equilibrium values. This in turn is maintained by heterotic selection and not selective neutrality. Kojima & Yarbrough (1967) and Huang, Singh & Kojima (1971) have observed selective neutrality of Esterase-6 genotypes in *D. m.* at the equilibrium value reached by action of frequency-dependent selection. Neutrality at equilibrium is considered by Gromko (1977) and by Gromko & Richmond (1978) as one of the features of frequency-dependent selection. In contrast, Kojima & Tobari (1969) have observed in *Drosophila ananassae* that the heterozygote for an inversion has the best survival value at the equilibrium approached by the action of frequency-dependent selection. The combined action of both selective mechanisms has been pointed out by Anxolabéhère (1976) at the *sepia* locus in *D. m.* Heterosis might be superimposed on frequency-dependent selection to maintain an enzymic polymorphism in equilibrium. On the other hand, there is nothing to be surprised that heterosis is effective only at values close to the equilibrium at which the advantage of either homozygote over the other two genotypes is not operating.

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