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Genetica. — *Phosphoglucomutase activity of three PGM electrophoretic phenotypes in a natural population of* Drosophila melanogaster (*). Nota di ROSARIA SCOZZARI (**), GIOVANNI TRIPPA (**), CARLO SANTOLAMAZZA (**), LAURA ULIZZI (**), CLAUDIA BARBERIO (**) e GUIDO MODIANO (**), presentata (***) dal Socio G. MONTALENTI.

RIASSUNTO. — È stata misurata l'attività PGM di 233 omogenati di *Drosophila melanogaster* PGM A, 55 PGM AB e 7 PGM B.

Sia il confronto tra le attività medie dei tre fenotipi PGM che il confronto tra la varianza interfenotipica con quella intrafenotipica permettono di concludere che l'allele Pgm^A e l'allele Pgm^B funzionano in modo additivo nell'eterozigote e che le attività ad essi associate sono uguali o quasi uguali.

Si può pertanto concludere che la variabilità elettroforetica del gene *Pgm* non contribuisce affatto o solo in misura minima alla variabilità globale dell'attività PGM osservabile nelle nostre condizioni sperimentali.

INTRODUCTION

The fraction or the extent of variation of enzyme activity due to polymorphism of enzyme structural genes (1) depends, *for a given population* and in a given environment, on two factors only, and it is, in a way, their product (2). These two factors are: *a*) the extent of polymorphism of these genes (expressed, for example, as the mean degree of heterozygosity), and *b*) the mean magnitude of the differences between the activities associated with common alleles of these genes. Both factors appear large indeed from recent data [1, 2, 3, 4].

However, only the estimate of the first of these two factors is reasonably well substantiated: it is based on results obtained in various species and cells (3)

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(1) That is, *how much* of the *observed* variation of enzyme activity can be ascribed to the polymorphism of their structural genes, *irrespective* of the involved mechanisms.

(2) The variance of anyone enzyme activity contributed by this source of variation (the polymorphism of the enzyme structural gene) is for a *single* enzyme with two alleles with frequencies p and q, and activities 1 and $(1 + d)$ respectively, approximately equal to $2pq \cdot d^2$ [4].

(3) As a matter of fact, the measure which is actually made concerns the extent of that portion of the structural gene's polymorphism which is electrophoretically detectable. Then the estimate of the total extent of the polymorphism of these genes is achieved by multiplying by 3 the extent of the electrophoretically detectable polymorphism, on the assumption that the electrophoresis is a method of analysis able to pick up one structural difference out of three between allelic polypeptide chains.

(see for a review, [2]). On the contrary, the estimate available at present regarding the second of these factors is based on data concerning almost (4) exclusively electrophoretic alleles in *a single species* (4), Man, and, in six cases out of seven, *a single type of "cell"*, the erythrocyte (for a discussion, see [4]). Data obtained on the red cells are, moreover, particularly liable to yield estimates biased in excess. In fact, owing to the lack of protein synthesis in mature erythrocytes, allelic enzymes having different rates of decline in aging red cells may express with quantitative differences in erythrocytes but in other tissues. One of such cases has been indeed found [5].

The possibility of comparing enzyme activities associated with different common alleles in a whole organism is given by the study of enzyme polymorphisms in another species, *Drosophila melanogaster*. An enzyme suitable for this purpose is the phosphoglucomutase (PGM), because it shows a polymorphism in *Drosophila melanogaster*. At least two common codominant electrophoretic alleles (Pgm^A and Pgm^B) exist at the *Pgm* locus [6, 7] and all the molecules of the homozygote Pgm^A/Pgm^A are different from those of the homozygote Pgm^B/Pgm^B.

In the present paper PGM activities associated with Pgm^A and Pgm^B have been compared by comparing, as it is commonly done, the mean activities of different common electrophoretic phenotypes. Since we were interested in *natural* variability the flies were examined within a short time (3–4 days) after they were trapped.

MATERIALS AND METHODS

THE SAMPLE.

The sample consists of 295 random *Drosophila melanogaster* females collected in a large fruit store in Rome. The Pgm^A and Pgm^B gene frequencies were 0.883 and 0.117, respectively. The frequencies of the three PGM phenotypes (see fig. 1) were in Hardy-Weinberg equilibrium ($\chi^2_{1\,df\,(Yates)} = 2.12$; $P > .10$).

DETERMINATION OF THE ELECTROPHORETIC PGM PHENOTYPE.

It was determined according to the technique of Spencer *et al.*, [10] as modified by Trippa *et al.*, [7].

(4) The activities associated with two electrophoretic alleles of alcoholdehydrogenase have been compared in extracts of adult [8] and of third instar larvae [9] of *Drosophila melanogaster*. In both the cases the activity associated with the fast allele Adh^F appeared higher than that of the slow allele Adh^S.

DETERMINATION OF PGM ACTIVITY AND OF NUCLEIC ACIDS [5].

The PGM activity was defined as the rate of production of G6P in a system consisting of a measured volume of clear *Drosophila* homogenate incubated at 37° C with G1P and GI6P in presence of NADP and G6PD in excess.

Solutions.

1) Tris-maleate buffer pH 7.4
   - Tris 10^{-2} M
   - maleic acid 10^{-2} M
   - EDTA 10^{-3} M
   - $MgCl_2 \cdot 6H_2O$ 10^{-3} M

2) Tris–HCl buffer pH 9.0
   - Tris 10^{-1} M
   - $MgCl_2 \cdot 6H_2O$ 1.5×10^{-2} M
   - Imidazole 4.0×10^{-2} M

3) [6] G1P 6.7×10^{-3} M [7]; G16P 10^{-4} M } in solution 2

4) 5.0×10^{-7} moles of NADP and 0.67 µl of G6PD (Boehringer; 140 U/ml) per ml of solution 3.

Procedure.

An homogenate was prepared from each single fly to be examined by squashing it in 50 µl of solution 1. A square of filter paper Whatman 3 MM, 4×4 mm was dipped in the homogenate in order to determine the electrophoretic PGM phenotype. 4 ml [8] of solution 2 was added to the remaining homogenate, and the clear supernatant obtained by centrifugation (20′) was used for the determination of nucleic acid content and for PGM assay.

Supernatants were incubated about 5′ in a waterbath at 37° C within 30′ from squashing [9] (i.e. within 5′ after centrifugation). 1.5 ml of the

(5) It has been assumed for convenience that the whole $E_{2600\,Å}$ of the supernatant was made up of nucleic acids; moreover the nucleic acid concentration (mg/ml) has been expressed by the product $E_{2600\,Å} \times .05$. Obviously, the results of the comparisons between the relative mean activities of the PGM phenotypes do not depend on the accuracy of the estimate of the nucleic acid concentration.

(6) This solution was freshly prepared from a stock solution 7.5 times more concentrated which was the same throughout this work and kept as small aliquots at —20° C.

(7) This concentration was found to be optimal in our experimental conditions.

(8) The rate of G6P production is proportional to the supernatant concentration, at least, in a range from 0.05 to 0.5 *Drosophila* per ml.

(9) Supernatants must neither be frozen (because afterwards activity is lost) or stored in a refrigerator (the enzyme activity of both the homozygous phenotypes was found to decrease steadily at a rate of about 10% per hour at 4° C).

preincubated freshly prepared solution 4 was added to 0.5 ml of the supernatant to be tested. The $E_{3400Å}$ of the resulting solution was determined 1′ and 11′ [10] later by a Beckman DB spectrophotometer.

Calculation of the PGM activities.

The PGM activities were expressed either as *μmoles of* G6P *produced per hr at 37° C per ml of supernatant* and as *μmoles of* G6P *produced per hr at 37° C per mg of nucleic acids* [11] *in the supernatant.* They were calculated by the following formulas:

$$\text{PGM activity per ml} = (\bar{E}_{3400Å(11')} - \bar{E}_{3400Å(1')}) \times 3.859 \quad [12]$$

$$\text{PGM activity per mg of nucleic acids} = \frac{\bar{E}_{3400Å(11')} - \bar{E}_{3400Å(1')}}{E_{2600Å}} \times 77.17 \quad [12]$$

$\bar{E}_{3400Å(11')}$ and $\bar{E}_{3400Å(1')}$ are the mean $E_{3400Å}$ of two solutions containing 0.5 ml of the same supernatant plus 1.5 ml of solution 4, both incubated at 37° C for 11′ and 1′ respectively; $E_{2600Å}$ is the $E_{2600Å}$ of the supernatant.

This method was found to give consistent and reproducible results when aliquots of the same supernatant were examined. Moreover all the procedure is very quick.

RESULTS [13]

Since the PGM activities have been measured in supernatants obtained from whole flies so many biological factors, besides the PGM activity per cell, were involved as to make the *absolute* values of the single determinations, of their means and of their variances, practically meaningless from a biological standpoint. We have, however, compared the mean activities of the three PGM phenotypes on the reasonable assumption that they were, on the average, equally affected by the above mentioned biological factors.

No significant differences were found between the three means of the PGM activities, expressed per ml, obtained from 233 PGM A, 55 PGM AB and 7 PGM B supernatants. As might have been expected, however, the

(10) The rate of G6P production remains constant for at least 20′.

(11) Alternative terms of reference, such as proteins and body weight, have not been used for technical reasons.

(12) Under the conditions of the present assay the NADPH production is equimolar to the G6P present in the final solution (0.5 ml + 1.5 ml). This equivalence was observed even when a known amount of G6P was incubated for 10′ with only supernatant and then measured by a further incubation in presence of NADP and G6PD in excess. Thus, a significant consumption of the produced G6P (e.g. by PGI) could be excluded.

(13) The same activities and range of variations as those reported in this section have been found in a sample of about one hundred flies collected one year later in the same season and fruit store. On the contrary, activities measured in laboratory stocks flies turned out to be much lower (~ 30) and much more variable.

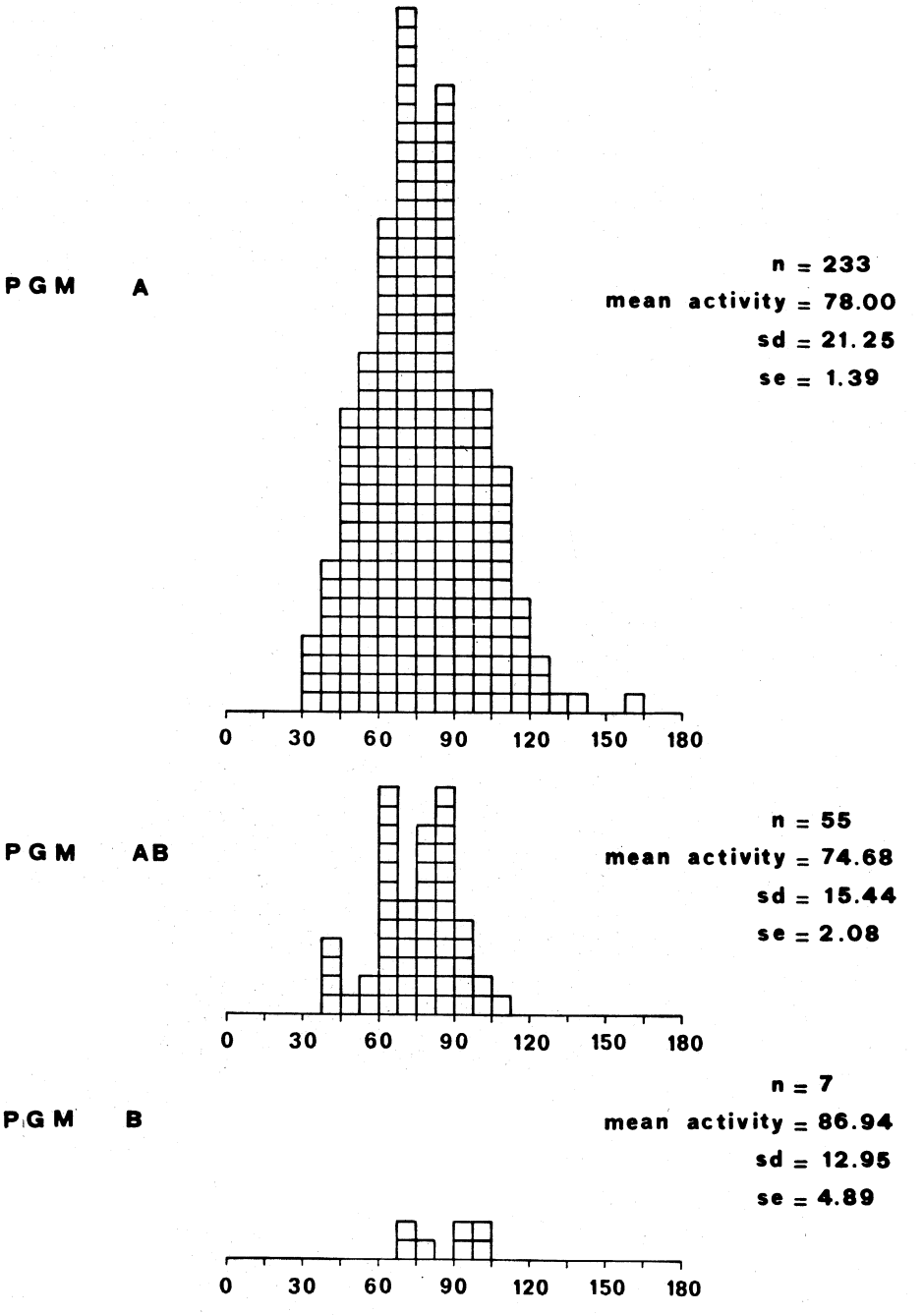

Fig. 1. – Distributions of the PGM activities in three PGM phenotypes of *Drosophila melanogaster*. The activities are expressed as μmoles of G6P produced per mg of nucleic acids per hour at 37° C. Each square represents the mean of two determinations carried out on a single fly supernatant (the few cases less than 5 % in which the results of the two replicate determinations differed more than 10 % from their mean, have been discarded).

sd = mean square deviation; *se* = standard error;

$t_{(A-AB)} = 1.092$, $P > .20$; $t_{(A-B)} = 1.106$, $P > .20$; $t_{(AB-B)} = 2.008$, $P \sim .05$.

activities expressed in this way were quite variable. Therefore, in order to obtain a more sensitive comparison, the PGM activities have been made much less variable by expressing them in terms of nucleic acids (it has been verified that the mean nucleic acid content of the three PGM phenotypes was the same). These comparisons showed little or no differences between the mean activities of the three PGM phenotypes (see fig. 1 and Table I).

TABLE I.

Analysis of variance of PGM *activities observed in 295* Drosophila melanogaster *females.*

SOURCE OF VARIATION	Degrees of freedom	Sum of squares	Variance or Mean square
Within phenotypes	292	118717.70	406.57
Between phenotypes	2	1121.73	560.86
TOTAL . . .	294	119839.43	407.62

$F = \frac{560.86}{406.57} = 1.38$; $F_{.25} = 1.39$.

DISCUSSION

Fig. 1 and Table I show that, under our experimental conditions and within the population examined, the enzyme activities associated with Pgm^A and Pgm^B do not show a statistically detectable difference; they appear additive in the PGM AB heterozygote [14] (this finding, of course, does not necessarily imply that these two *Pgm* alleles are physiologically equivalent).

Table I shows that the variance between phenotypes is not significantly larger than that observed within phenotypes so that the variance in the whole population is the same as that within phenotypes.

It can thus be concluded that, if both Pgm^A and Pgm^B are homogeneous, (that is, if all the Pgm^A alleles are identical to each other and the same applies for the Pgm^B alleles) [15] little, if any, contribution to the observed total variation of PGM activity, can be given by the polymorphism of the *Pgm* gene [16].

(14) This is what one would have expected considering that in the PGM AB heterozygote no hybrid molecules are found.

(15) It can be easily realized that, if this is the case, all the Pgm^A alleles differ in the same way from all the Pgm^B alleles.

(16) If the two *Pgm* alleles are homogeneous the whole polymorphism of the *Pgm* gene is that electrophoretically detectable, which by itself appears to give little, if any, contribution to the observed total variation of the PGM activity.

The PGM polymorphism in *Drosophila melanogaster* is the second example of allelic genes whose products, although electrophoretically different, show the same enzyme activity (the first example is the red cell PGM_1 polymorphism in Man [11, 12]). It appears fruitless to try to evaluate this finding until data on other enzymes of *Drosophila melanogaster* and other species become available.

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