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Polìmorphism for heat-sensitivity isoelectrophoretic alleles at the phosphoglucomutase (PGM) locus in laboratory populations of Drosophila m elanogaster

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Genetica. — *Polimorphism for heat-sensitivity isoelectrophoretic alleles at the phosphoglucomutase* (PGM) *locus in laboratory populations of* Drosophila melanogaster (*). Nota di GIOVANNI TRIPPA, ADA LOVERRE, ALDO CATAMO, ANTONIO LOMBARDOZZI, ROSADELE CICCHETTI e ALDO MICHELI, presentata (**) dal Socio G. MONTALENTI.

RIASSUNTO. — Il grado di eterogeneità genetica di un gene strutturale è senza dubbio maggiore di quello finora noto usando le sole tecniche elettroforetiche. Allo scopo di svelare una ulteriore quota di questa variabilità genetica non elettroforetica sono stati saggiati con studi di denaturazione al calore della fosfoglucomutasi, omogenati di singole drosofile derivate da quattro popolazioni naturali dell'Italia meridionale, mantenute in laboratorio per circa un anno. Tutte le popolazioni esaminate presentano un elevato grado di polimorfismo per alleli isoelettroforetici temperatura-sensibili. L'incremento nel grado di variabilità genetica è notevole come indicato dalle stime del grado di eterozigosi delle popolazioni, tenendo conto una prima volta dei soli alleli elettroforetici e successivamente anche degli alleli isoelettroforetici.

INTRODUCTION

Electrophoresis is a method of analysis which is expected — considering genetic code and chemical proporties of the aminoacids—to reveal approximately one out of three structural differences between allelic proteins. Since about 30 % of the proteins examined by this method in Man [1], Drosophila [2–4] and several other species [9–17] exhibit a genetic polymorphism, it is believed that about two thirds of the structural genes do have common isoelectrophoretic alleles [18], that is polymorphic alleles whose structural differences are not detectable by electrophoresis. The experimental demonstration of the existence of this great amount of genetic variability per locus is certainly one of the most challenging problems in the field of evolutionary biology. It is reasonable to assume that the use of different techniques could reveal further genetic variability in a number of loci. It is important to point out that these techniques should be relatively simple and of known efficiency.

Heat-denaturation turned out to be a very simple method for the detection of isoelectrophoretic alleles at the phosphoglucomutase locus (*Pgm* = 3 : 43.6) in *Drosophila melanogaster* [19], because it reveals the presence of temperature-resistant (*tr*) and temperature sensitive (*ts*) isozymes with the same electrophoretic mobility. At this locus six electrophoretic alleles

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have so far been described, two of them being common, Pgm^{A} and Pgm^{B}, and four being rare, Pgm^{C}, Pgm^{D}, Pgm^{E} and Pgm^{F} [20].

We report data on frequencies of heat-resistant and heat-sensitive alleles in four laboratory populations of *Drosophila melanogaster*.

MATERIALS AND METHODS

The sample.

Samples of four populations collected in south Italy were kept in the laboratory for about one year. Single males from these populations were crossed with $Pgm^{B,ts}$ (1) homozygous females and one of each F_1 progeny was tested according to the scheme reported below. Thus only one allele from the laboratory population *per* each crossed male was examined.

$$\delta\ \frac{Pgm\ ?}{Pgm\ ?} \times \frac{Pgm^{B,ts}}{Pgm^{B,ts}}\ \varphi$$

$$\downarrow$$

$$F_1\ \frac{Pgm\ ?}{Pgm^{B,ts}}$$

Determination of the electrophoretic PGM *phenotype and the heat sensitivity behaviour of* Pgm *alleles.*

Samples of single fly homogenates (about 40 µl) were electrophorised on starch gel and stained according to the technique of Spencer *et al.* [21]. After electrophoresis the gel was cut into two slices, which were put into thin plastic bags. One of them was incubated at 37 °C and the other one at 60 °C. The 15′ incubation was performed in a waterbath. After incubation the slices were stained according to the standard technique [21].

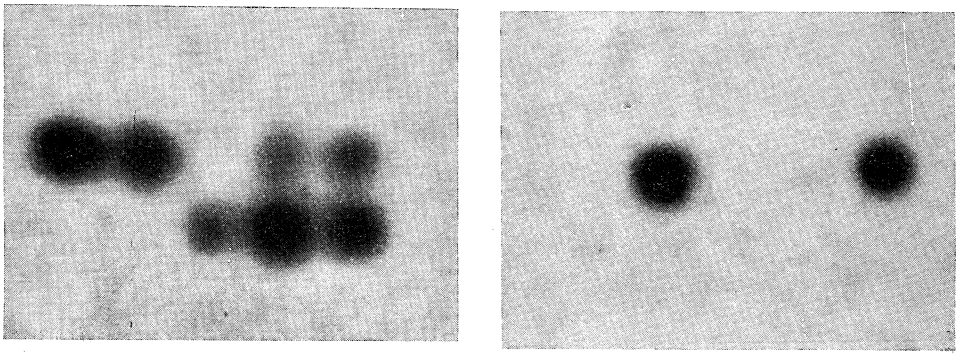

Fig. 1. – Starch gel electrophoretic patterns of homogenates of single flies on two slices of the same gel, incubated in a waterbath for 15′, one (*a*) at 37 °C and the other one (*b*) at 60 °C. The genotypes of the single flies are as follows: 1) $Pgm^{A,tr}/Pgm^{A,tr}$; 2) $Pgm^{A,ts}/Pgm^{A,ts}$; 3) $Pgm^{B,ts}/Pgm^{B,ts}$; 4) $Pgm^{A,tr}/Pgm^{B,ts}$; 5) $Pgm^{A,ts}/Pgm^{B,ts}$.

(1) We indicate with the superscripts *tr* and *ts*, added to the name of the various *Pgm* electrophoretic alleles or PGM phenotypes, the corresponding heat-resistance and heat-sensitivity, respectively.

Following this procedure it was possible to classify heat resistant (presence of PGM activity after the 60 °C treatment) and heat-sensitive (no PGM activity after the 60 °C treatment) bands. Fig. 1 shows the electrophoretic patterns of different homogenates on the two slices of the same gel after incubation at the two temperatures.

Results and discussion

The results are reported in Table I.

Table I.

Percent frequencies of heat-sensitivity Pgm *isoelectrophoretic alleles and degree of heterozygosity for the* Pgm *locus in samples of four laboratory populations of* Drosophila menalogaster.

Origin of the populations	Tot. alleles examined	$Pgm^{A,tr}$	$Pgm^{A,ts}$	$Pgm^{B,ts}$	Degree of heterozygosity	
					Electrophoretic alleles	Electroph. isoelectroph. alleles
Castellaneta . . .	187	175 (93.58)(*)	6 (3.20)	6 (3.20)	6.19	12.18
Corato	209	177 (84.68)	18 (8.61)	14 (6.69)	12.48	27.06
Ranna	223	198 (88.76)	3 (1.34)	22 (9.86)	17.77	20.15
Archi	123	113 (91.86)	8 (6.50)	2 (1.62)	3.19	15.13

(*) The gene frequencies are given in parenthesis.

Table I shows the pattern of genetic electrophoretic and heat-sensitivity isoelectrophoretic variations and the corresponding gene frequencies found in the examined populations. All the samples exhibit only two out of the six electrophoretic alleles, that is Pgm^{A} and Pgm^{B}. This could be due both to the limited size of the samples examined and to changes of the gene frequencies in the populations maintained in laboratory conditions. Moreover in all the populations within the series of alleles that behave electrophoretically as Pgm^{A} it is possible to identify alleles with different degrees of heat-sensitivity, $Pgm^{A,tr}$ and $Pgm^{A,ts}$. Both the heat-resistant and the heat-sensitive isoelectrophoretic alleles attain polymorphic frequencies: in fact the frequencies of the less common one (the heat-sensitive) were found to be 0.03, 0.09, 0.01

and 0.07 in the Castellaneta, Corato, Ranna and Archi populations respectively. The Pgm^B electrophoretic allele, on the contrary, appears to consist of an homogeneous heat-sensitive class in all the samples studied.

Thus, by using the heat-denaturation method it has been possible to discover a further great extent of genetic heterogeneity at the *Pgm* locus in *Drosophila melanogaster*. The identified variability of this gene increases considerably, as indicated by the estimates of the degrees of heterozygosity in all the populations, considering the electrophoretic variations only or the isolectrophoretic alleles too.

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