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Distribution of phosphoglucomutase (PGM) isoelectrophoretic alleles temperature-resistant (tr) and temperature-sensitive (ts) in two laboratory populations of Aedes aegypti

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

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Genetica. — *Distribution of phosphoglucomutase* (PGM) *isoelectrophoretic alleles temperature-resistant* (tr) *and temperature-sensitive* (ts) *in two laboratory populations of* Aedes aegypti. Nota di ROSADELE CICCHETTI (*), GABRIELLA CANCRINI (**), GABRIELLA ARGENTIN (***) e ROSARIA SCOZZARI (****), presentata (*****) dal Socio G. MONTALENTI.

ABSTRACT. — Phosphoglucomutase phenotypes of 440 individuals from two laboratory populations of *Aedes aegypti* have been determined by combining standard electrophoresis with a heat denaturation technique. This study has revealed the existence of two different PGM 0.90 isozymes, which were termed thermoresistant (tr) and thermosensitive (ts) depending on whether after heat treatment they were electrophoretically detectable or not.

The analysis of the progeny phenotypes obtained in informative crosses involving both electrophoretic and thermostability variants showed a segregation pattern which conformed very closely to the Mendelian expectations on the hypothesis that the observed differences in heat sensitivity reside at the *Pgm* structural locus. Therefore, two different $Pgm^{0.90}$ alleles were identified: $Pgm^{0.90.tr}$ and $Pgm^{0.90,ts}$, whose products—isoelectrophoretic in standard conditions—can be distinguished at high temperature.

The two samples examined could be well differentiated from each other for both the frequencies of the shared *Pgm* alleles and the presence or absence of particular *Pgm* alleles.

KEY WORDS: Aedes aegypti; Phosphoglucomutase; Isoelectrophoretic alleles.

RIASSUNTO. — *Distribuzione di alleli isoelettroforetici, temperatura-resistenti* (tr) *e temperatura-sensibili* (ts), *per la fosfoglucomutasi in due popolazioni di laboratorio di* Aedes aegypti. Mediante l'uso combinato di una tecnica elettroforetica standard e di un metodo di denaturazione al calore, si è determinato il fenotipo per la fosfoglucomutasi di 440 individui di *Aedes aegypti*, appartenenti a due popolazioni di laboratorio.

Questo studio ha rivelato, all'interno della classe elettroforetica $Pgm^{0,90}$, l'esistenza di due differenti isozimi, che sono stati classificati come termoresistenti (*tr*) e termosensibili (*ts*) sulla base del loro diverso comportamento al calore.

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L'analisi del fenotipo della progenie di una serie di incroci eseguiti utilizzando due linee isogeniche di laboratorio, caratterizzate sia dal punto di vista elettroforetico che da quello per la termosensibilità, ci ha permesso di concludere che la base genetica della differente termostabilità risiede al locus strutturale PGM. Due alleli differenti sono stati quindi identificati all'interno della classe $Pgm^{0,90}$, i cui prodotti, isoelettroforetici in condizioni standard, mostrano un diverso comportamento al trattamento con il calore.

Poiché le due popolazioni esaminate sono risultate ciascuna « monomorfica » per l'uno o per l'altro dei due sottotipi $Pgm^{0,90}$, i dati ottenuti, pur dimostrando l'esistenza di una ulteriore variabilità genetica, non sono sufficienti a dimostrare che si tratti di un nuovo esempio di polimorfismo genetico. Pertanto nessun incremento nella stima del grado di eterogeneità intra-popolazione è possibile ottenere quando, oltre alle variazioni per la mobilità elettroforetica, si considerino anche quelle per il comportamento al calore.

I risultati di questo lavoro, d'altro canto, dimostrano che lo studio di variazioni genetiche elettroforeticamente silenti può rivelarsi uno strumento molto utile per discriminare ulteriormente tra differenti popolazioni.

Le due popolazioni esaminate possono infatti essere ben differenziate non solo in base alle frequenze relative degli alleli che esse hanno in comune, ma anche per i differenti alleli *Pgm* che esse possiedono.

Introduction

During the past twenty years, a considerable amount of data has been collected about the incidence of genetically determined variation in natural populations. This information has been available since the advent of inexpensive and efficient techniques such as one-dimensional electrophoresis, which quickly led to the demonstration of far more variability than had previously been assumed. In most sexually reproducing organisms, an average individual is heterozygous at 5 to 15 percent of its loci [1 and 2]. The extent to which this value is underestimated is not precisely known, but since standard electrophoresis detects only those aminoacid substitutions that result in charge differences in the protein, it has been suggested that electrophoretic techniques overlook 1/2 to 2/3 of the genetic variability [3].

A variety of techniques involving the determination of characteristics such as heat or urea stability, isoelectric point, pH optima, etc. have been applied in recent years, mostly in *Drosophila*, in order to uncover electrophoretically cryptic variation. Among them, the heat denaturation technique has proved a valuable tool in detecting thermolability variants whose electrophoretic behaviour remains unchanged and the data collected so far suggest that the amount of genetic variability which goes undetected by routine electrophoresis may be very large [4-12], at least for the loci already known to be electrophoretically polymorphic [13].

The present study was performed to assess the possible existence of a hidden genetic variability for thermolabile and thermoresistant variants among electrophoretic alleles in one species of *Culicidae* never examined before from this viewpoint. Two laboratory populations of *Aedes aegypti* and the phosphoglucomutase (PGM) locus were chosen to this purpose.

Aedes aegypti is certainly one of *Culicidae* species most interesting from a parasitological point of view and also for this reason one of the mosquito species more studied and perhaps more known. Many advances made in the genetics of *Aedes aegypti* have been well documented in two reviews [14 and 15]. The localization on the three linkage groups of factors for lethality, susceptibility to parasites and various morphological and enzyme markers, have been also reported [16]. The enzyme markers especially seem to be suitable either to formal genetic studies, or mechanisms analyses of new species isolation and formation, or study of problems concerning the biological struggle.

PGM locus has proved to be highly polymorphic in this species from an electrophoretic point of view and the data collected so far have shown the existence of seven codominant alleles [17].

MATERIALS AND METHODS

The sample.

The sample consisted of 440 individuals from two laboratory populations (*A* and *B*) of *Aedes aegypti*. The population *A*, derived from egg samples collected in water reservoirs near Shaury Moyo' village (Kenia), was kept in the laboratory for about four years; population *B*, of unknown provenience, was maintained for at least thirty years.

Methods.

Both populations were mass reared in standard environmental conditions, at 28 ± 1 °C, with relative humidity around 80% and in the absence of light: in these conditions, the life cycle is 6-8 days. The adults, transferred to standard cubic cages ($18 \times 18 \times 18$ cm), were supplied with a 5% sucrose solution and the females were fed on albino mice.

Electrophoretic and thermosensitivity phenotypes were obtained according to the following method. Single individuals have been homogenized in 50 µl of distilled water and submitted to electrophoresis on starch-gel, according to Spencer *et al.*, 1964 [18]. After the electrophoretical run the gel was cut horizontally in two slices. One was incubated at 37 °C to develop the electrophoretic pattern and the other at 60 °C for 15', in order to reveal the presence of thermosensitive PGM isozymes, according to the method of Trippa *et al.*, 1976 [9]. Both slices were subsequently stained for PGM activity as described by Spencer *et al.*, 1964 [18].

RESULTS AND DISCUSSION

Four out of the seven electrophoretic alleles previously described [17] were encountered in this study. The alleles previously named *Pgm* A_2, A_1, B_2 and

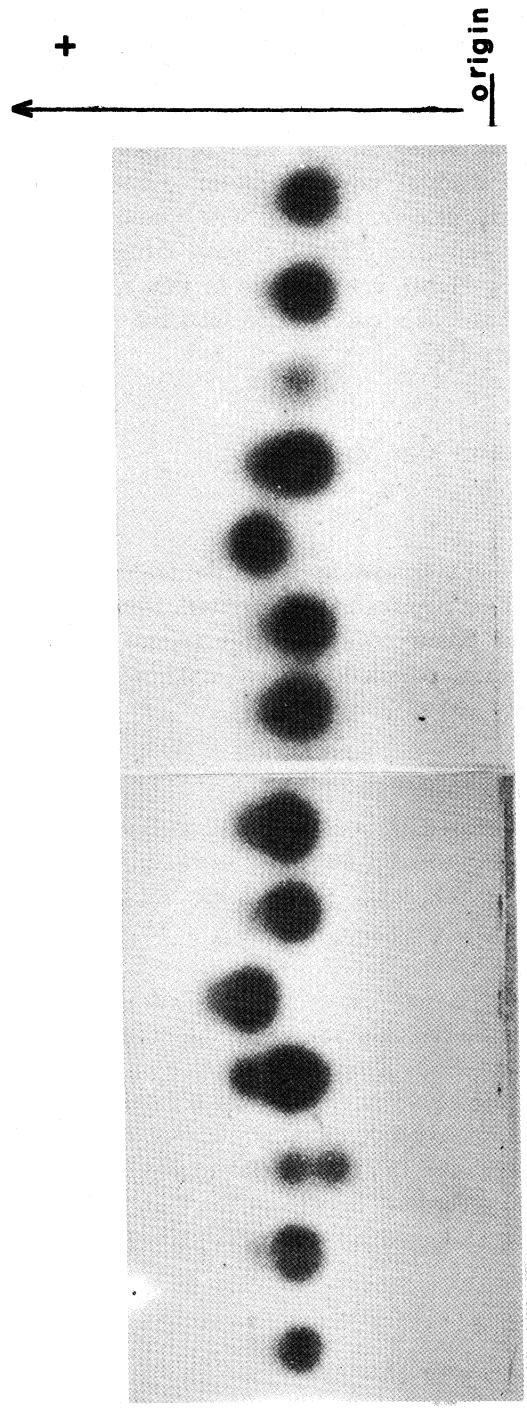

Fig. 1. - PGM phenotype of single individual homogenates of *Aedes aegypti*, obtained by combining electrophoresis and heat denaturation techniques. Two gel slices of the same gel were incubated at 37 °C (*a*) and 60 °C (*b*). The following PGM phenotypes are shown: 1 and 2 = PGM 1.00, tr; 3 = PGM 0.90, ts-1.00, tr; 4 = PGM 1.00, tr; 5 = PGM 1.10, tr; 6 and 7 = PGM 1.00, tr.

B_1 are here termed 0.90, 1.0, 1.10 and 1.20 respectively, following the criterion already adopted for the PGM system in *Drosophila melanogaster* [19].

Analysis of the electrophoretic patterns of single mosquito homogenates after incubation at the temperatures of 37 °C and 60 °C allowed for the classification of temperature-resistant (*tr*) and temperature-sensitive (*ts*) PGM isozymes, depending on whether after heat treatment they were electrophoretically detectable. Fig. 1 shows gels stained for PGM activity with (*b*) or without (*a*) heat treatment. The isozyme with the mobility PGM 1.00 appears to be non-sensitive to heat treatment, whereas the band corresponding to the allele $Pgm^{0,90}$, present at 37 °C in the individual 3 with the phenotype PGM 0.90-1.00, is seen to disappear in gel (*b*), indicating that it is temperature-sensitive.

To ascertain whether the observed variation in heat sensitivity is, indeed, due to variation at the PGM structural locus, reciprocal crosses were made between two laboratory lines isogenic for 0.90, *ts* and 1.00, *tr* alleles (Table I). The

TABLE I.

Mendelian segregation of thermosensitivity and electrophoretic behaviour of $Pgm^{0,90}$ *and* $Pgm^{1,00}$ *alleles. Reciprocal crosses were pooled (number in parentheses).*

Parental PGM genotype	Offspring PGM phenotype		
	0.90, ts	0.90, ts — 1.00, tr	1.00, tr
$\frac{0.90, ts}{0.90, ts} \times \frac{1.00, tr}{1.00, tr}$ [6]	—	53	—
$\frac{0.90, ts}{1.00, tr} \times \frac{0.90, ts}{1.00, tr}$ [8]	16	42	16
$\frac{0.90, ts}{1.00, tr} \times \frac{1.00, tr}{1.00, tr}$ [5]	—	31	32

following lines of evidence are consistent with such a hypothesis: (1) F_1 progeny of the crosses 0.90, ts/0.90, ts × 1.00, tr/1.00, tr, and their reciprocals, all show the phenotype 0.90, ts-1.00, tr; (2) F_2 progeny shows co-segregation of heat sensitivity with electrophoretic mobility not significantly different from the 1 : 2 : 1 ratio expected if heat sensitivity is a property of the $Pgm^{0,90}$ allelic product ($\chi^2_{2df} = 1.35$; $P > 0.50$); finally, (3) the progeny resulting from the crosses 0.90, ts/1.00, tr × 1.00, tr/1.00, tr is in complete agreement with the hypothesis of the $Pgm^{0,90}$ allelic product being heat labile (the overall genotype ratio of 0.90, ts/1.00, tr : 1.00, tr/1.00, tr is 31 : 32). An alternative hypothesis is that of a strictly linked modifier locus. However, out of 13 informative crosses with a total progeny of 137 individuals, not one instance of recombination of heat sensitivity and electrophoretic behaviour was found; i.e., heat sensi-

TABLE II.

Distribution of PGM *electrophoretic and heat-sensitivity phenotypes in the two populations examined. Expected numbers in parentheses.*

PGM phenotype	Population	
	A	B
0.90, tr	—	14 (10.0)
0.90, ts	— (1.6)	—
0.90, tr/1.00, tr	—	45 (56.3)
0.90, ts/1.00, tr	34 (33.7)	—
0.90, tr/1.10, tr	—	8 (5.9)
0.90, ts/1.10, tr	6 (3.1)	—
0.90, tr/1.20, tr	—	7 (5.9)
1.00, tr	177 (174.2)	86 (79.2)
1.00, tr/1.10, tr	26 (31.9)	14 (16.6)
1.10, tr	3 (1.5)	2 (0.9)
1.00, tr/1.20, tr	—	17 (16.6)
1.10, tr/1.20, tr	—	— (1.7)
1.20, tr	—	1 (0.9)
Total	246	194
P (*) >	10 %	5 %

(*) For Hardy-Weinberg equilibrium. For the calculation of chi-square, rare alleles were pooled.

tivity was always seen to be transmitted together with the eletcrophoretic character with which it was originally associated.

Table II shows the PGM phenotype frequencies observed in the two populations analyzed, when electrophoretic mobility and heat sensitivity are considered, together with those expected under the Hardy-Weinberg equilibrium. The *Pgm* allele frequencies observed in the two samples are given in Table III. No significant differences between expected and observed phenotype frequencies were found in either sample.

There is a total of five different alleles detected under our conditions in the whole sample: three of them were found in population *A*, and four in population *B*. Both populations display the same general pattern of allele frequencies: one numerically dominant class, which is the same for the two populations ($Pgm^{1,00,tr}$), together with some subsidiary classes with faster and slower mobilities. The two populations, however, differ in their extent of genetic variability. This is apparent by comparing their effective numbers of alleles (n_e) (Table III). It may be observed that population *B* has the higher value. If this difference is expressed in terms of proportion of heterozygous individuals in each population, the corresponding values will be 0.531 (*B*) *versus* 0.297 (*A*). The larger variability in population *B* as compared with population *A*, is the result of the significantly lower frequency of the most common allele (0.639 *vs.* 0.842; $\chi^2_{1df} = 47.6$; $P \ll 0.0001$), almost fully compensated by the relatively high frequency of the $Pgm^{0,90,tr}$.

Comparing population *A* and population *B*, further differences are observed in the proportion of alleles in common between them which can be detected before and after heat-treatment. In fact, after the heat denaturation criterion

TABLE III.

Pgm *allele frequencies found in the two populations examined.*

Pgm allele	Population	
	A	*B*
0.90, tr	—	0.227 ± 0.021
0.90, ts	0.081 ± 0.012	—
1.00, tr	0.842 ± 0.016	0.639 ± 0.024
1.10, tr	0.077 ± 0.012	0.067 ± 0.013
1.20, tr	—	0.067 ± 0.013
n_e (*)	1.39	2.13

(*) n_e (effective number of alleles) is defined as the number of equally frequent alleles that would produce the same homozygosity as in the actual population and is calculated as $1/\Sigma x_i^2$, where x_i is the frequency of any *i*-th allele.

has been employed, $Pgm^{0,90}$ allele is subdivided into two classes, each of them unique to one population: population *A* appears to be " monomorphic " for the $Pgm^{0,90,ts}$ and population *B* for the $Pgm^{0,90,tr}$.

On the reasonable assumption that the variation responsible for the electrophoretic mobility (0.90/non-0.90) and that responsible for heat sensitivity reside at two different sites within the PGM structural locus, it may be observed that whereas no variation at the *tr*/*ts* site was detectable in population *B*, both sites turned out to be polymorphic in population *A*. In this sample the heat sensitivity has been always found associated with mobility 0.90. Thus, if different mutations are responsible for the two properties, they must be in absolute linkage disequilibrium. Alternatively, there is only one mutation causing them simultaneously; this, in turn, would imply that the mutation responsible for the 0.90 electrophoretic property in population *A* is not the same as that of population *B*.

Heat denaturation studies of phosphoglucomutase in natural populations of different species of *Culicidae* have revealed that the existence of common electrophoretically cryptic alleles is a general phenomenon [10, 11]. Indeed, by the analysis of a natural population of *Culiseta litorea*, which proved to be electrophoretically monomorphic for PGM locus, Scozzari *et al.*, 1977 [20] were able to subtype the *Pgm* common variant recognized by standard electrophoresis into two " silent " alleles, both having polymorphic frequencies.

As is shown in Table III analysis of the thermostability of PGM electrophoretic variants in *Aedes aegypti* has resulted in a subdivision of one electromorph into two distinct types on the basis of their response to heat. In the populations examined, $Pgm^{0,90}$ is a class of two alleles which, although sharing the same electrophoretic mobility, could be differentiated with respect to their heat stability. No evidence, however, was obtained for additional alleles within populations; therefore, no increase in the estimate of the intra-population heterozygosities was obtained by combining the two methods.

By contrast, an increased amount of genetic differentiation between the populations was apparent when both electrophoretic mobility and heat stability criterion were employed. With regard to both electrophoretic and isoelectrophoretic PGM variants, the two samples differ not only in *Pgm* allele frequencies but also in the presence or absence of particular alleles.

It seems likely that this is the result of random drift. The laboratory populations here examined originated from localities which are presumably different and were established at different times: thus, the probability that random sampling caused deviations in the allelic frequencies may be very high.

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