NOTES AND COMMENTS

MULTIPLE PHOSPHOGLUCOMUTASE ALLELES IN *ANOPHELES STEPHENSI*

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1. INTRODUCTION

Anopheles stephensi Liston is an important malaria vector in the Indo-Persian zone with a distribution ranging from Saudi Arabia eastward across India to Burma. This is one of the best *Anopheles* mosquitoes for genetical studies as it can be fairly easily maintained in the laboratory and it shows good polytene chromosomes both in the larva and in the adult (Sharma *et al.*, 1969; Coluzzi *et al.*, 1970). However, the formal genetics of *A. stephensi* has been investigated essentially in connection with the genetical aspects of insecticide resistance and apparently only one morphological mutant has been isolated (Davidson and Mason, 1963; Mason and Davidson, 1966). As regards biochemical mutants two esterase isoenzymes were shown electrophoretically by Bianchi (1968). In the present paper we report the evidence for a polymorphism involving phosphoglucomutase (PGM). This enzyme catalyzes the transfer of glucose 1-phosphate to glucose 6-phosphate, and it has an important role in carbohydrate metabolism.

2. MATERIAL AND METHODS

The material examined consists of four strains of *A. stephensi*, kindly supplied by Dr G. Davidson, Ross Institute, London, and having the following geographical origins: Mamlaha, Iraq; Rahguerd, Iran; Karachi, Pakistan; Delhi, India. The mosquitoes were reared and maintained in a constant temperature/humidity room (temperature: $27 \pm 1^\circ \text{C}$.; relative humidity: 70 ± 5 per cent.) with a fixed photoperiod of 15 hours' light. Larval rearing was carried out in plastic bowls (cm. $40 \times 25 \times 10$) filled with well water to a depth of 3 cm. The larval density was adjusted to provide a minimum of 3 sq. cm. of water surface per larva. The food, given daily, consisted of a mixture of equal parts of Farex, finely minced rat pellets and bran. The adults, maintained in 25 cm. cubic cages, were supplied with sucrose solution and the females were blood fed on guinea-pigs.

Studies on the PGM were carried out utilizing, with minor modifications, the electrophoretic method described by Spencer *et al.* (1964). For each individual examined a small piece of filter paper (Whatman No. 3) of about 5×3 mm. was impregnated with the adult mosquito homogenate (obtained by grinding the mosquito in 0.01 ml. of the gel buffer) and inserted in starch-gel. This was performed in plexiglass trays ($0.4 \times 10 \times 20$ cm.) with a gel composed of 10 per cent. starch dissolved in a 1:10

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dilution of the maleic acid-Tris-EDTA-MgCl₂ buffer at pH = 7.4 as described in the original technique. Horizontal starch-gel electrophoresis was carried out at +5° C. at 4 v/cm. for 14 hr. or 9 v/cm. for 4 hr. The gel was sliced and developed for PGM as described by Spencer *et al.* (1964), with the modification that the developing solution was solidified by agar at a final concentration of 0.8 per cent. The PGM patterns appear after about 30 minutes of incubation at 37° C. in the dark.

TABLE 1
Distribution of the PGM phenotypes in the offspring from 120 crosses

Type of mating	Number of crosses	Offspring PGM phenotypes						Total	χ^2	Probability
		A	AB	B	AC	BC	C			
A × A	7	107	—	—	—	—	—	107	—	—
B × B	7	—	—	130	—	—	—	130	—	—
C × C	7	—	—	—	—	—	196	196	—	—
A × B	4	—	98	—	—	—	—	98	—	—
AB × AB	10	93	168	87	—	—	—	348	0.619	>0.70
A × AB	5	70	80	—	—	—	—	150	0.666	>0.40
B × AB	6	—	119	128	—	—	—	247	0.326	>0.50
C × AB	4	—	—	—	103	100	—	203	0.044	>0.80
A × C	8	—	—	—	158	—	—	158	—	—
AC × AC	6	91	—	—	194	—	87	372	0.774	>0.60
A × AC	7	78	—	—	68	—	—	146	0.684	>0.40
B × AC	5	—	55	—	—	70	—	125	1.800	>0.15
C × AC	8	—	—	—	143	—	148	291	0.084	>0.70
B × C	7	—	—	—	—	130	—	130	—	—
BC × BC	5	—	—	75	—	183	70	328	4.554	>0.10
A × BC	3	—	99	—	96	—	—	195	0.046	>0.80
B × BC	5	—	—	102	—	105	—	207	0.042	>0.80
C × BC	4	—	—	—	—	89	78	167	0.724	>0.30
AB × AC	3	58	53	—	65	51	—	227	2.055	>0.50
AB × BC	4	—	55	60	65	58	—	238	0.889	>0.80
AC × BC	5	—	37	—	48	47	36	168	2.904	>0.30

3. RESULTS AND CONCLUSIONS

The adult mosquito homogenates can be classified into six distinct types according to the banding pattern produced (fig. 1). Three of the phenotypes show only one anodic band and are named A (band 1), B (band 2) and C (band 3). The remaining three phenotypes are produced by combinations of bands 1 and 2 (AB), 2 and 3 (BC) and 1 and 3 (AC). These double-band phenotypes are also produced by an artificial mixture of approximately equal amounts of homogenates from two mosquitoes having the corresponding single band phenotypes. The following evidence supports the conclusion that the bands shown in fig. 1 really represent PGM activity: (1) if glucose 1-phosphate is omitted from the reaction mixture, none of the bands develop; (2) if glucose 6-phosphate dehydrogenase is omitted from the reaction mixture, patterns appear similar to those shown in fig. 1 but fainter and more diffuse. Such a pattern is presumably due to endogenous G6PD present in the homogenate.

The formal genetics of the enzyme variation was studied by using three

TABLE 2
Distribution of the PGM phenotypes in samples of Anopheles stephensi from four laboratory populations of different geographic origin

Strain	Number tested	Phenotypes *							Frequency of PGM alleles		
		A	AB	B	AC	BC	C	P_{gmA}	P_{gmB}	P_{gmC}	
Iraq	164	13 (11.54)	10 (9.02)	1 (1.76)	51 (54.91)	22 (21.44)	67 (65.32)	26.52 ± 2.44	10.37 ± 1.68	63.11 ± 2.66	
Iran	145	72 (76.03)	—	—	66 (57.93)	—	7 (11.03)	72.41 ± 2.62	—	27.59 ± 2.62	
Pakistan	177	44 (43.26)	87 (88.49)	46 (45.26)	—	—	—	49.44 ± 2.66	50.56 ± 2.66	—	
India	126	15 (16.80)	62 (58.41)	49 (50.79)	—	—	—	36.51 ± 3.03	63.49 ± 3.03	—	

* In brackets the numbers expected from a Hardy-Weinberg equilibrium involving three codominant alleles in the Iraq strain and two in the other strains.

pure lines selected from the progenies of single females homogeneous for the banding patterns A, B and C respectively. Mass crosses were carried out in laboratory cages, some with males and females from the pure lines, some with males from the pure lines and females from the strains. After insemination, the females were blood fed, isolated to obtain single ovi-positions and then examined for their PGM phenotypes if these were unknown. This procedure was followed to overcome some difficulty existing in obtaining spontaneous insemination with single males and to avoid the use of the time-consuming induced-copulation technique.

Table 1 shows the results of the examination of 120 progenies from all the 21 possible crosses among the six PGM phenotypes. Both reciprocal crosses have been made, but the data are combined in the table as the sex has been found irrelevant. The simplest explanation of these figures involves the assumption of an autosomal gene (*Pgm*) with three codominant alleles (Pgm^A , Pgm^B , Pgm^C). The homozygotes Pgm^A/Pgm^A , Pgm^B/Pgm^B and Pgm^C/Pgm^C correspond to the phenotypes A, B and C respectively, while the double-band phenotypes AB, BC, AC are the corresponding heterozygotes.

Table 2 shows the distribution of the six PGM phenotypes and the corresponding frequencies of the three alleles in the four laboratory populations studied. The distribution of the phenotypes agrees well with the frequencies expected from a Hardy-Weinberg equilibrium involving respectively two or three codominant alleles. The three PGM alleles are variably distributed in the four laboratory populations. The Iraq strain shows all the three alleles while the strains from Karachi and India show only Pgm^A and Pgm^B and the Iran strain only Pgm^A and Pgm^C . Such differences, taking into account the fact that the strains were mass bred from the beginning, presumably reflect genetical differences among different populations of *Anopheles stephensi*. On the other hand, the existence of a genetical differentiation among these four laboratory populations has been recently shown also by cytogenetical observations on polytene chromosomes (Coluzzi *et al.*, 1970).

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Plate

FIG. 1.—Photograph of the PGM zymograms of adult mosquitoes of *A. stephensi* showing the six electrophoretic phenotypes observed in the present study.