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Genetics of Glucose Phosphate Isomerase and Phosphoglucomutase in *Aedes albopictus* (Diptera: Culicidae)

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Summary. Glucose phosphate isomerase (E.C. 5.3.1.9) and phosphoglucomutase (E.C. 2.7.5.1) were found to be polymorphic in a laboratory colony of *Aedes albopictus*. The glucose phosphate isomerase locus is represented by two alleles resulting in three genotypes, while the phosphoglucomutase locus is represented by at least five alleles giving rise to a total of 15 genotypes. The inheritance of these two enzymes is of the Mendelian type with co-dominant alleles. Present data indicate that these genes are not linked.

Of 105 mosquitoes analysed for these two gene-enzyme systems, the frequencies for glucose phosphate isomerase alleles are Gpi^S = 0.68 and Gpi^F = 0.32, while the frequencies for phosphoglucomutase alleles are Pgm^A = 0.16, Pgm^B = 0.11, Pgm^C = 0.19, Pgm^D = 0.30 and Pgm^F = 0.24. The frequencies of the three glucose phosphate isomerase genotypes are in accord with Hardy-Weinberg expectations (X^2_1 = 2.74). Similarly, the frequencies of the 15 phosphoglucomutase genotypes probably do not differ significantly from Hardy-Weinberg expectations (X^2_{10} = 18.45).

Key words: Mosquito – Biochemical genetics – Gene-enzyme systems – *Aedes*

Introduction

The mosquito *Aedes albopictus* is one of the two principal vectors of dengue virus in Malaysia. It is a far more common species than *Aedes aegypti*, the other principal vector of dengue. It is found in both city and countryside, in areas with a good deal of such vegetation as gardens, parks, plantations and forest fringe (Rudnick 1965). Records of *A. albopictus* being involved as the principal vector of dengue virus go back as far as August 1942 when an epidemic of explosive incidence of dengue occurred suddenly in Nagasaki, Japan (Hotta 1969). In addition dengue virus has also been isolated from this mosquito.

Although *A. albopictus* may be as important a vector as *A. aegypti*, its biochemical genetics has not been intensively and extensively studied. The first electrophoretic study on gene-enzyme systems of *Aedes mosquitoes* was probably initiated by Trebatoski & Haynes (1969). Subsequent work has centered largely on *A. aegypti* (for reviews see Bullini & Coluzzi 1973; Kitzmiller 1976; Munstermann and Craig 1979; Steiner and Joslyn 1979).

The present report deals with the formal genetics of phosphoglucomutase (E.C. 2.7.5.1) and glucose phosphate isomerase (E.C. 5.3.1.9) in *Aedes albopictus*.

Materials and Methods

The mosquitoes used for the present study were obtained from the laboratory colony maintained at the Institute for Medical Research, Kuala Lumpur, Malaysia.

Paired matings of the mosquitoes were carried out at random. The parental flies and their offspring were kept in liquid nitrogen or −70°C deep freeze until required for electrophoretic study. 105 mosquitoes were also sampled from the F129 generation for estimating the gene frequencies.

Individual mosquitoes were homogenized in a drop of distilled water immediately before the electrophoretic run. The homogenate was adsorbed onto a 6 × 10 mm filter paper. Horizontal electrophoresis, using a 12% hydrolysed starch, was carried out for 16 hr at 4°C at a constant voltage of 80 V. The 'TEMM' buffer system (Spencer et al. 1964) was utilised. The gel buffer was prepared by diluting the bridge buffer 1 in 10 and the pH adjusted to 7.4.

The method used for visualization of glucose phosphate isomerase was that of Detter et al. (1968), while that for phosphoglucomutase was that of Spencer et al. (1964), with slight modification.

Results

The glucose phosphate isomerase (GPI, E.C. 5.3.1.9) and the phosphoglucomutase (PGM, E.C. 2.7.5.1) zymograms

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of *Aedes albopictus* did not exhibit differences attributable due to sex.

Glucose Phosphate Isomerase

Homogenates of single mosquitoes belonged to one of three distinct electrophoretic phenotypes (Fig. 1). These had either a slow-moving band (S), or a fast-moving band (F), or one with three bands (FS) consisting of the slow- and fast-moving bands and a band with intermediate mobility. The electrophoretic patterns indicated that glucose phosphate isomerase occurred as a dimer.

When FS individuals were mated with each other, three phenotypes (S, FS, F) were found in the offspring (Table 1). The proportions of these three phenotypes were in accordance with Mendelian inheritance for codominant genes. That the FS individuals were heterozygotes were confirmed by the mating of FS with S individuals, which produced FS and S offspring in about equal proportions (Table 1).

In a sample of 105 individuals from the F129 generation, the gene frequency ($Gpi^S = 0.68$) was in good accordance with the Hardy-Weinberg expectation ($X_1^2 = 2.74$, Table 2).

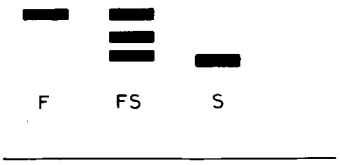

Fig. 1. Electrophoretic phenotypes of glucose phosphate isomerase in *Aedes albopictus*

Table 1. Mendelian inheritance of glucose phosphate isomerase in *Aedes albopictus*

Mating	Progeny			X^2
	S	FS	F	
FS × FS	5	23	7	3.69
FS × S	46	36	0	1.22

Table 2. Distribution of glucose phosphate isomerase electrophoretic phenotypes in the F129 generation of *Aedes albopictus*

	GPI phenotype			Allele frequency	
	S	FS	F	Gpi^S	Gpi^F
Observed no.	45	53	7	0.68	0.32
Expected no.	48.55	45.70	10.75		

$x_1^2 = 2.74$

Phosphoglucomutase

A total of 15 phenotypes were observed in a sample of 105 individuals from the F129 generation. These phenotypes were attributable to the occurrence of five codominant phosphoglucomutase (PGM) alleles (Fig. 2) and their proportions were shown in Table 3. Each *Pgm* allele determined a two-band electrophoretic pattern. The allele frequencies were $Pgm^A = 0.16$, $Pgm^B = 0.11$, $Pgm^C = 0.19$, $Pgm^D = 0.30$ and $Pgm^F = 0.24$. Although $X_{10}^2 = 18.45$, the frequencies of the 15 phenotypes probably did not differ significantly from Hardy-Weinberg expectations. The Mendelian inheritance of this enzyme was indicated

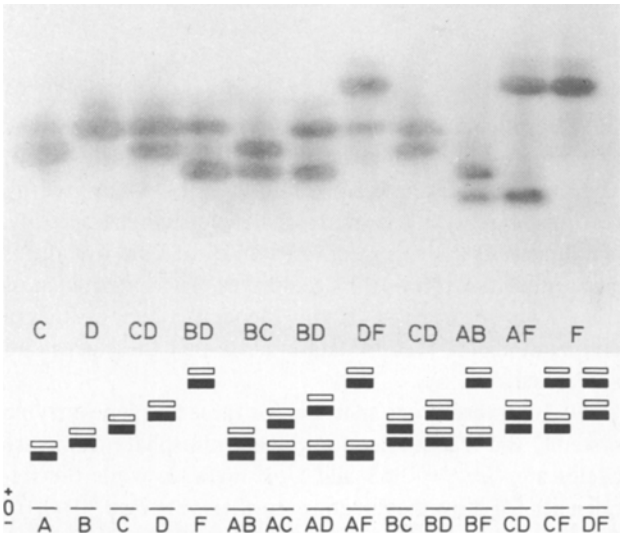

Fig. 2. Electrophoretic phenotypes of phosphoglucomutase in *Aedes albopictus*. The Pgm^E allele is not found in the present sample but has been detected in a small sample from the University of Malaya campus

Table 3. Distribution of phosphoglucomutase electrophoretic phenotypes in the F129 generation of *Aedes albopictus*

Phenotype	Observed no.	Expected no.	Allele frequency
A	6	2.69	0.16
B	1	1.27	0.11
C	8	3.79	0.19
D	12	9.45	0.30
F	7	6.05	0.24
AB	4	3.70	
AC	6	6.38	
AD	9	10.08	
AF	3	8.06	
BC	4	4.39	
BD	5	6.93	
BF	8	5.54	
CD	7	11.97	
CF	6	9.58	
DF	19	15.12	($x_{10}^2 = 18.45$)

Table 4. Mendelian inheritance of phosphoglucomutase in *Aedes albopictus*

Mating	Progeny	Test for Mendelian ratio
1. DF ♀ × DF ♂	6D : 12DF : 4F	$X_2^2 = 0.55$
2. AF ♀ × DF ♂	19AD : 15AF : 12DF : 14F	$X_3^2 = 1.72$
3. D ♀ × AD ♂	10AD : 11D	$X_1^2 = 0.04$
4. BC ♀ × DE ♂	4BD : 2CD : 6BE : 6CE	$X_3^2 = 1.56$
5. AD ♀ × BC ♂	17AB : 22AC : 10BD : 15CD	$X_3^2 = 4.62$

in paired matings involving various combinations of the five codominant alleles (Table 4).

Independent Assortment of Glucose Phosphate Isomerase and Phosphoglucomutase

The results obtained from matings between PGM $\overline{AD}$, GPI $\overline{FS}$ females and PGM $\overline{BC}$, GPI $\overline{S}$ males (Table 5) indicated that the genes governing glucose phosphate isomerase and phosphoglucomutase segregated independently. Similar results were obtained for other crosses involving different combinations of these two genes, but the number of offspring in each case was too small and hence not presented here.

Discussion

Electrophoretic studies of gene-enzyme systems have contributed one of the most significant advances in mosquito genetics in recent years. Of the *Aedes* mosquitoes, only *A. aegypti* appears to have received much attention (Bullini et al. 1973; Kitzmiller 1976; Steiner et al. 1979). Although a number of gene-enzyme systems have been widely studied in mosquitoes, they have not been critically studied in *Aedes albopictus*. The first study appears to be that of Trebatoski et al. (1969), who studied the 4th-instar larvae of *A. albopictus* together with 9 other species of *Aedes*, 1 species of *Anopheles* and 1 species of *Culex*. This study concerned primarily on interspecific comparison, and did not include genetic analyses. The enzymes studied were acetate and butyrate esterases, alkaline and acid phosphatase, and malic and lactic dehydrogenase. More recently, glucose phosphate isomerase and hexokinase have also been reported (Yong et al. 1979, in press).

Table 5. F_1 progeny of PGM $\overline{AD}$/GPI $\overline{FS}$ ♀ × PGM $\overline{BC}$/GPI $\overline{S}$ ♂

		AB	AC	BD	CD	Total
S	M	7	6	5	6	24
	F	3	5	2	3	13
FS	M	4	6	1	2	13
	F	3	5	2	4	14
Total		17	22	10	15	64

Test for independent assortment between PGM and GPI, $X_7^2 = 6.78$; Test for Mendelian inheritance of PGM, $X_3^2 = 4.62$; Test for Mendelian inheritance of GPI, $X_1^2 = 1.56$

The present study reports for the first time the formal genetics of glucose phosphate isomerase and phosphoglucomutase in *Aedes albopictus*. These two enzymes show characteristic Mendelian inheritance. This concurs with studies in other mosquitoes which show non-linkage between glucose phosphate isomerase and phosphoglucomutase (Munstersmann et al. 1979). In *Aedes aegypti* glucose phosphate isomerase is assigned to Linkage Group III while phosphoglucomutase is assigned to Linkage Group II. On this basis, the two genes in *A. albopictus* may be provisionally assigned to similar linkage groups as in *A. aegypti*. Glucose phosphate isomerase in *A. albopictus* has been demonstrated to be a dimer. This is consistent with the findings in other organisms, including other species of mosquitoes. So far only a pair of codominant alleles have been found, and their frequencies are in good agreement with Hardy-Weinberg expectations. The allele frequency ($Gpi^S = 0.68$) in the present study (F129 generation) is similar to that reported for the F123 generation of the same laboratory colony reported by Yong et al. (in press). As with the glucose phosphate isomerase, the frequencies of the 15 phosphoglucomutase phenotypes in the present sample of 105 *A. albopictus* mosquitoes also probably do not differ significantly from Hardy-Weinberg expectations ($X_{10}^2 = 18.45$). In the absence of other evidence, the high X^2 value may be reasonably attributed to the small sample size.

As with most culicine mosquitoes, each *Pgm* allele in *A. albopictus* determines a two-band electrophoretic pattern. The allele with the highest frequency ($Pgm^D = 0.30$) in the present study also agrees with earlier reports in other mosquitoes that the most frequent allele is generally the one controlling a phenotype with an intermediate electrophoretic mobility (Bullini et al. 1973). This has been taken as supporting evidence for the idea that protein polymorphism is not primarily influenced by random genetic drift acting on a number of neutral isoalleles (Bullini & Coluzzi 1972, Bulmer 1971).

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