GENETIC MAPPING OF A PHOSPHOGLUCOMUTASE LOCUS IN *AEDES TOGOI*

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ABSTRACT. An electrophoretic survey of the phosphoglucomutase (PGM) enzyme was performed using agar gels in 6 strains (3 Japanese strains and 3 strains from Taiwan, Thailand and Canada) of *Aedes togoi*. The survey revealed at least 3 alleles involved at the *Pgm* locus among the 6 strains examined. Backcross experiments showed that the *Pgm* locus was located on the sex chromosome in the following order: *Odh* (octanol dehydrogenase)—*M*(sex)—(13.8 map units)—*Pgm*—(17.0 map units)—*h*(hooked leg)—s(straw-colored larva).

INTRODUCTION

Polytene chromosome preparations are not useful tools for the study of chromosomal relationships among the *Aedes* mosquito species since the polytene chromosomes of these species cannot be satisfactorily prepared. Chromosomal rearrangements in the aedine mosquitoes have been studied, instead, on the basis of their linkage groups marked with mainly enzyme loci; by this method Munstermann (1981), Munstermann et al. (1982), and Pashley and Rai (1983) have revealed some evidence of chromosomal translocations and inversions in the course of speciation, as well as for presence of chromosomal homology among *Aedes (Stegomyia) aegypti* (Linn.), the *Ae. (Stg.) scutellaris* group, the *Ae. (Protomacleaya) triseriatus* group, and *Ae. (Ochlerotatus) atropalpus* (Coquillett).

All three linkage groups of *Ae. (Finlaya) togoi* (Theobald), a vector of several filarial species, have been marked with 14 morphological mutant loci and 7 enzyme loci, with all 7 enzyme loci located on linkage group 1 (sex chromosome) (Tadano 1984). This paper adds another enzyme, phosphoglucomutase (E. C. 2.7.5.1), locus (*Pgm*), to the genetic linkage map of *Ae. togoi*, providing further information on the comparative genetics of aedine mosquitoes.

MATERIALS AND METHODS

The following 7 lines of *Ae. togoi*, all isolated from laboratory strains, were used in this study: (1) Pgm^S (homozygous for phosphoglucomutase slow allele); (2) Pgm^F (homozygous for *Pgm* fast allele); (3) *bl*, Pgm^I (homozygous for *Pgm* intermediate allele, isolated from the *bl* mutant strain); (4) *s*, Pgm^S (isolated from the *s* mutant strain); (5) *h*, Pgm^S (isolated from the *h* strain); (6) $Odh\text{-}2^S$, Pgm^I (homozygous for both octanol dehydrogenase-2 slow allele and Pgm^I allele); (7) $Odh\text{-}2^I$, Pgm^S (homozygous for both *Odh-2* intermediate allele and Pgm^S allele).

The mosquitoes were reared and crossed as reported in Tadano (1977). The morphological mutants, *s*(straw-colored larva) and *h* (hooked leg), are linkage group-1 markers, arranged in the following order: *Odh-2*—(24 map units)—*M*(sex locus)—(17 map units)—*h*—(24 map units)—*s*. The *bl*(bleached pupa) is a linkage group-3 marker (Tadano 1984).

The electrophoresis procedure for octanol dehydrogenase (ODH) in agar gels has been described previously (Tadano 1983). Phosphoglucomutase (PGM) procedures were as follows: the electrode buffer was 0.0125 M potassium phosphate solution (pH 6.8), and 0.0031 M potassium phosphate solution (pH 6.8) was used as a gel buffer. The gel solution consisted of 2 g PVP (polyvinyl pyrrolidone) and 0.75 g agar (Wako Co.) per 100 ml of the gel buffer. The dimension of the agar gel was 0.7 mm × 12 cm × 17 cm. Only adult homogenates were electrophoresed, since neither pupae nor larvae gave clear electrophoretic patterns. Electrophoresis was performed at 30 mA/17 cm for 2 hours. The incubation solution for electrophoresed gels was: 50 ml 0.05 M Tris-HCl (pH 7.1), 25 mg α-D-glucose 1-phosphate, 12 units glucose 6-phosphate dehydrogenase, 5 mg $NADP^+$, 10 mg nitroblue tetrazolium, 10 mg EDTA, and 25 mg $MgCl_2$. After 30 minutes of incubation at 37°C, 1.2 mg PMS was added to this incubation solution and then incubation was continued for about 1 hour. For other details of this agar gel electrophoresis, refer to Tadano (1982).

In every backcross experiment, single female parents were isolated into separate plastic cups for oviposition. Each family was separately reared and scored for the phenotypes studied. Backcross data were examined for the expected 1:1 segregation ratio of each phenotype by chi-square tests at the 5% probability. Linkage between the genes was tested by chi-square tests at the 1% level.

RESULTS AND DISCUSSION

A preliminary survey of phosphoglucomutase (PGM) was conducted in 6 wild-type strains (3 Japanese strains and 3 strains from

Taiwan, Thailand, and Canada). During the survey, at least 3 alleles were detected at this enzyme locus (*Pgm*) and named fast (*F*), intermediate (*I*), and slow (*S*) alleles according to their mobilities. Electrophoretic patterns showed that each allele produced two bands, with a faint band migrating anodic to a intensely stained band. Heterozygotes $Pgm^{I/S}$ produced two intense bands and one faint band, since one faint band of *S* allele overlapped with the principal band of *I* allele; the same was true for the $Pgm^{F/I}$ heterozygotes. This situation of one allele-two bands has been reported in PGM of *Anopheles claviger* (Meigen), *An. maculipennis* Meigen (Bullini and Coluzzi 1973), *Culex quinquefasciatus* Say (Cheng and Hacker 1976), and *Aedes albopictus* (Skuse) (Yong et al. 1981). Particularly, in *Cx. quinquefasciatus*, the two bands produced by one allele were almost equally intense. In many anopheline mosquitoes so far electrophoresed, heterozygotes for *Pgm* alleles displayed simply two bands corresponding to the respective alleles (Narang et al. 1981, Dubash et al. 1981), suggesting the monomeric structure of this enzyme.

With a rare exception (noted below), all the phenotypes of backcross offspring segregated at the 1:1 ratio. In addition to the PGM activity zone mentioned above of *Ae. togoi*, very occasionally one additional zone of PGM activity was observed on the gels, which was very faintly stained more on the anodal side. This suggested existence of a second isozyme locus of PGM.

Two preliminary backcrosses involving *M* (sex locus), *bl*, and *s* were made (Table 1) to locate the *Pgm* locus. The result of cross A, yielding only one family, indicated lack of linkage ($P > 0.01$) between *Pgm* and *bl*, but presence of linkage of *Pgm* to *M* with a recombination distance (± S. E.) of 10.7±3.6 map units. Again cross B suggested that *Pgm* is sex-linked in the order of *M—Pgm—s*. Here, the recombination distance (±S. E.) between *Pgm* and *M* was estimated at 23.2±4.8 map units.

Since the gene arrangement on linkage group 1 has been established as *Odh—M—h—s* (Tadano 1984), four more backcrosses, C through F (Tables 2 and 3), were undertaken to determine the position of *Pgm* in this gene sequence. Recombination units among *M*, *Odh* and *Pgm*, obtained from crosses C and D supported the conclusion of crosses A and B, and indicated the gene arrangement of *Odh—M—Pgm—s*. The recombination distance for *M—Pgm* varied from 12.4±1.4 to 13.3±1.7 units, with an average of 12.8 units.

Crosses E and F positioned *Pgm* between *M* and *h*. Thus, the gene arrangement on linkage group 1 was concluded to be: *Odh—M—Pgm—h—s*. The distance between *M* and *Pgm* ranges from 11.3±1.8 to 18.2±1.9 units, with the weighted mean units being 15.2, while the distance units for *Pgm—h* was 15.0±2.0 to 18.5±1.9 with an average of 17.0.

Table 1. Genetic relationships among *bl*, *s*, sex(*M*) and *Pgm*.

Cross A: (*bl*; Pgm^{I}; *m*/*bl*; Pgm^{I}; *m*) × (*bl*; Pgm^{I}; *m*/+; Pgm^{S}; *M*)

Phenotypes of offspring			
Female + $Pgm^{I/I}$	21	Male + $Pgm^{I/I}$	1
(*m*/*m*) + $Pgm^{I/S}$	1	(*M*/*m*) + $Pgm^{I/S}$	11
bl $Pgm^{I/I}$	16	*bl*$Pgm^{I/I}$	3
bl $Pgm^{I/S}$	3	*bl*$Pgm^{I/S}$	19

Total = 75
Families examined = 1
Recombination units
between *M* and *Pgm* = 10.7 ± 3.6
between *bl* and *Pgm* = no linkage

Cross B: (*s*; Pgm^{S}; *m*/*s*; Pgm^{S}; *m*) × (s; Pgm^{S}; *m*/+; Pgm^{I}; *M*)

Phenotypes of offspring			
Female + $Pgm^{S/S}$	5	Male + $Pgm^{S/S}$	0
(*m*/*m*) + $Pgm^{I/S}$	9	(*M*/*m*) + $Pgm^{I/S}$	24
s $Pgm^{S/S}$	24	*s* $Pgm^{S/S}$	7
s $Pgm^{I/S}$	3	*s* $Pgm^{I/S}$	10

Total = 82
families examined = 1
Recombination units
between *M* and *s* = 37.8 ± 5.4
between *M* and *Pgm* = 23.2 ± 4.8
between *s* and *Pgm* = 22.0 ± 4.6

Table 2. Genetic relationships among sex (*M*), *Odh* and *Pgm*.

Cross C: (Odh^{S}; Pgm^{I}; *m*/Odh^{S}; Pgm^{I}; *m*) × (Odh^{S}; Pgm^{I}; *m*/Odh^{I}; Pgm^{S}; *M*)
Cross D: (Odh^{S}; Pgm^{I}; *m*/Odh^{S}; Pgm^{I}; *m*) × (Odh^{I}; Pgm^{S}; *m*/Odh^{S}; Pgm^{I}; *M*)

Phenotypes of offspring		C	D
Female	$Pgm^{I/I}$ $Odh^{S/S}$	191	2
(*m*/*m*)	$Pgm^{I/I}$ $Odh^{I/S}$	68	25
	$Pgm^{I/S}$ $Odh^{S/S}$	25	37
	$Pgm^{I/S}$ $Odh^{I/S}$	7	141
Male	$Pgm^{I/I}$ $Odh^{S/S}$	5	127
(*M*/*m*)	$Pgm^{I/I}$ $Odh^{I/S}$	35	54
	$Pgm^{I/S}$ $Odh^{S/S}$	69	25
	$Pgm^{I/S}$ $Odh^{I/S}$	181	3
Total		581	414
Families examined		6	4
Recombination units between:			
	Odh–*Pgm*	33.9 ± 2.0	34.1 ± 2.3
	M–*Odh*	25.7 ± 1.8	23.2 ± 2.1
	M–*Pgm*	12.4 ± 1.4	13.3 ± 1.7

Table 3. Genetic relationships among sex (*M*), *h* and *Pgm*.

Cross E: *(h; Pgm^S; m/h; Pgm^S; m)* × *(+; Pgm^F; m/h; Pgm^S; M)*
Cross F: *(h; Pgm^S; m/h; Pgm^S; m)* × *(h; Pgm^F; m/+; Pgm^S; M)*

Phenotypes of offspring		E	F
Female	+ $Pgm^{S\|S}$	2	15
(m/m)	+ $Pgm^{F\|S}$	130	31
	h $Pgm^{S\|S}$	7	3
	h $Pgm^{F\|S}$	19	172
Male	+ $Pgm^{S\|S}$	17	131
(M/m)	+ $Pgm^{F\|S}$	17	29
	h $Pgm^{S\|S}$	124	16
	h $Pgm^{F\|S}$	11	31
Total		327	428
Families examined		3	4
Recombination units between:			
	M–h	18.4±1.9	21.7±2.0
	h–Pgm	15.0±2.0	18.5±1.9
	M–Pgm	11.3±1.8	18.2±1.9

It should be noted that in cross F more $Pgm^{F/S}$ heterzygotes (263 individuals) were produced than $Pgm^{S/S}$ homozygotes (165 individuals) (χ^2= 22.4, $P < 0.01$), and that the average recombination distance for *M—Pgm,* based on the data pooled from four crosses C to F, was approximately 13.8 map units.

The *Pgm* locus has been found only on linkage group 2 (autosomes) in all the *Aedes* species so far studied, i.e , in *Ae. aegypti,* the *Ae. (Stegomyia) scutellaris* group, and *Ae. (Protomacleaya) triseriatus* (Munstermann 1981, Pashley and Rai 1983). The placement of *Pgm* on the sex chromosome in *Ae. togoi,* a member of the *Finlaya* subgenus, suggests that a translocation event has occurred in the chromosomal evolution of *Aedes* subgenera. A comparison of linkage groups of enzyme loci in the above *Aedes* species indicates that the gene arrangement of *Odh*—sex(*M*)—*Pgm*—*α-Gpdh*—*Idh-I*—*Est-2* in *Ae. togoi* is the most similar to that in *Ae. triseriatus,* which has the arrangement of *Odh*—*Pgm*—*Idh-2*—*Est-5* on linkage group 2. In the case of *Ae. togoi,* the sex locus appears to have been inserted into the chromosomal region between *Odh* and *Pgm* of *Ae. triseriatus.*

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References Cited

Bullini, L. and M. Coluzzi. 1973. Electrophoretic studies on gene-enzyme systems in mosquitoes (Diptera: Culicidae). Parassitologia 15:221–248.

Cheng, M. L. and C. S. Hacker. 1976. Inheritance of 6-phosphogluconate dehydrogenase variants in *Culex pipiens quinquefasciatus* Say. J. Hered. 67:215–219.

Dubash, C. J., R. K. Sakai and R. H. Baker. 1981. A phosphoglucomutase polymorphism in the mosquito, *Anopheles culicifacies.* J. Hered. 72:136–138.

Munstermann, L. E. 1981. Enzyme linkage map for tracing chromosomal evolution in *Aedes* mosquitoes, pp. 129–140. *In:* M. W. Stock (ed.), Application of genetics and cytology in insect systematics and evolution. Univ. Idaho Press, Moscow.

Munstermann, L. E., D. B. Taylor and T. C. Matthews. 1982. Population genetics and speciation in the *Aedes triseriatus* group, pp. 433–453. *In:* W. W. M. Steiner, W. J. Tabachnick, K. S. Rai and S. Narang (eds.). Recent developments in the genetics of insect disease vectors. Stipes, Champaign, IL.

Narang, S., J. A. Seawright and D. J. Joslyn. 1981. Inheritance and mapping of hexokinase-1 and phosphoglucomutase in *Anopheles albimanus* Wiedemann. Mosq. News 41:99–106.

Pashley, D. P. and K. S. Rai. 1983. Linkage relationships of eleven enzyme loci in the *Aedes scutellaris* group. Biochem. Genet. 21:1195–1201.

Tadano, T. 1977. Genetics of three new mutants, straw-colored larva, ruby eye and pigmented pupa, in *Aedes (Finlaya) togoi* (Diptera: Culicidae). J. Med. Entomol. 14:33–37.

Tadano, T. 1982. Linkage studies on an esterase locus in the mosquito *Aedes togoi.* Biochem. Genet. 20:711–721.

Tadano, T. 1983. Genetics of octanol and α-glycerophosphate dehydrogenases in the mosquito *Aedes (Finlaya) togoi.* Biochem. Genet. 21:1167–1174.

Tadano, T. 1984. A genetic linkage map of the mosquito *Aedes togoi.* Jpn. J. Genet. 59:165–176.

Yong, H. S., K. L. Chan, S. S. Dhaliwal, W. H. Cheong, G. L. Chiang and J. W. Mak. 1981. Genetics of glucose phosphate isomerase and phosphoglucomutase in *Aedes albopictus* (Diptera: Culicidae). Theor. Appl. Genet. 59:345–348.