INHERITANCE OF GLUCOSEPHOSPHATE ISOMERASE IN *AEDES TOGOI*

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ABSTRACT. Two isozymes of glucosephosphate isomerase (GPI:E.C. 5.3.1.9.) were observed in the mosquito *Aedes togoi* by means of agar gel electrophoresis. The locus (*Gpi-1*) controlling the more anodally migrated isozyme (GPI-1) was located on linkage group 1 (sex chromosome) of this species; the gene arrangement being *Gpi-1* — (18.2 map units) — *To-2* (tetrazolium oxidase-2) — (27.3 map units) — *M/m*(sex) — (about 40 map units, as estimated by previous studies) — *s* (straw-colored larva). Linkage homologies concerning *Gpi* and *Odh* (octanol dehydrogenase) are compared among three species: *Ae. aegypti*, the *Ae. scutellaris* group, and the *Ae. triseriatus* group in terms of chromosomal evolution.

INTRODUCTION

Comparisons of genetic linkage maps among genera or species provide a remarkable insight into the evolutionary divergence of their chromosomes. Foster et al. (1981), for example, examined the linkage relationships of biochemically or morphologically similar mutants in three Diptera, *Lucilia cuprina* (Wiedemann), *Musca domestica* Linn., and *Drosophila melanogaster* (Meigen); they found that the major linkage groups have survived largely intact during the evolution of these Diptera. Since polytene chromosomes cannot be successfully prepared in the *Aedes* mosquito species (Pashley and Rai 1983), linkage groups mapped with enzyme loci in *Aedes (Stegomyia aegypti* (Linn.), the *Ae. (Stg.) scutellaris* group, the *Ae. (Protomacleaya) triseriatus* group, *Ae. (Ochlerotatus) atropalpus* (Coquillett), and *Ae. (Finlaya) togoi* (Theobald) were compared from the point of view of the chromosomal evolution (Munstermann 1981, Munstermann et al. 1982, Pashley and Rai 1983; Tadano 1983, 1986). These studies on chromosomes indicated the occurrence of chromosomal translocations and inversions during the evolution of the *Aedes* species, but still there is great scarcity of mapped loci on linkage groups in these species as well as in other genera of mosquitoes.

This study gives genetic mapping of the locus (*Gpi-1*) for one of two isozymes of glucosephosphate isomerase (E. C. 5.3.1.9.) in the mosquito *Ae. togoi*, which is a vector of human filarial worms.

MATERIALS AND METHODS

The following seven lines of *Ae. togoi* were employed in this research: (1) *bl, pm, Gpi-1*^{S}, (2) *p, Gpi-1*^{S}, (3) *s, ru, Gpi-1*^{S}, (4) *w, Gpi-1*^{S}, (5) *Gpi-1*^{I}, (6) *To-2*^{S}, *Gpi-1*^{I}, and (7) *To-2*^{F}, *Gpi-1*^{S}. The sex in culicine mosquitoes (haploid chromosomes = 3) including this species is determined by a pair of alleles, *m* and *M*, males and females having the *m/M* and *m/m* sex genotypes, respectively. The *bl* (bleached pupa) and *pm* (plum eye) loci are on linkage group 3 with a recombination distance of over 40 map units (Tadano 1982a). The *p* (pigmented pupa), *ru* (ruby eye), and *w* (white eye) are on linkage group 2, and they are arranged in the order of *ru* — (30 map units) — *p* — (45 map units) — *w* (Tadano 1977, 1980). The *s* (straw-colored larva) and *To-2*$^{S \text{ or } F}$ (tetrazolium oxidase-2 slow or fast allele) are linkage group-1 (sex chromosome)—markers, with a gene order: *s* — (40 units)—*M/m*(sex) — (35 units) — *To-2* (Tadano 1984). The *Gpi-1*$^{S \text{ or } I}$ represents the slow or intermediate allele of glucosephosphate isomerase-1.

The mosquitoes were reared and crossed as previously reported (Tadano 1977). In backcross experiments single females were separately isolated into plastic cups for oviposition. Each egg batch was reared as a family and the phenotypes were scored in each family. Chi-square tests were applied to examine the 1 : 1 segregation ratio of each allele in the pooled data at the 5% level, as well as to examine linkage relationships between the loci at the 1% level.

Electrophoresis was performed using agar gels as outlined in earlier studies (Tadano 1982b, 1984). For electrophoresis of glucosephosphate isomerase, a 0.0125 M potassium phosphate buffer (pH 6.8) was used for both the electrode buffer and gel buffer. Gels consisted of 0.75 g agar and 2 g polyvinylpyrrolidone/100 ml of the gel buffer. After electrophoresis, the gels were incubated at 37° C in the solution of 10 mg D-fructose-6-phosphate, 50 mg $MgCl_2$, 10 mg $NADP^+$, 20 mg NBT(nitro blue tetrazolium), and 20 units of glucose-6-phosphate dehydrogenase/100 ml 0.05 M Tris-HCl (pH 8.0). Approximately 3 mg phenazine methosulfate was added to this solution after 20 min of incubation, and further incubation was continued for about 40 min.

Table 1. Two backcrosses (A and B) involving the *bl, pm,* and *Gpi*-1 alleles.

A: (*bl;pm;Gpi*-1^S/+;+;*Gpi*-1^I) ♀x (*bl;pm;Gpi*-1^S/*bl;pm;Gpi*-1^S)♂
B: (*bl;pm;Gpi*-1^S/*bl;pm;Gpi*-1^S)♀x (*bl;pm;Gpi*-1^S/+;+;*Gpi*-1^I)♂

Phenotype	A	B
+ + *Gpi*-1$^{S/S}$	4	45
+ + " I/S	7	32
+*pm* " S/S	9	21
+*pm* " I/S	13	31
bl+ " S/S	8	23
bl+ " I/S	12	37
blpm " S/S	7	42
blpm " I/S	15	49
Total	75	280
Families examined	1	3

χ^2 for linkage	A	B
between *bl* and *Gpi*-1 :	0.33	2.06
between *pm* and *Gpi*-1 :	0.33	0.91

RESULTS AND DISCUSSION

Although both larval and pupal homogenates gave trailed bands of glucose-phosphate isomerase (GPI) on the gels, one day or older adults displayed clear banding patterns of this enzyme. Thus, only adult homogenates were used throughout this study. Two isozymes of GPI were detected on the gels, one (GPI-1) migrated more anodally (5–6 cm from the origin) and the other (GPI-2) only 5 mm from the origin. This study was focused only on GPI-1. An electrophoretic survey of the laboratory strains and lines showed that at least three codominant alleles, named *F*(fast), *I*(intermediate), and *S*(slow) according to their relative mobilities, were involved at the locus (*Gpi-1*) for GPI-1, and that this isozyme is dimeric in structure, as reported by Yong et al. (1980) who used the Malaysian and Taiwan strains of this species.

Seven backcrosses were made to locate *Gpi-1* utilizing representative genetic markers for all the three linkage groups in this species. Crosses A and B (Table 1) involved two linkage group-3 markers, *b1* and *pm,* and provided evidence that *Gpi-1* is not linked to either of the two markers (P>>0.01). Although cross A produced more *Gpi-1*$^{I/S}$ heterozygotes (47 individuals) than the homozygotes (28 individuals) ($\chi^2 = 4.82$, $0.05 > P > 0.02$), all other alleles in the two crosses exhibited a 1 : 1 segregation ratio (P > 0.05).

Next, crosses C and D (Table 2), in which two linkage group-2 markers, *w* and *p,* were involved, revealed that all the alleles concerned segregated at the 1 : 1 ratio (P>0.05), and that there is no linkage between *Gpi-1* and either *w* or *p* (P>0.05). The fifth cross E (Table 3) yielded information on genetic relationships among *Gpi-1, ru,* and *s,* but again this backcross failed to locate the *Gpi-1* locus since the data indicated that there is no linkage of *Gpi-1* with either of *ru* (linkage group-2 marker) and *s* (linkage group-1 marker) (P>0.05).

Two more crosses, F and G (Table 4) showed that the *Gpi-1* locus is on linkage group 1. All the alleles involved in these two crosses, except for the *Gpi-1* alleles in cross G, showed the 1 : 1 segregation ratio (P > 0.05); there were more Gpi-1$^{I/S}$ heterozygotes (237 individuals) than the homozygotes (193 individuals) produced by G ($\chi^2 = 4.50$, $0.05 > P > 0.02$), most probably due to excessive mortality of the homozygotes. Data obtained from F and G strongly suggested existence of linkage between *Gpi-1, M/m,* and *To-2* (P<0.01). Thus, recombination values among these three loci were calculated as given in Table 4. Map units between *To-2* and *Gpi-1* were 11.5±3.0 to 20.0±1.9 in F and G, respectively, and those between *M/m To-2* were

Table 2. Two backcrosses (C and D) involving the *w, p,* and *Gpi*-1 alleles.

C: (*Gpi*-1^I;+/*Gpi*-1^S;*w*)♀x (*Gpi*-1^S;*w*/*Gpi*-1^S; *w*)♂
D: (*p;Gpi*-1^S/+;*Gpi*-1^I)♀x (*p;Gpi*-1^S/*p;Gpi*-1^S)♂

Score in C		Score in D	
+ *Gpi*-1$^{S/S}$	115	+ *Gpi*-1$^{S/S}$	100
+ " I/S	127	+ " I/S	131
w " S/S	100	*p* " S/S	134
w " I/S	118	*p* " I/S	133
Total	460	Total	498
Families examined	3	Families examined	4

χ^2 for linkage	C	D
between *w* and *Gpi*-1:	0.08	None
between *p* and *Gpi*-1:	None	2.06

Table 3. A backcross (E) involving the *s*, *ru*, and *Gpi*-1 alleles.

E: (*s;ru;Gpi*-1^S/+;+;*Gpi*-1^I) ♀ X (*s;ru;Gpi*-1^S/*s;ru;Gpi*-1^S)♂

Phenotype	Score	Phenotype	Score	
+ + *Gpi*-1$^{S/S}$	26	*s* + *Gpi*-1$^{S/S}$	28	
+ + 〃 $^{I/S}$	25	*s* + 〃 $^{I/S}$	30	χ^2 for linkage between
+ *ru* 〃 $^{S/S}$	21	*s ru* 〃 $^{S/S}$	17	*ru* and *Gpi*-1 = 0.73
+ *ru* 〃 $^{I/S}$	28	*s ru* 〃 $^{I/S}$	23	*s* and *Gpi*-1 = 0.02
		Total	198	
		Families examined	2	

26.3±2.1 to 31.0±4.4, while the units for the *M/m* — *Gpi-1* segment ranged from 33.6±4.5 to 34.±2.3. Therefore, the gene arrangement must be *Gpi-1* — *To—-2* — *M/m*, and the weighted average map units were 18.2 for *Gpi-1* — *To-2*, 27.3 for *To-2* —*M/m*, and 34.1 for Gpi-1 — *M/m*.

Since the octanol dehydrogenase locus (*Odh-2*) is situated between *To-2* and *M/m* in *Ae. togoi* (Tadano 1984), the sex chromosome (linkage group 1) of this species bears the gene arrangement of *Gpi-1* — *To-2* — *Odh-2* — *M/m*. This arrangement is similar to linkage group 3 (autosomes) of both *Ae. aegypti* and the *Ae. scutellaris* group, as well as to linkage group 2 (autosome) of the *Ae. triseriatus* group, in that each of these linkage groups contains *Gpi* and *Odh* (Munstermann 1981; Pashley and Rai 1983). This suggests that some chromosomal translocations might have occurred among the autosomes and sex chromosomes of these subgenera during the course of evolution. As more gene loci are mapped on these linkage groups, it should be possible to determine more precise chromosomal changes during their evolution. This kind of research may give a significant insight into our understanding of chromosomal evolution in mosquito species.

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Table 4. Two backcrosses (F and G) involving the *sex(m, M)*, *To-2*, and *Gpi*-1 alleles.

F: (*To-2*^{S};*Gpi-1*^{I};*m*/*To-2*^{S};*Gpi-1*^{I};;*m*) x (*To-2*^{F};Gpi-1^S;*m*/*To-2*^{S};*Gpi-1*^{I};*M*)
G: (*To-2*^{F};*Gpi*-1^S;*m*/*To-2*^{F};Gpi-1^S;*m*) x (*To-2*^{F};Gpi-1^S;*m*/*To-2*^{S};*Gpi-1*^{I};*M*)

Phenotypic score in F		Phenotypic score in G	
♀ *To-2* $^{S/S}$ *Gpi*-1$^{I/I}$	13	♀ *To-2* $^{F/F}$ *Gpi*-1$^{S/S}$	113
♀ 〃 $^{S/S}$ 〃 $^{I/S}$	3	♀ 〃 $^{F/F}$ 〃 $^{I/S}$	37
♀ 〃 $^{F/S}$ 〃 $^{I/I}$	4	♀ 〃 $^{F/S}$ 〃 $^{S/S}$	9
♀ 〃 $^{F/S}$ 〃 $^{I/S}$	40	♀ 〃 $^{F/S}$ 〃 $^{I/S}$	39
♂ 〃 $^{S/S}$ 〃 $^{I/I}$	30	♂ 〃 $^{F/F}$ 〃 $^{S/S}$	48
♂ 〃 $^{S/S}$ 〃 $^{I/S}$	4	♂ 〃 $^{F/F}$ 〃 $^{I/S}$	17
♂ 〃 $^{F/S}$ 〃 $^{I/I}$	2	♂ 〃 $^{F/S}$ 〃 $^{S/S}$	23
♂ 〃 $^{F/S}$ 〃 $^{I/S}$	17	♂ 〃 $^{F/S}$ 〃 $^{I/S}$	144
Total	113	Total	430
Families examined	2	Families examined	3
Map units (±SE) between		Map units (±SE) between	
To-2 and *Gpi*-1 = 11.5±3.0		*To-2* and *Gpi*-1 = 20.0±1.9	
M and *To-2* = 31.0±4.4		*M* and *To-2* = 26.3±2.1	
M and *Gpi*-1 = 33.6±4.5		*M* and *Gpi*-1 = 34.2±2.3	

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