INHERITANCE OF PHOSPHOGLUCONATE AND XANTHINE DEHYDROGENASES IN *AEDES (FINLAYA) TOGOI*[1]

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ABSTRACT. Phosphogluconate dehydrogenase (PGD) and xanthine dehydrogenase (XDH) were genetically studied by agar gel electrophoresis in the mosquito *Aedes togoi*. Adult homogenates displayed banding patterns with one zone of activity of either enzyme. Eight backcrosses were conducted to map the two loci, *Pgd* and *Xdh*, to linkage group III with the following arrangement: *Pgd*—(ca. 15 map units)—*bl* (bleached pupa)—(ca. 5)—*y* (yellow larva)—(38.0 ± 1.7)—*pm* (plum eye)—(6.1 ± 0.9)—*Xdh*. All *Pgd* and *Xdh* loci thus far mapped in other mosquito species are reviewed for a comparison with those in this species.

INTRODUCTION

The phosphogluconate dehydrogenase locus (*Pgd*; E.C. 1.1.1.44) has been mapped to linkage group III in *Aedes aegypti* (Linn.), the *Ae. scutellaris* group, and the *Ae. triseriatus* group, to linkage group I (sex chromosome) in *Ae. atropalpus* (Coquillett) (Munstermann 1981, Pashley and Rai 1983), to chromosome II in *Anopheles albimanus* Wiedemann (Narang and Seawright 1987) and to chromosome III in *Culex quinquefasciatus* Say (Cheng and Hacker 1976). No other genetic mapping study on this enzyme in other mosquito species has been reported.

Although genetic studies on the xanthine dehydrogenase locus (*Xdh*; E.C. 2.1.37) are more scant, allozymes at the *Xdh* loci have been reported in the *Cx. pipiens* complex (Miles and Paterson 1979) and in a few *Anopheles* species (Steiner et al. 1981). To the author's knowledge, among anophelines *An. punctipennis* (Say) is the only mosquito species in which linkage group studies on *Xdh* have been reported (Narang and Kitzmiller 1972).

A genetic map of *Aedes togoi* (Theobald) has been constructed by use of enzyme and visible markers (Tadano 1984). This study presents linkage maps of two more loci, *Pgd* and *Xdh*, which will provide some information on chromosomal changes in the *Aedes* evolution.

MATERIALS AND METHODS

The mosquito rearing method and crossing experiments were undertaken as described in an earlier study (Tadano 1977). Electrophoresis was performed using agar gel as in Tadano (1986). Of 17 colonized strains of this mosquito electrophoretically surveyed, the following 8 strains were used for crossing experiments: (1) s,Pgd^S, (2) p,ru,Pgd^S, (3) y,Pgd^S, (4) y,pm,Pgd^S, (5) Pgd^F, (6) ru,y,Xdh^I, (7) bl,pm,Xdh^I, and (8) Xdh^S. The linkage groups of the mutants were: *s* (straw-colored larva) and *M/m* (sex) on linkage group I; *p* (pigmented pupa) and *ru* (ruby eye) on linkage group II; *y* (yellow larva), *pm* (plum eye), and *bl* (bleached pupa) on linkage group III (Tadano 1984). The allelic designations of the superscripts *S* (slow), *I* (intermediate), and *F* (fast) for *Pgd* or *Xdh* are given below.

In backcross experiments, offspring from each parental female were separately reared in a single container and were scored for mutants and allozymes. Data from families which showed a 1:1 segregation ratio of each allele at 5% level were pooled. Linkage between various loci was also tested by chi-square at 1% level.

RESULTS AND DISCUSSION

Electrophoresis of adults exhibited only one zone of activity of phosphogluconate dehydrogenase. Two electromorphs were detected by an electrophoretic survey of 10 strains, of which the most frequent allele migrated 10 mm from the origin, and the other 14 mm from the origin under the electrophoretic conditions employed. The slow (*S*) allele (Pgd^{100}) and the fast (*F*) one (Pgd^{104}) have been designated as Pgd^S and Pgd^F in Tables 1 and 2. The heterozygotes for these showed a three-band pattern typical of a dimeric molecule. In contrast to PGD zymogram in *Ae. aegypti* which showed an additional lighter band antecedent to each major band (Munstermann and Craig 1979), no such subbands were observed in *Ae. togoi*.

Five backcrosses were made to map the *Pgd* locus (Tables 1 and 2). Crosses A and B involve the sex (*M/m*) and *s* markers for linkage group I, and *p* and *ru* for linkage group II. Their results indicated that *Pgd* is not linked to either of those markers ($P > 0.01$). Then, crosses C, D, and E, involving *y* and *pm* as linkage group III

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Table 1. Results of backcrosses showing linkage relationships among *Pgd* (phosphogluconate dehydrogenase), *M/m* (sex), *s* (straw-colored larva), *p* (pigmented pupa), and *ru* (ruby eye).

Cross A: s,Pgd^S ♀ × (s,Pgd^S ♀ × Pgd^F ♂)♂
Cross B: p,ru,Pgd^S ♀ × (p,ru,Pgd^S ♀ × Pgd^F ♂)♂

Offspring from cross A		Offspring from cross B	
♀ + $Pgd^{S/S}$	17	+ + $Pgd^{S/S}$	44
♀ + $Pgd^{F/S}$	23	+ + $Pgd^{F/S}$	40
♀ *s* $Pgd^{S/S}$	42	+ *ru* $Pgd^{S/S}$	15
♀ *s* $Pgd^{F/S}$	40	+ *ru* $Pgd^{F/S}$	20
♂ + $Pgd^{S/S}$	40	*p* + $Pgd^{S/S}$	13
♂ + $Pgd^{F/S}$	37	*p* + $Pgd^{F/S}$	24
♂ *s* $Pgd^{S/S}$	11	*p ru* $Pgd^{S/S}$	41
♂ *s* $Pgd^{F/S}$	19	*p ru* $Pgd^{F/S}$	41
Sum	229	Sum	238
Families tested	2	Families tested	3
χ^2 values testing for linkage		χ^2 values testing for linkage	
between *M/m* and *Pgd* = 0.004		between *p* and *Pgd* = 0.42	
between *s* and *Pgd* = 0.04		between *ru* and *Pgd* = 0.02	

Table 2. Results of backcrosses showing linkage relationships among *Pgd* (phosphogluconate dehydrogenase), *y* (yellow larva), and *pm* (plum eye).

Cross C: (y,Pgd^S ♀ × Pgd^F ♂)♀ × y,Pgd^S ♂
Cross D: (y,pm,Pgd^S ♀ × Pgd^F ♂)♀ × y,pm,Pgd^S ♂
Cross E: y,pm,Pgd^S ♀ × (y,pm,Pgd^S ♀ × Pgd^F ♂)♂

Offspring from cross C		Offspring from cross D		Offspring from cross E	
+ $Pgd^{S/S}$	20	+ + $Pgd^{S/S}$	31	+ + $Pgd^{S/S}$	19
+ $Pgd^{F/S}$	130	+ + $Pgd^{F/S}$	123	+ + $Pgd^{F/S}$	68
y $Pgd^{S/S}$	119	+ *pm* $Pgd^{S/S}$	22	+ *pm* $Pgd^{S/S}$	7
y $Pgd^{F/S}$	35	+ *pm* $Pgd^{F/S}$	107	+ *pm* $Pgd^{F/S}$	38
Sum	304	*y* + $Pgd^{S/S}$	70	*y* + $Pgd^{S/S}$	43
Families tested	3	*y* + $Pgd^{F/S}$	19	*y* + $Pgd^{F/S}$	7
Map units between					
y and *Pgd* = 18.1 ± 2.2		*y pm* $Pgd^{S/S}$	130	*y pm* $Pgd^{S/S}$	69
		y pm $Pgd^{F/S}$	45	*y pm* $Pgd^{F/S}$	26
		Sum	547	Sum	277
		Families tested	6	Families tested	3
		Map units between		Map units between	
		y and *Pgd* = 21.4 ± 1.8		*y* and *Pgd* = 21.3 ± 2.5	
		y and *pm* = 39.9 ± 2.1		*y* and *pm* = 34.3 ± 2.9	
		pm and *Pgd* = 46.3 ± 2.1		*pm* and *Pgd* = 45.5 ± 3.0	

markers were conducted (Table 2). In cross D more *pm* individuals were observed than wild-type individuals ($0.01 > P > 0.001$), but all other alleles in the three backcrosses segregated at a 1:1 ratio ($P > 0.05$).

Chi-square values testing for linkage between *Pgd* and the linkage group III markers clearly suggested genetic linkage of *Pgd* with these markers ($P < 0.01$). The map units calculated between the three loci, as given in Table 2, indicated a gene order of *pm—y—Pgd*. Weighted averages of the map units were 38.0 for *pm—y*, 20.5 for *y—Pgd*, and 46.0 for *pm—Pgd*. The average map units estimated for *pm—y* are nearly identical with 40 map units previously reported (Tadano 1984).

Xanthine dehydrogenase could be resolved electrophoretically only by using adult homogenates. Homogenates of pupae or larvae did not reveal distinct activity bands on gels. Electrophoresis of seven strains showed only three alleles present at the *Xdh* locus. This was the only zone observed of XDH activity on gels. The three electromorphs migrated only 10, 12, and 14 mm to the anode from the origin, and so were designated Xdh^{98}, Xdh^{100}, and Xdh^{102}, respectively, since the electromorph with a 12-mm-migration distance was most frequently ob-

Table 3. Backcrosses involving *Xdh* (xanthine dehydrogenase), *M/m* (sex), *ru* (ruby eye), *y* (yellow larva), *bl* (bleached pupa), and *pm* (plum eye).

Cross F: *ru,y,Xdh*^{I} ♀ × (*ru,y,Xdh*^{I} ♀ × *Xdh*^{S} ♂)♂
Cross G: (*bl,pm,*Xdh^{I} ♀ × *Xdh*^{S} ♂)♀ × *bl,pm,Xdh*^{I} ♂
Cross H: *bl,pm,Xdh*^{I} ♀ × (*bl,pm,Xdh*^{I} ♀ × *Xdh*^{S} ♂)♂

Offspring from cross F		Offspring from cross G		Offspring from cross H	
♀ + + *Xdh*$^{I/I}$	26	+ + *Xdh*$^{I/I}$	1	+ + *Xdh*$^{I/I}$	6
♀ + + *Xdh*$^{I/S}$	24	+ + *Xdh*$^{I/S}$	103	+ + *Xdh*$^{I/S}$	91
♀ + *ru Xdh*$^{I/I}$	18	+ *pm Xdh*$^{I/I}$	70	+ *pm Xdh*$^{I/I}$	61
♀ + *ru Xdh*$^{I/S}$	25	+ *pm Xdh*$^{I/S}$	6	+ *pm Xdh*$^{I/S}$	6
♀ *y* + *Xdh*$^{I/I}$	29	*bl* + *Xdh*$^{I/I}$	2	*bl* + *Xdh*$^{I/I}$	4
♀ *y* + *Xdh*$^{I/S}$	19	*bl* + *Xdh*$^{I/S}$	81	*bl* + *Xdh*$^{I/S}$	67
♀ *y ru Xdh*$^{I/I}$	29	*bl pm Xdh*$^{I/I}$	79	*bl pm Xdh*$^{I/I}$	94
♀ *y ru Xdh*$^{I/S}$	22	*bl pm Xdh*$^{I/S}$	10	*bl pm Xdh*$^{I/S}$	7
♂ + + *Xdh*$^{I/I}$	20	Sum	352	Sum	336
♂ + + *Xdh*$^{I/S}$	27	Families tested	3	Families tested	3
♂ + *ru Xdh*$^{I/I}$	18	Map units between		Map units between	
♂ + *ru Xdh*$^{I/S}$	30				
♂ *y* + *Xdh*$^{I/I}$	33	*bl* and *Xdh* = 46.3 ± 2.7		*bl* and *Xdh* = 42.0 ± 2.7	
♂ *y* + *Xdh*$^{I/S}$	22	*bl* and *pm* = 45.2 ± 2.7		*bl* and *pm* = 41.1 ± 2.7	
♂ *y ru Xdh*$^{I/I}$	23	*pm* and *Xdh* = 5.4 ± 1.2		*pm* and *Xdh* = 6.9 ± 1.4	
♂ *y ru Xdh*$^{I/S}$	15				
Sum	380				
Families tested	3				
Map units between					
y and *Xdh* = 42.1 ± 2.5					

served. But in Table 3 *Xdh*^{98} was designated as *Xdh*^{S}, and *Xdh*^{100} as *Xdh*^{I}. Heterozygotes for these alleles displayed wider, fainter bands than those of the homozygotes without appearance of clear banding patterns. Since *S* and *F* alleles migrated closely, the hybrid band was not evident in heterozygotes.

The results from three backcrosses (Table 3) showed that *Xdh* was on linkage group III marked with *pm*, *y*, and *bl* ($P < 0.01$). All alleles involved in the three backcrosses segregated at a 1:1 ratio ($P > 0.05$), with only one exception of *Xdh* in cross G that yielded more heterozygotes (*Xdh*$^{I/S}$) than the homozygotes ($0.02 > P > 0.01$). Since map units estimated from crosses G and H gave a gene order of *bl—pm—Xdh*, and a previous study (Tadano 1984) mapped *y* between *bl* and *pm*, the gene order should be *bl—y—pm—Xdh*. Average map units were computed from G and H to be 44.2 ± 1.9 for *bl—Xdh*, 43.2 ± 1.9 for *bl—pm*, and 6.1 ± 0.9 for *pm—Xdh*. Also crosses D and E (Table 2), as well as cross F provided average map units of 38.0 ± 1.7 for *y—pm*, of 20.5 ± 1.2 for *y—Pgd*, and of 42.1 ± 2.5 for *y—Xdh*. Therefore, the five loci on linkage group III are arranged in the following order: *Pgd*—(ca. 15 map units)—*bl*—(ca. 5)—*y*—(38.0 ± 1.7)—*pm*—(6.1 ± 0.9)—*Xdh*. Thus, linkage group III has now approached to a length of about 70 map units. Another gene, *c* (curved wing), is also situated around the *Pgd* locus, being at about 16 map units from *bl* (Tadano 1984).

This is the first study to map a xanthine dehydrogenase locus in the genus *Aedes*. A phosphogluconate dehydrogenase locus was located on linkage group III of *Ae.* (*Stegomyia*) *aegypti*, the *Ae.* (*Stg.*) *scutellaris* group, *Ae.* (*Protomacleaya*) *triseriatus* (Munstermann 1981, Pashley and Rai 1983), as is the case in *Ae. togoi* shown during this study. Thus, probably this locus is situated on "linkage group III" as in other species of the subgenera, *Stegomyia*, *Protomacleaya* and *Finlaya*.

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