LINKAGE STUDIES ON A NEW MUTANT, BLEACHED PUPA, IN *AEDES (FINLAYA) TOGOI*

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ABSTRACT. A recessive mutant, bleached *pupa* (*bl*), was isolated from the Taipei (Taiwan) strain of *Aedes* (*Finlaya*) *togoi*. The *bl* pupae are yellowish but brighter than the *y* (yellow larva) pupae. Although the 2 phenotypes cannot be readily distinguished in their pupal stage, they are not identical nor allelic. The *bl* allele is linked to *c*(curved wing) and *pm* (plum eye) in linkage group III. The *bl-c* map distance is 14.2 map units in females and 17.8 units in males. The gene sequence in linkage group III appears to be: *pm* - (40 units) -*y*- ? -*bl*- (14-18)- *c*.

In the review of genetics of *Aedes* (*Stegomyia*) *aegypti* (Linn.) made by Craig and Hickey (1967), all 3 linkage groups of this mosquito had been marked with 28 mutant loci, and a total length of these linkage groups was 110 map units. Twelve years later, a new linkage map for this species was reconstructed with a total of 60 marker loci including 14 enzyme markers; the total map length was 156 units (Munstermann and Craig 1979).

About 15 genetic markers have been placed in all 3 linkage groups of *Aedes* (*Finlaya*) *togoi* (Theobald), a vector of filarial worms in Asia (Tadano 1980). The map lengths of linkage groups I, II and III are approximately 60, 80 and 60 units, respectively; thus far a total length in this species has become about 200 map units. Therefore, it can be speculated that the total map length in *Ae. togoi* may be longer than that in *Ae. aegypti*.

A new recessive body color mutant, bleached pupa (*bl*), was recovered from an inbred line of the Taipei (Taiwan) strain. A similar mutant named 'bleached' (*b*) has been described in *Culex pipiens* Linn. by McClelland (1978), but the *bl* mutant is not homologous to the *b* mutant in that *b* affects all developmental stages while *bl* only affects the larval and pupal color.

This paper presents results of linkage studies on the *bl* mutant.

MATERIALS AND METHODS

The following strains were used for the genetic crosses. The 2 wild-type strains were MNZR (Manazuru) and NGSK (Nagasaki). The marker strains employed were a) linkage group II marker strains ruby eye (*ru*) and pigmented pupa (*p*) described by Tadano (1977a) and b) linkage group III marker strains plum eye (*pm*) and curved wing (*c*) (Tadano 1977b).

The rearing methods have been previously reported (Tadano 1976, 1977a). In each experiment, mass matings were used; subsequently females were isolated from the experiment cage into individual plastic cups for oviposition. Each egg batch was separately hatched and reared as a family. Phenotypes were recorded for each family, and then were pooled. Statistic calculations were made according to the formulae given by Bailey (1961).

RESULTS AND DISCUSSION

Twelve sets of crosses were made, 4 involving linkage group II markers (*ru* or *p*) and 8 crosses involving linkage group III markers (*pm* and *c*) (Tables 1 and 2). Crosses 5 to 8 are $F_1 \times F_1$ crosses, while 1 to 4 and 9 to 12 are testcrosses. The pooled results of each cross were examined by chi-square (χ^2) to test for segregation of the alleles and for linkage. Significant χ^2 values ($P < 0.05$) for the allele segregation were obtained for the sex allele (*m*) in crosses 8 ($\chi^2 = 7.22$), 9 ($\chi^2 = 4.60$), and 12 ($\chi^2 = 5.88$), for *bl* in cross I ($\chi^2 = 4.64$), and for *pm* in cross 12 ($\chi^2 = 11.78$); that is, the segregation ratios of these alleles deviated from the 1 : 1 or 1 : 3. But all other alleles involved in all crosses (1 to 12) segregated at the 1 : 1 or 1: 3 ratio expected.

The χ^2 values testing for linkage (Table 3) indicated clearly ($P < 0.01$) that the *bl* allele is linked to *c*; the χ^2 value for *bl-pm* obtained from cross 10 suggested linkage between *bl* and *pm* at the 5% level. Recombination units for *bl-c* from data in crosses 6 through 12 are given in parentheses in Table 3. There was a wide range of variation in these recombination units, from 10.2 (cross 8) to 27.2 ± 2.9 (cross 10). Such variation in crossover frequency may not be unusual since examples of this kind have been reported

Table 1. Scores from crosses between *bl*, *ru*, and *p*.

Cross	Parental genotype ♀(*m*/*m*)	×	♂(*m*/*M*)	Progeny phenotype: Female + / +	Female + / *ru*	Female *bl* / +	Female *bl* / *ru*	Male + / +	Male + / *ru*	Male *bl* / +	Male *bl* / *ru*	Families pooled	Total
1	$\frac{+}{ru}$ $\frac{bl}{+}$	×	$\frac{ru}{ru}$ $\frac{bl}{bl}$	164	172	179	178	149	147	189	165	9	1343
2	$\frac{ru}{ru}$ $\frac{bl}{bl}$	×	$\frac{+}{ru}$ $\frac{bl}{+}$	66	63	62	59	61	61	82	60	7	514
				+ / +	+ / *p*	*bl* / +	*bl* / *p*	+ / +	+ / *p*	*bl* / +	*bl* / *p*		
3	$\frac{p}{+}$ $\frac{+}{bl}$	×	$\frac{p}{p}$ $\frac{bl}{bl}$	136	139	149	154	140	129	140	165	9	1152
4	$\frac{p}{p}$ $\frac{bl}{bl}$	×	$\frac{p}{+}$ $\frac{+}{bl}$	143	138	134	155	160	129	108	163	13	1130

The alleles above the lines in the genotypes are of maternal origin and those below the lines of paternal origin.

Table 2. Scores from crosses among *bl*, *c*, and *pm*.

Cross*	Parental genotype ♀ (*m*/*m*) × ♂ (*m*/*M*)	Progeny phenotype: Female								Male								Families pooled	Total
		+ + / + +	+ + / + *c*	+ *pm* / + +	+ *pm* / + *c*	*bl* + / + +	*bl* + / + *c*	*bl pm* / + +	*bl pm* / + *c*	+ + / + +	+ + / + *c*	+ *pm* / + +	+ *pm* / + *c*	*bl* + / + +	*bl* + / + *c*	*bl pm* / + +	*bl pm* / + *c*		
5	*pm* + / + *bl* × *pm* + / + *bl*	137	—	71	—	52	—	16	—	173	—	42	—	58	—	24	—	4	573
6	*c* + / + *bl* × *c* + / + *bl*	56	22	—	—	32	0	—	—	56	30	—	—	27	3	—	—	4	226
7	+ + *c* / *pm bl* + × + + *c* / *pm bl* +	132	63	28	16	52	0	17	0	103	61	29	18	54	0	17	5	10	595
8	+ + + / *pm bl c* × + + + / *pm bl c*	232	17	83	9	10	79	8	20	311	15	77	9	17	76	12	26	8	1001
9	*pm bl c* / *pm bl c* × *pm bl c* / + + +	40	5	44	2	5	34	12	28	54	3	42	7	2	39	9	56	6	382
10	*pm bl c* / *pm bl c* × + + + / *pm bl c*	20	17	19	7	9	21	7	21	32	6	16	5	3	25	12	23	4	243
11	*pm bl c* / + + + × *pm bl c* / *pm bl c*	85	13	82	16	15	84	27	92	71	12	95	11	9	74	21	72	8	779
12	+ + + / *pm bl c* × *pm bl c* / *pm bl c*	65	11	41	1	6	42	7	39	67	3	51	6	12	70	8	48	5	477

The alleles above the lines in the genotypes are of maternal origin and those below the lines of paternal origin.
* Crosses 5 and 6 are 2-point crosses; 7–12 are 3-point crosses.

Table 3. Chi-square values testing for linkage among the alleles.

Cross	Type	Combinations of alleles*					
		bl–M	*bl–ru*	*bl–p*	*bl–pm*	*bl–c***	*c–pm*
1	Backcross		0.72				
2	"	1.54	0.94				
3	"			1.25			
4	"	0.60		1.10			
5	$F_1 \times F_1$				0.00		
6	"					18.50 (22.0)	
7	"				2.64	57.94 (17.5)	0.41
8	"				0.88	547.55 (10.2)	0.23
9	Backcross				2.68	223.20 (11.8±1.7)	0.09
10	"				4.48***	50.70 (27.2±2.9)	0.04
11	"				0.06	360.00 (15.9±1.3)	1.76
12	"				0.76	285.45 (11.3±1.5)	0.25

* Figures in parentheses are percent recombination ± standard errors. No standard errors were calculated for $F_1 \times F_1$ data.

** All χ^2 values estimated for *bl-c* are significant ($P<0.01$).

*** Significant at $P = 0.05$.

in other mosquito species (e.g. Craig and Hickey 1967, Baker and Sakai 1972, McClelland 1978).

The weighted average of the recombination units for *bl-c*, calculated from the backcross data, was 17.8 in the heterozygous males (crosses 9 and 10) and 14.2 in the heterozygous females (crosses 11 and 12). Since the recombination distances and gene sequence on linkage group III are: *pm* – (40 units) –*y*(yellow larva) – (18 units) –*c*, the total distance between *pm* and *c* should be approximately 60 units (Tadano 1977b). But no strong evidence for linkage between *pm* and *bl* has been found among the χ^2 values in any cross except cross 10 ($\chi^2 = 4.48$, $P < 0.05$). Therefore, the gene sequence of *pm, c,* and *bl* cannot be definitely determined.

However, frequencies of double crossovers do give some information on the gene sequence. If the sequence was *pm-bl-c*, the double crossover classes in crosses 9 through 12 would be *pm*, bl^+, *c* and pm^+, *bl*, c^+; if it was *pm-c-bl*, the double crossover classes must be *pm*, c^+, *bl* and pm^+, *c*, bl^+. In crosses 9 and 10, 40 individuals have phenotypes of either *pm*, bl^+, *c* or pm^+, *bl*, c^+; and in crosses 11 and 12, 76 individuals have these phenotypes. On the other hand, 71 individuals from crosses 9 to 10 belong to the *pm*, c^+, *bl* or pm^+, *c*, bl^+ class, while 102 from crosses 11 and 12 are in these classes. Since double crossovers are usually the least frequent, the gene sequence is most likely *pm-bl-c*.

Although the *bl* pupae are brighter than the *y* pupae, the *bl* and *y* pupae (or larvae) greatly resemble each other. Therefore, the 2 phenotypes cannot be distinguished and, moreover, the adults of both mutants appear the same as the wild type. But reciprocal allelism tests between *bl* and *y* have demonstrated that the 2 genes are not allelic, although backcrosses involving *bl* and *y* could hardly be done because of the phenotypic similarity.

Since *y* is located approximately 40 map

units far from *pm* as mentioned above, the gene sequence in linkage group III must be *pm-y-bl-c*. Thus, it is of much interest that the *y* and *bl* alleles, which produce very similar phenotypes, are closely linked to each other.

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