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INHERITANCE OF A NEW MUTANT, PLUM EYE, IN THE MOSQUITO *AEDES TOGOI*

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ABSTRACT. A new recessive mutant, plum eye (*pm*), of the mosquito *Aedes* (*Finlaya*) *togoi* expresses dark brown eyes prominently in pupae, though difficulty is encountered in detection of this phenotype in larvae and adults. This allele has been located in the same linkage group as yellow larva (*y*) and curved wing (*c*), which have been tentatively assigned to linkage group 3. The gene sequence and the map units among them were: *c*— (17–18 units)—*y*—(40–41 units)—*pm*. There was positive interference between the two segments, the coincidence coefficient being 0.542–0.636 in the females and 0.685–0.743 in the males.

Six genetic markers thus far known for *Aedes* (*Finlaya*) *togoi* (Theobald), a vector of various species of filariae, have been tentatively assigned to the expected three linkage groups (Tadano, in preparation), since the haploid chromosome number in this mosquito is three (Suzuki 1939; Sinoto and Suzuki 1943; Rai 1963; Kanda 1968); two of these mutants, yellow larva (*y*) and curved wing (*c*), have been placed in linkage group 3, and 16–20 map units exist between the two alleles.

A new mutant plum eye (*pm*) expresses dark brown eyes most prominently in pupae in contrast to black eyes of wild-type pupae but can be hardly distinguished in larvae and it darkens on emergence. This allele is recessive, highly penetrant, and as viable as the wild phenotype. This paper presents results of the cross experiments to elucidate the linkage relationship among *pm*, *y*, and *c*.

MATERIALS AND METHODS. The yellow larva (*y*) strain was found in the NGSK strain provided by Department of Medical Zoology, School of Medicine, Nagasaki University, Kyushu (Tadano 1977a). Both curved wing (*c*) and plum eye (*pm*) mutants were isolated from the MUR strain collected at Miura City, Kanagawa, Japan, and the MUR strain was utilized as a wild type whenever needed for cross experiments.

Rearing of the mosquitoes and procedures for experiments were the same as those described by Tadano (1976, 1977b). Although mass crosses were done for all experiments, single blood-fed females were isolated into each of plastic cups for oviposition. Three or four days after oviposition, these egg batches were hatched in separate plastic containers in which thereafter the larvae were reared as single families. Classification of phenotypes was made in 3rd or 4th instar for *y*, in pupae for *pm*, and in adults for sex and *c*; the proportions of phenotypes including sex in each family were tested by chi² to see whether the expected ratios (1:1 or 1:3) were obtained. All families in which all the proportions did not show a significant deviation ($P>0.05$) from the expected ratios were pooled for the subsequent statistical treatment, from which the other families were excluded. Chi² - tests for genetic linkage were performed according to Bailey (1961) and Serra (1965).

RESULTS AND DISCUSSION. Of 13 crosses undertaken in this study (Table 1), 8 crosses (A through H) are dihybrid experiments and the rest (I through M) are trihybrid crosses; phenotypic scores from dihybrid crosses are tabulated together with those from trihybrid crosses. Four crosses (A, F, G, and I) were F_1x F_1 and all others were test crosses.

Genetic linkage between the three mutant alleles was examined by the chi²-test for one degree of freedom (table 2). Chi² values for *c y* ranged from 82.81 (I) to 431.13 (H), which indicate significantly ($P<0.01$) the existence of linkage between the loci. The values for *y pm* were much greater than 6.635, which is the chi² value at $P=0.01$ for one degree of freedom, though only the value from cross A (5.63) was exceptionally low ($0.02>P>0.01$). Thus *y* and *pm* have been proved to be linked together. The maximum of linkage chi² values for *y pm* was 65.19 (I), though even a minimum value for *c y* was 82.81 (I), and so it is very likely that length of the *c-y* segment is shorter than that of the *y-pm* segment.

However, the chi² values for *c pm* were 2.44 or much less and indicate that a free recombination occurred ($P>0.05$). Therefore, all the facts mentioned above suggest that the gene sequence is *c-y-pm*.

Recombination distances among three loci and the standard errors were calculated for each cross; these are also shown in Table 2, where the standard errors were not estimated from the F_1x F_1 data. Recombination values between *c* and *y*, estimated from testcross data (H, J, K, L, and M), were 15.98±1.41 to 19.53±1.18%, while the estimation from an F_1x F_1 cross (I) was 23.24%. On the other hand, recombination units in the *y-pm* sgement ranged from 34.74 (I) to 42.80±1.73 (J). Thus it is clear that the *c-pm* segment is so long as to experience a free recombination, as indicated by the recombination value of 47.29±1.73 (L) to 50.14% (G).

Female heterozygotes were employed in crosses C, E, H. K, and M, whereas male heterozygotes were used for crosses B, D, J, and L; from these recombination values no evidence has been obtained that differences in recombination units exist between sexes. Recombination units in the *c-y* segment were 15.98±1.41 to 19.53±1.18 in females, and 16.10±1.27 to 16.85±2.22 in males, the average units being 18.23±0.92 in females and

Table 1. Scores from crosses to elucidate the relationship among *c*, *y*, and *pm*

Cross	Parental genotype* Female		Parental genotype* Male	Progeny phenotype: Female +	Female *c*	Female *pm*	Female *c pm*	Female *y*	Female *c y*	Female *pm y*	Female *c pm y*	Male +	Male *c*	Male *pm*	Male *c pm*	Male *y*	Male *c y*	Male *pm y*	Male *c pm y*	Families pooled
A	y + / + pm	x	y + / + pm	89	..	37	..	29	..	5	..	100	..	37	..	44	..	8	..	3
B	y pm / y pm	x	y pm / + +	196	..	125	..	122	..	208	..	192	..	140	..	145	..	233	..	12
C	y pm / + +	x	y pm / y pm	216	..	155	..	126	..	212	..	211	..	151	..	146	..	238	..	10
D	y pm / y pm	x	+ + / y pm	107	..	85	..	74	..	121	..	144	..	81	..	81	..	118	..	7
E	+ + / y pm	x	y pm / y pm	221	..	137	..	168	..	230	..	255	..	169	..	171	..	252	..	8
F	+ + / y pm	x	+ + / y pm	85	..	22	..	26	..	17	..	96	..	33	..	31	..	21	..	3
G	+ c / pm +	x	+ c / pm +	102	53	43	13	..	..	..	..	137	34	47	20	..	..	..	..	4
H	y c / + +	x	y c / y c	199	41	..	..	46	208	..	..	190	35	..	..	35	202	..	..	10
I	+ y pm / c + +	x	+ y pm / c + +	221	105	58	32	63	3	46	1	231	96	40	23	102	1	66	11	8
J	c y pm / c y pm	x	+ + + / c y pm	86	29	93	8	16	59	25	99	101	16	97	11	7	60	20	93	11
K	+ + + / c y pm	x	c y pm / c y pm	125	42	88	10	16	87	57	141	125	33	96	16	11	102	30	122	9
L	c y pm / c y pm	x	c y pm / + + +	96	26	61	14	8	62	24	93	109	23	95	4	12	73	29	102	11
M	c y pm / + + +	x	c y pm / c y pm	75	19	58	6	9	70	26	90	65	21	67	5	4	58	18	85	7

* Alleles above the line are of maternal origin, those below the line of paternal origin.

Table 2. Chi-square values for genetic linkage and recombination values between *c*, *y* and *pm*.

Cross	(Type)	Chi-square value			Recombination value (%)		
		c:y	*y:pm*	*c:pm*	*c–y*	*y–m*	*c–pm*
A	(F_1 x F_1)	.....	5.63*	....	.....	39.00	.....
B	(Testcross)	.....	64.81	....	.....	39.09±1.30	.....
C	(Testcross)	.....	61.44	....	.....	39.73±1.27	.....
D	(Testcross)	.....	35.22	....	.....	39.58±1.73	.....
E	(Testcross)	.....	61.12	....	.....	40.24±1.23	.....
F	(F_1 x F_1)	.....	11.74	....	.....	39.20	.....
G	(F_1 x F_1)	.....	.....	0.01**	.....	.....	50.14
H	(Testcross)	431.13	.....	....	16.42±1.20	.....	.....
I	(F_1 x F_1)	82.81	65.19	0.06**	23.24	34.74	49.43
J	(Testcross)	377.00	16.98	0.59**	16.10±1.27	42.80±1.73	48.66±1.73
K	(Testcross)	408.94	55.91	0.87**	19.53±1.18	38.69±1.48	48.59±1.52
L	(Testcross)	365.34	36.02	2.44**	16.85±2.22	39.59±1.70	47.29±1.73
M	(Testcross)	313.02	22.02	0.01**	15.98±1.41	40.98±1.89	49.85±1.92

* $0.02>P>0.01$.
** Insignificant ($P>>0.01$).

16.48±0.91 in males. This confirms previous observations (Tadano, in preparation).

Recombination values between *y* and *pm* varied from 38.69±1.48 to 40.98±1.89% in females, and from 39.09±1.30 to 42.80±1.73% in males. The average value was 39.56±1.18% in females and 41.18±1.21% in males. Therefore, it can be concluded that the two segments, *c-y* and *y-pm*, are approximately 17–18 and 40–41 map units, respectively, and that the segment between *c* and *pm* is roughly 60 units.

Coincidence coefficients in the *c-pm* segment, calculated from data of test crosses J, K, L, and M, were 0.542 to 0.636 in females and 0.685 to 0.743 in males; they indicate the occurrence of positive interference between the two segments.

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