TWO NEW MUTANTS, HAIRLESS ANTENNA AND NOTCH WING, IN *AEDES TOGOI*

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ABSTRACT. Two recessive mutants, hairless antenna (*ha*) and notch wing (*n*), were recovered from inbreeding stocks of the mosquito *Aedes (Finlaya) togoi*; both mutant alleles exhibited incomplete penetrance and much variation in their expression. Linkage studies indicated that the *n* and *ha* alleles were located in linkage groups 1 and 2, respectively; the allele sequence and recombination distances in linkage group 1 were *s* (straw-colored larva)—(28.8±1.2 to 31.9±2.1%)—*M*(sex locus)—(17.6±2.2 to 41.2±1.5%)—*n*, and those in linkage group 2 were *ru*(ruby eye)—(26.2±1.5 to 29.2±1.1%)—*p*(pigmented pupa)—(17.8±1.8=17.8±1.8 to 23.2±2.2%)—*ha*.

During the course of genetic research on the mosquito *Aedes (Finlaya) togoi*, recessive mutants, hairless antenna (*ha*) and notch wing (*n*), could be isolated from inbreeding stocks of this species. Cross experiments were undertaken for placement of the two mutant alleles in the linkage groups; descriptions of the mutants and results of the crosses are presented in this paper.

MATERIALS AND METHODS

Three wild-type strains, which were collected at three localities (Nagasaki City, Miura City, and Manazuru) in Japan, were employed for composition of the heterozygotes for marker alleles used in this investigation; here, 4 doubly-or triply-marked strains as described below were utilized for localization of the mutant alleles, *ha* and *n*, in the linkage groups. The multiple-marker strains are the *pruha*, the *haypm*, the *sn*, and the *snru* strains, in which the allele *s*(straw-colored larva) is a linkage group 1 marker, *p*(pigmented pupa) and *ru*(ruby eye) are group 2 markers, and both *y*(yellow larva) and *pm*(plum eye) represent linkage group 3 (Tadano 1977a,b,c).

Since the *ha* and *n* alleles, as will be seen, showed incomplete penetrance, selection for both traits had been attempted for several generations to improve their penetrance before cross experiments were initiated. This selection was remarkably effective for improvement of penetrance of the *ha* but not for the *n*.

Mass crosses were carried out in all experiments, but single blooded females which had likely mated were isolated in individual plastic cups for egg-laying; each egg batch was hatched in a plastic container and the larvae were reared as a family. Sex genotypes can be designated *m/m* for femaleness and *M/m* for maleness as in other species of mosquitoes (Tadano 1976). Procedures for rearing mosquitoes were similar to those described before (Tadano 1976, 1977a). Cross data were analyzed by the methods of Bailey (1961); linkage chi-square tests and calculation of recombination frequencies concerning the *ha* and *n* alleles were made especially by the formulae described by him for partially manifested traits.

RESULTS AND DISCUSSION

As shown in Fig. 1, a number of antennal hairs are missing in hairless antenna (*ha*) males and the remaining are stuck on the antennae; in *ha* females the hairs are shorter or lost especially on the anterior antennal segments which have become swollen like beads. Almost naked antennae were frequently observed in *ha* males and nearly all of the antennal segments in *ha* females appeared to be occasionally like strings of beads. Thus, the expressivity is variable. Fig. 2 exhibits notch wing(*n*) in both females and males together with normal wings; the *n* wings are incised at the tips and various spots especially along the posterior edges. Four pairs of the wings displayed here were sampled from 4 individuals of each sex, a pair of right and left wings being from the same individual; the expression of the *n* trait was sometimes quite different even in both wings of one individual, and mosquitoes bearing notch and wild-type wings frequently came out of the *n* strain. This suggests high variability in the expressivity of *n*.

Six testcrosses involving *ha* were made (Table 1) using the *p*, *ru*, *y*, *pm*, and *M*(sex) alleles; another 6 testcrosses were undertaken to elucidate genetic relationships of the *n* allele with *ru*, *s*, and *M* (Table 2). Families indicated in the 2 tables are numbers of the families scored in each cross.

Before these testcrosses were started, the following two $F_1 \times F_1$ crosses had been made as a preliminary experiment:

$$\left(\frac{m\ ha+}{m\ +p}\right)+\left(\frac{m\ ha+}{M\ +p}\right)\text{and}\left(\frac{sm+}{+mn}\right)+\left(\frac{sm+}{+Mn}\right),$$

the former intercross produced 191 ++: 17 +*ha*: 80 *p*+: 0 *pha* (of females), and 198 ++: 11 +*ha*: 84 *p*+: 1*pha* (of males) in 7 families examined; and the phenotypic score from the latter intercross was 335 + +: 14 +*n*: 153 *s*+:27 *sn* (of females), and 345 ++: 83 +*n*: 52 *s*+: 28 *sn* (of males) in 11 families pooled. Linkage chi-square tests were performed on the above scores by the formula

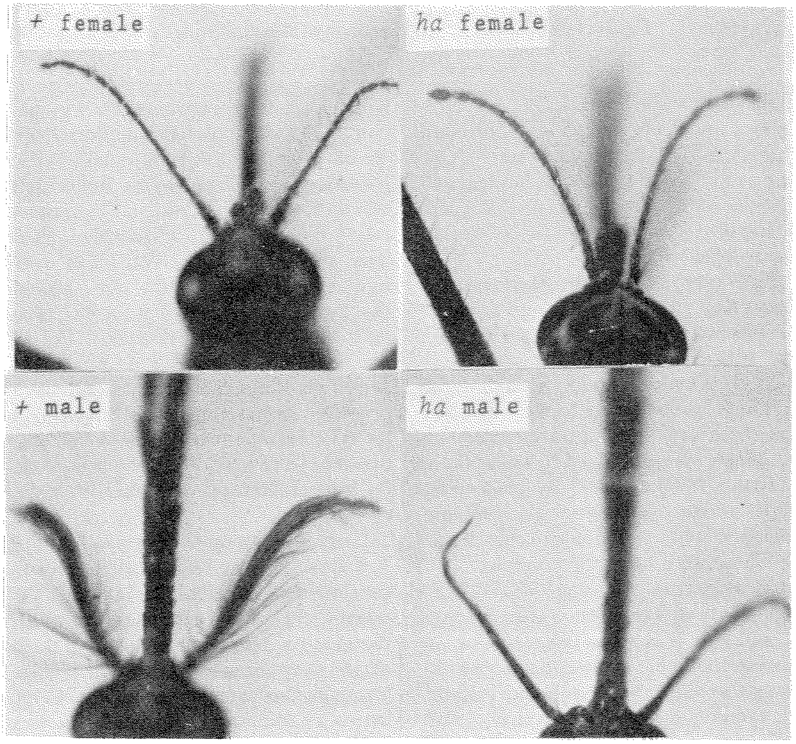

Fig. 1. Normal antenna (+) and hairless antenna (*ha*) in female and male mosquitoes.

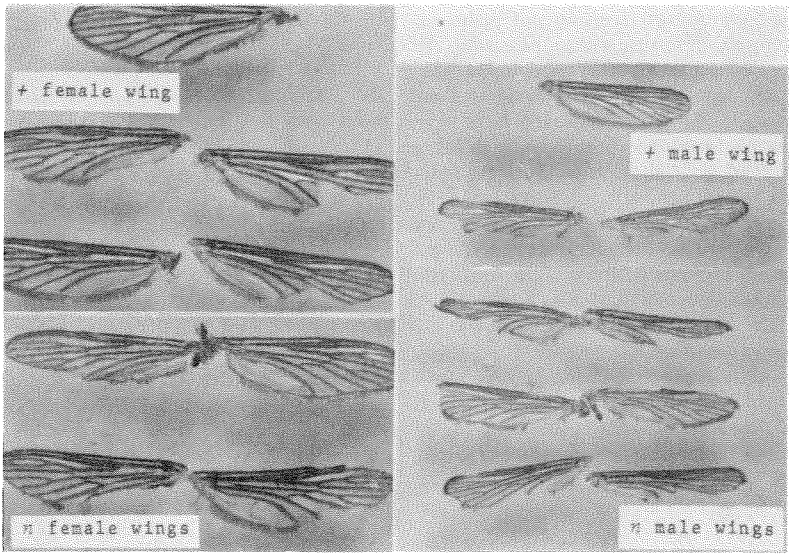

Fig. 2. Normal wings (+) and variation of notch (*n*) wings.

(Bailey 1961) which was devised for intercrosses involving partially manifested characters. The chi-squares were 2.35 for *ha-p*, and 7.81 for *n-s*, which suggested that the *ha* allele was inherited independently of *p* while *n* was linked to *s* (probability level <0.01).

Testcrosses Involving Hairless Antenna (*ha*). In crosses A, B, and E female parents were heterozygous and so were males in all other crosses mentioned in Table 1; in every cross (A through F) the 1: 1 segregation of each allele involved and the independent assortment among the alleles were examined by chi-squares, and also the heterogeneity chi-squares were computed to test for the variation among families within each cross (Table 3). There were significant shortages ($P < 0.05$) of female offspring (738♀:922♂) and of *p* individuals (884+: 776♂) in cross A, and also of females (609♀: 689♂) in cross D. In A more males and fewer *p* individuals were produced in 3 and 1 out of 12 families scored, respectively, and moreover, 3 of 12 families pooled in D produced more male offspring than females. On the other hand, the segregation of +: *ha* departed significantly ($P < 0.05$) from the 1: 1 ratio in all crosses, indicating a shortage of *ha* individuals and thus, poor penetrance of this allele; 4 out of 6 heterogeneity chi-squares calculated in the 1: 1 segregation for *ha* were significant ($P < 0.05$). Raw data showed that the +: *ha* segregated at the 1: 1 ratio only in 2 of 12 families in cross A, 1 of 10 families in B, 1 of 5 in C, 1 of 12 in D, and 1 of 3 in E, while all of 7 families in cross F produced less *ha* individuals. So selection for this trait might have still been effective although the selection had been done for 5 generations prior to initiation of the crosses.

Linkage chi-squares were significant ($P < 0.01$) for *p-ha* and *p-ru* in all the crosses concerned (A through D), and also significant for *ru-ha* in crosses C and D; therefore, it has been revealed that the 3 alleles were located together in linkage group 2. Also strong evidence for linkage between *y* and *pm* was obtained in crosses E and F, which confirms the previous finding (Tadano 1977c). Heterogeneity

Table 1. Testcrosses to elucidate the linkage relationship of hairless antenna (*ha*).

Cross	Genotype proposed			Offspring	Phenotype															Families
				Female progeny								Male progeny								
	Female		Male	+ + +	+ + *ha*	+ *ru* +	+ *ru ha*	*p* + +	*p* + *ha*	*p ru* +	*p ru ha*	+ + +	+ + *ha*	+ *ru* +	+ *ru ha*	*p* + +	*p* + *ha*	*p ru* +	*p ru ha*	
A	$\frac{m}{m}\ \frac{ha\ \ pru}{+\ +\ +}$	×	$\frac{m}{M}\ \frac{ha\ \ pru}{ha\ \ pru}$	214	53	133	4	25	72	136	101	291	66	116	7	40	87	171	144	12
B	$\frac{m}{m}\ \frac{+\ +\ +}{ha\ \ pru}$	×	$\frac{m}{M}\ \frac{ha\ \ pru}{ha\ \ pru}$	171	41	61	1	23	39	96	68	147	43	80	0	34	35	102	97	10
C	$\frac{m}{m}\ \frac{ha\ \ pru}{ha\ \ pru}$	×	$\frac{m}{M}\ \frac{+\ +\ +}{ha\ \ pru}$	34	4	13	0	10	1	21	9	25	1	8	3	9	3	22	15	5
D	$\frac{m}{m}\ \frac{ha\ \ pru}{ha\ \ pru}$	×	$\frac{m}{M}\ \frac{ha\ \ pru}{+\ +\ +}$	205	27	86	2	41	40	110	98	230	31	75	6	46	56	146	99	12
				Female progeny								Male progeny								
				+ + +	+ + *ha*	+ *pm* +	+ *pm ha*	*y* + +	*y* + *ha*	*y pm* +	*y pm ha*	+ + +	+ + *ha*	+ *pm* +	+ *pm ha*	*y* + +	*y* + *ha*	*y pm* +	*y pm ha*	
E	$\frac{m}{m}\ \frac{ha\ \ y\ pm}{+\ \ +\ +}$	×	$\frac{m}{M}\ \frac{ha\ \ y\ pm}{ha\ \ y\ pm}$	50	22	34	9	20	13	47	24	46	17	31	21	25	23	45	24	3
F	$\frac{m}{m}\ \frac{ha\ \ ypm}{ha\ \ ypm}$	×	$\frac{m}{M}\ \frac{ha\ \ y\ pm}{+\ \ +\ +}$	110	23	60	17	55	17	100	34	98	26	68	17	57	24	108	21	7

Table 2. Testcrosses to elucidate the linkage relationship of notch (n).

Cross	Genotype proposed		Offspring								Phenotype								Families
	Female	× Male	Female progeny								Male progeny								
			+ + +	+ + n	+ ru +	+ ru n	s + +	s + n	s ru +	s ru n	+ + +	+ + n	+ ru +	+ ru n	s + +	s + n	s ru +	s ru n	
G	$\frac{+m+}{smn}$	× $\frac{smn}{sMn}$	264	110			283	118			237	47			215	81			12
H	$\frac{+m+}{smn}\ \frac{+}{ru}$	× $\frac{smn}{sMn}\ \frac{ru}{ru}$	160	29	159	29	168	45	162	39	195	48	167	41	188	42	152	52	11
I	$\frac{+m+}{smn}\ \frac{+}{ru}$	× $\frac{smn}{sMn}\ \frac{ru}{ru}$	136	51	136	55	113	49	129	37	109	49	110	62	98	57	122	85	12
J	$\frac{smn}{smn}\ \frac{ru}{ru}$	× $\frac{+m+}{sMn}\ \frac{+}{ru}$	171	68	181	68	78	16	64	26	55	52	54	48	138	89	159	96	16
K	$\frac{smn}{smn}\ \frac{ru}{ru}$	× $\frac{+m+}{sMn}\ \frac{+}{ru}$	33	6	35	9	17	3	17	4	4	5	6	12	17	12	25	21	5
L	$\frac{smn}{smn}\ \frac{ru}{ru}$	× $\frac{smn}{+M+}\ \frac{ru}{+}$	9	26	4	25	30	52	43	48	71	9	82	7	43	6	40	6	8

Table 3. Chi-square analysis of testcrosses involving hairless antenna (*ha*).

Cross	A	B	C	D	E	F
1:1 for						
M	20.40* (23.28)*	1.66 (10.86)	0.20 (2.59)	4.94* (28.04)*	0.38 (5.60)	0.02 (7.8)
p	7.02* (12.59)	1.50 (10.71)	0.02 (4.51)	0.52 (16.47)		
ru	0.78 (5.29)	0.00 (7.67)	0.10 (1.10)	2.24 (11.04)		
ha	211.12* (49.75)*	146.26* (37.05)*	63.12* (8.05)	259.16* (45.68)*	46.62* (24.06)*	272.50* (10.22)
y					0.18 (2.60)	0.02 (13.30)*
pm					0.80 (3.80)	0.28 (4.52)
linkage between						
p & *ha*	263.16**(26.90)*	108.89**(5.92)	13.36**(5.68)	210.76**(13.18)		
ru & *ha*	0.30 (9.19)	1.43 (7.00)	10.32**(4.20)	16.74** (8.88)		
p & *ru*	288.47** (9.22)	193.93**(5.18)	39.64**(1.37)	271.83**(6.71)		
M & *ha*			1.92 (4.53)	0.03 (7.31)		
y & *ha*					3.22 (2.88)	1.32 (5.49)
pm & *ha*					0.01 (0.61)	0.13 (3.13)
y & *pm*					21.73**(0.08)	50.33**(17.46)*
families	12	10	5	12	3	7

Figures in parentheses are heterogeneity chi-square values.

* Significant at 5% level.

** Significant at 1% level.

values were significant in the linkage chi-squares for *p-ha* in cross A (26.90) and for *y-pm* in F (17.46); in A 2 families showed relatively lower linkage chi-squares of 8.21 and 8.28, but all other families gave much higher values, whereas in F 2 out of 7 families showed low linkage chi-squares of 0.07 and 2.46.

Percent recombination values and their standard errors were estimated for all the combinations of alleles which had given significant linkage chi-squares as above. They are shown in Table 4, where recombination units between *ha* and either *p* or *ru* were calculated by the method of maximum likelihood; the units in the *p-ha* segment varied from 17.8±1.8 (cross D) to 23.2±2.2 (B), while those in the *p-ru* segment were 26.2±1.5 (B) to 29.2±1.1 (A), which is in agreement with the values previously estimated (Tadano 1977a). The recombination units for *ru-ha* showed almost free recombination distance ranging from 40.9±2.2 (D) to 51% (A). Thus, the allele sequence in linkage group 2 can be concluded to be *ru-p-ha*.

TESTCROSSES INVOLVING NOTCH WING (*n*). Female parents employed in crosses G, H, and I, and male parents in crosses J, K, and L were in the heterozygous state as seen in Table 2; crosses J and K are replicates, but the heterozygotes in J were composed by a cross Miura (wild-type strain) ♀ x *snru* ♂ while those in K were made by Manazuru (wild-type) ♀ x *snru* ♂.

Table 4. Recombination values among the alleles hairless antenna (*ha*), pigmented pupa (*p*), ruby eye (*ru*), yellow larva (*y*), and plum eye (*pm*).

Cross	*p–ha*	*p–ru*	*ru–ha*	*y–pm*
A	22.0±1.6	29.2±1.1	51.0*	
B	23.2±2.2	26.2±1.5	47.9*	
C	22.6±6.5	26.4±3.3	41.0±3.7	
D	17.8±1.8	27.1±1.2	40.9±2.2	
E				39.0±2.3
F				37.7±1.7

* Linkage chi-square values were not significant.

From data of the testcrosses, chi-square tests were made for the 1: 1 segregation for *M*, *s*, *n*, and *ru*, and also for the independent assortment among these alleles; besides, the heterogeneity chi-square values were estimated for the above functions from raw cross data. This chi-square analysis is given in Table 5. Cross G (731♀: 535♂) and H (791♀: 885♂) showed significant departures ($P<0.05$) from the 1: 1 sex ratio, since more females came out in 5 out of 12 families pooled in G and more males were produced in 3 out of 11 families in H. Furthermore, there was a significant excess of the *ru* individuals in crosses I (662 + : 736 *ru*) and K (97 + : 129 *ru*); but the *s* allele segregated at the 1 : 1 ratio in all crosses. However, a highly significant shortage of the *n* individuals was yielded in all crosses G through L because of poor penetrance of the *n* allele and, very likely, of increased pupal deaths of the *n* individuals shortly before their emergence; the *n* allele segregated at the 1: 1 ratio only in 4 of 12 families pooled in cross I, 8 of 16 families in J, 3 of 5 families in K, and 3 of 8 families in L. Four heterogeneity values in the *n* segregation (H, I, J, and K) were significant ($P < 0.05$).

All linkage chi-square values for *M–n* and *s–M*, which were calculated in crosses J, K, and L, were significant at the 1% level; but the value for *s–n* was significant only in cross L, although there was a heterogeneity in this value. The above values indicated that the *M* allele was located in between *s* and *n* in linkage group 1. Consequently, the allele order should be *s–M–n*. All other linkage chi-squares given in Table 5 provide evidence for the independent assortment among other combinations of alleles; this fact confirms the previous results (Tadano 1977a). Here, percent recombination values were estimated for the *s–M–n* segment, and the values and their standard errors are arranged in Table 6; the *s–M* was calculated to be 28.8±1.2% (J) to 31.9±2.1% (L) and this range of the value is consistent with the results obtained before (Tadano 1977a). Meanwhile, units for the

Table 5. Chi-square analysis of testcrosses involving notch (*n*).

Cross	G		H		I		J		K		L	
1:1 for												
M	30.34*	(13.32)	5.28*	(16.24)	0.14	(38.36)*	0.26	(12.96)	2.14	(2.02)	1.46	(5.52)
s	0.38	(13.04)	0.24	(17.73)	0.23	(12.05)	0.70	(18.02)	0.16	(10.38)*	2.44	(9.18)
n	245.94*	(7.66)	628.08*	(29.58)*	184.60*	(38.82)*	140.10*	(46.16)*	29.76*	(11.28)*	40.82*	(8.42)
ru			3.26	(8.45)	3.92*	(4.58)	0.62	(20.50)	4.54*	(3.12)	0.16	(10.06)
Linkage between												
M & *n*							29.66**	(7.11)	21.90**	(4.29)	140.88**	(8.27)
s & *M*							244.26**	(17.48)	35.84**	(5.78)	65.39**	(10.39)
s & *n*	0.01	(16.88)	2.81	(6.00)	0.92	(12.65)	0.01	(27.55)*	0.76	(3.05)	8.47**	(19.39)*
ru & *n*			0.01	(10.06)	0.29	(17.13)	0.03	(13.92)	1.92	(7.88)	0.91	(4.16)
s & *ru*			0.00	(7.39)	1.03	(10.83)	0.27	(9.12)	0.08	(4.16)	0.02	(9.07)
M & *ru*							0.21	(14.53)	1.77	(8.61)	0.05	(5.95)
Families		12		11		12		16		5		8

Figures in parentheses are heterogeneity chi-square values.
* Significant at 5% level.
** Significant at 1 % level.

Table 6. Recombination values among the alleles notch (*n*), straw-colored larva (*s*), and sex (*M*).

Cross	*s–M*	*M–n*	*s–n*
J	28.8±1.2	41.2±1.5	free
K	30.1±3.1	31.6±1.1	free
L	31.9±2.1	17.6±2.2	42.1±2.2

M–n segment varied between 17.6±2.2 (L) and 41.2±1.5 (J) as estimated by the maximum likelihood, which displays a very wide range in the recombination units for unknown reasons. Only one thing known is that heterozygous male parents in crosses J and K had the *s* and *n* alleles on the *M* chromosomes while the males in L included these alleles on the *m* chromosomes. The recombination distance between *s* and *n* was as distant as over 42.1±2.2%.

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