Linkage studies on a new dominant mutant, extra-jointed palp, and three other mutants in the mosquito *Aedes togoi*

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Abstract: Two mutants, hairless-antenna (*ha*) and white-eye (*w*), in *Aedes togoi* had been previously reported to be located on the same side of the pigmented pupa (*p*) locus in linkage group 2, but the gene sequence of these three alleles had not been determined by the three-point experiments. This study confirmed that the gene sequence is *p-ha-w*, and showed that the recombination units between *p* and *ha* range from 11.9 to 38.4 and the units between *ha* and *w* are more than 32.4.

A new mutant, extra-jointed palp (*Ex*) is the first dominant mutant described in *Aedes togoi*. The *Ex* allele exhibited very low penetrance of 34% and was found to be located over 32.5 map units far from the *ru* (ruby-eye) locus in linkage group 2. The *Ex* trait is sex-limited, expressed in only female palps; in the *Ex* female there is an additional segment extended from a normal palp. This extra segment is pointed and covered with white scales instead of black ones.

The white-eye allele (*w*) has been placed on the same side as the hairless-antenna allele (*ha*) in respect to the pigmented pupa (*p*) locus of linkage group 2 in *Aedes togoi* (Tadano, 1979, 1980); the *ha* allele was located as far as 18 to 23 map units from the *p* locus and there existed a still farther recombination distance of 31 to 47 map units between *p* and *w*. It, thus, appears that the gene sequence is *p-ha-w*, but this sequence had not been confirmed by three-point backcrosses in the previous study (Tadano, 1980). This confirmation has been made by this study.

Meanwhile, a dominant mutant, extra-jointed palp (*Ex*), was found in an inbreeding subcolony of the Miura strain in April, 1977 and this is the first dominant mutant to be described in this species.

MATERIALS AND METHODS

The methods for rearing and crossing the mosquitoes were nearly the same as previously reported (Tadano, 1977), but the mosquito larvae obtained by experimental crosses were reared in salted tap water (ca. 2% NaCl), which gave higher survival rates of the larvae. Four wild-type strains from Taipei (Taiwan), Nagasaki, Manazuru and Miura cities (the last two cities are in Kanagawa Prefecture), were utilized throughout this study to make heterozygotes for the alleles studied.

In heterozygous genotypes given in Tables of RESULTS AND DISCUSSION, the alleles above lines were derived from female parents and the alleles below the lines were from male parents. Since the penetrance of *ha* and *Ex* is incomplete as will be seen later, the existence of linkage of these partially manifesting alleles was examined by the χ^2 formula mentioned by Bailey (1961) especially for this purpose; the recombination units were calcu-

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lated by the method of maximum-likelihood.

RESULTS AND DISCUSSION

1. Determination of gene sequence of white-eye (*w*), hairless-antenna (*ha*) and pigmented pupa (*p*)

The *w* (Fig. 1) and *p* alleles are completely penetrant as shown in the previous results (Tadano, 1977, 1980), but the *ha* trait is partially manifested as in Fig. 2, where one antenna is of wild type in the *ha* homozygote.

Four backcrosses containing *p*, *ha* and *w* were performed, and their results are given in Table 1. Since all three alleles are markers of linkage group 2 exhibiting no sex

linkage, the cross data were not assorted according to sexes in this table. In each of the four crosses, the segregation ratio of each of *w* and *p* was tested for 1:1 by χ^2 from the data pooled from the families (Table 2). Only in cross B the segregation ratio of *w* showed a significant departure from the 1:1 ratio; here less *w* individuals were produced than its wild-type ones. But the *p* allele segregated at the 1:1 ratio in all crosses, which is indicated by insignificant χ^2 values ($P>0.05$). On the other hand the *ha* allele did not show the 1:1 ratio of segregation in any of four backcrosses, since much less *ha* individuals were produced. Observed numbers of the *ha* and its wild-

Table 1 Results of crosses among white-eye (*w*), hairless-antenna (*ha*) and pigmented pupa (*p*)

Cross	Parental genotype		Offspring phenotype								<i>F</i> *
	Female×Male		+	+	+	+	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>	
			+	+	<i>w</i>	<i>w</i>	+	+	<i>w</i>	<i>w</i>	
			+	<i>ha</i>	+	<i>ha</i>	+	<i>ha</i>	+	<i>ha</i>	
A.	$\frac{+ + +}{w\ ha\ p}$	$\frac{w\ ha\ p}{w\ ha\ p}$	62	8	43	3	52	14	44	22	3
B.	$\frac{w\ ha\ p}{+ + +}$	$\frac{w\ ha\ p}{w\ ha\ p}$	63	3	40	3	55	10	47	9	3
C.	$\frac{w\ ha\ p}{w\ ha\ p}$	$\frac{+ + +}{w\ ha\ p}$	186	4	134	10	146	28	131	54	10
D.	$\frac{w\ ha\ p}{w\ ha\ p}$	$\frac{w\ ha\ p}{+ + +}$	255	4	199	5	162	26	190	33	11

* Families pooled.

Table 2 χ^2 analysis of crosses A through D and recombination units between *w*, *ha* and *p*

	Cross			
	A	B	C	D
1 : 1 segregation (χ^2 values)				
+ : <i>w</i>	2.32	4.46*	0.30	0.46
+ : <i>p</i>	1.04	0.62	0.04	3.10
χ^2 values for linkage between				
<i>p</i> - <i>ha</i>	7.83**	6.12	54.46**	46.56**
<i>ha</i> - <i>w</i>	1.50	0.28	13.93**	9.27**
<i>p</i> - <i>w</i>	2.29	0.85	1.92	1.46
Recombination units between				
<i>p</i> - <i>ha</i>	38.4	26.0	14.8	11.9
<i>ha</i> - <i>w</i>	free	free	32.4	44.8
<i>p</i> - <i>w</i>	free	free	free	free

* Significant at the 5% level.

** Significant at the 1% level.

Fig. 1 Wild-type (+) and white (*w*) eyes

Fig. 2 Hairless-antenna (*ha*), and wild-type antenna (+) in the male with partial manifestation of *ha*

Figs. 3 and 4 Wild-type palp (+) and extra-jointed palp (*Ex*)

type (*ha*⁺) individuals were: 47 *ha*/201 *ha*⁺ (cross A), 25 *ha*/205 *ha*⁺ (B), 96 *ha*/617 *ha*⁺ (C) and 68 *ha*/806 *ha*⁺ (D). Total numbers of the *ha* and wild-type individuals, accumulated from the four crosses, were 236 and 1,829, respectively. Therefore, the penetrance of *ha* is roughly $236/1,829=13\%$.

The presence of linkage between *w*, *ha* and *p* was examined by χ^2 -tests (Table 2). Strong linkage between *p* and *ha* is clearly indicated by the χ^2 values in all four crosses, and also the χ^2 values in crosses C and D suggest the existence of linkage between *ha* and *w*. However, all χ^2 values testing for linkage between *p* and *w* are not significant ($P>0.10$, d. f. = 1). Therefore, these χ^2 values indicate that the gene sequence is *p-ha-w* and free recombination can occur between *p* and *w*. Also the recombination units calculated (Table 2) from data of crosses C and D gave another evidence for the gene sequence of *p-ha-w*. The map units for *p-ha*

were very various, ranging from 11.9 (D) to 38.4 (A) and those for *ha-w* were over 32.4.

2. *Linkage studies on a new dominant mutant, extra-jointed palp (Ex)*

In the *Ex* female palp there is an additional pointed segment extending beyond the apical segment (Figs. 3 and 4); this extra segment is covered with white scales and therefore appears like a white point. The *Ex* phenotype is not expressed in the male palp and so shows sex-limited inheritance.

The phenotypes very similar to the *Ex* trait have been described in *Aedes aegypti* by Craig and Hickey (1967) who called it 5-jointed (*5-j*), and also in *Culex tritaeniorhynchus* by Baker and Sakai (1974) who gave the mutant nomenclature of extra jointed palpi (*ex*). Both *5-j* and *ex* are recessive. The penetrance of the *5-j* allele in *Ae. aegypti* was very poor and then was discarded; whereas *ex* is a completely penetrant allele located in linkage group 1 of *C. tritaenio-*

Table 3 Results of crosses between ruby-eye (*ru*) and extra-jointed palp (*Ex*)

Cross	Parental genotype		Offspring phenotype						Families pooled	
			Female				Male			
	Female × Male	+	+	+	<i>Ex</i>	<i>ru</i> +	<i>ruEx</i>	++		<i>ru</i> +
1	$\frac{ru}{ru} \frac{+}{+}$	$\frac{ru}{+} \frac{Ex}{+}$	160		41	128	46	177	168	9
2	$\frac{ru}{ru} \frac{+}{+}$	$\frac{ru}{+} \frac{Ex}{+}$	128		19	135	23	167	162	6
3	$\frac{ru}{ru} \frac{+}{+}$	$\frac{+}{ru} \frac{Ex}{+}$	92		111	157	77	227	198	8
4	$\frac{ru}{+} \frac{Ex}{+}$	$\frac{ru}{ru} \frac{+}{+}$	236		36	198	75	302	303	10
5	$\frac{ru}{+} \frac{Ex}{+}$	$\frac{ru}{ru} \frac{+}{+}$	144		37	181	43	176	181	6
6	$\frac{ru}{+} \frac{Ex}{+}$	$\frac{ru}{ru} \frac{+}{+}$	161		37	72	40	244	289	9
7	$\frac{+}{ru} \frac{Ex}{+}$	$\frac{ru}{ru} \frac{+}{+}$	166		82	194	71	307	249	8

Table 4 χ^2 analysis of cross data and recombination units between *ru* and *Ex*

Cross	1	2	3	4	5	6	7
χ^2 values for <i>ru</i> : + segregation (1:1)	0.36	0.03	0.00	0.00	3.02	0.96	1.58
χ^2 values for linkage between <i>ru</i> and <i>Ex</i>	1.90	0.17	20.92**	17.03	0.10	10.24**	2.41
Recombination units	free	free	37.6	32.5	free	34.4	free

** Significant at the 1% level.

rhynchus.

Since a preliminary cross done prior to this study had provided evidence for linkage between *Ex* and *ru* (ruby-eye) in linkage group 2, seven crosses involving only *Ex* and *ru* were made as given in Table 3. Crosses 1 and 2 are of the same type, but the heterozygous male parents in the two crosses were produced by hybridization with two different wild-type strains; similarly, crosses 4, 5, and 6 are replicates, employing the heterozygous female parents that were made by hybridization with three wild-type strains.

No *Ex* male progeny appeared in any of the seven crosses, which indicated sex-limited inheritance of *Ex*. Although the *Ex* allele is obviously dominant, the segregation ratios of this allele departed far from the 1:1 ratio in each cross and actual numbers of the individuals obtained were as follows:

87 *Ex*/288 wild type (*Ex*⁺) from cross 1
42 " /263 " 2

188 *Ex*/249 wild type (*Ex*⁺) from cross 3
111 " /438 " 4
80 " /325 " 5
77 " /233 " 6
153 " /360 " 7

Then, total numbers of the *Ex* and its wild-type individuals scored from all crosses are 738 and 2,156, respectively. Thus, the penetrance of *Ex* was 738/2,156=34%.

Meanwhile, the *ru* allele segregated at the 1:1 ratio in each of all crosses. This is certified by the χ^2 values for segregation of *ru*: + (1:1), all values being insignificant at the 5% level (Table 4). It was again examined by χ^2 whether *Ex* was linked to *ru* or not; three χ^2 values for linkage, calculated from crosses 3, 4 and 6, were significant at the 1% level, confirming the presence of linkage between *ru* and *Ex*, although the χ^2 values from crosses 1, 2, 5 and 7 were not significant because of a far distance between the *ru* and *Ex* loci, and

probably because of poor penetrance of *Ex*. The recombination units between *ru* and *Ex*, which were estimated from the results of crosses 3, 4 and 6, were as many as 32.5 to 37.6 (Table 4). But further cross experiments to determine the *Ex* locus more precisely could not be undertaken since there was no more suitable marker available for this intention.

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摘 要

トウゴウヤブカにおける white-eye, hairless-antenna, pigmented pupa の遺伝子配列と優性突然変異体 extra-jointed palp について

トウゴウヤブカ *Aedes togoi* の突然変異体のうち, hairless-antenna (*ha*) と white-eye (*w*) とは第2連鎖群の pigmented pupa (*p*) 遺伝子座位から同じ方向に連鎖していることをすでに報告した. それによると, *ha* は *p* から 18~23 map unit の距離に存在し, また, *w* は *p* から 31~47 map unit の位置に存在する. この組換え距離からみれば, 遺伝子配列は *p-ha-w* の順であるように考えられる. しかし, 前報では3点実験でその配列は確かめられていない.

そこで, 今回, *p*, *ha*, *w* を用いた3点実験で遺伝子配列が *p-ha-w* であることを確認した. *p* と *ha* との組換え距離は 11.9~38.4 map unit であった. このように交配実験によって組換え価に差ができたのは, *ha* の浸透度が非常に低いことに起因する.

この蚊では, extra-jointed palp (*Ex*) ははじめて記載される優性突然変異である. これは雌の触しゅ (palp) にだけ現れる表現型であり, 一種の限性遺伝である. 野生型の palp にさらにもう1節 (segment) が追加されたもので, この余分な1節は白鱗でおおわれている. *Ex* も浸透度が悪いが, 第2連鎖群の *ru* (ruby-eye) から 32.5 map unit 以上離れたところに位置していることがわかった.