# INHERITANCE OF WHITE-BODY AND BROWN-EYE IN AEDES ALBOPICTUS

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ABSTRACT. A recessive mutant, brown-eye (b), and a dominant mutant, White-body (Wb), were isolated from a strain of Aedes (Stegomyia) albopictus a vector of dengue. The b phenotype can be best distinguished in the pupal eyes, and the expressivity is rather variable but with complete penetrance. The whole abdomen, scutum and vertex of the Wb mutant are

covered with white scales; Wb homozygotes are lethal most probably in their egg stage, although they may rarely survive to the adult stage. Backcrosses involving Wb and b alleles revealed absence of genetic linkage among Wb, b and the sex allele (M); the 3 alleles might be markers of all 3 linkage groups in this mosquito.

Aedes albopictus (Skuse), subgenus Stegomyia, occurs widely in the Oriental Region, Oceania and Australia, and is a vector of viral diseases such as dengue. Formal genetics of this species have been reported in only a few papers; Bat-Miriam and Craig (1966) described several mutants including a homeotic mutant proboscipedia, which is located about 20 recombination units far from the sex locus (Quinn and Craig 1971). No further linkage study has been made in this mosquito.

A new dominant mutant White-body (Wb) was recovered from the Nagasaki strain of this species by two of us (Mori and Wada). We had selected for the Wb trait for 6 generations of this mutant strain before they supplied the eggs to the senior author. Selection for the trait was continued by the senior author who could

later isolate another new mutant browneyed pupae (b) from the Wb strain. But it appeared that homozygosity for Wb was not made in this strain, since many wild-type individuals came out even after 8 generations of selection. As will be seen below, homozygotes for Wb are lethal most probably in the egg stage. This paper gives a description of the two mutants Wb and b, and their mode of inheritance.

## MATERIALS AND METHODS

The rearing methods and facilities employed for this study were similar to those mentioned by Tadano (1977) for *Aedes* (Finlaya) togoi. Tap water was used for rearing. Eggs were dried 3 days after deposition before storage. Mass crosses

were made in a cage  $(20 \times 20 \times 20 \text{cm})$  for each experiment, but single blood-fed females were isolated into individual plastic cups for single ovipositions. The cups were lined with small pieces of moistened filter paper, on which eggs were laid. Each egg batch was reared separately as a family.

Adults were maintained in an insectary with an almost constant temperature (25±1°C) and relative humidity (70 to 75%), while larvae were reared in a laboratory where only the temperature (25±1°C) was controlled. In this paper alleles above lines in heterozygous genotypes were of maternal origin and those below the lines of paternal origin.

## RESULTS AND DISCUSSION

As seen in Figs. 2 and 4 the vertex, scutum and abdomen of the Wb mosquito are wholly covered with white scales;

therefore, a median longitudinal white stripe on the scutum, which is characteristic of this species (Figs. 1 and 3), is not distinct in the mutant. Even after continuous selection for the Wb trait through 8 generations, the Wb strain still produced many wild-type as well as Wb individuals. Then, 5 crosses A to E given in Table 1 were performed; the raw data showed that each of 53, 12 and 69 families scored respectively in crosses A. B and C yielded individuals of both Wb and wild type. This reveals that every Wbparent used in the crosses was heterozygous for the Wb allele with dominance and no Wb homozygotes were present among the parents. Furthermore, the data pooled in the table seem to indicate that + : Wb segregation ratios were 1 : 1 in crosses A, B and D, and 1: 2 in C and E. Chi2 tests were made on the pooled data in A through E to verify this assumption; the chi<sup>2</sup> values indicated that + : Wb

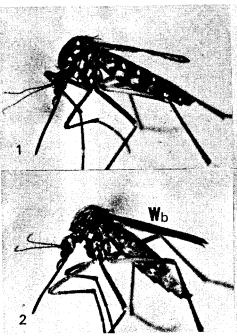


Fig. 1. Aedes albopictus wild-type female.

Fig. 2. Aedes albopictus Wb female.

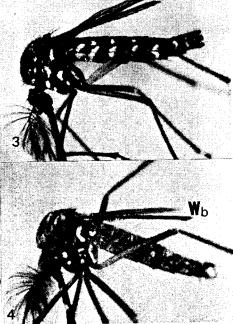


Fig. 3. Aedes albopictus wild-type male.

Fig. 4. Aedes albopictus Wb male.

Table 1. Pooled data obtained from five crosses concerning the Wb allele.

	-					Of	fspring	Pheno			
						_	φ	ੋਂ		Chi² value	
Cross		φ		ð	Families pooled	+	Wb	+	Wb	(+ : Wb)	(sex ratio)
A		+	×	Wb	53	618	629	774	757	0.02 (1:1)*	29.04
В		Wb	×	+	12	220	169	232	217	5.20 (1:1)	4.30
C		Wb	×	Wb	69	492	1079	532	1126	3.94(1:2)	2.30
D	$\frac{m}{m}$	++	×	$\frac{m}{MWb}$	14	282	260	305	300	0.64 (1:1)	3.46
E	$\frac{m}{m}$	$W_b^+$	×	$\frac{m}{MWb}$	12	151	270 1	472711	.75	(1:2)	0.000

<sup>\*1:1</sup> and 1:2 in parentheses are hypothetic ratios for chi² tests.

segregated at 1: 1 ratio in A (P>0.80), B (0.05>P>0.02) and D (0.50>P>0.30) and at 1: 2 in C (0.05>P>0.02) and E (0.20>P>0.10). From the above it can be concluded that the 1: 2 (+: Wb) ratio in C and E resulted from loss of Wb homozygous offspring. The chi² tests for sex ratios suggested a significant departure (P<0.05) from 1: 1 in A and B where more male offspring came out.

No differential mortalities were observed in adults of the Wb strain throughout this study. In order to find out in which stage of the life cycle the Wbhomozygotes are lethal, egg hatchability and survival rates of the immature stages were examined in crosses A, B and C (Table 2). Since some egg batches hatch sluggishly for as long as a week after immersion in water, hatchability of each batch was observed for 7 or 8 days. A total of 2703 eggs laid by 37 females in A and 978 eggs from B gave the same hatchability of 87%, whereas C showed a lowered hatchability of 65% among 5769 eggs. Meanwhile, survival rates (1st instar to adult stage) were nearly the same in all 3 crosses, ranging between 81 and 90%. In addition to the above percentages, if the hatchability of normal eggs is assumed to be about 87% in this study, the hatchability of eggs from C should be  $\frac{3}{4}$  of the general hatchability on the assumption that all Wb homozygous eggs are lethal; that is,  $87\% \times \frac{3}{4} = 65.3\%$  which is approximately equal to the actual hatchability obtained in C. Thus, the hatchabilities observed above are quite compatible with the conclusion that Wb homozygous eggs laid in crosses C and E did not hatch and were lethal.

However, 3 families out of over 130 families that were scored in crosses A, B and C yielded only Wb individuals; and so the 3 families were excluded from the data of Tables 1 and 2. This may indicate that Wb homozygotes could survive to the adult stage on rare occasions, and adults homozygous for Wb might exist with extremely low frequency in the Wb strain.

Sex in this species is determined by a pair of alleles, M and m, females being m/m, males M/m as in other culicine mosquitoes (Bat-Miriam and Craig 1966).

Table 2. Hatchability and survival rate of offspring from crosses A, B and C.

ç		ੋ	(Cross)	Families examined	Egg no.	Hatched larvae	Adult no.	HA*(%)	SR**(%)
+	×	Wb	(A)	37	2703	2343	1900	87	81
$\dot{Wh}$	×		(B)	12	978	855	772	87	90
Wb	×	Wb	$(\mathbf{C})$	69	5769	3735	3229	65	87

<sup>\*</sup> HA: Hatchability, \*\*SR: Survival Rate.

Table 3. Crosses involving the Wb and b alleles.

				Offs							fspring	spring Phenotype				
									· · · · · · · · · · · · · · · · · · ·				₫			
Cross		Ş				♂		F*	++	+ Wb	<i>b</i> +	$b \\ Wb$	++	Wb	<i>b</i> +	b Wb
F	$\frac{m}{m}$	$\frac{+}{Wb}$	$\frac{+}{b}$	×	$\frac{m}{M}$	+++++++++++++++++++++++++++++++++++++++	<u>b</u>	7	58	42	41	46	59	56	42	49
G	$\frac{m}{m}$	$\frac{Wb}{+}$	<u>b</u> +	×	$\frac{m}{M}$	+++++++++++++++++++++++++++++++++++++++	$\frac{b}{b}$	7	66	57	47	70	60	64	58	63
Н	$\frac{m}{m}$	₩b +	$\frac{+}{b}$	×	$\frac{m}{M}$	+ + +	$\frac{b}{b}$	4	25	27	18	23	23	36	23	21
I	$\frac{m}{m}$	$\stackrel{+}{\overset{-}{Wb}}$	<i>b</i>	×	$\frac{m}{M}$	+++++	<u>b</u>	5	16	13	11	6	16	18	9	13
J	$\frac{m}{m}$	+ - +	$\frac{b}{b}$	×	$\frac{m}{M}$	$\frac{+}{Wb}$	$\frac{+}{b}$	7	41	43	32	35	45	42	42	38
K	$\frac{m}{m}$	+++++++++++++++++++++++++++++++++++++++	$\frac{b}{b}$	×	$\frac{m}{M}$	$\frac{Wb}{+}$	$\frac{b}{+}$	7	47	37	28	31	43	53	46	39
L	$\frac{m}{m}$	+++++++++++++++++++++++++++++++++++++++	$\frac{b}{b}$	×	$\frac{m}{M}$	W_b +	$\frac{+}{b}$	6	42	46	34	39	37	35	33	36

F\*: Families pooled.

Pooled data in cross D suggest an independent assortment between Wb and M by a linkage chi<sup>2</sup> value (Bailey 1961) of 0.25 (0.90 > P > 0.80).

The *brown-eye* (b) mutant has dark red, occasionally more reddish, eyes, and the eye color can be distinguished in the larval stage but best in pupae, although it darkens after emergence. It is our experience that b larvae generally pupated later than wild-type ones (black eye), and thus many more wild-type pupae came out first in scoring the backcross off-spring. The b allele is recessive and the penetrance appeared to be complete.

Seven crosses (Table 3) were carried out to obtain data on the genetic relationship between Wb and b alleles. Every time when heterozygotes for Wb were made by crossing the Wb strain to wild-type, normal adults also appeared in the  $F_1$  offspring, suggesting heterozygosity involved in the Wb strain used. Chi² values (Bailey 1961) were estimated from the pooled data in crosses F through L to determine whether the alleles segregated at the expected I:I ratio and whether they were linked with each other (Table 4). A significant deficiency of b (P<0.05) occurred in crosses I and K, and more

Table 4. Chi<sup>2</sup> analysis of crosses F through L.

(Cross)	F	G	Н	1	J	K	L
sex ratio	0.46	0.05	0.52	1.00	0.40	4.46*	1.32
+:Wb	0.12	1.10	1.66	0.04	0.01	0.04	0.34
+: b	3.48	0.16	3.44	5.64*	0.91	4.00*	1.60
Wb-b	2.45	2.25	0.74	0.00	0.00	0.05	0.12
M - Wb					0.45	0.31	0.21
M-b					0.32	0.61	0.48
	sex ratio +: Wb +: b Wb-b M-Wb	sex ratio 0.46 +: Wb 0.12 +: b 3.48 Wb-b 2.45 M-Wb	sex ratio 0.46 0.05 +: Wb 0.12 1.10 +: b 3.48 0.16 Wb-b 2.45 2.25 M-Wb	sex ratio 0.46 0.05 0.52 +: Wb 0.12 1.10 1.66 +: b 3.48 0.16 3.44 Wb-b 2.45 2.25 0.74 M-Wb	sex ratio 0.46 0.05 0.52 1.00 +: Wb 0.12 1.10 1.66 0.04 +: b 3.48 0.16 3.44 5.64* Wb-b 2.45 2.25 0.74 0.00 M-Wb	sex ratio         0.46         0.05         0.52         1.00         0.40           + : Wb         0.12         1.10         1.66         0.04         0.01           + : b         3.48         0.16         3.44         5.64*         0.91           Wb-b         2.45         2.25         0.74         0.00         0.00           M-Wb         0.45	sex ratio $0.46$ $0.05$ $0.52$ $1.00$ $0.40$ $4.46*$ $+: Wb$ $0.12$ $1.10$ $1.66$ $0.04$ $0.01$ $0.04$ $+: b$ $3.48$ $0.16$ $3.44$ $5.64*$ $0.91$ $4.00*$ $Wb-b$ $2.45$ $2.25$ $0.74$ $0.00$ $0.00$ $0.05$ $M-Wb$ $0.45$ $0.31$

<sup>\*</sup> Significant at the 5% probability.

male offspring were produced in K; but all alleles in other crosses showed the 1:1 segregation ratio. Meanwhile, all linkage chi2 values estimated were not significant at the 5% level, which reveals that M. Wb and b were inherited independently of each other. The 3 alleles may be separately located in all 3 linkage groups in this species. Before this can be proved linkage studies on more mutants are required.

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