

LINKAGE STUDIES ON TWO NEW MUTANTS, FROSTY-BODY AND PIGMENTED PUPA, IN THE MOSQUITO *Aedes albopictus*

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ABSTRACT. A dominant mutant, *Frosty-body* (*F*), and a recessive mutant, *pigmented pupa* (*p*), were discovered from the Nagasaki strain of *Aedes (Stegomyia) albopictus*. The *F* mutant has numerous white scales dispersed on its scutum and abdomen; this trait is very distinct from that of *White-body* (*Wb*), another dominant mutant earlier reported. The *F* allele is allelic to *Wb*, and both dominant alleles are lethal in the homozygous conditions and, similarly, the *F/Wb* genotype is also lethal. For this reason, the dominance relationship between *F* and *Wb* could not be revealed in this work.

Although about 10 mutants have so far been described in *Aedes (Stegomyia) albopictus*, a vector of dengue fever, linkage studies have been reported only on *proboscipedia* (*prb*), *White-body* (*Wb*) and *brown-eye* (*b*) (Bat-Miriam and Craig 1966, Quinn and Craig 1971, Tadano et al. 1980). The *prb*, which is a homeotic mutant allele, is located on the sex chromosome, while the *Wb* and *b* alleles are inherited independently of one another and neither of them shows sex-linkage. Moreover, the *Wb* is a dominant allele and lethal in its homozygous state.

Another dominant mutant, *Frosty-body* (*F*), and a new recessive mutant, *pigmented pupa* (*p*), were recovered from the Nagasaki strain of *Ae. albopictus* during routine work in this laboratory. This paper describes the 2 new mutants as well as genetic studies on them.

MATERIALS AND METHODS

All mosquito strains utilized for this study were derived from the Nagasaki strain collected in a suburb of Nagasaki City, Kyushu, Japan. Procedures and methods for rearing mosquitoes and for cross experiments were the same as those mentioned in the previous study (Tadano

The locus for these dominant alleles is situated at a recombination distance of $3.5 \pm 1.1\%$ or less from the *p* locus. The *p* homozygotes become pigmented (dark brown) a few hours after pupation; its penetrance is good with a little variation in the expressivity. The *F* and *p* alleles, as well as *Wb*, are inherited independently of either *b* (*brown-eye*) or sex allele; furthermore, the *b* allele shows an independent assortment with sex. Therefore, these 5 alleles might be representative markers of all 3 linkage groups for this mosquito species.

et al. 1980). In all experiments mass crosses were made in 20 cm³ cages, but blood-fed females were singly isolated into individual plastic cups for oviposition; and thereafter each egg batch laid by single females was hatched and reared in a separate plastic box as a family. All phenotypes and numbers of individuals were scored in each family.

As in other culicine species, sex in this mosquito is determined by a pair of alleles, *M* and *m*, and so females have the *m/m* genotype and males the *m/M* genotype (Bat-Miriam and Craig 1966).

In tables shown below in this paper, all alleles of maternal origin are given above the lines of genotypes, those of paternal origin being below the lines, and, for simplicity, all sex alleles are omitted in the genotypes.

RESULTS AND DISCUSSION

The vertex, scutum and abdomen of a *White-body* (*Wb*) mosquito are entirely covered with white scales, whereas the scutum and abdomen of a *Frosty-body* (*F*) individual have many dispersed white scales (Figs. 1-3). Both traits are distinct from each other, and no expression intermediate between the *F* and *Wb*

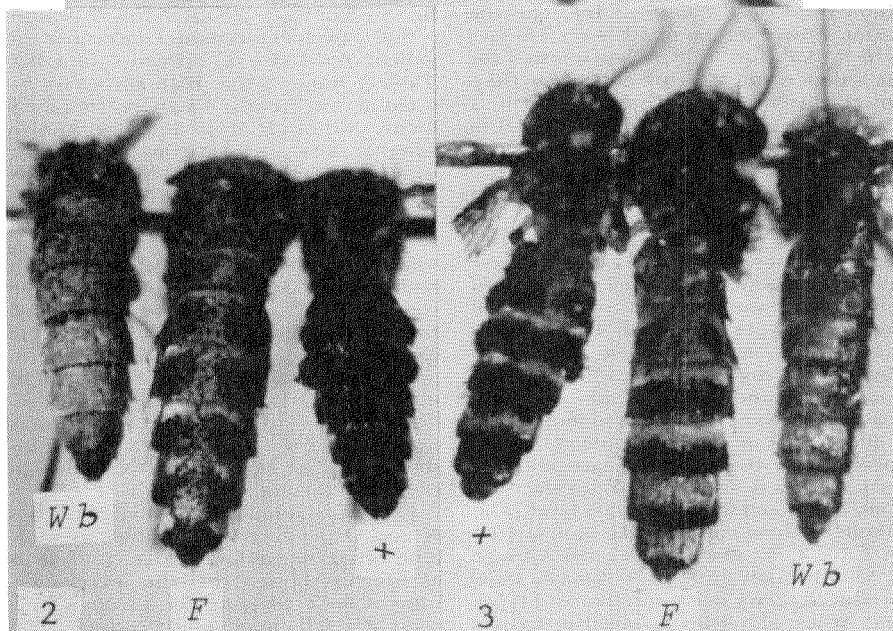
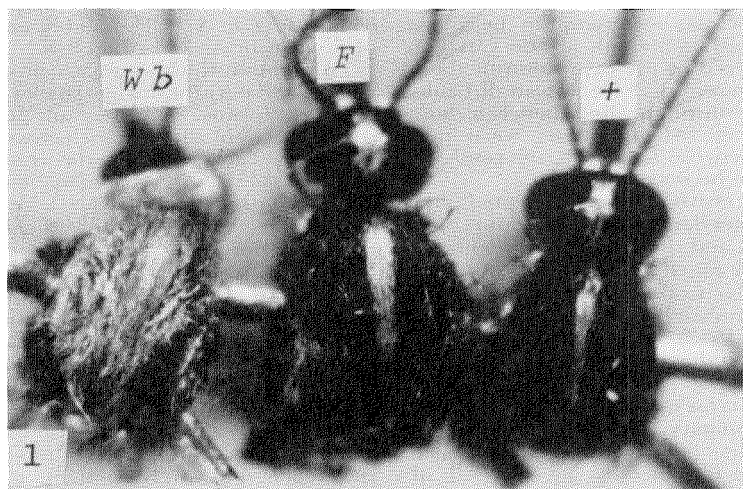


Fig. 1. Dorsal views of female thoraxes showing *Wb*, *F*, and wild type.
 Fig. 2. Dorsal views of female abdomens showing *Wb*, *F*, and wild type.
 Fig. 3. Ventral views of female abdomens showing wild type, *F*, and *Wb*.

phenotypes has been found in the course of this study, although a little variation was observed in the expression of the *F* allele. Therefore, there was no difficulty at all in distinguishing between both phenotypes. Allelism tests were performed between *F* and *Wb*, the results of which will be detailed below.

The recessive mutant, pigmented pupa (*p*), becomes dark brown a few hours after pupation, and this trait can be identified only in the pupal stage. This phenotype is very similar to that of the *p* (pigmented pupa) mutant of *Ae. (Finlaya) togoi* (Tadano 1977), and so the same gene designation has been adopted for this mutant.

Before cross experiments were started, two preliminary crosses: $p+/+++♀ \times p+/+F♂$ and $p+/+F♀ \times p+/+++♂$ had been made; the former cross gave a result of $100++ : 137+F : 65P+ : 1pF$, and the latter showed a ratio of $191++ : 313+F : 71p+ : 3pF$. χ^2 values, which tested for an independent assortment between the *p* and *F* alleles on the basis of a 3 : 3 : 1 : 1 ratio, were significant at the 1% level in both the former ($\chi^2=61.7$) and latter ($\chi^2=112.1$) crosses. Thus, these two crosses yielded first evidence for presence of linkage between the 2 alleles.

All other crosses performed in this study are shown in Tables 1 and 2, where all families which produced all expected phenotypes were pooled together. All male genotypes described in the tables conceal the maleness allele (*M*) below their lines. Crosses in Table 1 involve the *p* *b* (brown-eye) and *Wb* alleles, while those in Table 2 involve the *F* allele together with *p* and *b*. Since all crosses exhibited in the 2 tables are testcrosses, the segregation of each allele was tested by χ^2 for the 1 : 1 ratio on pooled data at the 5% level. The results of these χ^2 tests are summarized in Table 3. For example, in cross A fewer *p*, *b* individuals and fewer females were produced than their respective wild types and males; all alleles involved in crosses C, E, G, L and M, segregated at the 1 : 1 ratio, although there were deficiencies of

b individuals in A, B, H, I and K, and also of females in A, D, F, I, and K.

To find out which alleles are linked together, χ^2 tests for linkage (Bailey 1961) were performed between all possible combinations of alleles in each cross at the 1% level; Table 4 shows the results of these tests along with recombination units between linked alleles. This table indicates that *p* is very closely linked to *F* with recombination units (\pm standard errors) of 0 (cross L) to 3.5 ± 1.1 (J), and that there is also very close linkage between *p* and *Wb* with recombination units of 2.5 ± 0.9 (E) to 8.0 ± 1.9 (cross F). But there is no evidence for linkage among all other combinations of alleles. Therefore, the sex and *b* alleles are inherited independently of each other and also show an independent assortment with the 3 linked alleles, *p*, *F*, and *Wb*; they might be members of 3 linkage groups in this mosquito.

Still a problem remains concerning the genetic relationship between *F* and *Wb*. Table 4 suggests that both alleles are located at nearly equal distances from the *p* locus, although it is not yet known whether these alleles are on the same side with respect to the *p* locus. Nine crosses N through V, given in Table 5, were made to solve this problem.

The *F* strain reproduced numerous wild-type individuals even after this strain had been selected for the *F* trait during 8 consecutive generations, and so it seemed that the *F* homozygotes were lethal in the early developmental stage. All crosses involving the *F* allele (N through T) were initiated after the 8 generations of selection for the *F* trait. Egg hatchabilities as well as survival rates between the 1st instar and adult stages were examined in crosses N through R.

Each of all families examined in crosses N and O produced both *F* and its wild-type offspring, although data pooled in each cross showed that the segregation ratio of the *F* allele deviated from a 1 : 1 ratio ($P < 0.01$ in N and $0.05 > P > 0.02$ in O). This suggests that the *F* parents employed were all heterozygous. The

Table 1. Crosses involving *p* (pigmented pupa), *b* (brown eye) and *Wb* (white-body).

Cross	Parental genotype		Phenotypes of offspring												F.*
	♀	♂	Female						Male						
			+	+	+	+	+	+	+	+	+	+	+	+	
A	$\frac{p}{p}$	$\frac{p}{p}$	34	18	11	6	46	28	23	16	6	6	6	6	
B	$\frac{p}{p}$	$\frac{b}{b}$	87	75	60	40	78	79	68	41	10	10	10	10	
C	$\frac{p}{p}$	$\frac{b}{b}$	15	9	13	15	19	8	14	1	1	1	1	1	
D	$\frac{p}{p}$	$\frac{p}{p}$	0	31	0	25	2	0	62	0	57	51	5	4	
E	$\frac{p}{p}$	$\frac{b}{b}$	0	30	0	44	1	0	40	0	35	39	3	3	
F	$\frac{p}{p}$	$\frac{b}{b}$	3	19	2	12	0	3	42	4	35	29	2	1	
G	$\frac{p}{p}$	$\frac{b}{b}$	1	39	0	43	3	44	0	0	40	2	50	1	

* F. are numbers of families pooled.

heterozygosity for *F* of the *F* strain was reconfirmed by cross P, where individuals of the *F* and its wild phenotypes were produced by each family and also at a 2 : 1 ratio in the pooled score instead of the 3 : 1 because of lethality of the *F* homozygotes. This lethality occurs most likely in their egg stage, since egg hatchability appears to be lower in cross P than in N or O. This kind of lethality has also been reported in the *Wb* homozygotes (Tadano et al. 1980). Reciprocal crosses between the *F* and *Wb* individuals (Q and R) gave an unexpected result; that is, the *F*, *Wb*

and wild-type individuals were reproduced in each family at almost same frequencies and no phenotype intermediate between the *F* and *Wb* phenotypes appeared in all 30 and 25 families examined in Q and R, respectively. This provided good evidence that the two dominant alleles are allelic to each other and the *F/Wb* genotype must also be lethal. This evidence is further strengthened by data obtained from another 4 crosses S through V.

All parents used in S and T were offspring themselves reproduced by Q, and,

Table 3. Results of χ^2 tests ($P=0.05$) for the 1:1 ratio among offspring of each backcross.

Cross	+ : <i>p</i>	+ : <i>b</i>	+ : <i>F</i>	+ : <i>Wb</i>	Females : males
A	* >	>			<
B	>	>			=
C	* =	=			=
D	=	=		=	<
E	=	=		=	=
F	>	=		<	<
G	=	=		=	=
H		>	<		=
I		>	<		<
J	<	=	>		=
K	>	>	<		<
L	=	=	=		=
M	=	=	=		=

* > indicates that fewer *p* individuals were produced than its wild-type ones, while < indicates the reverse result; = suggests statistically equal numbers of individuals produced.

Table 4. Results of χ^2 tests ($P=0.01$) for linkage between alleles involved in each cross, and recombination units between linked alleles.

Cross	Combinations of alleles involved								
	<i>p-b</i>	<i>p-F</i>	<i>p-Wb</i>	<i>p-sex</i>	<i>b-F</i>	<i>p-Wb</i>	<i>b-sex</i>	<i>F-sex</i>	<i>Wb-sex</i>
A	N. L.								
B	N. L.			N. L.			N. L.		
C	N. L.			N. L.			N. L.		
D	N. L.		L.(3.1 ± 0.9)			N. L.			
E	N. L.		L.(2.5 ± 0.9)			N. L.			
F	N. L.		L.(8.0 ± 1.9)	N. L.		N. L.	N. L.		N. L.
G	N. L.		L.(2.6 ± 0.9)	N. L.		N. L.	N. L.		N. L.
H					N. L.				
I					N. L.		N. L.	N. L.	
J	N. L.	L.(3.5 ± 1.1)			N. L.				
K	N. L.	L.(2.7 ± 0.7)			N. L.				
L	N. L.	L.(0)		N. L.	N. L.		N. L.	N. L.	
M	N. L.	L.(1.1 ± 0.6)		N. L.	N. L.		N. L.	N. L.	

N. L. = No linkage exists; L. = Linkage exists; figures in parentheses are recombination units ± standard errors.

Table 5. Crosses to investigate the relationship between the *F* and *Wb* alleles.

Cross	♀ × ♂	Families examined	Egg % hatchability	*Larval % survival	Phenotype				Hypothetical phenotype ratio	**Chi ²
					Female		Male			
					+ <i>Wb</i>	<i>F</i>	+ <i>Wb</i>	<i>F</i>		
N	+ × <i>F</i>	35	65.2	73.5	392	298	468	391	+ : <i>F</i> = 1 : 1	18.9 ^o
O	<i>F</i> × +	18	62.9	73.7	124	152	186	209	+ : <i>F</i> = 1 : 1	3.86 ^{ss}
P	<i>F</i> × <i>F</i>	11	49.9	74.9	61	104	85	146	+ : <i>F</i> = 1 : 2	1.23
Q	<i>F</i> × <i>Wb</i>	30	51.1	76.6	187	169	180	204	+ : <i>Wb</i> : <i>F</i> = 1 : 1 : 1	7.02 ^{ss}
R	<i>Wb</i> × <i>F</i>	25	46.4	88.0	130	123	101	142	+ : <i>Wb</i> ; <i>F</i> = 1 : 1	0.74
S ^o	<i>F</i> × +	20	Not examined		358	0	389	395	+ : <i>F</i> = 1 : 1	1.54
T ^o	+ × <i>F</i>	20	Not examined		371	0	382	364	+ : <i>F</i> = 1 : 1	1.64
U ^{oo}	<i>Wb</i> × +	19	Not examined		317	339	0	388	+ : <i>Wb</i> = 1 : 1	2.20
V ^{oo}	+ × <i>Wb</i>	24	Not examined		368	363	0	422	+ : <i>Wb</i> = 1 : 1	1.86

* Survival rates between the first instar and adult stages. ** Chi² values testing for the hypothetical phenotype ratios. ^o: Significant ($P < 0.01$), ^{ss}: Significant ($0.05 > P > 0.02$).

^o: All parents employed in crosses, S and T, were obtained from the offspring produced by cross Q.
^{oo}: All parents employed in crosses, U and V, were obtained from the offspring produced by cross R.

similarly, all parents in U and V were individuals obtained by R. Reciprocal crosses S and T, from which 20 families each were examined, resulted in the 1 : 1 segregation of $F : +$ without production of any single Wb individual; also in this case each of 40 families produced only the F and wild phenotypes. Reversely, crosses U and V yielded only the Wb and wild phenotypes in each of 19 and 24 families examined, respectively. Moreover, the Wb allele showed a 1 : 1 segregation in each cross. Thus, it was proved by crosses S to V that the offspring produced by either Q or R did not have the F/Wb genotype. All progeny with the F/Wb genotype which must have been reproduced by Q and R are considered to have been lethal in their egg stage, since larval survival rates in P, Q and R, both parents of which involved the dominant traits, were almost identical with those in N and O, and egg hatchabilities in P, Q and R (46.4% to 51.1%) were somewhat lower than those in N and O (62.9% to 65.2%).

Consequently, the dominance relation-

ship between F and Wb could not be determined in this investigation.

ACKNOWLEDGMENT

The author thanks Mrs. S. Sugiyama of this laboratory for technical assistance in rearing mosquitoes. This work was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture, Japan.

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