A NEW MUTANT, *WHITE LARVA*, OF THE MOSQUITO *TOXORHYNCHITES SPLENDENS*: GENETICS AND CANNIBALISM[1]

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ABSTRACT. A strain of a new body-color mutant, *white larva* (*wl*), was established from a field-collected wild-type strain of *Toxorhynchites splendens*. The mutant can be distinguished from the wild type in both the larval and pupal stages, but not in the adult. Crossing experiments confirmed its mode of inheritance to be a single recessive system. This is the first visible mutant found in *Tx. splendens*. Larvae of the *wl* phenotype seem to be recognized as prey by other individuals in mass larvae rearing.

INTRODUCTION

At present, 76 species and subspecies of the genus *Toxorhynchites* Theobald are known (Knight and Stone 1977, Steffan and Evenhuis 1985, Evenhuis and Steffan 1986, Tsukamoto 1989). Among them, *Toxorhynchites splendens* (Theobald) occurs widely in Asia from Kashmir to the South Pacific. This species is considered to be one of the most useful agents for the biological control of aedine vectors of various diseases such as filariasis, dengue and yellow fever.

The benefits of *Toxorhynchites* as a laboratory animal have also recently been recognized for use in the isolation of arboviruses and filariae. Comparative studies have demonstrated this procedure is more sensitive for primary isolation of arboviruses than is the inoculation of newborn mice or mammalian cell culture (Rosen 1981). Advantages of *Toxorhynchites* adults lie in their safety (nonbiting) and large size (Eshita et al. 1982).

No genetical information has been previously available on *Tx. splendens* except for a report on its karyotype by Bhat (1976).

During the laboratory colonization of a population of *Tx. splendens* collected from Palawan Island, Philippines, a colorless larva was found among the usual dark-colored larvae. Isolation of this mutant was attempted from the same population in succeeding generations. After several generations of trials, this colorless larval mutant was established as a fixed colony. The mutant strain has been maintained for 53 generations up to the present (August 1989), in a laboratory of the University of Occupational and Environmental Health, Japan. This paper presents the results of genetic analysis of a new mutant, *white larva* (*wl*), and the effect of larval body color on cannibalism.

MATERIALS AND METHODS

The following strains were used: 1) Wild type: Colonized in the laboratory from field collections of *Tx. splendens* from leaf axils of *Colocasia* plants and ovitraps on Palawan Island, Philippines, in October 1982. 2) *White larva* (*wl*): A visible mutant strain isolated from the Palawan strain in August 1983.

Maintenance of mosquitoes: Immature and adult mosquitoes were maintained in an insectary at a temperature of 26 ± 1°C, RH 75 ± 5%, and light:dark regime of 14:10 h.

For rearing *Tx. splendens*, about 50 adults were kept in each cage (20 × 20 × 30 cm) with a stainless wire frame enclosed with white nylon mesh with a sleeve at a lateral end. Adults were fed on an 8% sucrose solution, in a 150-ml Ehrlenmeyer flask with a cellulose wick. For oviposition, a black plastic cup was provided containing distilled water. For crossing experiments, larvae were reared individually in 100-ml plastic vials each containing 50 ml of distilled water. For routine maintenance, however, larvae were reared en masse in a plastic tray (20 × 30 × 6.5 cm) containing 2 liters of distilled water. They were fed on larvae of *Aedes aegypti* (Linn.) with a prey:predator ratio of 15:1. Pupae were sexed and placed individually into small capsules. The mean duration from egg hatch to adult emergence was 18.9 days with a range of 17.6–20.2 days (Horio and Tsukamoto 1985).

Crossing experiments: Upon emergence, 20 virgin females and 20 males were put together in each cage for the crossing experiments as shown in Table 1.

Scoring for the white or dark larval phenotypes was done at the 4th instar, and sex determination was done in pupae 2- to 3-days-old under a binocular microscope. Divergence of data from the expected segregation ratios for sexes and mutant phenotypes was examined by chi-square tests.

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Table 1. Results of crosses to elucidate the mode of inheritance of white larvae.

Parental genotypes**					Phenotypes of progeny				Chi-square values*		
					♀♀		♂♂		♀♂	+♀ wl♀ +♂ wl♂	+ wl
Cross	♀♀		♂♂	Hatching rate (%)	+	wl	+	wl	1:1	1:1:1:1	3:1
1	+/+	×	+/+	92.2	80	—	76	—	0.103	—	—
2	wl/wl	×	wl/wl	77.8	—	85	—	73	0.911	—	—
3	wl/wl	×	+/+	73.8	60	—	51	—	0.730	—	—
4	+/+	×	wl/wl	77.5	60	—	78	—	2.348	—	—
5	wl/+	×	wl/+	93.7	71	23	89	19	0.970	—	1.908
6	+/wl	×	+/wl	83.3	79	31	75	28	0.230	—	0.828
7	wl/+	×	wl/wl	82.9	35	46	40	36	0.159	1.904	—
8	+/wl	×	wl/wl	90.3	30	36	31	29	0.286	0.921	—
9	wl/wl	×	wl/+	83.5	34	25	27	37	0.203	3.146	—
10	wl/wl	×	+/wl	79.2	29	29	34	28	0.133	0.733	—

* $P > 0.05$ for all values.
** Alleles above the lines in heterozygous genotypes are of maternal origin.
+: wild, *wl*: *white larva*.

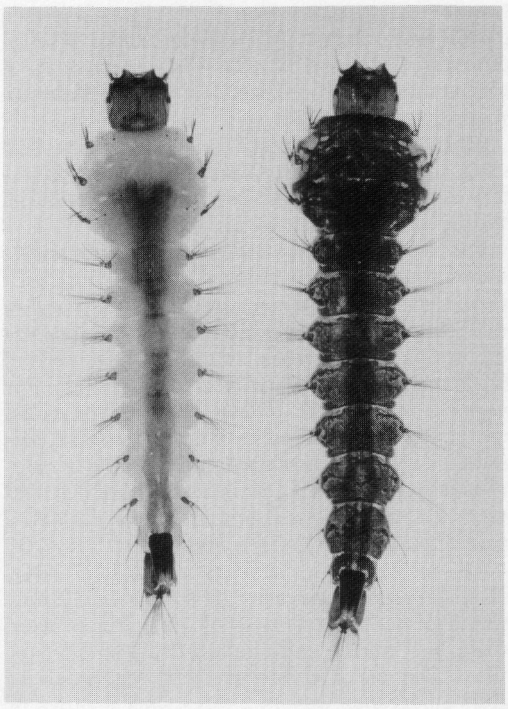

Fig. 1. *Toxorhynchites splendens:* left, *white larva* mutant (*wl*); right, wild type.

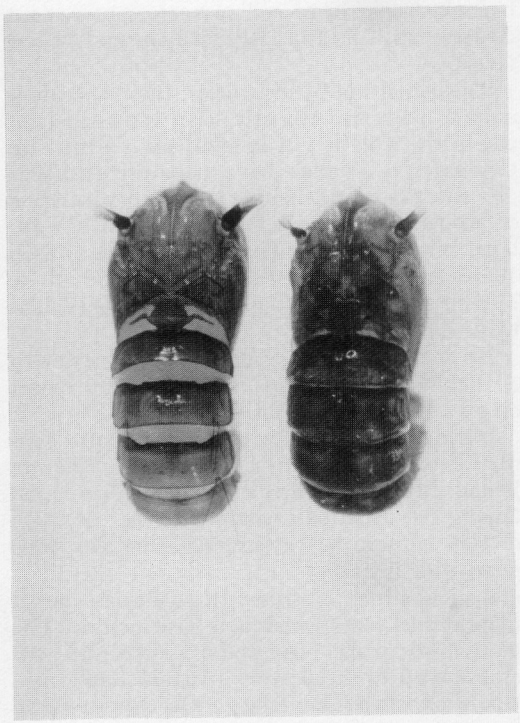

Fig. 2. *Toxorhynchites splendens* pupa: left, *white larva*; right, wild type.

Survival rate under individual and mass rearing conditions: Wild type strains of *Tx. splendens* are usually very easy to rear even under mass rearing conditions. However, the *white larva* strain was difficult to rear en masse because of its decreasing number. Experiments were carried out to determine whether the *white larva* is cannibalistic or has a lower viability. The following 4 aspects were studied: 1) How many larvae survived after individual rearing started with 100 larvae from the *wl* strain? 2) How many larvae survived after mass rearing of 100 larvae of the *wl* strain? 3) Which type of larva survived in greater numbers after mass rearing beginning with a mixture of 50 *wl* and 50 wild type larvae? 4) Were there any differences in number of

survivors between the mutant type and the wild type by either the individual or mass rearing method? If the difference is significant among these conditions, the results may provide an answer to the question of whether or not the dark body color contributes to protection from attacks by other individuals.

RESULTS AND DISCUSSION

Description of the mutant: The normal color of larval skin in *Tx. splendens* is dark mahogany-red on the dorsal half with a grayish-white ventral half. The head capsule, siphon, saddle and sclerotized setal plates are dark chitinous brown (Fig. 1, right). Pigments of dark color are deposited as particles in the fat bodies of the wild type, whereas only the alimentary canal and tracheal tubes can be seen through the transparent skin in the white larval mutant (Fig. 1, left).

This pigment is not produced in the white larval mutant even after the eating of prey. In the wild type larvae the chitinous plate is initially colorless just after ecdysis, but it becomes brown within several hours. In contrast, the dark color of the larval skin can even be observed immediately after ecdysis.

The normal pupal color in *Tx. splendens* is entirely dark brown in the wild phenotype type (Fig. 2, right). Pupae of the mutant show a dark color but can easily be distinguished from the wild type pupae because their intersegmental membranes are still grayish-white (Fig. 2, left).

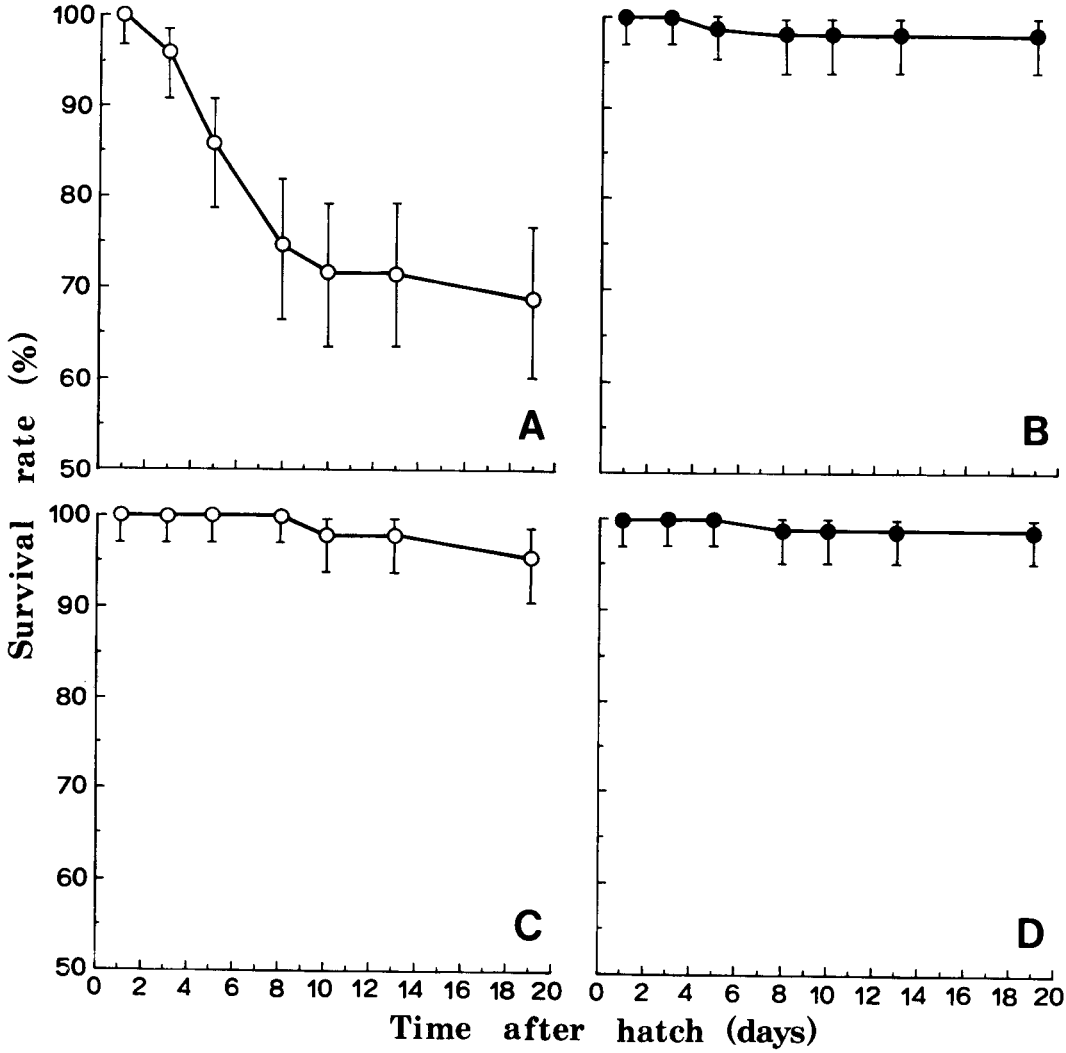

Fig. 3. Survival rate after mass (A and B) and individual (C and D) rearings from 100 of each larval type. Open circles indicate *white larva* and solid circles wild type.

There were no detectable differences in the pigmentation of adults between the mutant and wild type strains.

Hartberg (1978) reported a white larva mutant in a strain of *Tx. brevipalpis* (Theobald) originating from Tanzania, Africa. Females of this mutant produced only a few eggs with poor hatch, indicating the difficulty in maintenance of the homozygous *wl* strain. On the contrary, the present mutant strain of *Tx. splendens* has normal fecundity and hatchability.

Genetic analysis: To determine the mode of inheritance of the *white larva* (*wl*), results of crosses are summarized in Table 1. When females of the wild type strain were crossed with males of the wild type (Cross 1), females of the *wl* strain were crossed with males of the wild type (Cross 3), and females of wild type strain were crossed with the mutant males (Cross 4), all the F_1 progenies showed the wild phenotype. The *wl* mutant stock produced only white larvae (Cross 2). Crosses 5 and 6 are combinations between females and males of the F_1 heterozygotes (from crosses 3 and 4), respectively, and produced the F_2 progenies of both *wl* and wild phenotypes; in these crosses segregation of + and *wl* phenotypes was 3:1 and the overall sex ratio was 1:1. Furthermore, reciprocal backcrosses (crosses 7–10) produced progenies of 1:1 segregation for both the sex ratio and for larval color phenotypes. Therefore, it is obvious that the *wl* mutant has a recessive character. If the mutant *wl* is closely linked to the sex-determining locus, the progenies from Cross 9 may be all the *wl* type females and all the wild type males, and from Cross 10 may be all the wild type females and all the *wl* type males, respectively. However, wild type females were counted in the 1:1:1:1 ratio. Since chromsomes of *Tx. splendens* are known to be homomorphic (Bhat 1976; Horio, unpublished data), it may be assumed that either the *wl* gene is linked to an autosome or the mutant locus is a long distance from the sex-determining locus.

Effect of larval body color on cannibalism: When 100 larvae of the *wl* strain of *Tx. splendens* were mass reared, there was some decrease in number at the 2nd and 3rd stages, due to cannibalism which was frequently observed (final pupation rate was about 69% as shown in Fig. 3A). When the mutant larvae were individually reared in separate containers, however, mutant strains showed a high pupation rate (96%, Fig. 3C). The wild type larvae produced many pupae (about 98%) either in mass (Fig. 3B) or in separate (Fig. 3D) rearings. These results clearly suggest that decrease in the pupation rate in the *wl* mutant strain of the mass rearing was due to cannibalism and not to low viability of the mutant. This assumption was further confirmed by the next experiment in which 50 white larvae and 50 wild type larvae were mixed together. Here again, only *wl* larvae decreased in number, and the final pupation rate was 74% whereas the rate for the wild type was 98% (Fig. 4). Larvae of dark color thus did not fight each other, but some parts of white larvae became

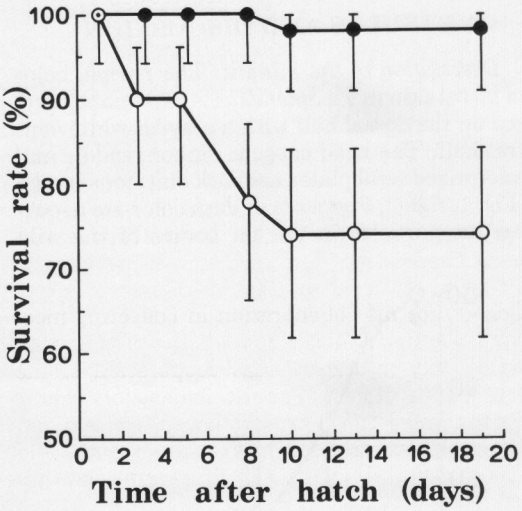

Fig. 4. Survival rate after mass rearing from a starting mixture of 50 *wl* and 50 wild type larvae. Open circles indicate *white larva* and solid circles wild type.

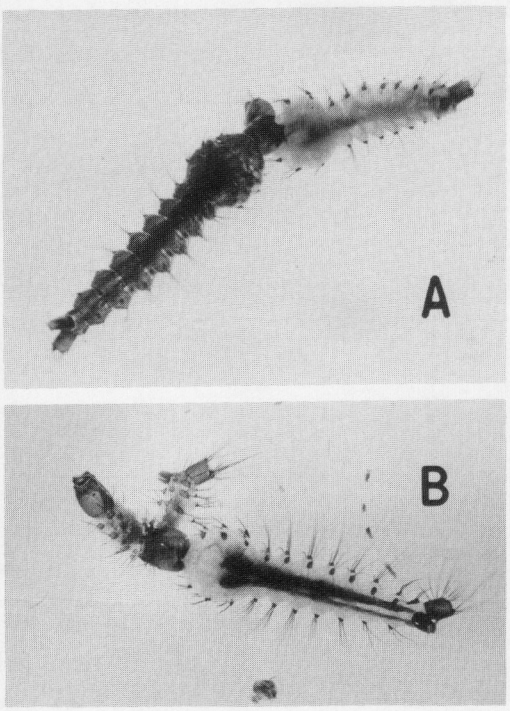

Fig. 5. Cannibalism between wild type and *wl* larvae (A) or *wl* by *wl* (B).

prey of both dark and white larvae even though the food provided (*Ae. aegypti*) was adequate. In other words, white larvae may not be recognized as companions but attacked as prey either by another white larva or by a dark larva (Fig. 5). It is frequently found that in trees holes many larvae of *Orthopodomyia anopheloides* Theobald (the body color is dark reddish-brown) can survive with larvae of *Toxorhynchites*, whereas larvae of other species are not found (Tanaka et al. 1979).

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