GENETIC ANALYSIS OF A LARVAL COLOR MUTANT, *GREEN LARVA*, IN *ANOPHELES STEPHENSI*

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ABSTRACT. A larval color mutant *green larva* was isolated from *Anopheles stephensi* Liston from a laboratory colony. Genetic crosses have shown that it is an autosomal recessive mutant with complete penetrance. Another autosomal recessive mutant *colorless-eye* showed random segregation with *green larva*.

INTRODUCTION

A larval color mutant, *green larva* (*g*), with greenish-black color phenotype has been isolated from a laboratory colony of *Anopheles stephensi* Liston (type form). It expresses its phenotype in instar II and the color is visible even in pupae. Adults of this genotype are slightly darker than the wild type. This is the first larval color mutant isolated and studied in *Anopheles stephensi* since Mason and Davidson (1966) reported a black larval color mutant. Linkage studies have shown that it is an autosomal recessive mutant. This paper describes the mode of inheritance of *green larva* and its linkage relationship with another autosomal mutant *colorless-eye* (Sharma et al. 1977).

MATERIALS AND METHODS

The new mutant, *green larva*, was observed in a male-linked translocation line and subsequently a pure line of mutant type was established. Initially fewer

females laid eggs and there was heavy mortality (50–60%) during eclosion. During emergence the right hind leg gets stuck in the pupal case, and in trying to pull the leg out mosquitoes die on the water. This problem has been overcome to a great extent (30%) after 6–8 generations of selection and inbreeding.

The stocks used in the crosses were:

WILD TYPE. A laboratory colony established from a pupal collection made from Sonepat, Haryana, India in the year 1975. Since then it has been maintained as a routine laboratory colony.

COLORLESS-EYE (c/c). An autosomal recessive mutant (Sharma et al. 1977). Mosquitoes homozygous for *colorless-eye* gene have completely white eyes. In addition to this, larvae are lighter in color compared to wild type and are distinguishable as early as in instar II with the naked eye.

EXPERIMENTAL PROCEDURE. All experiments were carried out in a laboratory maintained at 27–28°C and 70–80% RH. Larvae were reared in enamel basins following standard procedures evolved at this laboratory. Virgin females were obtained by microscopic examination of pupal genitalia. Crosses were set up in standard laboratory cages of 30 × 30 × 30 cm size. Rabbits were provided as a blood source on the 5th day of the cross and eggs were collected on the 9th day.

In F_2 progeny of genetic crosses, different phenotypes were observed. Since these phenotypes could be distinguished at instar II, F_2 larvae were separated at instar II and were rechecked at instar IV. This procedure provided correct numbers in each category and helped in avoiding errors due to occasional mortality in the later instars. In dihybrid crosses (Table I cross No. 3 and 4) a light green phenotypic category was observed. Light green color is visible only at instar IV, but these larvae could be distinguished at instar II due to sharp segmentation of the abdomen and also body color which is darker than the wild type. In instar IV light green color is present all over the body with a bright and shiny thorax.

RESULTS AND DISCUSSION

Results of crosses showing mode of inheritance and linkage relationship of *green larva* (*g*) with sex and *colorless-eye* (*c*) are given in Table 1. *Green larva,* and wild type were reciprocally crossed, and F_1 progeny were inbred to obtain F_2 progeny (crosses 1 and 2). Absence of mutant phenotype in F^1 progeny indicated that it is a recessive mutant. This was further supported by a 3:1 ratio of wild type to green observed in F_2 progeny of crosses 1 and 2. Absence of mutant phenotype in F_1 "sons" of cross

Table 1. Data showing mode of inheritance and linkage relationship of *green larva* with sex and *colorless-eye mutant*

Cross No.	Parental genotypes ♀♀		♂♂		Phenotypes of F_2 progeny: wild type	white eye	green	light green	Total	χ^2 Value P=0.05
1.	+/+	x	g/g		480 (472.5)	—	150 (157.5)	—	630	0.48 n.s.
2.	g/g	x	+/+		946 (972.75)	—	351 (324.25)	—	1297	2.94 n.s.
3.	+/+ c/c	x	g/g	+/+	638 (631.7)	217 (210.6)	213 (210.6)	55 (70.2)	1123	3.58 n.s.
4.	g/g +/+	x	+/+	c/c	343 (370.1)	139 (123.4)	140 (123.4)	36 (41.1)	658	6.82 n.s.
5.	g/g c/c	x	g/g	+/+	—	—	28	—	28	—

( ) Expected number
n.s. Not significant.

No. 2 indicated that it is not a sex linked mutant, since *An. stephensi* mosquitoes have the 'X' and 'Y' sex determination mechanism (Sharma et al. in preparation).

To determine its linkage relationship with *c*, *g* was reciprocally crossed with *c*, and in both the crosses F_1 progeny were inbred (crosses 3 and 4). Among the F_2 progeny 4 different larval phenotypes were observed viz. wild, green, white eye and light green with white eye (light green) in a ratio of 9:3:3:1 indicating that the 2 mutants were not linked. But the 4th category, light green, did not resemble the green parent in its phenotypic expression. In order to determine its genotype (cross No.5) light green females were crossed with green (*g*/*g*) males, the F_1 progeny were all green phenotype. This suggests that even though light green has *g*/*g* genotype, intensity of green color was reduced. This may be due to the presence of *c*/*c* genotype in light green mosquitoes which also reduces the intensity of body pigmentation. The interaction observed between *c*/*c and g*/*g* genotypes suggests that the *c* gene may be controlling the entire larval pigmentation mechanism.

ACKNOWLEDGMENT. We are thankful to Mr. Pritam Singh and Mr. K. B. Masiwal for their technical assistance. We are also grateful to Dr. V. P. Sharma for his critical comments and valuable suggestions on the manuscript.

References Cited

Mason, G. F. and Davidson, G. 1966. Morphological mutants in anopheline mosquitoes. Trans. Roy. Soc. Trop. Med. Hyg. 60:20.

Sharma, V. P., T. R. Mani, T. Adak and M. A. Ansari 1977. *Colorless-eye,* a recessive autosomal mutant of *Anopheles stephensi.* Mosquito News 37:667–669.

Sharma, V. P., Sarala K. Subbarao and R. K. Razdan. Red-eye, a recessive sex linked mutant of *Anopheles stephensi.* (in preparation).