BLACK BODY, A LETHAL MUTANT IN *ANOPHELES QUADRIMACULATUS* SAY

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ABSTRACT. A recessive autosomal lethal mutant (black body, bl) was found in *Anopheles quadrimaculatus* Say. Mosquitoes with this genotype usually died in the late fourth instar or pupal stages. Electron micrographs showed a large number of black granules present in the cytoplasm, of the fat body and cuticular cells of larvae homozygous for bl.

Kitzmiller and Mason (1967) summarized the genetic studies of *Anopheles quadrimaculatus* Say and listed the morphological mutants that have been described; the genetic basis for only two had been established (French and Kitzmiller, 1964). A new mutant, black body (bl) is described in the present paper, which is particularly interesting because it is a recessive lethal. The inheritance of this gene was studied, and cytological observations were made with conventional and electron miscroscopy.

METHODS. The black body (bl) mutant was found in a laboratory colony of *A. quadrimaculatus* during a study of DDT resistance. It appeared in the F_2 progeny from a single pair cross: F_1 female (DDT-resistant female X DDT-susceptible male) X F_1 male (DDT-resistant female X DDT-susceptible male). All bl larvae died before or shortly after pupation. We therefore inbred the normal sibs to obtain a stock that was maintained by sib matings for 8 generations. During each generation, we collected eggs from individual females, hatched them, and reared the larvae to the fourth instar at which time they were categorized by phenotype. The mutant was difficult to maintain because of a decrease in vigor in the stock, problems in rearing the larvae, and our failure to achieve a high percentage of mating in the laboratory.

In addition, fourth-instar larvae of the bl and normal genotypes were embedded in paraffin, and sections 6 μ thick were stained with Delafield's hematoxylin, counterstained with eosin (Humason, 1967), and observed by conventional light microscopy. For the electron microscope studies, the fourth-instar larvae were cut into small pieces, fixed for 3 hours in 3 percent gluteraldehyde in 0.10 M phosphate buffer (pH 7.2), rinsed in buffer, postfixed in 1 percent OsO_4 in phosphate buffer, and dehydrated through ascending concentrations of ethanol. Then they were embedded in epon-araldite (Mollenhauer, 1964), and sections 60–80 nm thick were placed on uncoated grids and stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965). Examinations and photographs were made with an electron miscroscope operating at an accelerating voltage of 50 kV.

RESULTS. The normal color of larvae of *A. quadrimaculatus* in our laboratory colonies (DDT-susceptible and DDT-resistant) is light tan; however, some are brownish to reddish, and some are bright green. Also, laboratory personnel recalled that the black phenotype had previously appeared occasionally; however, observations during our investigations indicated that its occurrence was rare.

The larvae homozygous for bl are black except for two regions, one in the thorax and the other in the fourth abdominal segment (Fig. 1); these light areas appear to result from the presence of tracheolar bundles that are not pigmented. The black color was evident in all larval instars, in pupae, and in the single adult examined. The larvae developed normally except for the color until the late fourth instar, but about 90 percent died before pupation. All pupae that were obtained died except for a single male specimen that completed pupation and emerged. This male

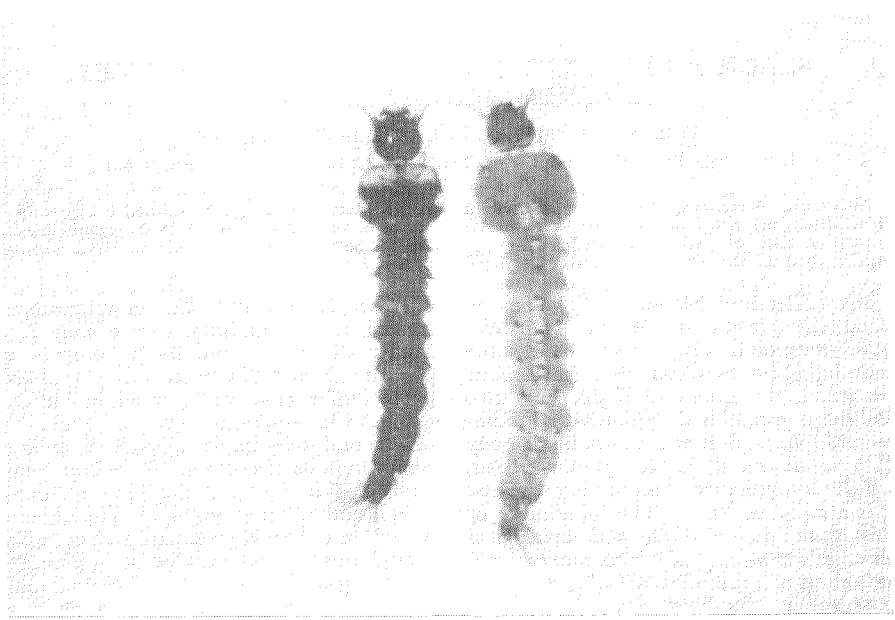

FIG. 1.—A comparison of the mutant (blbl) (left), and the normal (bl^+bl^+) phenotypes in *A. quadrimaculatus*.

was very weak and died after a few hours. The bl gene was not manifest in the heterozygotes.

From the crosses of normal sibs of the black larvae, we obtained 10 progenies which produced 1600 larvae; 380 of the larvae were bl ($X^2=1.50$, $P=0.2-0.3$). Therefore, the bl gene is inherited as a recessive and there is no indication of sex-linkage because the normal sibs were about equally distributed between both sexes.

The examinations of serial sections of bl larvae with brightfield microscopy revealed that the fat body and cuticular tissues contained dark granules though the other tissues appeared normal. Also, the fat body cells were much smaller in the bl larvae. The electron micrographs revealed that the black granules were variable in structure and density and had no membrane (see Figures 2a and 2b). All were spherical with a maximum diameter of 1 micron, and most appeared smooth though the intensity of the stain varied, but some had a coarse appearance, and some were more diffuse than others. Also, the number of granules per fat body cell was variable, but all were confined to the cytoplasm. In some sections, the mitochondria were larger than normal and appeared to be degenerating. The relatively large fat droplets that occur in normal fat body cells were absent in the sections from bl larvae, which indicates that the larvae might die from lack of necessary food reserves. Examination of normal larvae which lacked the bl character showed some black granules in the cytoplasm of the fat body, but their occurrence was rare. Lack of hormonal control or feedback mechanisms could be responsible for the black granules, which appeared to be melanin pigment. The normal growth of the bl larvae precludes a lack of necessary enzyme reac-

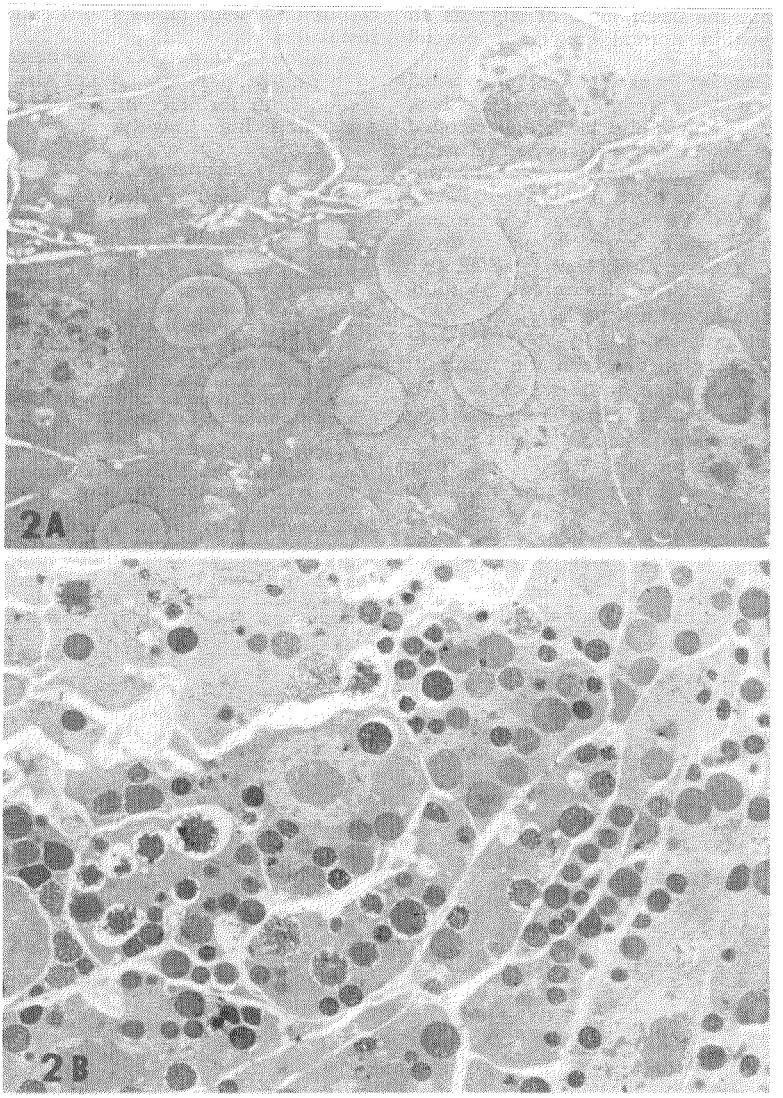

FIG. 2A.—Electron micrograph of fat body tissue from a normal (bl^+bl^+) larva of *A. quadrimaculatus*, ×5,200.

FIG. 2B.—Electron micrograph of fat body tissue from a black body (blbl) larva of *A. quadrimaculatus*, ×5,200.

tions, but the black pigment suggests an imbalance in normal enzymatic control mechanism(s).

The mutant is very similar to a lethal mutant in *Culex pipiens* (Laven and Chen, 1956) which was also black and resulted from an autosomal, recessive, lethal gene. These mosquitoes homozygous for this lethal gene also died in the fourth larval instar or in the pupal stage. Laven and Chen used paper chomatography to determine that these black larvae lacked two free amino acids, β-alanine and γ-aminobutyric acid, and a peptide.

Lethal mutants of the bl type might be used in conjunction with other aberrant genetic mechanisms in the genetic suppression of insects. LaChance and Knipling (1962) presented a control model for the utilization of recessive lethal mutants in suppression of insect populations. The bl mutant does not fit their model because the homozygote is not viable, even in the laboratory, but an application of lethal mutants such as bl can be envisaged, possibly in a balanced lethal system or in conjunction with either translocations or inversions.

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