PUPAL PLEOMORPHISM IN A STRAIN OF *ANOPHELES ALBIMANUS* FROM EL SALVADOR

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ABSTRACT. Studies on a single strain of *Anopheles albimanus* from El Salvador have revealed a high degree of genetic complexity. Based on pupal morphology, two alleomorphic pairs comprising four morphologic phenotypes have been derived from this strain by selection pressure, i.e., striped, nonstriped, green, and brown. The epidemiologic implications of genetic variations in a localized population of a malaria vector are briefly discussed.

INTRODUCTION. The presence of morphologic variants has been reported within many species of anophelines. Such differences may occur at any developmental stage of the mosquito. In some situations these variations have been the basis for the eventual separation of species and subspecies within a species complex. This has been particularly true where a complex extends over a broad geographic range. *Anopheles albimanus* Wiedemann has been reported from a variety of habitats from the Florida Keys and Southeast Texas to northern South America, including many of the islands of the Caribbean. Although considered to be a single species throughout this extensive distribution area, variations in both habits and morphology have been reported. This mosquito is an important transmitter of malaria through most of its range and is considered to be the primary vector from Guatemala to Panama. Differences in physiology and behavior, possibly associated with morphologic variations, in populations of *A. albimanus* are important in investigations of malarial epidemiology. This is a report of studies on morphologic variants in a population of *A. albimanus* isolated from a single site in the interior of El Salvador.

MATERIALS AND METHODS. The Apastepeque strain of *A. albimanus* was established in September–October 1971 from female mosquitoes collected in stables near Lake Apastepeque, El Salvador (Dame et al. 1974). The adult colony was maintained at the Central America Research Station by daily feedings on guinea pigs and honey-soaked sponges. Larvae were reared with finely ground commercial pig food which had been sifted through a 50 mesh screen. Pupae were returned to the colony cage on a random basis and no selection pressure was exerted.

In 1972, a collection of eggs from this colony was transported to the NIH laboratories in Chamblee, Georgia. There, the colony was maintained by daily feedings on rabbits supplemented by sponges soaked in 5% Karo syrup. Larval rearing was accomplished with finely ground commercial dog food (sifted through a 50 mesh screen). In July 1974, the colony in Chamblee was taken over by the Bureau of Tropical Diseases, Vector Biology and Control Division (CDC) where it has remained. Prior to this time there had been no selective breeding of this colony at any time. The colony had been consistently maintained at a temperature of 26° C and relative humidity of 80%.

In July 1974, it was noted that pupae in this colony were not morphologically homogeneous. Striped forms similar to those reported by Keppler (1965) and by Georghiou et al. (1967) were seen. This phenotype has also been noted in at least 2 strains of *A. albimanus* isolated from different areas of El Salvador. In addition to the striped phenotype, an occasional specimen with bright green pigmentation was also observed. Green specimens had been noted in this species from wild material collected in El Salvador, but this phenomenon was attributed to dietary influences such as the presence of algae in the environment. However, it was clear that the same explanation would not apply to colony mosquitoes maintained with a controlled diet in the laboratory.

Initial selection of these phenotypes was carried out using the pupae collected each day from the base colony. Specimens were macroscopically selected and the particular phenotypic character was confirmed under a stereoscopic microscope. Specimens with a specific character were collected daily over a period of 10 to 14 days after which the succeeding generation was derived from the selected line. Similar selections were made from each successive generation. The number of selections required to produce pure lines varied with the specific phenotype. Striped and nonstriped lines were selected first. The 2 pigment phenotypes (green and brown) were derived from the nonstriped line.

RESULTS. The nonstriped line was easily established in pure form. The stripe character was completely absent from this line in the second generation. The striped line continued to produce both nonstriped and partially striped types for 4 generations, after which only striped forms were seen. Selections from the clearest phenotype of green and dark brown specimens were made from the nonstriped form. Clear green females began to appear in the second generation but some yellow-green specimens were produced for 4 generations. Selection pressure has been maintained with this isolate and rare olive to yellow-green forms are still seen after 7 months of selection. The clear green phenotype has been consistently less clear in males than in females.

The brown phenotype has proved to be the most difficult line to establish. This is partly due to the fact that the character is less definitive and, therefore, the selection of breeding stock was less consistent than with the other variants. Adopting a policy of rigid adherence to the selection of a dark brown form for breeding has now clarified the line and this type now produces rare specimens with light brown pigment. No green or olive forms have been seen in this line for approximately 8 generations.

The range of pupal morphology in the base colony of the Apastepeque strain of *A. albimanus* is illustrated in Fig. 1. Striped or partially striped forms constitute from 12 to 15% of the specimens. Distinct dark brown forms are primarily females and have a 4% frequency. True green specimens are rare, usually females, and make up 1% or less of the pupae from the base colony. The pure striped phenotype is seen in Fig. 2. The green and brown alleomorphs are illustrated in Fig. 3 and 4. The three types currently under study are compared in Fig. 5.

DISCUSSION. The epidemiology of malaria in areas where *A. albimanus* is the vector presents some peculiar features. Blood meal source studies on wild specimens captured in El Salvador indicate that this species is not primarily anthropophilic. Even with indoor catches of adult mosquitoes, only 44% had fed on man, and in outdoor catches more than 86% contained cattle blood (Breeland, 1972). Dissection records on mosquitoes collected from areas endemic for malaria have generally shown extremely low natural malaria infection rates (Rachou et al. 1973; Breeland, 1972). However, in particular areas, at specific times high anthropophilic indices (Breeland, 1972) and high natural malaria infection rates (Warren et al. 1975) have been reported. These findings suggest considerable variations in mosquito populations, not only geographic but also quite possibly temporal as well.

The studies reported here on a single strain of *A. albimanus* indicate a high level of genetic complexity even in a small geographic area. The presence of 4 true-breeding morphologic forms in a single isolate of an anopheline is interesting and suggests the possibility of significant behavioral or physiologic variability. It is clear that epidemiologic studies on malaria

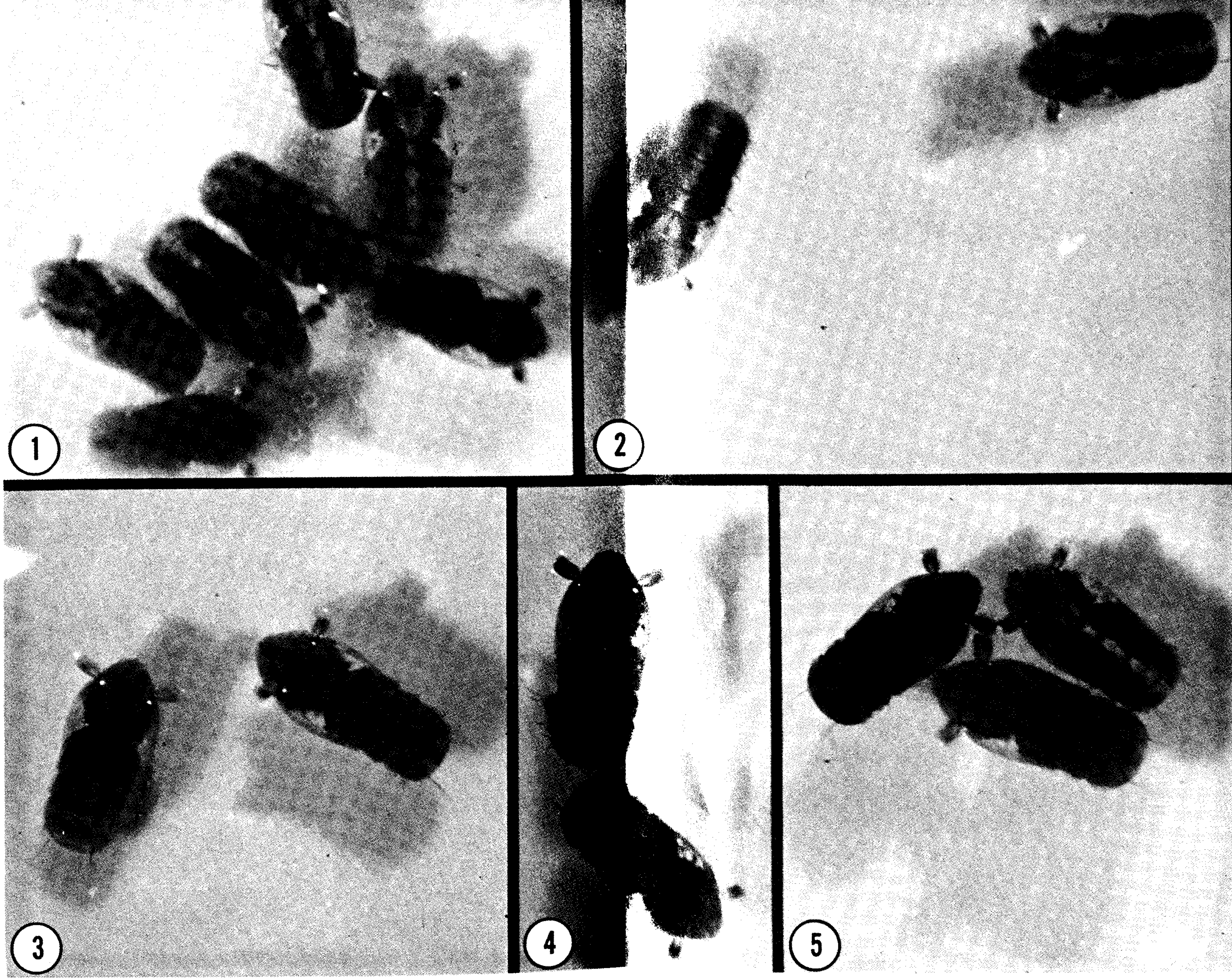

Fig. 1. Morphologic range of pupae from the Apastepeque strain of *Anopheles albimanus*. Note the presence of green, brown, striped and nonstriped forms. This figure presents variations, but not naturally occurring proportions.
Fig. 2. The striped phenotype after seven generations of selection.

Fig. 3. The green (nonstriped) phenotype after seven generations of selection.
Fig. 4. The brown (nonstriped) phenotype after seven generations of selection.
Fig. 5. Comparison of the phenotypes of *Anopheles albimanus* (Apastepeque strain) under study.

and *A. albimanus* must consider not only variations in behavior and physiology of the mosquito in different parts of its range but also the possibility of differences within a localized population related to time and/or environmental pressures. Initial studies are underway on the possible association of these morphologic variations with physiologic characteristics and will include the susceptibility to malaria and the response to insecticides of the several types.

References Cited

Breeland, S. G. 1972. Studies on the ecology of *Anopheles albimanus*. Am. J. Trop. Med. Hyg. 21:751–754.

Dame, D. A., C. S. Lofgren, H. R. Ford, M. D. Boston, K. F. Baldwin and G. M. Jeffery. 1974. Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. II. Methods of rearing, sterilization, and distribution. Am. J. Trop. Med. Hyg. 23:282–287.

Georghiou, G. P., F. E. Gidden and J. W. Cameron. 1967. A stripe character in *Anopheles albimanus* (Diptera:Culicidae) and its linkage relationships to sex and dieldrin resistance. Ann. Entomol. Soc. Am. 60:323–328.

Keppler, W. J. 1965. Genetic, cytological and chemical studies on *Anopheles albimanus*. Thesis, University of Illinois, Urbana, Illinois.

Rachou, R. G., L. A. Schinazi and M. Moura-Lima. 1973. An intensive epidemiological study of the causes for the failure of residual DDT-spraying to interrupt the transmission of malaria in Atalaya and Falla, two villages of the coastal plain of El Salvador, Central America. Rev. Brasil. Malariol. Doencas Trop. 25:1–293.

Warren, McW., J. Mason and J. Hobbs. 1975. Natural infections of *Anopheles albimanus* with *Plasmodium* in a small malaria focus. Am. J. Trop. Med. Hyg. 24:545–546.