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ESTABLISHMENT OF A FREE-MATING COLONY OF *ANOPHELES ALBITARSIS* FROM BRAZIL[1]

STEVE HOROSKO III,[2] JOSE B. P. LIMA[2] AND MAURO B. BRANDOLINI[3]

ABSTRACT. The establishment of a free-mating colony of *Anopheles albitarsis sensu stricto*, a member of the *Anopheles albitarsis* complex, is described. Groups of females from the F_5,F_6, and F_{11} generations were examined, and the percent inseminated, mean number of eggs oviposited, and percent hatch discussed. The colony has been continued through 18 generations, with larval development averaging 10 days, and larval mortality ranging between 20 and 30%.

Anopheles (*Nyssorhynchus*) *albitarsis* Lynch-Arribalzaga is recognized as a complex of 3 to 5 species (Kreutzer et al. 1976, Rosa-Freitas et al. 1990). Wilkerson et al. (1995), using random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR), recently demonstrated 4 species in the complex, which they designated A, B, C, and D. These were hypothesized to correspond respectively to *An.* (*Nys.*) *albitarsis sensu stricto* (*s.s.*), an undescribed species, *An.* (*Nys.*) *marajoara* Galvao and Damasceno, and *An.* (*Nys.*) *deaneorum* Rosa-Freitas. The range of *An.* (*Nys.*) *albitarsis s.s.* (species A) appears to be Paraguay, southern Brazil, and Argentina, and although the other 3 species are sympatric with *An. albitarsis*, their ranges extend northward over much of lowland South America and into southern Central America. However, detailed distributions are unknown. Members of this complex have been incriminated as important vectors of malaria in South America (Arruda et al. 1986, Klein and Lima 1990; with others reviewed by Rosa-Freitas et al. 1990), but due to taxonomic confusion, the vector status of the various species in the complex is unknown.

We describe here the first establishment of a self-mating colony of *An. albitarsis s.s.* Past attempts to colonize members of the *An. albitarsis* complex have been partially successful. Klein et al. (1990) established a colony of *An. deaneorum* in Costa Marques, Rondonia, Brazil, using an induced-mating technique. Galvao et al. (1944) maintained a free-mating colony of *Anopheles albitarsis domesticus* Galvao and Damasceno through 6 generations; however, Linthicum (1988) considered *An. albitarsis domesticus* to be conspecific with *An. marajoara* and not a subspecies of *An. albitarsis*. Rios et al. (1984) also could not justify this subspecies as a valid taxon.

A colony of *An. albitarsis* was established in our laboratory in 1993 with specimens collected from Massaranduba (26°35′S, 48°58′W), Santa Catarina, Brazil. This species has been confirmed to be *An. albitarsis s.s.* using the RAPD-PCR markers from Wilkerson et al. (1995). The colony was maintained until May 1995 using a forced-mating technique similar to that described by Ow Yang et al. (1963). From May 1995 through August 1996 we successfully reared 18 free-mating generations.

Larvae were reared in 18-cm-diam × 8-cm-deep white plastic basins. Approximately 100 newly hatched larvae were placed in each basin with *ca.* 150 ml dechlorinated tap water. Through the 2nd instar, larvae were fed once per day with a 1:1 mixture of TetraMin® (TetraWerke, Melle, Germany) type L and E baby fish food. The fish food mixture was ground into a fine powder and spread evenly onto the water surface. Beginning at the 3rd instar, and continuing until pupation, larvae were fed the same mixture twice daily. This type of diet required little maintenance to keep the basins clean. Only at the beginning of the 3rd instar was it necessary to transfer the larvae to a new basin with clean water.

Development from the 1st instar to pupa took *ca.* 10 days. Pupae were transferred into cups and placed into metal BioQuip® (BioQuip Products, Gardena, CA, USA) mosquito cages (60 × 60 × 60 cm). Newly emerged adults were provided a 10% sucrose solution in 100-ml Erlenmeyer flasks with cotton gauze wicks. Four flasks were placed in each cage and changed twice weekly. The temperature in the insectary ranged between 25 and 29°C, and the relative humidity varied between 80 and 90%. Windows in the insectary provided natural light, which was supplemented with fluorescent lamps during the day. The sex ratio was generally 1:1. Mating was not observed and presumably occurred at night.

When there were *ca.* 500 adults at least 3 days old in a cage (*ca.* 1,000 adults total) a guinea pig was provided for blood meals. The sucrose solution was removed from the cage *ca.* 8 h prior to introducing the guinea pig. The guinea pig was restrained in a metal screened cylinder, and a black plastic sheet was placed over the entire cage, as the mosquitoes appeared to feed better in darkness. After 2 h the guinea pig was removed from the cage and the sugar solution returned.

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[2] U.S. Army Medical Research Unit–Brazil, American Consulate–Rio, APO AA 34030 (outside the USA, use the address below).

[3] Instituto de Biologia do Exercito, Rua Francisco Manuel 102–Benfica, CEP 20911-270–Rio de Janeiro, RJ, Brazil.

Table 1. Percentage of female *Anopheles albitarsis* inseminated, mean number of eggs produced, and percent hatch from samples of 3 generations.

Generation	No. females bloodfed	% females inseminated[1]	% females oviposited	Mean no. eggs oviposited	% eggs hatched
F_5	100	69	66	54.7	69
F_6	100	63	57	116.7	76
F_{11}	100	60	56	93.5	79

[1] Females were dissected 2 days after oviposition.

On the 3rd day after the blood meal, a white plastic basin (15 × 8 × 4 cm) was introduced into the cage. Strips of filter paper (3 cm wide) were placed along the sides of the basin, and *ca.* 100 ml of water was added to the basin. The adults freely oviposited on the water surface, primarily at night. After 2 days the basin was removed from the rearing cage, and a new basin was introduced for an additional 2 days. When the first eggs began to hatch, food was added to the basin as described above. On the following day, the 1st-instar larvae were removed using plastic pipettes and placed into the rearing basins.

To quantify the mating success of the colony, tests were conducted with samples of females from the F_5, F_6, and F_{11} generations. For each test, 100 newly engorged females were removed from the large mosquito cage and separated into 10 equal groups. After 3 days, each group of 10 females was placed into 500-ml-capacity cylindrical screened containers (9 cm wide), which were subsequently placed into white plastic oviposition basins containing 100 ml water, as described above. Two days after oviposition the spermathecae were removed from each female and examined for sperm by light microscopy at 400×. The ovaries were also examined for the presence of eggs. The results of these observations are shown in Table 1. The mean number of eggs oviposited by the F_5 generation was relatively low. However, through the F_6 and F_{11} generations as the colony adapted, the number of eggs and percent hatching increased. Generally, larval mortality ranged between 20 and 30%.

Individuals from this colony have been used to facilitate taxonomic and malaria parasite susceptibility studies. The development of a continuous colony of *An. albitarsis* will also allow increased experimentation in vector abatement as a means of disease control.

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