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COLONIZATION OF *ANOPHELES PSEUDOPUNCTIPENNIS* IN PANAMA[1]

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Abstract: A Panamanian strain of *Anopheles pseudopunctipennis* was colonized and has been sustained for 40 generations. Immature stages were mass reared applying usual insectary techniques. Average pupal yields have reached over 1000 per day. Adults were maintained in large screened cages and exposed to natural cycles of photoperiod, temperature, and relative humidity. Periodic modifications of these factors facilitated adaptation of the colony.

Anopheles pseudopunctipennis is one of the most widely distributed mosquitoes in the Western Hemisphere, having been recorded from Argentina and Chile north to the central United States (Lane 1953, Carpenter & LaCasse 1955, Vargas & Martínez Palacios 1956). The field biology of *A. pseudopunctipennis* is well known, particularly in tropical America where this anopheline is an important vector of human malaria (Foote & Cook 1959). However, attempts to obtain experimental data have been limited by the absence of productive insectary cultures. As summarized by Trembley (1955), and more recently by Gerberg (1970), there are few references on adaptation of this species to a laboratory environment. Downs & Arizmendi (1951) cited unpublished records of an *A. pseudopunctipennis* colony of Colombian origin which had been carried at a low level for a period of 6 months, although they failed to colonize a strain from Mexico using similar techniques. In Mexico, Prof. Amado Martínez Palacios was able to rear this mosquito for 4 generations (pers. commun., 1968). Artificial copulation has been used to maintain *A. pseudopunctipennis* in temporary laboratory populations (Martínez Palacios & Davidson 1967).

The purpose of this paper is to report the successful colonization of an indigenous strain of *A. pseudopunctipennis* at the Gorgas Memorial Laboratory in Panama.

MATERIALS AND METHODS

Aquatic stages of *A. pseudopunctipennis* were obtained for colonization from breeding areas near the localities of San Carlos and Santa Clara, approximately 90 km southwest of Panama City. Weekly collections were made, totalling over 10,000 larvae, from March to May of 1968. All material was processed and maintained in the Rand Insectary of Gorgas Memorial Laboratory.

Larvae were grown in white enameled trays (41.6 × 24.8 × 6.4 cm) with tap water at controlled temperatures of 27–30 °C. A 12-hr light cycle was employed over the holding racks utilizing 60-watt incandescent bulbs (FIG. 1). Small patches of filamentous green algae (primarily *Spirogyra*) from local watercourses were placed in each pan to encourage microfloral populations and to increase larval feeding surfaces. Pulverized food, consisting of equal weights of wheat germ, Brewer's yeast, and high protein breakfast cereal (Kellogg's Concentrate®), was sprinkled on the water as needed 3 or more times daily. A glass slide coated with a paste of Baker's yeast was suspended in each rearing medium. The larvae were diluted serially during growth to prevent overcrowding; larval concentrations ranged from several thousand per pan as first instars to less than 500 as fourth instars. Water was not changed during larval development except in those pans showing a surface pellicle or contamination as indicated by odor or diseased larvae. Bacterial and fungal infections were reduced by adding a few drops of sodium hypochlorite (Purex®) to each affected pan for one or more days.

Pupae were gathered with a wide-mouthed pipette or filter pump aspirator and transferred to 350-ml

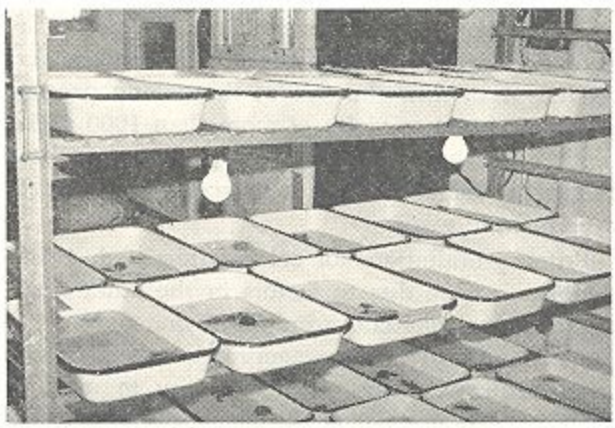

FIG. 1. *Anopheles pseudopunctipennis* larval rearing trays illuminated in holding racks.

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plastic dishes. Adults emerged into screened 9.5-liter ice cream cartons or directly into 76 × 38 × 38 cm stock cages. The latter units routinely housed totals of 1000–5000 specimens. Insectary windows provided natural photoperiod exposure as shown in FIG. 2. During the course of laboratory adaptation and in periods of low colony yield, night and morning (8:00–9:00 PM and 6:00–7:00 AM) red light cycles were implemented to stimulate swarming and mating. Optimum low light intensities were selected by using a variable transformer. Temperature and relative humidity in the cages fluctuated with the ambient cycles; room hygrothermograph readings normally ranged from 22 to 29°C and from 60 to 85%, respectively. Nutriment for the adults was furnished by raisins and by cotton pads saturated with 5–10% solutions of honey or corn syrup. A shaved guinea pig, restrained on the cage floor for several hours each morning, served as a source of blood. Occasionally, a human blood meal was made available.

Eggs were deposited in tap water containing surface algae or other organic floatage. Oviposition trays were replaced each morning and retained in the holding racks until hatching of the eggs. A filter paper lining in the trays prevented desiccation of laterally stranded eggs during incubation.

TABLE 1. Yields of *Anopheles pseudopunctipennis* during the 4-month period following colonization.

MONTH (1968)	NO. OF OVIPOSITIONS	NO. OF PUPAE
June	89	1,987
July	343	17,770
August	385	19,094
September	528	32,601

RESULTS AND DISCUSSION

First generation progeny of *A. pseudopunctipennis* from field collections were pooled into a single insectary population over a 3-month period to establish the reproducing strain. During the succeeding 4 months the size of the colony progressively increased to yields of more than 1000 pupae per day (TABLE 1). The colony since has been carried at these levels as a source of material for experimental work.

Primary limitations for colonization of *A. pseudopunctipennis* were due to factors in the adult stage. Spermathecal dissections indicated that only a low proportion (5–10%) of unengorged or gravid females were fertilized. This reduced ability to mate in captivity was not changed appreciably after more than 2 years in laboratory culture. Although

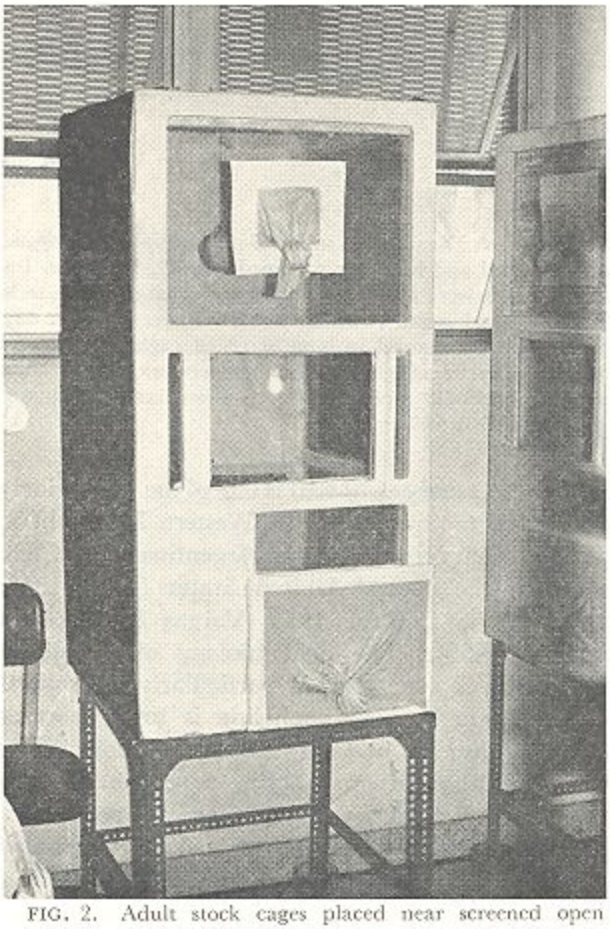

FIG. 2. Adult stock cages placed near screened open windows.

mating took place in standard sized units (38 × 38 × 38 cm), it has been necessary to maintain several of the large stock cages to achieve continuous mass production. Exposure to additional cycles of low intensity light did help to induce mating, but was not continued as a standard procedure. Red or blue light was more effective than yellow or white light for these trials. Spontaneous swarming and pairing were observed in the colony during crepuscular hours, and during daylight hours (afternoon) when natural photoperiod intensities were reduced by cloud cover. The life span of adult *A. pseudopunctipennis* averaged less than 2 weeks in the colony cages. A maximum survival time of 20 and 25 days was recorded for males and females, respectively, at a mean temperature of 24°C.

As noted by Davis (1928), more than one complete blood meal often is necessary to initiate ovarian development for *A. pseudopunctipennis*. In the present study repeated blood meals also were taken by mated and unmated blood-engorged and gravid individuals. Females, when unmated, tended to withhold (and resorb) their eggs, even when oviposi-

tion sites were made more attractive with floating organic material (algae, grass clippings, leaves). The numbers of eggs deposited per parous cycle were variable; the majority of females laid fewer than 200 eggs, although in some instances more than 300 were observed. Rate of hatch among viable clutches was irregular (<10–100%), and averaged under 70%. All eggs conformed to the configuration described for *A. pseudopunctipennis* in Panama by Rozeboom (1937).

Minimal mortality (<10%) and a rapid and uniform larval growth rate (8–11 days to pupation) were achieved using the described procedures. Both embryonic and pupal development required 2–3 days at the given temperatures. Adult broods normally deposited eggs within 7–10 days after emergence, thus giving a period of 3 weeks for the complete life cycle. Approximately 40 generations have been produced by this colony since it was initiated in 1968.

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