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COLONIZATION OF *ANOPHELES PSEUDOPUNCTIPENNIS* FROM MEXICO

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ABSTRACT. Two colonies of *Anopheles pseudopunctipennis*, Tapachula and Abasolo strains, were established under laboratory conditions with a thermoperiod (29°C during the day; 24°C during the night) and artificial dusk. To stimulate mating, a light beam from a flashlight was shone on the cage shortly after lights off. This procedure was repeated for the first 6 mosquito generations (parental to F_6) and thereafter light stimulation was unnecessary for mating. The Tapachula colony has been maintained for 24 generations in 24 months, with insemination rates in females >80% since the F_3, and a monthly production of 30,000 pupae since the F_7. Using the same procedure, the Abasolo colony from northeastern Mexico has been maintained for 13 generations in 14 months, with insemination rates of 26–52%.

KEY WORDS *Anopheles pseudopunctipennis*, laboratory colony, flashlight plus thermoperiod method, malaria, Mexico

INTRODUCTION

Anopheles pseudopunctipennis Theobald is the most widely distributed anopheline species in the Neotropical region (Bruce-Chwatt 1985). In Mexico, *An. pseudopunctipennis* is the principal vector in nearly two thirds of endemic malaria areas (Rodríguez and Loyola 1989). Because of the unavailability of laboratory colonies, basic biological studies of this vector have been limited to insecticide resistance (Martínez-Palacios and Davidson 1967), genetics (Estrada-Franco et al. 1993), and susceptibility to *Plasmodium* parasites (Warren et al. 1980).

One of the principal problems in establishing a colony is the failure of this species to mate under caged conditions (Martínez-Palacios and Davidson 1967, Baerg 1971). To establish and maintain colonies of this species, force-mating is normally used (Baker et al. 1962, World Health Organization 1975, Darsie and Lopez 1980, Estrada-Franco et al. 1993), a tedious, costly, and time-consuming technique. A previous attempt succeeded in stimulating mating among *An. pseudopunctipennis* in captivity by exposing caged mosquitoes to crepuscular light through a window (Baerg 1971), but these results have not been repeated. We report here the establishment and maintenance of 2 colonies of *An. pseudopunctipennis* by artificial stimulation of mating under insectary conditions.

MATERIALS AND METHODS

Immature *An. pseudopunctipennis* larvae were collected during the dry season, when peak larval abundance occurs (Fernández-Salas et al. 1994), in 2 areas of Mexico. The first colony (Tapachula) was established in February 1996, from mosquito larvae collected along the Coatán River in the Foothills area (altitude 450 m) (15°04′N, 92°13′W) of Tapachula, Chiapas, in southern Mexico. The 2nd colony (Abasolo) was obtained from mosquito larvae collected in November 1996 along the margins of the Salinas River (altitude 550 m) in Abasolo, Nuevo León (25°58′N, 100°24′W), in northeastern Mexico.

In both habitats, *An. pseudopunctipennis* larvae were collected from a slow-moving river containing dense mats of filamentous green algae (*Spirogyra* sp. and/or *Cladophora* sp.). The larvae were transported to the Centro de Investigación de Paludismo insectary, and placed at concentrations of 700–1,000 in plastic trays (43 × 37 × 7 cm) containing 1.5–2 liters of purified tap water. Larvae were fed finely ground mouse food (Lab Diet the Richmond Standard®, MPI Nutrition International, St. Louis, MO). Pupae were collected using a vacuum pump (Gerberg et al. 1994) and placed into small bowls in 60-cc screened cages for adult emergence. Adults (1,000–3,000 per cage) were supplied with cotton soaked in a 10% sucrose solution and maintained in a temperature-controlled room at 70–90% relative humidity with a temperature of 29 ± 1°C during the day and 24 ± 1°C at night and a 12-h light 12-h dark photoperiod. Temperature changes were controlled with 3 halogen incandescent bulbs (500 W) oriented towards the larval trays in the 5 × 4 × 2.35-m room.

A flashlight with a 1.2-W incandescent bulb was used to induce mating, after the halogen lamps were turned off and the temperature started cooling. The light beam was applied for 30 min at 3 different angles: 0°, 45°, and 90°. Three replicates for each light angle were carried out using 1,000 adults (500 males and 500 females). Light-induced mating was conducted each day for 10 days in the first 4 mosquito generations, and for 5 days in generations

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F_5 and F_6, but no light was needed after generation F_6. Insemination rates were determined by examining spermathecae of 20–83 females from each generation on day 25 after emergence. As a control for this experiment, *An. pseudopunctipennis* larvae collected from the same site along the Coatán River were exposed to the same insectary conditions, including cooling at crepuscular hours, but without flashlight treatment.

Human and/or rabbit blood was offered every 3rd day, and at 3 days postfeeding, a plastic bowl (20 cm diameter) containing 600 ml of water and lined with a strip of filter paper was placed in the cage as an oviposition substrate. Eggs were separated from bowls (attached to paper and floating on water) and counted daily under a dissecting microscope. Egg hatching was measured by counting 1-instar larvae.

Statistical analysis included a *G*-test of independence on the insemination rates between mosquitoes stimulated at different light beam angles; a chi-square test was applied comparing thermoperiod-plus light-stimulated mosquitoes and field-collected mosquitoes that were subjected to thermoperiod changes but no light stimulation. Finally, Student's *t*-test was conducted to compare the insemination rates between the Tapachula and Abasolo strains after data were $\log(x + 1)$ transformed (Zar 1984).

RESULTS

Applying a beam of light along with changes in room temperature stimulated mating in *An. pseudopunctipennis* from both sites. Sexual encounters started on day 2 after adult emergence. When the stimulus was applied daily, these encounters were observed up to day 25. After 5 generations, spontaneous swarming and pairing without the application of light was observed.

The *G*-test of independence between insemination rates and the light beam at different angles showed no significant positive association (mean insemination rates of 78, 72, and 69%, with 0°, 45°, and 90°, respectively; $G = 4.013$, $P = 0.4048$). These results were obtained with the Tapachula colony in the F_4, F_5, and F_6 generations. The insemination rate in the Tapachula colony was higher than in the Abasolo colony in all generations (Table 1; $t = 9.393$, $P = 0.0001$). In the Tapachula colony, the insemination rate was 70% from F_1 to F_3, and from F_4 thereafter the insemination rate was ≥80%. In the Abasolo colony insemination rates were less than 50% in all generations except for F_4.

Cooling without applying the light beam produced an insemination rate of 29% ($n = 119$ mosquitoes dissected) after 12 days in mosquitoes grown from field-collected larvae and pupae, but no eggs were obtained from these mosquitoes, even after 25 days of observation. These insemination rates were significantly lower compared with those of thermoperiod- plus light-stimulated F_1 Tapachula strain mosquitoes (70%; $\chi^2 = 12.999$, $P = 0.0003$). In contrast, no differences in insemination were seen between mosquitoes grown without light stimuli and the thermoperiod- plus light-stimulated Abasolo strain F_1 mosquitoes (35%; $\chi^2 = 0.492$, $P = 0.4829$).

Table 1. Insemination rates of 25-day-old *Anopheles pseudopunctipennis* in the Tapachula and Abasolo colonies under laboratory conditions.

Light beam stimulation (days)	Generation	% insemination Control[1] (*n*)	Tapachula colony (*n*)	Abasolo colony (*n*)
0	Parental	29 (119)		
10	F_1		70 (20)	35 (50)
10	F_2		70 (20)	39 (56)
10	F_3		70 (20)	38 (50)
10	F_4		82 (83)	53 (57)
5	F_5		88 (50)	26 (27)
5	F_6		80 (63)	40 (20)
0	F_7		80 (58)	41 (20)

[1] Control, mosquitoes stimulated only with temperature change but no flashlight; *n*, number dissected.

Egg hatch was variable (Table 2). In the Tapachula colony, it was 70.5% in the F_1, reached 99.1% in the F_4, but it decreased slightly in the F_5. In the Abasolo colony, egg hatching was 98.7 and 95.6% in the F_1 and F_2, respectively, but in the following generations decreased, ranging between 53.9 and 75.8%. The mean rearing successes of hatched eggs that produced adults in the Tapachula and Abasolo colonies were 85.6% (72.1–97.2%) and 55.6% (23.0–84.6%), respectively. The average adult production in the Tapachula colony was 12,249 from F_1 to F_7 and thereafter increased to approximately 30,000 and this production was maintained in the F_{24}. In Abasolo colony adult production was lower, with an average of 2,874 from F_1 to F_7.

DISCUSSION

Many attempts to colonize *An. pseudopunctipennis* have been unsuccessful. The best results were obtained in Panama, where 40 generations over 2 years were reported (Baerg 1971). However, the exposure of our field-collected *An. pseudopunctipennis* mosquitoes to natural light and to photoperiod conditions similar to those used in the Panama colony did not result in mating, nor did the application of a red light (Baerg 1971). On the other hand, simulation of dusk using a light beam along with a rapid environmental temperature drop proved adequate to stimulate natural mating. Flight activity during mating was similar to that reported previously (Rodríguez-Pérez et al. 1991), but more sexual encounters (approximately 250 per 30 min) and sexual activity (up to 25 days) were observed. The absence of loud noises during stimulation is additionally important.

Table 2. *Anopheles pseudopunctipennis* production in 7 generations by natural mating in the Tapachula and Abasolo colonies.

	Tapachula colony					Abasolo colony				
	Eggs				Rearing success[1]	Eggs				Rearing success
Generation	Laid	Hatched	%	Adults	(%)	Laid	Hatched	%	Adults	(%)
Parental[2]				772					272	
F_1	2,087	1,471	70.5	1,062	72.1	310	306	98.7	259	84.6
F_2	5,553	4,953	89.2	4,299	86.8	1,154	1,103	95.6	867	78.6
F_3	15,767	15,420	97.8	12,747	82.7	9,326	6,447	74.5	4,521	70.1
F_4	14,449	14,318	99.1	13,445	93.9	18,576	11,015	59.3	4,152	37.7
F_5	16,830	14,389	85.5	13,993	97.2	17,305	13,117	75.8	7,968	60.7
F_6	15,200	13,452	88.5	10,300	76.6	12,084	6,513	53.9	1,495	23.0
F_7	33,017	—	—	≈30,000	>90.0	4,415	2,746	62.2	862	31.4

[1] Rearing success = (number of adults produced/number of hatched eggs) × 100.

[2] Originated from 1,100 and 750 larvae of different instars and pupae collected, respectively, in Tapachula, Chiapas (Tapachula colony) and Abasolo, Nuevo León (Abasolo colony) México.

In one experiment in which temperature alone was lowered at the time of darkening, 29% insemination was observed among females obtained from field-collected larvae, but no oviposition was recorded. On the other hand, application of the light beam with temperature change stimulated 88% insemination. Our data, based on the total numbers of eggs collected per cage and the average number of eggs laid per female (data not shown), indicate that the number of inseminated field-collected females that laid eggs was only about 6.3%, and it is possible that the lack of oviposition in the temperature-alone treatment resulted from the low proportion of females treated and inseminated. Nevertheless, although the light beam is necessary to initiate colonization, temperature change seems to be an important mating stimulus over time, even when the light beam was no longer necessary. Filamentous green algae or mud and larvae extracts were not necessary as oviposition substrates or for larval rearing, as previously reported (Baerg 1971, Darsie and López 1980, Rodríguez-Pérez et al. 1991).

The success obtained with the northern strain (Abasolo) was more modest. Although a self-mating colony has been established using this strain, insemination rates and adult production have been consistently lower that those of the Tapachula colony. Climatic conditions in our insectary have been set to resemble those of the foothills from which the Tapachula strain was collected. It is possible that the Abasolo strain, adapted to different environmental conditions (temperatures ranging between 0°C in winter and 45°C in the summer), has had difficulties adapting to our insectary conditions. Also, it is possible that unknown intrinsic mosquito factors are playing a role in this adaptation.

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