STUDIES OF COLONIZATION OF EL SALVADOR STRAINS OF *ANOPHELES PSEUDOPUNCTIPENNIS PSEUDOPUNCTIPENNIS*

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ABSTRACT. Two colonies of *Anopheles pseudopunctipennis pseudopunctipennis* Theobald have been established in El Salvador using induced copulation. The Ilopango strain was initiated using immatures collected from a highland lake. The Huiza strain was started with blooded females captured near a coastal river. A study of stenogamy began with holding male and female adults together in emergence cages. Natural mating has been achieved and oviposition of viable eggs has continued on a substrate of moist earth wetted with larval essence.

Komp (1942) has stated that *Anopheles (Anopheles) pseudopunctipennis pseudopunctipennis* Theobald 1901 is the most widely distributed anopheline species in the Neotropical Region. Its range extends from Kansas (Hill 1939) to the Provinces of Tarapacá in Chile and Córdoba and San Luis in Argentina (Forattini 1962) and has also been reported from: Colombia and Paraguay (Shannon et al. 1927), Peru (Shannon 1930), Lesser Antilles (Earle 1933), Costa Rica (Kumm et al. 1940), Guatemala (De Leon 1940), El Salvador (Kumm & Zuniga 1942), Belize, Honduras and Trinidad and Tobago (Komp 1942), Ecuador (Levi Castillo 1944), Bolivia (Hackett 1945), Nicaragua (Woke 1947), Brazil (Levi Castillo 1949), Panama (Baxter & Zetek 1944), Venezuela (Cova Garcia 1951) and Mexico (Vargas & Martínez Palacios 1956).

The most successful colonization of this species to date was performed by Baerg (1971). At least 40 generations of a Panama strain were produced without using induced copulation. Other efforts to isolate a cage-mating strain have been largely unsuccessful. Downs and Arizmendi (1951) noted a personal communication from Bates of six months' maintenance of a poorly producing colony in Colombia. More recently, Martínez-Palacios and Davidson (1967) have maintained five strains from Mexico by forced mating. Insectary techniques given by Davidson (WHO 1961) were used through an unstated number of generations in this colony rearing. Baerg (1971) reported a personal communication from Martínez-Palacios in 1968 on the rearing of a Mexican strain for four generations.

The need for a colony arose from a desire to investigate its role in malaria transmission in Central America; this paper reports results of efforts to colonize two strains from El Salvador, C.A. See Warren et al. (1979) for an account of malaria investigations with this species.

NATURAL HABITATS IN EL SALVADOR

The immature stages of *An. pseudopunctipennis* are found in two principal habitats. In the highlands, a dense mat of aquatic plants of varying widths grows in the shallows near the shoreline of fresh-water lakes. This biotope, which provides an ideal larval habitat, has been described by Breeland et al. (1974). The lowland habitat occurs in rivers of the coastal zone where, during the dry season December to May, water flow decreases and many isolated pools are formed. Mats of algae develop into suitable habitats in

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the more persistent pools. Hobbs (1973) and Breeland (1974) indicated that peak numbers of larvae occur in such sites in February and breeding continues until June.

As differences were anticipated in the behavior of highland and lowland strains, separate colonies were established with specimens from representative habitats. Between January 18 and March 31, 1977, 10 lots of larvae and pupae, totalling about 7500 immatures, were collected from Lake Ilopango (480 meters above mean sea level). This highland strain was designated the "Ilopango" strain. To colonize *An. pseudopunctipennis* occurring in the lowlands, information given by Breeland (1972) was useful. He described the diurnal resting sites in the coastal area, and from one of these, underneath bridges, 423 blooded females were captured between March 1 and April 21, 1977, and transported to the laboratory for oviposition. The wild-caught inseminated females were employed to start this colony, named the "Huiza" strain, because they obviated protracted rearing of immatures.

REARING PROCEDURE DURING INITIAL PHASE. The field-collected larvae and pupae to start the Ilopango colony were separated by instars in the insectary which was maintained at 27°C and 80% relative humidity. Pupae were placed in 37 × 32 × 32 cm cages for emergence, and the larval instars were reared in 33 × 23 × 5.7 cm enamelware trays on a standard diet, developed by Dame et al. (1978), of pulverized food containing 2 parts powdered liver, 1 part powdered yeast and 1 part hog supplement prepared as a slurry. This same larval food has been used for all colony rearing. Each rearing pan was provided with a section of the aquatic plant, *Hydrilla* spp., about 25 cm long. Adults obtained from field-collected larvae and pupae constituted the P_1 generation of the Ilopango colony.

The P_1 generation of the Huiza colony was composed of the sanguineous females captured in the field and immediately set for oviposition in the insectary.

From this point, both colonies were manipulated in the same manner, following the procedure described by Darsie and Cagampang-Ramos (1970). The induced copulation technique of McDaniel and Horsfall (1957) and Baker et al. (1962) was used as the basic procedure to manage adult reproduction. Except for the P_1 generation of the Huiza colony, adults, after emergence in cages, were separated by sexes. Males were held in lots of varying number by age and generation with wicks of cotton soaked in 10 percent sucrose solution as a food source. Males used for induced copulation were at least four days of age. Females were transferred to individual vials and given sugar solution the first day, held fasting the second day and offered blood meals on succeeding days. No female was used for induced copulation unless it had taken 2 blood meals. At first, human blood was offered. This source was changed, however, when females gradually adapted to guinea pig blood, offered from the shaved stomach of unanesthesized animals. When females became gravid, about 2 ml of larval essence (water in which larvae had been reared) was added to the bottom of the vial for an oviposition medium. Females readily laid eggs in these vials. Initially, glass shell vials, 85 × 21 mm, covered by nylon netting and containing a 7– × 70-mm strip of damp filter paper on the vertical wall were used. They were later replaced by 64- × 25-mm plastic, snap-cap vials, each with a hole 22 mm in diameter cut in the cap and the hole covered by 16-mesh aluminum screening.

After oviposition, each lot of eggs was washed onto a 9-cm circle of filter paper and counted under a stereoscopic microscope. The lot was then placed in a hatching dish (11.4 cm diameter × 5.4 cm deep) lined on the inside perimeter with filter paper and half filled with seasoned water (tap water allowed to sit for 48 hr to dissipate chlorine). After hatching, larvae were counted and transferred to enamelware pans containing seasoned water. Lots of similar age were combined

and distributed so as not to exceed 250 larvae per pan. Pupae were hand picked daily and placed in emergence cages.

RESULTS THROUGH F_6 GENERATION

The above procedure was followed unchanged through the first 6 generations. Details were recorded for a 100-female sample in each generation (Table 1). In the first 6 generations a range of 153 to 589 females were inseminated by induced copulation with approximately 29% of these becoming gravid in each colony. Egg fertility averaged about 57% in the Ilopango colony and 56% in the Huiza colony; moreover, 91% of the former successfully pupated, while only 59% of the latter reached the pupal stage.

The length of time for development from egg to adult averaged 14 days. Six additional days were required for blood feeding, induced copulation and oviposition, so that the total time from egg to egg was a minimum of 20 days. No observations were made on oviposition without insemination.

One factor affected the oviposition by females. For the first 4 generations of the Ilopango and the F_1 and F_2, of the Huiza colony, the lights in the insectary were inadvertently allowed to burn all night. When they were placed on a 12-hour: 12-hour day-night cycle, there was a marked increase in egg production (Table 1).

REARING PROCEDURE AFTER F_6 GENERATION. The method of handling adults was changed after the F_6 generation. Pupae emerged into cages, as before, but the males and females were held together for a minimum of 6 days. Feeding procedures remained the same except that an animal was placed in the cage daily from the 4th day. After the 6th day females were isolated from each cage and inseminated by induced copulation with males from the same generation. This method of reproduction was continued with an average of nearly 500 females being inseminated each generation.

Two or three generations have been maintained concurrently for each colony, because the collection of field specimens for starting each colony extended over such a long time, i.e., 73 days for Ilopango and 52 days for Huiza. It was possible to keep several generations developing simultaneously so the practice was adopted as a routine safeguard against losing the colonies.

The success of induced copulation continues to be limited (Table 2). From the F_7 to F_9 generation there was a problem of pupal mortality, traced to contamination of pupal emergence containers.

The oviposition pattern of 80 females from the Huiza colony in the F_{11} generation was followed. In all 46, or 57.5%, successfully oviposited. Of these, 26 laid eggs after 2 blood meals; 8, after 3; 7, after 4; and 5, after 5. An average of 2.8 blood meals was required for the 1st oviposition. This proved to be very similar to the record of Darsie and Cagampang-Ramos (1970) in the case of *An. litoralis* King, a Philippine anopheline.

DEVELOPMENT OF STENOGAMY. A study of stenogamy began with holding male and female adults together in emergence cages. Various types of oviposition depositories were tried. Use of petri dishes containing a layer of cotton covered with filter paper wetted with seasoned water or larval essence and plastic cups, 7.6 cm in diameter and 4.4 cm deep, half full or either of the same two liquids proved unsuccessful. The use of mud, as proposed by Breeland et al. (1970) was then tried. Females of both colonies readily deposited eggs on unsterilized, but previously dried, mud which was wetted with larval essence and maintained in a solid state. This method was adopted in the F_8 generation of both colonies. With the use of mud for oviposition, natural insemination was achieved and fertile eggs were produced. To date a total of 3772 viable eggs from the Ilopango colony have hatched, of which 73% have pupated (Table 3). Only the first generation has been produced by natural pairing to date. The stenogamous portions have now been

Table 1. Results of induced copulation and insectary rearing of the first six generations of *An. pseudopunctipennis* in El Salvador, 1977.

Generation	Total ♀♀ Artificially Inseminated	Total ♀♀ Number Gravid	Record for 100—Female Sample: Total Ova Oviposited	Total Ova Hatched	Total Ova % Fertile	Total Pupated	% 1st Instar Lar. Pupating
			Ilopango Colony				
P_1/F_1	262	21	815	596	73.1	435	72.9
F_1/F_2	231	27	1250	841	67.3	821	97.6
F_2/F_3	344	31	793	649	81.8	602	92.7
F_3/F_4	589	24	569	448	78.7	379	84.5
F_4/F_5	430	31	2413*	1509	62.5	1462	96.8
F_5/F_6	572	40	4584	1918	41.8	1739	90.6
Total	2428	174	10,424	5961	57.1	5438	91.2
			Huiza Colony				
P_1/F_1	423	48	2400	2100	87.5	1540	73.3
F_1/F_2	276	13	563	411	73.0	323	78.5
F_2/F_3	153	21	1940*	1193	61.4	984	82.4
F_3/F_4	188	39	6036	1909	31.6	1009	52.8
F_4/F_5	274	35	3401	1044	30.6	807	77.2
F_5/F_6	356	49	4960	3224	65.0	1209	37.5
Total	1670	205	19,300	9859	55.9	5872	59.5

* Started 12 hour: 12 hour day-night cycle.

Table 2. Results of induced copulation and insectary rearing of generations subsequent to F_6 for two colonies of *An. pseudopunctipennis*, El Salvador, 1977–78.

Generation	♀♀ Inseminated	Total Eggs Laid	Total Eggs Hatched	Total Pupae	% First Instar Larvae Pupating
		Ilopango Colony			
F_6/F_7	690	27,548	6,196	4,836	78.0
F_7/F_8	570	29,572	10,672	3,531	33.0
F_8/F_9	534	23,475	8,570	4,745	55.3
F_9/F_{10}	640	40,742	10,613	3,744	35.2
F_{10}/F_{11}	648	37,180	9,565	5,417	56.6
F_{11}/F_{12}	533	34,475	5,690	4,779	83.9
F_{12}/F_{13}	448	30,216	6,734	6,639	98.6
F_{13}/F_{14}	499	35,991	8,130	6,980	85.9
F_{14}/F_{15}	583	50,134	7,035	5,769	82.0
F_{15}/F_{16}*	480	35,548	5,805	4,015	—
F_{16}/F_{17}*	434	30,526	6,725	2,727	—
		Huiza Colony			
F_6/F_7	412	15,037	4,037	2,372	58.7
F_7/F_8	445	19,802	5,357	2,712	50.6
F_8/F_9	318	17,721	3,100	2,144	69.1
F_9/F_{10}	298	9,736	1,762	1,735	98.4
F_{10}/F_{11}	288	18,429	4,362	4,269	97.9
F_{11}/F_{12}	432	37,005	6,250	5,627	90.0
F_{12}/F_{13}	273	19,846	4,198	3,566	85.0
F_{13}/F_{14}*	455	25,849	7,235	3,238	—

* Generations incomplete.

isolated and establishment of a subcolony is being attempted.

The Huiza colony started stenogamic reproduction in the F_8 generation and continued in the F_9 (Table 3); the two subsequent generations have failed to reproduce in this manner. Stenogamous individuals were not continued as separate lines. Recently, however, the F_{12} and F_{13} generations have again shown stenogamy and larvae have been produced. This is apparently the 1st report of stenogamy in *An. pseudopunctipennis*.

Table 3. Results of stenogamic reproduction in cages of *An. pseudopunctipennis* colonies, El Salvador, October 1977–April 1978.

Generation	Number of Eggs			No.	%
	Laid	Hatched	% Hatched	Pupated	Pupated
Ilopango Colony					
F_8/F_9	30	18	60.0	17	94.4
F_9/F_{10}	112	76	67.8	53	69.7
F_{10}/F_{11}	1,980	1,219	61.5	1,096	89.9
F_{11}/F_{12}	644	443	68.7	320	72.2
F_{12}/F_{13}	4,823	547	11.3	449	82.0
F_{13}/F_{14}	4,177	320	7.6	314	98.1
F_{14}/F_{15}	4,962	954	19.2	367	38.4
F_{15}/F_{16}*	6,687	195	2.9	147	75.3
Totals	23,415	3,772	16.1	2,763	73.2
Huiza Colony					
F_8/F_9	1,229	959	78.0	591	61.6
F_9/F_{10}	80	67	83.7	55	82.0
F_{10}/F_{11}	0	0	0	0	0
F_{11}/F_{12}	2,070	0	0	0	0
F_{12}/F_{13}*	15,699	560	3.5	482	86.0
Totals	19,078	1,586	8.3	1,128	71.1

* Generations incomplete.

Baerg (1971) used cages 76 × 38 × 38 cm in size and observed mating swarms inside. No such activity has been noted in our colonies.

ACKNOWLEDGMENTS

The authors wish to acknowledge the faithful attention to the colony of Messrs. Carlos Rivera-López, Luis Salas-Aguilera & Alejandro Zetino; the helpful suggestions of Dr. Donald L. Bailey; and the review of the manuscript by Drs. G. D. Brooks and J. J. Sullivan.

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The Tenth International Congress on Tropical Medicine and Malaria is scheduled for November 9–15 in Manila, Philippines.