Bionomics of Adult *Anopheles pseudopunctipennis* (Diptera: Culicidae) in the Tapachula Foothills Area of Southern Mexico

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ABSTRACT Field studies on the bionomics of adult *Anopheles pseudopunctipennis* Theobald were conducted to assess its relative importance as a primary vector of *vivax* malaria in southern Mexico. In four malaria endemic villages in a foothill region near Tapachula, Mexico, population densities of *A. pseudopunctipennis* increased during the dry seasons of 1990 and 1991. The pattern of nocturnal host-seeking activity indoors was unimodal with a late night peak at 0100 hours enhancing its vectorial significance, because it occurred when most residents were asleep and fully exposed to the anophelines. Comparisons of trapping methods showed that a horse-baited trap was more effective than human landing catches or UV light traps. Pit shelters, on the other hand, were more effective than indoor and natural shelter resting collections. Results of enzyme-linked immunosorbent assays performed on wild-caught *A. pseudopunctipennis* specimens documented the presence of natural infections with the VK210 and new VK247 circumsporozoite polymorphs of *P. vivax*. These findings verify the importance of *A. pseudopunctipennis* as a major vector of *vivax* malaria at higher elevations and extend the geographical range of the VK247 *P. vivax* polymorph in Mexico.

KEY WORDS *Plasmodium vivax*, vector biology, malaria

VIVAX MALARIA IS CURRENTLY the most widely distributed vector-borne disease in Mexico (Rodriguez & Loyola 1989). *Anopheles albi-*

probably comprising a sibling species complex (Baker et al. 1965).

A. pseudopunctipennis is highly anthropo-

comparison of sampling methods, and malarial infection rates in wild-caught mosquitoes.

Materials and Methods

Description of the Study Areas. Our study was conducted in a rural area ≈20 km from Tapachula City at 17° 25' N and 92° 20 W on the Pacific slopes of the Sierra Madre mountain range (between the hypsometric curves of 400 and 600 m above the sea level). This area is a modified evergreen biotope with a hot-subhumid climate, mean annual relative humidity of 80%, and an average temperature of 25°C (Miranda 1952). There is a well-defined dry season, with the hottest months between November and May. The average rainfall is 3,800 mm, which is distributed between May and November. The landscape is characterized by a rugged terrain with slopes of varying degrees. Agriculture is devoted mainly to coffee plantations. The harvest between August and November is labor intensive and dependent on migrant workers. The study area consisted of four villages, each separated by about 5 km: El Plan (pop. 446 in 74 houses), El Retiro (pop. 341 in 57 houses), La Ceiba (pop. 236 in 67 houses), and La Concordia (pop. 240 in 52 houses). The houses in El Plan and El Retiro were scattered and often separated by more than 100 m. In the other two villages, houses were arranged along the main road and in close proximity to each other, often separated by less than 20 m.

The study sites were along the margins of the Coatan River where *A. pseudopunctipennis* larval habitats flourish during the dry season in pools with filamentous algae (Fernandez–Salas 1992). A few large permanent ground pools also serve as habitats. *A. pseudopunctipennis* populations quickly decline at the beginning of the wet season, after larval habitats in the river are inundated.

A malaria survey conducted in 1988 found that *A. pseudopunctipennis* was the most frequent indoor and outdoor biting an[illegible]

covering two dry seasons. Mosquito collections were conducted during one week per month in each village. The weekly effort consisted of four nights of 14-h collections (1700 to 0700 hours) during January, February, and March, which corresponded to the longest periods of darkness of the year and of 12-h collections (1800–0600 hours) during the remaining months. Mosquitoes were collected by two teams of four technicians per team each working 6–7 h per night. Collectors were protected from malaria by chemoprophylaxis.

Three methods were used to capture flying females: landing catches, horse-baited traps, and updraft ultraviolet light (UVL) traps (Sexton et al. 1986). For landing collections, technicians used oral aspirators to capture female mosquitoes as they attempted to feed after landing on their exposed feet and ankles. Landing collections were made by one collector per site. Collections were made inside a house, within 5 to 10 m of the house, and at a social gathering site within the village (20–50 m away from houses; e.g., along the road, soccer field). The latter collection was defined as extradomiciliary. Landing collections were conducted during 50 min each hour. Teams of collectors were changed at midnight, and starting times of teams were rotated each night. Additionally, individual team members changed collecting sites each night to avoid sampling bias.

Horse-baited trap collections were performed in the center of the village by tying a horse inside a nylon-screened trap (4 by 4 by 4 m, placed 30 cm above the ground). A technician opened the trap each hour and used an oral aspirator to collect all engorged and unengorged anopheline females resting on the screening.

Resting females were collected in house and outdoor natural resting sites and in artificial pit shelters. During 1990 and 1991 (January–May), a minimum of 10 houses per village was sampled for resting mosquitoes by four technicians for 2 h during 1 wk per month. Ten pit-shelters per vil-

***P. vivax* Circumsporozoite-Protein Assay.** Head-thorax portions of wild-caught females were removed, placed individually in a 1.5-ml tube, and stored in a glass container with silica-gel until processing. The head-thoraces were assayed individually by an ELISA for *P. vivax* VK210 and VK247 circumsporozoite (CS) proteins (Rosenberg et al. 1989), using monoclonal antibodies NSV-3 (Wirtz et al. 1985) and 182.1G12 (Wirtz et al. 1992), respectively. The lower detection limits of these assays were ≈25 and 50 sporozoites, respectively (Wirtz et al. 1992). Unfed, laboratory-reared *An. albimanus* served as negative controls. The cut-off value for a positive test was two times the mean absorbance value for seven negative controls. Confirmational tests were performed on positive wells.

Data Analyses. Analysis of variance (ANOVA) tests compared relative abundance of mosquitoes between villages, sample sites, and years (SYSTAT 1989). The Kolmogorov–Smirnov test (Siegel 1956) was used to compare patterns of host-seeking activities during the night. Fisher's protected least significant difference tests (LSD) were performed to compare parity rates during different quarters of the night (Zar 1984).

Monthly survival estimates were calculated for indoor and outdoor biting populations collected during the malaria transmission periods (January, February, and March) in both years. The probability of daily survival, based on the same period, was computed using Davidson's (1954) formula using a 3-d gonotrophic cycle (Fernandez–Salas 1992). An estimation also was made of the probability of females surviving for 9 d, the duration of the sporogonic cycle at 25° (Macdonald 1973). Only data from El Retiro and El Plan were used in these calculations.

Results

Population Dynamics. In addition to *A. pseudopunctipennis*, *A. albimanus*, *A. apicimacula* Dyar and Knab and *A. eiseni* Coquillett were captured in the four villages but accounted for <1% of the total during the 2-yr period.

The seasonal trend of *A. pseudopunctipennis* relative abundance was analyzed by pooling landing collections among villages (Fig. 1). Highest means of pooled mosquito collections were recorded from January (19.7 females per human per night) to March (11.2 females per human per night) 1990, and again January (11.9 females per human per night) to March (10.4 females per human per night) 1991. Heavy rains in April eliminated the larval habitats along the Coatan River and mosquitoes largely disappeared until the beginning of the next dry season in November.

Seasonal means of human landing collections inside houses for both years were greater in El Retiro (17.6 and 6.8 females per human per night) and El Plan (12.3 and 6.9 females per human per night) than in La Concordia (5.5 and 2.0 females per human per night) and La Ceiba (2.0 and 2.0 females per human per night); however, transformed data [ln (X + 1)] of females per human per night monthly means did not indicate significant differences among villages and years ($F = 1.424$; df = 7, 21; $P = 0.247$). In 1990, the highest monthly means for indoor biting catches were recorded during January (27 females per human per night) in El Retiro and February for El Plan (23 females per human per night), respectively. In 1991, biting peaks were observed in December for El Retiro (18 females per human per night) and January in El Plan (20 females per human per night) before villages were sprayed with DDT at the end of January. Monthly indoor biting means for La Concordia were highest during February (9 females per human per night) in 1990 and January (3 females per human per night) in 1991. Likewise, in La Ceiba, the highest monthly abundances were recorded during January (4 females per human per night) in 1990 and February (3 females per human per night) in 1991.

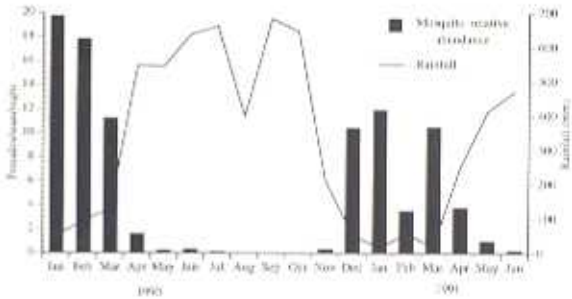

Fig. 1. Seasonal pattern of rainfall and *A. pseudopunctipennis* abundance (females/man/night) in the foothills near Tapachula, Mexico, from January 1990 to June 1991.

In concurrent collections during 1990, the numbers of *A. pseudopunctipennis* captured per human per night varied between peridomiciliary and extradomiciliary collections and inside houses in El Retiro (34.5 and 29.5 females per human per night), La Ceiba (2.0 and 2.7) and La Concordia (14.7 and 13.7 females per human per night). In 1991, these villages showed similar relative abundances for these types of collections; i.e., El Retiro (9.0 and 28.4 females per human per night), La Ceiba (2.6 and 0.8 females per human per night) and La Concordia (1.3 and 1.6 females per human per night). In 1990 for El Plan, the numbers collected indoors (12.3 females per human per night) were not statistically different than both outdoor collections (outdoor 7.0 and extradomiciliary 11.0 females per human per night, $F = 0.545$; df = 2, 6; $P = 0.6$). In 1991 indoor biting catches were lower (6.9 females per human per night) than outdoor (13.7 females per human per night) and extradomiciliary col-

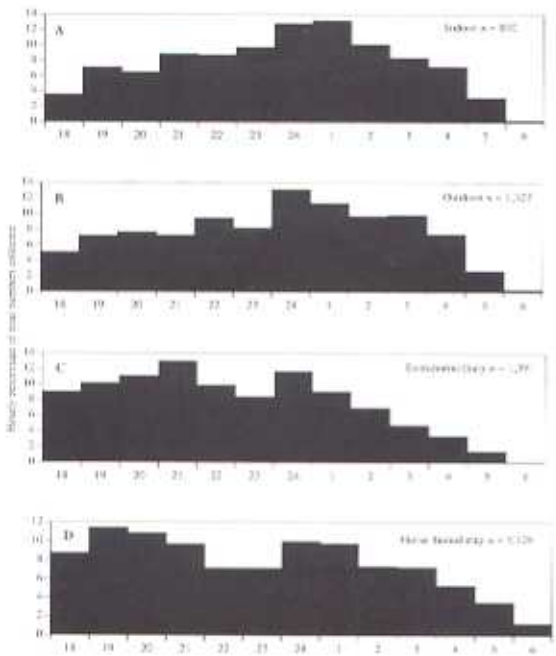

Fig. 2. Host-seeking patterns of *A. pseudopunctipennis* females at four villages in the foothills near Tapachula, Mexico (n = collected females).

lections, but did not differ significantly ($F = 0.51$; df = 2, 12; $P = 0.613$). Maximum landing counts were recorded before houses were sprayed with insecticide in December 1990 in El Retiro (18 females per human per night) and La Concordia (3 females per human per night) and in January 1991 for El Plan (20 females per human per night) and La Ceiba (2 females per human per night).

Biting Activity. The generalized pattern of nightly host-seeking activity was obtained by plotting arcsine-transformed percentages of total *A. pseudopunctipennis* collected during 126 collection nights in the four villages. The indoor activity cycle was unimodal (Fig. 2A) with a maximum at 0100 hours. Numbers collected continually increased during between 1900 h and 0100 h. A similar pattern was observed outdoors, with the largest peak one hour earlier at 2400 hours (Fig. 2B). In extradomiciliary landing and animal trap collections, host-seeking was bimodal with peaks between 2100 and 0100 hours (Fig. 2C) and at 1900 hours (Fig. 2D). Comparisons among these four patterns using the Kolmogorov–Smirnov test (Siegel 1956) indicated that the pattern for indoor activity was significantly different from patterns exhibited in the outdoor ($D = 0.571$, $P = 0.011$) and extradomiciliary ($D = 0.643$, $P = 0.002$) landing collections and horse-baited ($D = 0.857$, $P < 0.001$) trap collections. Significant differences were not found between patterns of peridomiciliary versus extradomiciliary ($D = 0.429$, $P = 0.111$) host-seeking activity, but versus the horse-baited trap ($D = 0.857$, $P < 0.001$). The Kolmogorov–Smirnov test also showed significant differences for extradomiciliary versus horse trap ($D = 0.786$, $P < 0.001$) female activity.

Population Age-Structure. During the two study years, 35.2% of 140 *A. pseudopunctipennis* females captured inside of houses in the first quarter of the night (1800–2100 hours) were parous, significantly less than 59.6% of 153 specimens (Fisher's LSD test, $P < 0.05$) captured in the fourth quarter (0300–0600 hours) (Table 1). The same increase in the parity rate of host-

Table 1. Numbers of *Anopheles pseudopunctipennis* females collected, dissected and percentage parous from indoor and outdoor landing collections in four villages in the foothills near Tapachula, Mexico (n = 117 nights of collecting)

Quarter (Q)	Collection hour	Indoor population			Outdoor population		
		No. caught	No. dissected	% Parous	No. caught	No. dissected	% Parous
Q1	18:00-19:00	29	27	33.3	68	24	50.0
	19:00-20:00	58	44	36.4	96	66	44.0
	20:00-21:00	53	39	36.0	102	68	56.0
	Total	140	110	35.2	266	158	50.0
Q2	21:00-22:00	72	43	51.0	97	71	38.0
	22:00-23:00	71	36	44.4	126	71	41.0
	23:00-24:00	79	47	45.0	109	63	38.0
	Total	222	126	46.8	332	205	39.0
Q3	24:00-01:00	104	50	40.0	174	75	44.0
	01:00-02:00	107	59	49.0	151	56	46.0
	02:00-03:00	82	41	39.0	129	56	27.0
	Total	293	150	42.6	454	187	39.0
Q4	03:00-04:00	68	28	50.0	131	48	42.0
	04:00-05:00	59	24	46.0	99	31	29.0
	05:00-06:00	26	12	83.0	36	15	47.0
	Total	153	64	59.6	266	94	39.3
	Totals	810	453	55.9	1,322	645	48.8

Table 2. Total numbers, seasonal means ± SD for *An. pseudopunctipennis* collected by different methods from the four study villages

Year		Human landing[a]	Light traps[b]	Horse trap[c]	House resting[d]	Natural shelters[d]	Pit shelters[d]
1990	*n*	2,068	83	2,969	129	237	—
		17.3 ± 4.0	2.3 ± 1.5	69.3 ± 29.3	42.3 ± 36.7	76.3 ± 63.7	—
1991	*n*	1,731	30	2,507	91	104	1,298
		8.2 ± 5.2	1 ± 0.1	30 ± 18.5	29.7 ± 42.1	33.7 ± 50.6	324.5 ± 335.2
Totals	*n*	3,799	113	5,476	220	341	1,298

[a] Combined indoor, outdoor, and extradomiciliary collections (females/man/night/season).
[b] Combined indoor and outdoor UV up-draft traps (females/trap/night/season).
[c] Females per night per season.
[d] Females per village per season.

seeking females was not observed for females collected outside houses.

Survivorship was estimated for females collected at El Retiro and El Plan from January to March. In 1990, daily survivorship ranged from 0.73 to 0.78 for the two villages including indoor and outdoor collections, greater than determined in 1991 (0.62 to 0.75). Overall, the theoretical percentage of indoor biting females surviving 9 d to complete sporogony of the malaria parasite successfully was low in El Retiro and El Plan for both years; 1990, 5.9% (n = 99) and 7.5% (n = 94), and 1991, 6.9% (n = 55) and 3.1% (n = 71), respectively. Percentages of outdoor females surviving were 7.5% (n = 144) and 7.5% (n = 60) for these villages in 1990 and 11.1% (n = 79) and 1.4% (n = 169), in 1991. Based on these samples, a significant reduction in survival by the insecticide spraying was only noticed for the outdoor population in El Plan; i.e., 0.75 (1990) to 0.62 (1991) (χ^2 = 3.92, df = 1, P = 0.048).

Trapping Method Comparison. The relative attractiveness of humans (in different environmental settings) versus horses in stable traps and UV light traps to *A. pseudopunctipennis* females was compared during 286 nights in the four villages. The horse-baited trap produced the highest catches (5,476 females) (Table 2) with seasonal means of 69.3 (±29.3) and 30.0 (±18.5) females per night in 1990 and 1991, respectively, followed by human landing captures, 3,799 [17.3 (±4.0) and 8.2 (±5.2) females per night], and UV up-draft traps, 113 [2.3 (±1.5) and 1.0 (±0.1) females per light trap]. Transformed data [ln (X + 1)] showed a significant effectiveness of horse trap versus human catches and up-draft UV light traps for this vector species (F = 34.58; df = 2, 11; P = 0.0001). Pit shelters were the best of the resting collection method tested. Pit shelters produced 1,298 females (324.5 [±335.2] females per village) only in the 1991 dry season, whereas natural shelters and houses produced 341 (76.3 [±63.7] and 33.7 [±50.6] females per village per season) and 220 (42.3 [±36.7] and 29.7 [±42.1] females per village per season) *A. pseudopunctipennis* females for the 2 yr (Table 2). Seasonal means of females collected by pit shelters were significantly greater than the remaining collection strategies in 1991 (F = 4.456, df = 5, 18, P < 0.001). In paired collections of *A. pseudopunctipennis* landing on the human host, indoors collections accounted for 21.7% (824 females) of the total number captured. Collections conducted outside, near houses, accounted for another 35.5% (1,349), and collections at the extradomiciliary site accounted for the remaining 42.8% (1,626).

CS-Protein Rates. Of 9,386 head-thoraces of *A. pseudopunctipennis* assayed for the presence of *P. vivax* four specimens (positivity rate of 0.04%) were positive for VK210 and six (0.06%) were positive for the VK247 CS protein. The overall infection rate for both CS proteins was 0.10%. Combined *P. vivax* CS-proteins accounted for positive rates in 1990 of 0.12% for El Plan and 0.04% at El Retiro (Table 3), whereas combined rates in 1991 where 0.11% for El Plan, and 0.25% in El Retiro. No specimens collected at La Ceiba or La Concordia were positive for *P. vivax* CS proteins.

Of the VK210 positive specimens, one was captured in El Retiro during 1990 (0.04%), two (0.08%) in 1991, and one from El Plan (0.05%) in 1991. Three of these positive females were from the horse-baited trap and one was from a pit-shelter collection. Of the six VK247 positive specimens, four (0.17%) were from El Retiro (in 1991) and two were from El Plan: one (0.12%) in 1990 and 1 (0.05%) in 1991. Two of the positive specimens were from horse-baited traps and four were from pit shelter collections.

Discussion

Anopheles pseudopunctipennis populations in the Tapachula area and throughout the malaria endemic countries of the Andean Region and Central America (Shannon 1930, Hackett 1945) are more abundant during dry season conditions encountered in mountain and foothill habitats above 200 m. Peak abundance is associated with pools formed in drying river beds (Hackett 1945, Savage et al. 1990).

Table 3. Prevalence of *Plasmodium vivax* VK210 and VK247 CS proteins detected by ELISA in *An. pseudopunctipennis* mosquitoes collected in the foothills of Tapachula, southern Mexico

Village	1990				1991			
	n[a]	VK210	VK247	% Positive[b]	n[a]	VK210	VK247	% Positive[b]
El Retiro	2,482	1	0	0.04	2,392	2	4	0.25
El Plan	859	0	1	0.12	1,837	1	1	0.11
La Ceiba	122	0	0	0.0	648	0	0	0.0
El Retiro	511	0	0	0.0	535	0	0	0.0
Totals	3,974	1	1	0.50	5,412	3	5	0.15

[a] Numbers tested pooling human landing, horse trap, and pit shelters collections.
[b] Includes both VK210 and VK247.

Human-vector contact was higher in the villages at higher elevations and closer to the Coatan River; *viz.*, El Plan and El Retiro. These villages also have presented higher malaria rates, 39% and 12.3% prevalence in 1988, than La Ceiba (1%) and La Concordia (6.7%) (M.H.R., unpublished data). Highest levels of *A. pseudopunctipennis* biting indoors in the former two villages were 23–27 females per human per night about 1 mo after the beginning of the dry season during both years of collections. Particularly, landing collections indoors, outside near the house, and outside away from houses in El Plan were similar, indicating an even distribution of human-biting. In a separate study, Fernandez-Salas (1992), found that 56.7–88.1% of *A. pseudopunctipennis* females resting indoors in El Plan had fed on human blood.

The effect of indoor spraying of DDT on *A. pseudopunctipennis* behavior is difficult to interpret. *A. pseudopunctipennis* in the study area was resistant to DDT (Centro de Investigación de Paludismo, unpublished data). Furthermore, a repellent effect of DDT has been documented for this species, which reduced the number of mosquitoes entering sprayed houses (Loyola et al. 1990). In a concurrent study, this repellent effect decreased the human blood index during the post-spray period in 1991, using outdoor resting females (Fernandez-Salas 1992). Regardless of the cause, decreases in the overall population coincided with indoor house spraying.

The biting cycle of *A. pseudopunctipennis* had a unimodal pattern similar to that seen in Ecuador (Elliot 1972) but different from the bimodal pattern in Peru (Elliot 1972). The peak occurred at late night (0100 hours) when most residents were sleeping and less easily disturbed by mosquito bites. Populations of *A. albimanus* [illegible] other malaria vector in Chiapas

tained malaria transmission in 1988 at much lower biting rates; i.e., <20 females per human per night or 1.7 females per human per hour (M.H.R., unpublished data). Other efficient vectors, such as *A. gambiae* Giles and *A. funestus* Giles, also feed late at night while the human hosts are sleeping (Gillies 1988). This information can be useful when considering the feasibility of using insecticide impregnated bednets for malaria control.

Significantly different unimodal patterns of indoor and outdoor activity were exhibited by the host-seeking populations of *A. pseudopunctipennis*. Differences in timing of peak activity, 2400 hours outdoors and 0100 hours indoors, might be explained as a lag of about 1 h from the time mosquitoes first arrive at outdoor sites until they enter the houses. On the other hand, the larger peak of females early-evening extradomiciliary (2100 hours) and horse-baited trap collections (1900 hours) might consist of dispersive females, newly emerged virgin females (Gillies 1988), or ovipositing females which probably will refeed later in the same night to produce the second peak at 2400 hours.

Of the three methods employed to collect biting *A. pseudopunctipennis*, the inexpensive horse-baited trap was strikingly more effective than either the UV light trap or the landing collections on humans. This reflects species preference for feeding on large domestic mammals. Similar preferences have been reported for this species in the Pacific northwestern coast of Mexico (Loyola et al. 1990) and in Peru (Sasse & Hackett 1950). We found UV light traps to be ineffective for collecting *A. pseudopunctipennis* populations. Light trap efficiency could be related to the trap light source, trap design, physi-

results provide additional evidence that *A. pseudopunctipennis* is a vector of *P. vivax* in the foothill areas of southern Mexico. After Warren et al. (1980) could not infect laboratory-reared *A. pseudopunctipennis* with *P. vivax* or *P. falciparum*, salivary gland sporozoite infection rates of 2.6% (3 out of 117) have been reported in Perú (Hayes et al. 1987) and VK210 CS protein rates of 0–3.16% (detected using ELISA) has been reported in northwest Mexico (Loyola et al. 1991). Second, our results provide the first evidence that the VK247 *P. vivax* allele is present in a Mexican vector and verifies its presence in Mexico. This finding supports the results of Kain et al. (1992), who found 3 out of 6 patients from the foothill region to be positive for the VK247 polymorph. The VK247 was first isolated in Thailand (Rosenberg et al. 1989) and antibody to the CS protein VK247 allele also has been reported in Brazil (Cochrane et al. 1990) and in *A. oswaldoi* (Peryassu) from Peru (Need et al. 1993).

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