Journal of the American Mosquito Control Association, 9(4):375–384, 1993

HOST SELECTION PATTERNS OF *ANOPHELES PSEUDOPUNCTIPENNIS* UNDER INSECTICIDE SPRAYING SITUATIONS IN SOUTHERN MEXICO

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ABSTRACT. Studies of host selection patterns of *Anopheles pseudopunctipennis* were conducted in villages in foothills near Tapachula, Mexico. Based on 2 years of collections, 53.8 and 86.1% of all engorged females resting inside houses were found to contain human blood. Estimates of weighted and unweighted human blood indices, including data from outdoor resting collections, varied from 29.5 to 54.7%. Humans and dogs were the more common blood sources for all *An. pseudopunctipennis* mosquitoes, accounting for 96% of blood meals tested. Results of analyses of host preference through estimates of forage ratios (FRs) indicated that the large numbers of blood meals from humans and dogs were more reflective of host availability than host preference. An FR of less than 1 indicated that, in terms of host availability, proportionately fewer *An. pseudopunctipennis* females fed on humans than other large animal hosts. In contrast, FRs of 15–20 and 5–7 revealed strong selective biases for horses and pigs as sources of blood meals, respectively. The proportion of outdoor-resting, blood-engorged females containing human blood declined markedly after houses were sprayed with DDT. This response to house spraying is attributed to an excito-repellency effect of DDT.

INTRODUCTION

Information on the feeding patterns of arthropod vectors is important to medical entomologists and epidemiologists for understanding host–vector relationships and the dynamics of disease transmission. Host selection patterns of malaria vectors have been quantified by the relative frequency of blood from different host types in samples of engorged mosquitoes by place (a locality or biotope) and time (Boreham and Garrett-Jones 1973). Within this framework, the human blood index (HBI) is defined as the proportion of freshly engorged anophelines found to contain human blood (World Health Organization 1963, Garrett-Jones 1964, Garrett-Jones and Shidrawi 1969). This index is an important component of vectorial capacity (Garrett-Jones and Shidrawi 1969) and is useful for epidemiological assessments of malaria control program effectiveness (Garrett-Jones 1964).

Included in this report are results of studies, conducted in southern Mexico, on the host selection patterns of *Anopheles pseudopunctipennis* Theobald, a primary vector of malaria in the area (Rodriguez and Loyola 1989). Because the villages are irregularly sprayed with insecticides by the National Malaria Control Program, data included host selection patterns under negligible (1990) and more intensive effects (1991) of residual insecticide indoor spraying.

MATERIALS AND METHODS

Description of study sites: The study area is located in the foothills of El Soconusco on the western Pacific slope of the Sierra Mountains of Chiapas in southern Mexico near Tapachula. The climate in this area is tropical with distinct wet (May–November) and dry (December–April) seasons.

The foothills comprise an area of rugged topography where raising coffee is the primary agricultural activity. The many small villages scattered throughout the foothills provide the labor pool for manually cultivating this crop. Dogs, cats, pigs, and chickens are common in the villages, and cows and horses are scarce.

Four villages, at elevations of 400–650 m and separated by an average of 3.5 km, were selected as sites for the study. Two villages, El Retiro and El Plan, were chosen for their high mosquito densities, and 2, La Ceiba and La Concordia, for low mosquito densities (Fernández-Salas 1992[4]). The number of people per village was 341 in El Retiro, 446 in El Plan, 236 in La Ceiba, and 240 in La Concordia. All 4 villages are located in similar ecological settings along the Coatan River. This river is the most important dry season breeding site of *An. pseudopunctipennis* (Fernán-

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dez-Salas 1992[4]). A census of animal populations was conducted in each village.

Insecticide treatments: The National Malaria Control Program in Chiapas State routinely applies insecticides to house walls in the malaria-endemic villages. In the foothills, DDT is sprayed at 6-month intervals in villages where more than 5 malaria cases are detected by passive surveillance. Bendiocarb is used if the outbreak seems to pose a hazard for surrounding villages. During the 2 years of the study, bendiocarb (0.4 g AI/m^2) was sprayed in the 4 study villages in September 1989 and again in May 1990. DDT (2 g AI/m^2) was sprayed between late January and early February 1991.

Field collections: During 1990, resting collections were conducted from mid-January to May. Each village was sampled during one week each month. Every other morning, a team of 4 people searched a minimum of 10 houses during 2 h. A different 10 houses per village were sampled during each day of collecting. A thorough examination was made of walls, cracks, and furniture using flashlights and mouth aspirators to spot and capture resting mosquitoes. On alternate days, shelters surrounding the human dwellings, such as chicken houses, coffee plants, orchards, ground depressions, rock piles, latrines, etc. were searched for resting adult anophelines during 2 h. Captured specimens were held in pint cardboard containers and taken to the laboratory where they were sorted by sex and classified as fed, unfed, or gravid. The abdomens of freshly fed females were smeared onto Whatman No. 2 filter paper. The papers were then dried, wrapped with glassine paper, and stored at 4°C until processed for blood meal identification at the end of the study.

The same procedures of indoor and outdoor collections of resting mosquitoes were continued during the dry season of 1991 (December 1990–May 1991). Additionally, in an attempt to increase the sample size of bloodfed mosquitoes, 10 1.5 × 1.2 × 1.0-m pit shelters were dug in each of the 4 villages; they were uncovered and located in the backyards of some houses and in the center of some neighboring coffee plantations. A separate team of collectors searched the pits for resting anophelines each morning during weekdays.

Blood meal identification: An indirect ELISA described by Beier et al. (1988) and modified by Loyola et al. (1990) was used to identify the blood meals smeared on filter papers. The samples were eluted overnight at 4°C with 200 µl of a phosphate buffer saline solution (PBS, pH 7.2). Five milliliters of each eluted sample were placed with 50 µl of coating buffer (sodium bicarbonate 35 mM, pH 9.6) in 6 wells of a polystyrene microtiter plate (Dynatech Laboratories, Inc., Alexandria, VA) and incubated for one hour at room temperature. After blocking unreacted sites with 2.5% dry skimmed milk in 7.2 pH PBS for 1 hour, the wells were screened against a bank of antibodies (Sigma Chemical Co., St. Louis, MO) for identifying blood from human, horse, dog, pig, or chicken; this was followed by horseradish peroxidase-conjugated goat serum anti-rabbit IgG. Color was developed using 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD) as a substrate. Blood samples of the same host species were dried on filter paper and used during tests as positive controls. A test was positive when its absorbance value was higher than 2 times the mean of 5 negative controls (consisting of male and unfed female *An. pseudopunctipennis* mosquitoes). Mixed blood meals represented 2 or more different hosts.

Data analysis: Two measurements of *An. pseudopunctipennis* bloodfeeding habits were calculated from blood meal identification data. Estimates of HBIs were calculated from specimens collected resting inside houses and from outdoor sites. An HBI was calculated as the unweighted mean of the proportion of specimens with human blood (HBP) that were collected from indoor and outdoor locations (unweighted HBI = [% indoor human bloodfed + % outdoor human bloodfed]/2). This index is considered an unbiased estimate because it uses percentages instead of raw numbers; it was also computed to account for bias introduced by disparities in the spatial distribution of resting females. The weighted HBI or crude mean was also computed. It was calculated as the sum of indoor and outdoor numbers containing a specific host blood meal divided by the total numbers of indoor and outdoor bloodfed mosquitoes (Garrett-Jones 1964). It was difficult to obtain adequate numbers of engorged resting specimens after houses were sprayed with DDT in 1991. This was a particular problem for those villages with low densities of resting mosquitoes (La Ceiba and La Concordia). In order to obtain a representative estimate of the overall HBI by host and year, blood meal data from all localities and months were pooled. These pooled values gave us a minimum of 50 blood smears or more per month, which is considered the minimum sample size

[4] Fernández-Salas, I. 1992. Bionomics of a primary malaria vector, *Anopheles pseudopunctipennis*, in the Tapachula foothill area of southern Mexico. Ph.D. dissertation, Uniformed Services University of the Health Sciences, Bethesda, MD.

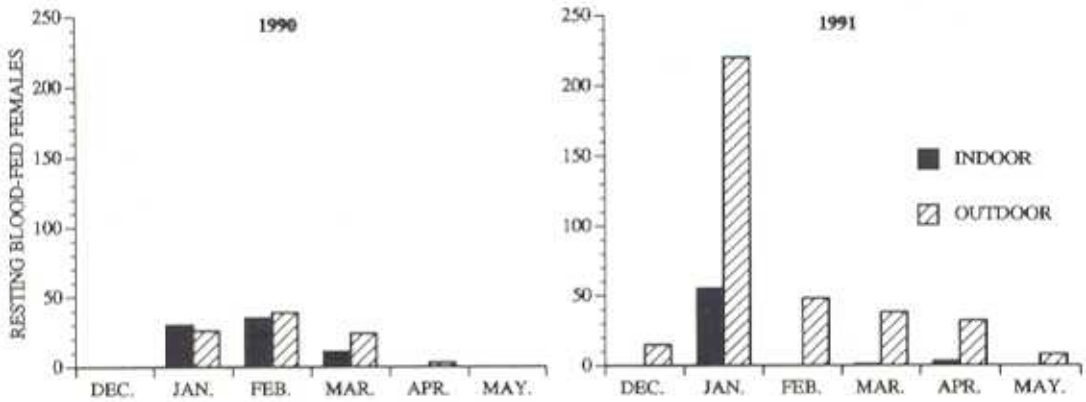

Fig. 1. Monthly distribution of resting, bloodfed *Anopheles pseudopunctipennis* females in 4 villages in the foothills near Tapachula, Mexico. Data were pooled for all indoor and outdoor collections of resting adults that were conducted during the dry seasons of 1990 and 1991. Numbers captured outdoors in 1991 include collections from pit shelters.

representative of a local population (Garrett-Jones 1964). The pooled data were used to calculate the weighted and unweighted mean HBI values.

The forage ratio (FR), which quantifies vector selection of a particular vertebrate host rather than other available hosts, was also measured (Boreham and Garrett-Jones 1973). The FRs were calculated by determining the percent of *An. pseudopunctipennis* females containing blood of a particular host, divided by the percent of the total available host population represented by that particular host (Hess et al. 1968). An FR of one, or near one, indicates neither a selective bias nor avoidance of a particular host animal; FRs significantly greater than one indicate a selective bias and values less than one indicate avoidance of a host in favor of other available hosts. Homogeneity chi-square tests were computed to analyze sample relative frequency comparisons (MacStats 1985).

RESULTS

Resting population densities: Anopheles pseudopunctipennis were most abundant from mid-December to May. Within this period of peak abundance, the monthly densities for indoor resting mosquitoes during 1990 were different from the 1991 season (Fig. 1). Sampling was initiated in January of 1990 and resting females were found on house walls from January to March. In contrast, in 1991 most resting females were found on house walls only in January.

A total of 1,859 (64.9%) *An. pseudopunctipennis* female and 1,005 (35.1%) male mosquitoes were obtained from all types of resting collections in the 4 study villages during the 2 years. In the first year only 366 (19.7%) female specimens were obtained, whereas 1,493 (80.3%) were captured the 2nd year. Of the total number of females captured during both years, 44.7% (832) were engorged. Approximately 33.9% (630) of the captured females did not contain blood and 21.4% (398) were gravid (Table 1).

The specimens processed for blood meal identification comprised 94% (784) of all 832 engorged females (Tables 1 and 2). Of the 784 specimens processed, 132 (16.8%) were collected indoors, 131 (16.7%) were collected from natural shelters, and 521 (66.5%) were collected from artificial pit shelters (from 1991) (Table 2).

As expected, the villages with overall higher densities of *An. pseudopunctipennis* in landing collections (Fernández-Salas 1992[a]) also yielded higher numbers in resting site collections (i.e., El Plan and El Retiro) (Table 1). In terms of numbers tested for blood meal identification, 30.4% (238) were from El Plan and 62.0% (486) were from El Retiro, whereas only 7.0% (55) were from La Concordia and less than 1% (5) were from La Ceiba (Table 2). There were no significant differences in overall numbers of females from indoor and natural shelter collections between 1990 and 1991 ($\chi^2 = 6.96$, $P < 0.05$); this statistic excludes data from pit-shelter collections.

Human blood index: Females captured inside dwellings showed higher human blood proportions (HBP) than those resting outdoors, either in natural shelters or pit shelters. During 1990, HBPs as high as 56.7 and 52.8% were obtained for indoor samples from El Plan and El Retiro (Table 2), whereas the natural shelter collection produced 18.2 and 19.2% HBPs in the same villages. Very few engorged specimens were cap-

Table 1. *Anopheles pseudopunctipennis* mosquitoes captured in indoor and outdoor resting collections in 4 villages in the foothills near Tapachula, Mexico, during the dry seasons of 1990 and 1991. Data are presented on numbers captured, tested for blood identification (host type), fed, unfed, gravid, and males.

	Indoor resting						Outdoor resting					
			Numbers						Numbers			
Village	Caught	% tested	Fed	Unfed	Gravid	Males	Caught	% tested	Fed	Unfed	Gravid	Males
1990								Natural shelters				
El Plan	56	53.6	30	23	3	5	124	8.9	45	43	36	3
El Retiro	70	51.4	43	20	7	0	101	60.4	43	57	1	11
La Concordia	1	100	1	0	0	0	4	0	2	1	1	0
La Ceiba	2	0.0	2	0	0	0	8	100	6	0	2	0
Totals	129	52.0	76	43	10	5	237	33.8	96	101	40	14
1991												
El Plan	78	75.6	51	14	13	1	8	12.5	4	2	2	4
El Retiro	10	50.0	7	3	0	1	92	50.0	53	38	1	1
La Concordia	1	100	1	0	0	0	1	100	1	0	0	0
La Ceiba	2	0.0	1	1	1	0	3	100	3	0	1	5
Totals	91	71.4	60	17	14	2	104	49.0	61	40	4	10
1991								Artificial pit shelters				
El Plan							412	33.3	145	142	125	479
El Retiro							757	44.7	343	253	161	418
La Concordia							124	35.6	49	33	42	66
La Ceiba							5	40	2	1	2	11
Totals							1,298	40.1	539	429	330	974

tured in La Concordia and La Ceiba, so no reliable data were generated for these villages. Again, in 1991, higher estimates of human bloodfed mosquitoes were found for indoor versus outdoor resting collections. In 1991, mosquitoes were collected from indoor resting sites only during this month (January) before house walls were sprayed with DDT. Those captured mosquitoes from indoor resting collections showed HBPs of 88.1 and 80.0% for El Plan and El Retiro, respectively (Table 2). Only El Retiro was positive for engorged mosquitoes resting in natural shelters. Of these, 23.9% contained human blood. The pit shelters were more productive; however, the HBPs were similar to those for the natural shelters (e.g., 26.3 and 22.8% for El Plan and El Retiro, respectively). On average, 53.8% of all specimens collected indoors in all villages during 1990 had fed on human blood, compared to 86.1% that had fed on humans during the single month of positive indoor resting collections in 1991 (Table 2). The differences between the 2 years were significant ($\chi^2 = 24.3$, $P < 0.01$). The rates of human blood meals in specimens from natural shelters were statistically similar ($\chi^2 = 0.74$, $P > 0.05$) (e.g., 18.6 and 23.6% for the first and 2nd years, respectively) (Table 3). The annual percent of specimens collected with human blood meals from pit shelters (23.0%) was similar to the annual rate for specimens from natural resting places (23.6%) (Table 2).

Weighted and unweighted HBI values were calculated using indoor and outdoor data from the proportions of human-fed mosquitoes. Weighted mean HBI estimates for the first and 2nd years were 34.0 and 29.5%, respectively (Table 3). The HBI values for the 2 years were not significantly different ($\chi^2 = 0.37$, $P < 0.05$). The unweighted HBI values were slightly higher than the weighted ones, 36.2% for the first year and 54.7% for the 2nd; again, the differences between these values were not significant ($\chi^2 = 0.37$, $P < 0.05$).

Of all available hosts, dogs were the second most frequent blood source for *An. pseudopunctipennis* mosquitoes (Table 2). During 2 years of collections, dogs were the blood source for 29.6–51.3% of all engorged *An. pseudopunctipennis* females collected outside in pit shelters and natural resting sites. Dogs were the blood source for 6.2 and 11.9% of the engorged females collected indoors in 1990 and 1991, respectively. These rates resulted in host blood indices, weighted or

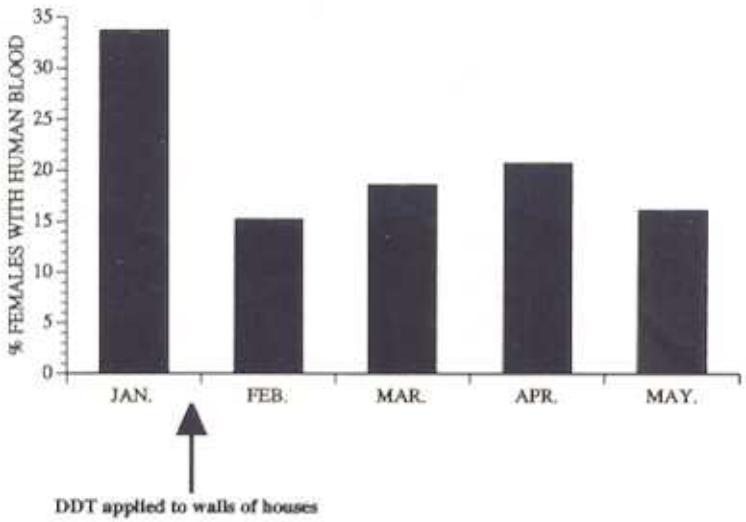

Fig. 2. Percentages of *Anopheles pseudopunctipennis* females that contained human blood in 4 villages before and after houses were sprayed with DDT. Data were compiled from routine resting collections from pit shelters during the dry season (January–May 1991) in the foothills near Tapachula, Mexico.

unweighted, of 33.3 and 31.6% in 1990, and 27.9 and 20.3% in 1991, respectively (Table 3). Horses, although relatively uncommon, were the third most important blood source. The horse blood indices were 13.6% (weighted) and 14.1% (unweighted) in 1990; in 1991 they were 18.2 and 11.5%, respectively (Table 3). Weighted pig blood indices were 15.0 and 18.4% in 1990 and 1991, respectively. The unweighted indices for pig blood were 14.9 and 8.7% for 1990 and 1991, respectively. Blood meals from chickens were rarely represented in the blood samples. The pit shelter collections from 1991 were used to assess the rate of mixed blood meals. We determined that only 2.1% or 11 of 521 specimens contained blood from more than one type of host.

Forage ratios: The patterns of host densities within the 4 villages were similar. Chickens constituted more than 50% of all vertebrate hosts within the village environments. Regardless, chickens accounted for less than 2.4% of all the identified blood meals (Table 4). Because chickens were clearly not a factor in the overall issue of *An. pseudopunctipennis* host preferences, statistics on chicken populations were not included in calculations of forage ratios.

Humans were 3 times more abundant (76.1%) in villages than domestic animals (excluding chickens). However, the FR for humans was less than one for both years, with (1.0) or without (0.4) numbers of chickens, which indicates that *An. pseudopunctipennis* females fed more frequently on other available hosts. Forage ratios greater than one were calculated for all other available hosts, *viz.*, dogs, pigs, and horses. The FRs for horses for the 2 years were 15.6 and 20.9 (Table 4). Although horses represented less than 1% of all available hosts, they were fed on frequently by *An. pseudopunctipennis* females. The second most frequently selected hosts were pigs, with FRs of 5.8 and 7.2 for the first and 2nd years, respectively. Forage ratios for dogs (FRs of 1.6 and 1.4) showed that these animals were less frequently selected for a blood meal than were horses and pigs.

Spraying effect on host selection: DDT was sprayed on house walls in late January 1991. Roughly 60% of all resting females (637) collected during the dry season of 1990–91 were collected in the December–January 1991 interval before the houses were sprayed with DDT. After spraying, densities of indoor-resting *An. pseudopunctipennis* females dropped precipitously. Only 4 specimens were collected indoors from February to May and, to a lesser extent, densities dropped in outdoor resting sites as well (Fig. 1). Under these circumstances, the pit shelters became an increasingly valuable source of blood-engorged specimens. Pit-shelter collections were used to determine the impact of DDT residues on host selection patterns in resting populations. A change in host selection occurred after the villages were sprayed with DDT. In engorged specimens from pit shelters, the proportions of human bloodfed females were high before treatment (33.7%), dropped after the houses were sprayed

Table 2. Blood meal identifications from *Anopheles pseudopunctipennis* mosquitoes captured in indoor and outdoor resting collections in 4 villages in the foothills near Tapachula, Mexico, during the dry seasons of 1990 and 1991.[1]

	Indoor resting								
	Numbers by host-type							Total	
Villages	Hu	Hr	Dg	Pg	Chk	Mx	Not ID	tested	HBP[2]
1990									
El Plan	17	6	4	2	1	0	0	30	56.7
El Retiro	19	7	3	7	0	0	0	36	52.8
La Concordia	0	0	1	0	0	0	0	1	0.0
La Ceiba	0	0	0	0	0	0	0	0	0.0
Totals	36	13	8	9	1	0	0	67	53.7
1991									
El Plan	52	1	3	0	2	1	0	59	88.1
El Retiro	4	1	0	0	0	0	0	5	80.0
La Concordia	0	0	1	0	0	0	0	1	0.0
La Ceiba	0	0	0	0	0	0	0	0	0.0
Totals	56	2	4	0	2	1	0	65	86.2

[1] Data are presented on numbers that had fed on humans (Hu), horses (Hr), dogs (Dg), pigs (Pg), chickens (Chk), mixed hosts (Mx), not identified (not ID), total tested, and the percent fed on humans.
[2] Human blood proportion or percent of engorged females containing human blood.

(15.2%), and increased slowly during the next 3 months (Fig. 2). The animal blood proportions were the inverse of the monthly human fed proportions and increased immediately after the houses were sprayed.

DISCUSSION

Resting populations: The study of host selection patterns of malaria vectors is a complex task, especially in areas where insecticides are continuously applied to house walls for malaria control. Dramatic changes in feeding patterns and in densities of resting mosquitoes are always expected under such conditions. Additionally, bloodfed anophelines tend to seek more protected resting places where they will not be irritated or affected by the insecticide, and this insecticide-induced population movement will be reflected in the results of the sampling program (Garrett-Jones 1964).

The patterns of monthly densities in the indoor resting populations of *An. pseudopunctipennis* in the study villages during 1990 and 1991 were different (Fig. 1). The differences might be explained, in part, by differences in timing of spraying as well as differences in insecticides employed. During the first year house walls were sprayed with bendiocarb in September 1989, in response to an early season occurrence of malaria cases. *Anopheles pseudopunctipennis* is the primary vector in this foothill region where its population densities begin to increase during mid-December. The wet-season outbreak in September was considered "early season" because it occurred when densities of *An. pseudopunctipennis* populations were at their lowest level. Bendiocarb was not sprayed on the house walls a second time until April 1990. Probably the 3-months residual action of bendiocarb (Pant 1988) on house walls degraded from September to mid-December 1989, and population densities of *An. pseudopunctipennis* increased during December 1989 and January 1990. In the following year (1991), DDT was sprayed on village house walls during the interval from late January to early February. This application of DDT was timed to correspond with the appearance of peak vector densities. There were no significant differences in numbers of females captured resting indoors and in natural resting sites between 1990 and 1991. However, in 1991 almost all females were captured before houses were sprayed with DDT. An inspection of the 1991 data shows that DDT residues essentially prevented indoor host-seeking and indoor resting by *An. pseudopunctipennis* mosquitoes. This effect lasted until late April 1991.

Overall, the intensive collections of resting *An. pseudopunctipennis* adults from indoors and natural sites outdoors were relatively unproductive during the 2 years, producing only 18.8 and 14.8% of the total number of engorged females processed for blood meal identification (Table 2). In

Table 2. Continued.

Villages	Outdoor resting								
	Numbers by host-type							Total tested	HBP
	Hu	Hr	Dg	Pg	Chk	Mx	Not ID		
	Natural shelters								
1990									
El Plan	2	3	5	0	1	0	0	11	18.2
El Retiro	12	1	35	10	1	0	2	61	19.2
La Concordia	1	3	1	3	0	0	0	8	12.5
La Ceiba	0	0	0	0	0	0	0	0	0.0
Totals	15	7	41	13	2	0	2	80	18.8
1991									
El Plan	0	0	1	0	0	0	0	1	0.0
El Retiro	11	10	17	6	1	1	0	46	23.9
La Concordia	0	0	1	0	0	0	0	1	0.0
La Ceiba	1	0	1	1	0	0	0	3	33.3
Totals	12	10	20	7	1	1	0	51	23.5
	Artificial pit shelters								
1991									
El Plan	36	40	50	6	1	1	3	137	26.3
El Retiro	77	42	93	100	10	10	6	338	22.8
La Concordia	7	21	10	4	1	0	1	44	15.9
La Ceiba	0	1	1	0	0	0	0	2	0.0
Totals	120	104	154	110	12	11	10	521	23.0

the 2nd year, the pit shelters produced about 4 times as many resting, engorged *An. pseudopunctipennis* females as were obtained in samples from more natural resting sites. However, data from pit shelters and natural resting sites indicate that samples from either site are representative of outdoor resting mosquito populations.

The Coatan River is the major larval habitat for *An. pseudopunctipennis* mosquitoes during the dry season (Fernández-Salas 1992[4]). Not surprisingly, the 2 villages located closest to the river, El Plan and El Retiro, also provided most of the engorged females (92%) in the resting collections.

Human blood index: In samples from the 4 study villages, both the weighted and unweighted HBIs for *An. pseudopunctipennis* characterized a relatively anthropophilic vector. The unbiased estimator, or unweighted mean, was always higher than the weighted HBI during the 2 years (36.2 and 54.7% versus 34.0–29.5%) (Table 3). The reason for this relationship between weighted and unweighted HBIs probably relates to the greater effect of large percentages of human bloodfed specimens in indoors collections on the unweighted HBI. For example, in the first year, 53.8% of indoor resting females contained human blood, whereas 86.1% had human blood meals during the prespray period of the 2nd year (Table 2). Similarly, high proportions of indoor resting *An. pseudopunctipennis* females with human blood have been found in other areas: Vargas (1938) found 67.6% in Temixco, Central Mexico; Davis and Shannon (1928) reported 50.0% with human blood in northern Argentina; and in Peru, Acosta (1960) found 80.6% with human blood. These data reveal a high degree of anthropophagy in indoor resting populations. Also, indications of endophily were provided by indoor resting mosquitoes containing blood meals from animal hosts (i.e., 19.4% horse [13], 11.9% dog [8], and 13.4% pig [9] [Table 2]). These results were better shown in 1990 than 1991.

The residual effects of insecticide sprayed on village house walls probably account for some differences in unweighted HBIs between 1990 (36.2%) and 1991 (54.7%). However, the influence of insecticide treatment was more apparent

Table 3. Weighted and unweighted HBIs and other hosts blood meals of *Anopheles pseudopunctipennis* from indoor and outdoor resting collections. Data were pooled by each year over all villages and months. The resting collections were conducted in 4 villages in the foothills near Tapachula, Mexico, during the dry seasons of 1990 and 1991.

	% blood meal[1]				
Hosts	Indoor	Outdoor (natural shelters)	Outdoor (pit shelters)	Weighted mean[2]	Unweighted mean[3]
1990					
Human	53.8	18.6	—	34.0[4]	36.2[4]
Animal[5]	46.2	81.4	—	66.0	63.8
Totals[6]	100 (67)	100 (80)		100 (147)	100 (147)
Horse	19.4	8.8	—	13.6	14.1
Dog	11.9	51.3	—	33.3	31.6
Pig	13.4	16.3	—	15.0	14.9
Chicken	1.5	2.5	—	2.0	2.0
Mixed	0.0	0.0	—	0.0	0.0
Not identified	0.0	2.5	—	2.1	1.3
1991					
Human	86.1	23.6	23.0	29.5[4]	44.2[4]
Animal	13.9	76.4	77.0	70.5	55.8
Totals[6]	100 (65)	100 (51)	100 (521)	100 (637)	100 (637)
Horse	3.1	19.6	20.0	18.2	14.2
Dog	6.2	39.2	29.6	27.9	25.0
Pig	0.0	13.7	21.1	18.4	11.6
Chicken	3.1	2.0	2.3	2.4	2.5
Mixed	1.5	2.0	2.1	2.0	1.9
Not identified	0.0	0.0	1.9	1.6	0.6

[1] Percent of blood meals by type of host. Blood meals were identified by ELISA.
[2] Weighted or crude mean: (numbers with human blood indoors + numbers with human blood outdoors)/(total numbers engorged indoors + total numbers engorged outdoors).
[3] Unweighted mean: (indoor percent with human blood + outdoor percent with human blood)/number of pooled samples; for example, 2 for 1990 and 3 for 1991.
[4] Human blood index (HBI).
[5] All animals other than humans plus "not identified" meals combined.
[6] Number of specimens tested for blood identification is given in parentheses.

Table 4. Forage ratio (FR)[1] estimates for *Anopheles pseudopunctipennis* females during 2 years of collections in the foothills near Tapachula, Mexico, during 1990 and 1991.

			1990			1991		
Hosts	% host population[2]	% host population[3]	% blood meals[4]	$FR^{1,2}$	$FR^{1,3}$	% blood meals[4]	$FR^{1,2}$	$FR^{1,3}$
Human	34.9	76.1	34.0	1.0	0.4	29.5	0.8	0.4
Animal[5]	65.1	23.9	66.0	1.0	2.8	70.5	1.1	2.9
Totals	100	100	100			100		
Horse	0.4	0.9	13.6	34.0	15.6	18.2	45.5	20.9
Dog	9.4	20.5	33.3	3.5	1.6	27.9	3.0	1.4
Pig	1.2	2.6	15.0	12.5	5.8	18.4	15.3	7.2
Chicken	54.2	—	2.0	0.0	—	2.4	0.0	—
Sample size	3,753	1,719	147			637		

[1] Forage ratio = percent host blood meals/percent host population.
[2] Chicken population included.
[3] Chicken population deleted.
[4] Weighted HBI, see Table 3.
[5] All animals other than humans plus not identified meals combined.

in comparisons of percentages of indoor resting females containing human blood. In 1991, approximately 86.1% of indoor resting females were positive for human blood, compared to 53.8% in 1990. The study houses were sprayed with bendiocarb in September 1989. Considering that residual effect of bendiocarb is estimated to be 3 months (Pant 1988), the overall effect of the insecticide was expected to be low during the sampling period of January–March in 1990. Clearly, the negligible effects of insecticide residues accounted for a high percentage of outdoor resting females with human blood during the dry season. Houses were sprayed with bendiocarb in April 1990, and were not treated again until late January–early February 1991. Consequently, during December 1990 and early January 1991 the vectors were feeding on humans inside houses with negligible residues of insecticide on the walls. However, after houses were sprayed with DDT in late January 1991, the indoor resting collections were negative. As a result, the percentage of human bloodfeeding detectable with indoor resting collections before and after DDT treatment was 86.7% (52 of 60) and 0%, respectively. The unweighted HBIs could not be used for these comparisons simply because no mosquitoes were caught resting indoors. Nevertheless, we can expect that the effects of bendiocarb and DDT will be different even in recently sprayed houses. Support for this conclusion is provided by the report of Loyola et al. (1990), in which they found a higher unweighted HBI (12.7–26.9%) in bendiocarb-sprayed villages vs. an unweighted HBI of 3.3–6.8% in DDT-sprayed villages in northwestern Mexico.

Dogs were second only to humans as a source of blood meals for *An. pseudopunctipennis* (Table 2). Furthermore, dogs were the second most common host (chickens excluded) within the village environment (Table 4). Humans and dogs accounted for more than 96.0% of the *An. pseudopunctipennis* blood meals. This skewed pattern in host selection reflects the local pattern of host availability; it also reveals a strong association of *An. pseudopunctipennis* with the domestic environment. Such an association clearly defines the human domicile as a high risk biotope for the transmission of malaria. Mixed blood meals, although found in very low proportion, represented combinations of man–dog, dog–horse, and man–pig. Mixed and cryptic (2 or more blood meals from the same type of host in a single mosquito) blood meals may be of epidemiological importance because of the increased risk of malaria transmission posed by a vector that takes multiple partial meals (Boreham and Garrett-Jones 1973).

Forage ratios: The HBIs were indicative of very high man–vector contact under the specific conditions of host availability at the study sites. In contrast, the FRs, which are measures of host selection patterns, clearly defined *An. pseudopunctipennis* as a zoophilic species (Table 4). The FRs for humans were equal to or less than one (with or without chicken populations); therefore when all factors are equal, it seems that these anophelines tend to feed on humans with less frequency than other large animals. Also, chicken blood was rarely encountered in engorged *An. pseudopunctipennis* specimens. Strong host selection was consistently demonstrated for the larger mammals, such as horses (FR > 15) and pigs (FR > 5.8), which were less abundant than humans in the study villages. Per animal, these large animal hosts presented large surface areas to host-seeking mosquitoes. Unfortunately, differences in quantity of surface areas are not included in the FR calculations. Some kind of adjustment for host surface area might better define the feeding relationships between the mosquito and its hosts. Larger hosts, such as horses and pigs, are known to attract most bites of *An. pseudopunctipennis* in a variety of ecological settings in Mexico (e.g., Sinaloa [Loyola et al. 1990]) and Temixco [Vargas 1938]). A similar preference by *An. pseudopunctipennis* for large mammals was demonstrated in Peru by Sasse and Hackett (1950). Sasse and Hackett used a stable trap and alternated exposures of hosts to blood-seeking populations; hosts included man, goat, pig, calf, and donkey. The result was that most blood meals (62.3%) were from donkey. In another comparison of horses versus cows and humans in experimental huts, horses received more than 70.0% of the bites (Vargas 1938). In our study villages, humans were more abundant than other hosts; consequently, feeding on humans seems to reflect host availability more than host preference. Host availability is possibly the dominant factor in most environments favoring transmission of human malaria by *An. pseudopunctipennis* mosquitoes.

Effects of insecticides on host selection: The pre- and postspraying changes in HBIs as a result of the behavioral responses of vectors to DDT have been documented for some malaria vectors (Garrett-Jones 1964). In our case, no *An. pseudopunctipennis* populations were found resting indoors for almost 3 months after the houses were sprayed with DDT; therefore, comparisons of pre- and postspraying HBIs were not possible. Regardless, the percent of specimens containing human blood in the pit shelter collection declined after the houses were sprayed with DDT. Similarly, the percent of specimens containing

animal blood increased. A change in host selection patterns occurred probably because a proportion of mosquitoes that fed indoors on humans were killed by the insecticide or because they were forced to feed on alternative hosts not protected by insecticide residues. The population-based reduction in human bloodfeeding as a result of DDT residues on house walls, as seen in Fig. 2, possibly indicates that the insecticide repellency produces an "area effect." In other words, presence of insecticide may reduce the numbers of bites on humans outside, as well as in indoor locations. Similar changes in the HBIs of populations collected from outdoor shelters have been reported for important vectors species in Africa, such as *Anopheles funestus* Giles and *Anopheles gambiae* Giles (Garrett-Jones 1964).

Insecticide repellency and irritability are involved in indoor resting behavior and the selection of blood meal hosts by *An. pseudopunctipennis* in DDT-sprayed villages. Indeed, behavioral avoidance of insecticide residues may be the primary mode of DDT action in controlling *An. pseudopunctipennis* transmitted malaria. Clearly, additional studies on the behavioral responses of *An. pseudopunctipennis* to insecticide residues are needed.

ACKNOWLEDGMENTS

This research was funded partly by project DIMOD of the National Aeronautics and Space Administration (NASA) through contract W16 306 to the USUHS. Funds were also provided by the Mexican Secretariat of Health and the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases. We appreciate the field assistance of Cresencio and Eufronio Diaz. This research is part of the senior author's doctoral dissertation at the Uniformed Services University of the Health Sciences, Bethesda, MD.

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