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PERI-/INTRADOMICILLARY BEHAVIOR IN RELATION TO HOST-SEEKING OF *ANOPHELES PSEUDOPUNCTIPENNIS* IN SOUTHERN MEXICO

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ABSTRACT. Peri-/intradomicillary resting and host-seeking behavior of *Anopheles pseudopunctipennis* was studied in an experimental house (surrounded by a curtain-net) that had not been sprayed with insecticide. Peak mosquito densities were recorded resting on vegetation 1 h earlier (1900–2000 h) than on the curtain-net, suggesting that a proportion of females prefer resting on adjacent vegetation prior to moving indoors. Between 2000 and 2100 h there was a marked decrease in numbers of mosquitoes resting on the exterior of the curtain-net. In separate experiments without the net, a single peak in numbers of mosquitoes resting on interior surfaces and/or biting human bait occurred between 2000 and 2100 h. Unfed mosquitoes had higher numbers of contacts with wall surfaces than with the roof. Moreover, a higher proportion of mosquitoes collected on human bait had ≥1 dilatation(s), and higher parity rates than those resting on walls. Higher proportions of parous mosquitoes more frequently fed from 1800 to 2100 h, whereas higher proportions of nulliparous mosquitoes fed during morning hours. Nearly 25% of all mosquitoes exited 1 h following their release inside the house, whereas nearly 7% remained indoors for >9 h. Overall results demonstrated that the behavior of *An. pseudopunctipennis* females depends on outdoor/indoor stimuli, being multivariable in nature. This includes contacts with a variety of biological (i.e., vegetation, human) and inert surfaces, which in part appears to be controlled by age structure.

INTRODUCTION

In recent years Mexico, Guatemala, and El Salvador have experienced a shift in the number of malaria cases away from some coastal lowlands, traditionally inhabited by *Anopheles albimanus* Wied., to the coastal foothills and interior lowlands (Centro de Investigación de Paludismo [CIP], unpublished data, annual reports of the malaria programs of Guatemala and El Salvador). As a result *Anopheles pseudopunctipennis* Theobald, predominating along the coastal foothills during the dry season, has taken on increased importance as a primary vector of malaria.

Vector control strategies should be based on a thorough understanding of mosquito behavior. Because there exists a potential for modification of behavior due to the presence of an insecticide, the evaluation of outdoor/indoor resting sites and behavioral patterns of anopheline vectors should be carried out prior to implementing control measures. Vector activity in time and space with respect to host-seeking and resting can be viewed as 2 essential components, pre- and postfeed indoor resting behavior (Elliott 1972, Bown et al. 1993, Casas 1993[3]), both of which represent a potential for contact with indoor surfaces. However, other temporal activity has bearing on vector/host relationships, for example, the gathering of mosquitoes on vegetation or on other surfaces around the outside of houses, followed by their movement indoors including a progression of resting and host biting, culminated by exiting outdoors. This sequence of events in general represents a more complete overview of vector behavior that may have an additional potential for intervention as part of an integrated vector control strategy. Considering this, the objectives of the present study were to evaluate the outdoor/indoor host-seeking behavior of *An. pseudopunctipennis,* having as a long-term goal the identification of those aspects with a potential use in alternative control schemes.

MATERIALS AND METHODS

Study area: The study was carried out in an experimental house, located approximately 20 km northeast of Tapachula, in the foothills of southern Chiapas, Mexico, during the dry sea-

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[3] Casas, M. M. 1993. Comportamiento peri e intradomiciliario de *Anopheles pseudopunctipennis* Theobald en el Sur de Chiapas, México. Informe de Servicio Social. Universidad Autónoma Metropolitana, México, D.F.

sons of January–April of 1992 and 1993. The experimental house (5 × 6 × 3m), situated within 100 m of the Coatan River, was constructed of wood siding and a corrugated metal roof. Similar houses are common in the surrounding communities. The nearest community, El Retiro (15°04'39"N, 92°13'20"W), located 1.5 km from the study site, is situated among coffee plantations at an altitude of 660 m and had a high of 13 malaria cases/1,000 inhabitants in 1991. The subhumid climate had an average rainfall of 3,800 mm, a mean annual temperature of 25°C, and an average humidity of 90–95%. DDT had been used to spray villages during the last 5 years, whereas bendiocarb was only sprayed during 1989. Although villages had been sprayed with DDT 6 months prior to the beginning of the study, the experimental house was insecticide free.

Outdoor resting (vegetation): Between January and March, 4 15-min observations each hour (1800–0600) were made by 2 technicians during a total of 11 nights. Numbers of fed and unfed *An. pseudopunctipennis* resting on vegetation adjacent to the experimental house as well as assessments of temperature and humidity were recorded.

Curtain-net (entrance/exit): A curtain-net that encircled the exterior of the experimental house, from the roof to the floor (Bown et al. 1986), was used to evaluate mosquito movements. During 19 experiments, 2 technicians collected unfed resting mosquitoes on the exterior of the curtain between 1800 and 2045 h, dusted them with fluorescent powder (Lumogen Yellow®, BASF, Holland, MI) and released them indoors at 2100 h. Two technicians acted as baits inside the house. After the marked mosquitoes were released indoors, they were subsequently identified using an ultraviolet lamp and captured while resting on the interior of the curtain. At 0600 h a final collection of all indoor resting mosquitoes was made. Technicians were offered weekly chloroquine chemoprophylaxis. Parity was determined hourly between 1800 and 0600 h from 10 mosquitoes collected resting on the outside of the curtain by ovarian dissection using methods suggested by Detinova (1962).

Human bait and indoor resting densities (without curtain-net): During 16 experiments, 2 techniques were employed to investigate human–vector contact and preferential resting sites of *An. pseudopunctipennis.* Between 1800 and 0600 h, one technician counted the number of mosquitoes resting on indoor surfaces (wall, roof, and other). During the same period, the 2nd technician acted as human bait while sitting indoors near the open front door. All landing mosquitoes were collected. Resting mosquitoes were continually monitored until they had contact with human bait. Depending on mosquito densities, 5–10 biting or resting mosquitoes per hour were collected and examined on the following morning to determine parity, numbers of ovarian dilatations (Detinova 1962), and Christophers's stages (Christophers 1911).

Data analysis: Statistical comparisons of parity rates of mosquitoes resting on the exterior of the curtain-net were evaluated using a one-way ANOVA whereas differences between age structure (parity rates, numbers of dilatations, and Christophers's stages) of mosquitoes collected resting indoors and from human bait were evaluated by χ^2 tests. Hourly parity rates were evaluated using a one-way ANOVA and a *t*-test following an arc-sin transformation (Zar 1984).

RESULTS

Outdoor resting (vegetation): Mosquitoes were first observed resting on vegetation adjacent to the house around 1830 h. Peak resting activity occurred between 1900 and 2100 h (Fig. 1), representing 32.5% of 1,326 *An. pseudopunctipennis* females observed during the study period. Resting densities gradually decreased to <4.0% between 0500 and 0600 h ($\bar{x}$ = 4.8 ± 5.5 [SD] mosquitoes/night). The overall mean was 120.5 ± 91.6 mosquitoes/night. In 1992, highly significant differences ($P < 0.01$) in the mean number of mosquitoes ($\bar{x}$ = 31.0 ± 17.7) were observed during the 2nd hour, 1900–2000 h, as compared to subsequent hours (i.e., 2300–0600 h) (mean <10.9 mosquitoes). No differences were found between hours during the 1993 sampling period. In 1993, maximum densities occurred between 2200 and 2300 h ($\bar{x}$ = 3.7 ± 5.2) and minimum densities between 1900–2000 h and 0100–0200 h ($\bar{x}$ = 0.7 ± 1.5 and $\bar{x}$ = 0.7 ± 0.5), respectively.

Curtain-net (entrance/exit): Through 19 collecting nights, mosquitoes were first noted landing on the curtain at 1830 h (Fig. 1). Peak numbers of resting mosquitoes appeared on the exterior of the curtain between 1900 and 2100 h, representing 38.0% ($\bar{x}$ = 135.8 ± 110.9 mosquitoes) of 6,798 mosquitoes collected between 1800 and 0600 h. At 2100 h a marked decrease (8.8%) in numbers of resting mosquitoes occurred ($\bar{x}$ = 31.6 ± 23.1), followed by a continued general decrease to <2.2% ($\bar{x}$ = 7.6 ± 6.2 mosquitoes/night) at 0600 h. In 1992, highly significant differences ($P < 0.01$) in the mean number of resting mosquitoes (95.5 ± 50.1 and 93.7 ± 61.6) were observed between 1900–2000 h and 2000–2100 h as compared to subsequent hours (mean < 39.5 mosquitoes). Significant differences ($P < 0.05$) were also found between 2000–2100 h and 0500–0600 h, respectively (31.9 ±

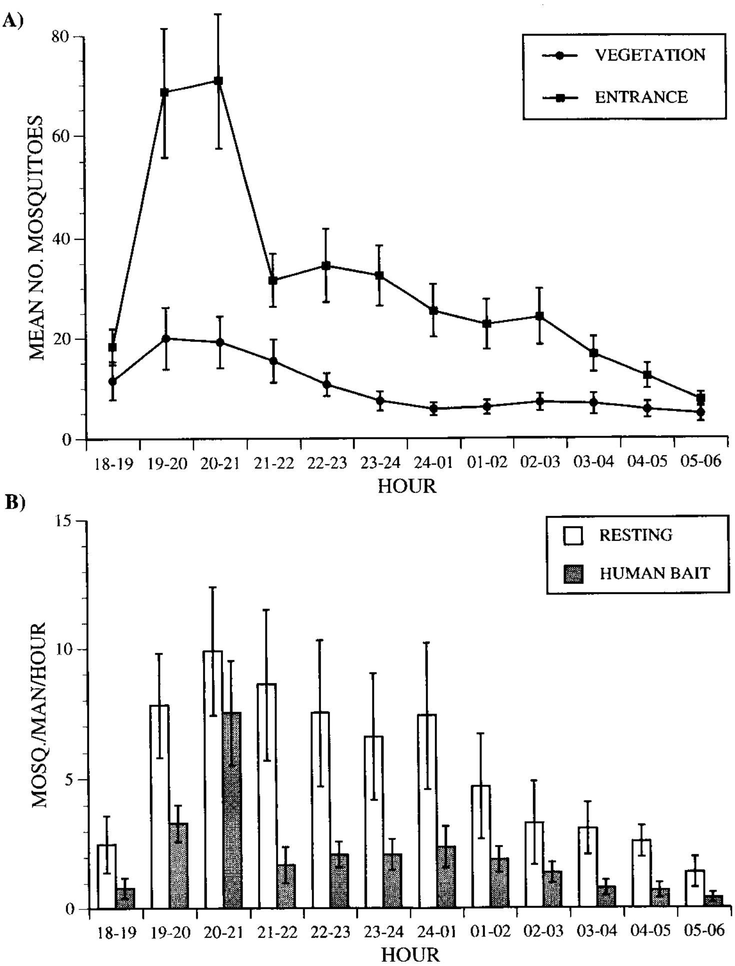

Fig. 1. *Anopheles pseudopunctipennis* observed and collected: (A) from vegetation and from the exterior surface of a curtain-net encircling the house; and (B) from human bait and from resting sites inside the house. Vertical lines represent standard errors (SE).

23.6 and 3.7 ± 4.8) during the 1993 sampling period.

A total of 2,888 unfed mosquitoes, having a mean parity of 35.0%, were released indoors (152 ± 108.5 mosquitoes/experiment) at 2100 h (Fig. 2). Of the marked and released mosquitoes, 36.2% were collected leaving the house during the first 2 h following their release (2100–2300 h). This was followed (2300–0600 h) by a gradual decrease in the number of exiting mosquitoes to 2/h by 0600 h. Although there were no significant differences in parity rates by hour of capture (mean 36.5%), the highest parity rate (53.8%) was observed between 2400 and 0100 h.

Human bait and indoor resting densities: Peak indoor biting (7.5 mosquitoes/man-hour) and resting densities (9.9 mosquitoes/man-hour) occurred between 2000 and 2100 h (Fig. 1). On the following hour (2100 h), biting densities sharply decreased to <1.7 mosquitoes/man-hour with a continued reduction to <0.5 mosquitoes/man-hour at 0600 h. However, peak resting densities on interior surfaces were observed to gradually decrease during subsequent hours to <1.5 mosquitoes/man-hour by 0600 h. An average of 25.2 and 65.2 mosquitoes/night were collected from human bait and observed resting on interior surfaces, respectively. During the first 3 h (Fig. 3), mosquitoes were observed resting in significantly higher densities ($P < 0.05$) on the roof (19.1% or 69 mosquitoes) as compared to the wall (11.9% or 43 mosquitoes) and other surfaces (3.6% or 13 mosquitoes). However, between 2100 and 0600 h, more mosquitoes (36.2% or 131 mos-

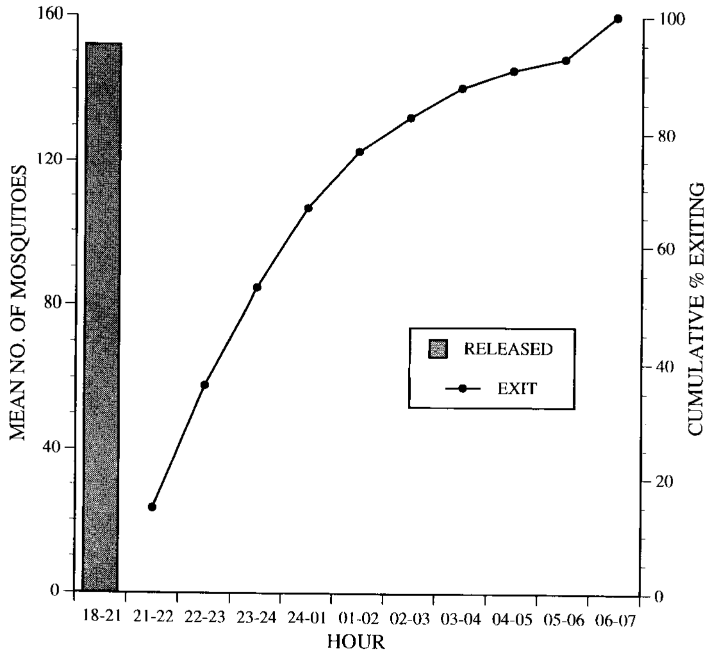

Fig. 2. *Anopheles pseudopunctipennis* that were marked and released indoors and later collected on the interior surface of the curtain-net surrounding the experimental house.

quitoes) were observed resting on walls (except between 0300 and 0400 h). During the 12-h period, 48.1% of 362 resting mosquitoes were found in significantly higher densities on walls ($P < 0.05$), followed by 37% on the roof and 14.9% on other surfaces.

Hourly parity rates of mosquitoes collected from human bait indoors and resting on indoor surfaces are given in Fig. 4. When comparing mean parity rates by 3-h intervals (e.g., 1800–2100 h), mosquitoes collected from human bait during the first interval (72.4%) had a significantly higher parity rate ($P < 0.05$) than those resting indoors (51.1%). No significant differences between human bait and resting mosquitoes were observed between 2100 and 0600 h. However, significant differences in parity rates of mosquitoes collected only from human bait were found during the first 3-h interval and that of the 3rd (47.7%) and 4th (40.6%) 3-h intervals. No significant differences were found between parity rates of resting mosquitoes during any time interval.

Tables 1 and 2 summarize dissection results of *An. pseudopunctipennis* females from human bait and resting collections. Results of parity rates and Christophers's stages, and dissections for numbers of dilatations must be evaluated separately because dissections were made between January–April and March–April, respectively. Overall, parity rates of females collected from human bait and those resting on indoor surfaces were found to be 60.9 and 57.6%, respectively. In both experiments, >60% of the mosquitoes collected from human bait and those resting were found to be in Christophers's stages 2′ and 2″. In total, 46.8% of the mosquitoes collected from human bait were found to have only one dilatation as compared to only 39.0% of indoor resting mosquitoes having one dilatation. Parity rates for human bait-collected mosquitoes were proportionally higher than those mosquitoes collected resting indoors, 56.1 and 46.0%, respectively.

DISCUSSION

Elliott (1972), Edman (1989), and Bown et al. (1993) reported that indoor resting behavior can be viewed as 2 separate components (pre- and postfeed), both associated with contact on indoor or outdoor surfaces. In Mexico it was demonstrated that prolonged use of DDT can provoke behavioral changes resulting in avoidance of indoor surfaces and subsequent lethal intoxication (Martínez-Palacios and De Zulueta 1964). This and similar studies underscore the importance of research on the behavior of potential and incriminated vectors and to further clarify their role in disease transmission and responses to control measures.

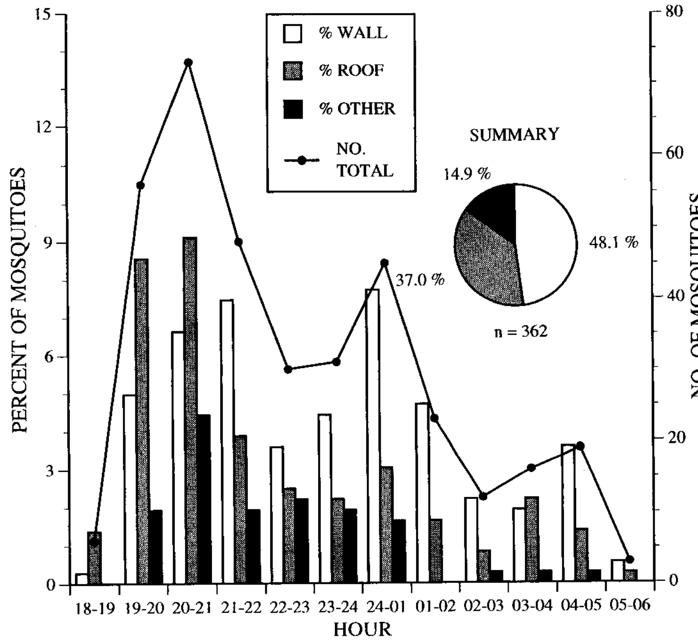

Fig. 3. Indoor resting preferences of unfed *Anopheles pseudopunctipennis* in the experimental house.

Mosquitoes were first observed to accumulate on adjacent vegetation of the experimental house as early as 1830–1845 h, followed by peak densities during the next 3 h. These observations correlate with those of Breeland (1972) in El Salvador, who noted that mass migration out of diurnal resting sites took place between 1800 and 1830 and no *An. pseudopunctipennis* were found again in natural resting sites until after 0500 h. Mosquitoes were observed to begin resting on the exterior of the curtain-net at nearly the same time as on vegetation. However, peak densities did not occur on the curtain-net until 1 h later than on vegetation, suggesting that a proportion of *An. pseudopunctipennis* females preferred resting on adjacent vegetation prior to moving indoors.

Between 2000 and 2100 h there was a marked decrease in numbers of mosquitoes resting on the exterior of the curtain-net. During the same hour (2000–2100 h), there was an increase in mosquitoes resting on interior surfaces and biting on human bait. This culminated in a peak of biting and resting occurring between 2000 and 2100 h, followed by a decrease in activity to <1.5 mosquito/man-hour at 0600 h. During an earlier study carried out in El Retiro, Fernández (1992[4]) described a bimodal biting activity, with the first peak also occurring between 2000 and 2100 h followed by a 2nd but higher peak taking place 5 h later. Although Elliott (1972) reported a similar bimodal biting activity in Peru, he found only a single biting peak to occur between 2200 and 2400 h in Ecuador. In our study, differences in peak biting activity were possibly a function of the proximity of the experimental house to larval habitats and to adult oviposition sites, resulting in high densities of nulliparous mosquitoes having less contact with humans (as a result of not being physiologically ready to feed), especially in the early morning.

At 2100 h when biting densities sharply decreased, resting densities on interior surfaces remained high until 0100 h, indicating a continuing influx of mosquitoes resting from outdoors. After 2100 h densities of resting mosquitoes decreased. Although the overall landing preference (12 h) was found to be on a wall, during the first 3 h the roof was the preferred landing site. Interestingly, Casas (1993[3]) reported a mark–release study (mosquitoes were observed for only 6 h, 1800–2400 h) that showed a stronger tendency for both fed and unfed mosquitoes to rest on the roof. It was further observed that in the absence of engorged mosquitoes or prefeed mos-

[4] Fernández, S. I. 1992. Bionomics of the primary malaria vector, *Anopheles pseudopunctipennis,* in the Tapachula foothills area of southern Mexico. Ph.D. thesis. Uniformed Services University of the Health Sciences, Bethesda, MD.

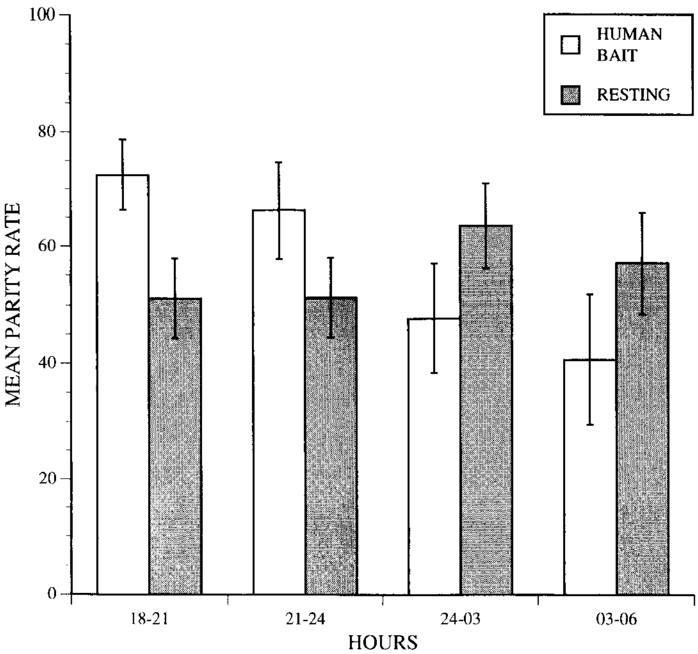

Fig. 4. Mean parity rates of *Anopheles pseudopunctipennis* collected indoors from human bait and resting. Vertical lines represent standard errors (SE).

quito behavior, unfed mosquitoes also had greater contact with the roof than with a wall or other surfaces. Although not recorded, it was observed that numerous mosquitoes (unfed) only rested on interior surfaces and later exited the house without contacting human bait.

Overall parity rates of females collected from human bait and those resting on indoor surfaces were nearly equal (Tables 1 and 2), however when they were evaluated according to 3-h intervals and type of collection, mosquitoes collected from human bait during the 1800–2100-h interval had significantly higher parity rates. This indicated that proportionally more older mosquitoes were biting in the early evening. This was followed by a transitional period from 2100 to 2400 h when there were no differences in parity rates between biting and resting mosquitoes. From 2400 to 0600 h, significantly fewer parous mosquitoes were found biting, whereas indoor-resting mosquitoes were found to have highest parity rates. Although parous mosquitoes continued to bite human bait later in the evening, they did so at a significantly reduced frequency. Most studies (primarily non-anopheline) have demonstrated little or no differences in parous rates during different periods of the night (Coz 1964, Gillies and Wilkes 1965). This may in part be because some anophelines may not return immediately to feed after oviposition (Spencer and Christian 1969), such that the influx of parous mosquitoes resulting from oviposition is diluted and has a limited effect on the biting curve.

The physiological age of biting and resting mosquitoes collected in the dry season (March–April) showed a higher proportion of parous mosquitoes collected on human bait than those resting on walls. According to Fernández (1992[4]), as many as 25% of the nulliparous *An. pseudopunctipennis* enter a pregravid stage and seek a 2nd blood meal before ovipositing at the end of their first ovarian cycle. In the present study, the majority of the resting mosquitoes were nulliparous. We suggest that the nullipars were less likely to bite because they were still not physiologically ready to feed and the pregravid females were not fully ready for a 2nd blood meal. Although these results were not statistically significant, they indicated that there were general differences between nulliparous and parous mosquitoes in their preparedness to feed.

Finally, in order to evaluate the pattern of house-exiting behavior, groups of *An. pseudopunctipennis* were released in the experimental house at 2100 h. Possibly as a result of the large proportion of nulliparous mosquitoes and their pregravid composition, nearly one-third of the mosquitoes exited 2 h following release and about 65% had exited 4 h after release. Similar results were obtained by Loyola et al. (1991), who noted

Table 1. Dissection of *Anopheles pseudopunctipennis* collected indoors on human bait.

	No. of mosquitoes	Parity[1]		Christophers's stages[1]								No. of mosquitoes	No. of dilatations[2]				
		Nulliparous	Parous	1	2	2′	2″	3	4	4–5	5		0	1	2	3	4
Total	279	109	170	42	51	92	78	12	0	0	4	139	61	65	12	0	1
%	100.0	39.1	60.9	15.0	18.3	33.0	28.0	4.3	0	0	1.4	100.0	43.9	46.8	8.6	0	0.7
SD	11.7	7.6	5.3	7.6	3.3	4.6	4.2	0.9	0	0	0.6	9.7	7.1	6.1	1.4	0	0.3

[1] Dissections made between January and April 1993.
[2] Dissections made between March and April 1993.

Table 2. Dissection of *Anopheles pseudopunctipennis* collected resting on indoor surfaces.

	No. of mosquitoes	Parity[1]		Christophers's stages[1]								No. of mosquitoes	No. of dilatations[2]				
		Nulliparous	Parous	1	2	2′	2″	3	4	4–5	5		0	1	2	3	4
Total	457	194	263	48	92	171	125	17	2	1	1	200	108	78	12	2	0
%	100.0	42.4	57.6	10.5	20.1	37.4	27.4	3.7	0.4	0.2	0.2	100.0	54.0	39.0	6.0	1.0	0
SD	14.3	9.1	9.5	6.3	5.1	7.4	4.4	1.0	0.3	0.2	0.2	12.5	11.2	7.4	1.6	0.4	0

[1] Dissections made between January and April 1993.
[2] Dissections made between March and April 1993.

that in the case of bloodfed *An. pseudopunctipennis*, >50% exited from insecticide-sprayed houses 2–4 h after release. In contrast, Bown et al. (1991) reported that nearly 80% of fed and unfed *An. albimanus* exited from sprayed houses during a similar period. Our results demonstrated that a highly nulliparous population of *An. pseudopunctipennis* readily exited after being released inside the experimental house; only about 7.0% remained indoors for more than 9 h. Interestingly, Breeland (1972) reported that *An. pseudopunctipennis* did not begin moving into daytime resting sites until after 0500 h, reaching peak densities between 0600 and 0800 h. It appears that early morning exiting from the house is the first step of a multibehavioral sequence of finding daytime resting sites.

In summary, it was determined that a proportion of *An. pseudopunctipennis* females prefer resting on adjacent vegetation prior to moving indoors. Once inside the house, peak numbers resting on interior surfaces and biting humans occurred during the same time interval. Proportionally more parous mosquitoes preferred to bite in the early evening following which the overall proportion of nulliparous and parous mosquitoes fed at about the same rate. During the morning hours the numbers of parous mosquitoes biting human hosts had been disproportionally reduced. An overall evaluation of these results indicates that the behavior of *An. pseudopunctipennis* females depends on outdoor/indoor stimuli, being multivariable in nature. This includes contacts with a variety of biological (i.e., vegetation, man) and inert surfaces, which in part appears to be controlled by age structure. When considered as a whole, vector behavior presents alternative possibilities where intervention techniques could be focused in order to improve the effectiveness and assessment of traditional control programs.

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