

## BIONOMIC STUDIES OF THE *ANOPHELES* MOSQUITOES OF DAJABON, DOMINICAN REPUBLIC<sup>1</sup>

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**ABSTRACT.** Field studies of Dajabon Province, Dominican Republic, revealed that *Anopheles albimanus* and *An. vestitipennis* were the most abundant anopheline species followed by *An. crucians* and *An. grabhamii*. Three of the species were collected as adults and larvae whereas *An. grabhamii* was found only in the adult stage. Perennial ponds and rice fields were the most important larval habitats. The anophelines were predominantly exophilic and exophagic; however, outdoor resting sites could not be identified. Large numbers of mosquitoes were collected from corrals at night and also by using an animal-baited net trap and UV light traps. Man-biting collections showed an early evening peak of biting activity by *An. albimanus* and *An. vestitipennis*. Only 23% and 13% of the bites by the 2 species, respectively, occurred indoors.

### INTRODUCTION

The *Anopheles* fauna of the Dominican Republic includes *An. (Nyssorhynchus) albimanus* Wied., *An. (Anopheles) crucians* Wied., *An. (Ano.) grabhamii* Theobald and *An. (Ano.) vestitipennis* Dyar and Knab (Belkin and Heinemann 1973). Although little is known about the bionomics and vector potential of the anophelines of the country, *An. albimanus* is the only one considered to be a vector of malaria (Martin et al. 1982,<sup>3</sup> Pan American Health Organization 1984).

A study was conducted between July 1987 and October 1988 to determine which *Anopheles* species occur in the Dajabon area of the Dominican Republic and to study their bionomics including their breeding habitats, feeding contact with man, resting behavior and relative abundance.

### MATERIALS AND METHODS

**Study site:** A general description of the area and the rationale for selecting it as a study site were given in Mekuria et al. (1990). Situated at an elevation of 35 m above sea level, the predominantly flat area of Dajabon has a mean annual temperature of 25.5°C and a mean annual rainfall of 1,316 mm (Garcia 1976). According to the National Malaria Eradication Service (SNEM), houses in the area have not been sprayed with residual DDT since 1983.

Localities with recent autochthonous malaria

cases were visited to determine the types of dwellings, their accessibility and their proximity to potential mosquito breeding sites. Based on preliminary surveys, the following 4 locations with relatively high numbers of anophelines were selected for continued sampling: Barrio La Fe (later abandoned), Calle Duarte, Colonia Japonesa and La Bomba, all within ca. 4 km of Dajabon Town.

**Larval sampling:** Searches for anopheline larvae were made in perennial and transient man-made or natural bodies of water using dippers. Third and fourth instar larvae were immediately preserved for later identification. Earlier instars were allowed to develop in water from the collection site before being preserved. At the time of collection, the temperature and pH of sampling waters were determined.

**Adult sampling:** Mosquitoes were collected using aspirators, aerial nets and the space spray or spray sheet method (World Health Organization 1975a, Service 1976). Intradomiciliary collections were conducted before 0900 h. Extradomiciliary collections from vegetation, river banks, tree trunks, piles of hollow blocks and corrals were made during the day and night. Man-biting anophelines were captured using flashlights and aspirators from the exposed legs of 2 or 3 subjects seated inside and 2 or 3 others seated in the yard of a house (Service 1976). Whenever possible, biting collections were made simultaneously indoors and outdoors. Collections were limited to the first 45 min of each hour, thus allowing a break for collectors. Mosquitoes obtained by hourly indoor and outdoor biting collections were held in cages until they were identified and counted. These collections were used to determine periodicity of biting activity, man-biting density, the relative abundance of *Anopheles* species and indoor/outdoor biting intensity. To determine man-biting density, each 45 min collection was multiplied by 1.33 in order to compensate for the 15 min break.

Three types of light traps equipped with re-

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<sup>3</sup> Martin, G. G., J. Ayalde, F. J. L. Antuñano, R. M. Rodriguez, D. Gañan, D. R. S. Cury, E. J. Medina, and P. Mencia, 1982. Informe (general) de la Evaluación del Programa de Malaria en La Republica Dominicana. 66 pp. + annexes (Trip report).

chargeable 12V, electrolyte batteries (manufactured by Globe Union) and ultraviolet (UV) fluorescent tubes (Sylvania, GTE, F4T5/BLB; ca. 320–420 nm) were used to sample mosquitoes at night. They included the miniature UV down-draft and updraft traps (Wilton 1975) and the Fay-Prince UV (FP-UV) trap (Model 812, J. W. Hock Co., Gainesville, FL). The traps were usually operated between 1800 and 0600 h. Insects captured in the traps were killed and the anophelines sorted and identified to species.

A modification of a trap originally employed by Mitchell et al. (1985) was used to collect anophelines attracted to animals. The 2 × 2 × 2 m trap was made of nylon mosquito netting. After tethering the bait animal inside the trap at sunset, the bottom corners were tied to pegs, leaving about 30 cm open above the ground as ingress for mosquitoes. In the morning, the animal was removed, the trap lowered and the anophelines aspirated from trap interior. Goats, pigs, burros and calves were used as baits.

Two exit window traps (World Health Organization 1975a) were used to capture anophelines leaving dwellings. The traps (60 × 60 × 60 cm) were installed at sunset on houses known to yield high numbers of anophelines.

## RESULTS

Four *Anopheles* species known to occur in the Dominican Republic were found in Dajabon (Table 1). *Anopheles albimanus*, *An. vestitipennis* and *An. crucians* were collected both in the larval and adult stages whereas *An. grabhamii* was found only in the adult stage.

*Larval studies:* Anopheline larvae were found in a variety of man-made, often partially shaded freshwater habitats including rice fields, drainage ditches and seepage or overflow pools (Table 2). Larvae were also found in rain pools and perennial ponds. Animal watering ponds and

rice fields were the 2 most productive sources. Of 1,249 larvae collected, 98.2% were *An. albimanus*, 1.1% *An. vestitipennis* and 0.6% *An. crucians*. The larvae of *An. grabhamii* were not encountered. Larvae of the other 3 species were found protected by algal mats and emergent vegetation at peripheral sites of water bodies which had *Gambusia affinis* (Baird and Girard).

Rice fields extended well into town and *An. albimanus* was found breeding well within the town limits. The most productive rice fields were those that were recently planted and in which sunlight reached the water with little obstruction. Older fields in which the rice stands were broken by open spaces were also highly productive of *An. albimanus*. Larvae were not found in tall, thickly growing stands of rice; only drainage ditches along the edges of such fields harbored larvae. Sites where larvae were found often had some algal growth.

*Anopheles vestitipennis* larvae, though few in number, were found in all types of habitats except transient rain pools, and frequently occurred in the same microhabitats as for *An. albimanus* larvae. *Anopheles crucians* was collected only once from a rice field and twice from a pond. Thus, it appears that ponds and rice fields served as breeding sites for 3 *Anopheles* species.

*Adult resting habits:* Although each of 25 houses in areas known to yield anophelines was searched for at least 30 man-minutes for resting mosquitoes, only 2 *An. albimanus* and 2 *An. vestitipennis* were found. Only 6 *An. albimanus* were recovered in space spray collections from 35 houses, although thousands of culicine mosquitoes were collected from the same houses. Using exit window traps, in 9 trap-nights of collection during September 1987, only 17 *An. albimanus*, 17 *An. vestitipennis* and 1 *An. crucians* were captured. In over 45 man-hours of work, not a single anopheline was collected from

Table 1. Collection sources and number of specimens of *Anopheles* mosquitoes from Dajabon, Dominican Republic, on which specific identifications were made (1987–88).

Source*	No. of specimens by species			
	<i>albimanus</i>	<i>vestitipennis</i>	<i>crucians</i>	<i>grabhamii</i>
Larval collections	1,227	14	8	0
Indoor colls. (L/trap)	11,806	2,075	225	10
Outdoor colls. (L/trap)	22,510	963	405	19
Corral colls. (L/trap and hand catch)	3,767	346	36	6
Animal baited trap catch	924	607	78	3
Indoor colls. (man-biting)	184	92	11	0
Outdoor colls. (man-biting)	2,546	1,441	39	2
Indoor (aspirator and spray) and window trap colls.	25	19	1	0
Totals	42,989	5,557	803	40

\*L/trap refers to UV light traps.

Table 2. *Anopheles* breeding habitats in Dajabon, Dominican Republic, physical characteristics of the habitats and the species and numbers of larvae found in them as determined by surveys carried out during 1987 and 1988.

Habitat	No. of coll. events	No. of dups	pH (range)	Mean temp (°C)	No. (%) of specimens*				Dens/dip AL
					AL	VE	CR	Total	
Ditch	7	696	6.8–8.2	27.8	84	1	0	85	0.12
Pond	32	4,866	6.2–7.9	28.1	795	10	7	812	0.16
Pool	8	982	6.8–8.4	25.8	71	0	0	71	0.07
Rice field	13	1,518	6.8–9.9	27.4	277	3	1	281	0.18
Summary	60	8,062	6.2–9.9	27.6	1,227	14	8	1,249	0.15

\*AL: *An. albimanus*; VE: *An. vestitipennis*; CR: *An. crucians*.

Table 3. *Anopheles* species and their abundance in samples taken by animal-baited trap in Dajabon, Dominican Republic, during 1988.

No. of nights	Type of bait	No. of specimens by species*				
		AL	VE	CR	GR	Total
5	Burro	353	186	25	1	565
8	Calf	359	198	16	0	573
7	Pig	210	213	32	2	457
Total		922	597	73	3	1,595

\*AL: *An. albimanus*; VE: *An. vestitipennis*; CR: *An. crucians*; GR: *An. grabhamii*.

daytime outdoor resting sites including tree trunks, corrals, posts, embankments, piles of hollow blocks, stone quarry sites, and plantations of cassava, banana and plantain. In October 1988 5 boxes (40 × 40 × 40 cm) placed in a cassava plantation for 5 days and in a peanut farm for another 5 days were found to harbor no anophelines on daily checking. *Anopheles* mosquitoes were found in corrals at night when animals were present. In 69.3 man-hours of collection from corrals during March–October 1988, 3,362 *An. albimanus*, 345 *An. vestitipennis*, 36 *An. crucians* and 6 *An. grabhamii* were collected. They were found resting on the stakes and barbed wire that formed the corrals or on shrubbery around them.

**Attraction to animal baits:** From March to October 1988 the animal-baited trap was installed in the La Bomba area within 30 m of a cattle corral. All 4 *Anopheles* species were represented in the trap samples (Table 3). Collections made using pigs as bait had relatively high proportions of *An. vestitipennis* and *An. crucians*.

**Man-biting habits:** *Anopheles albimanus* and *An. vestitipennis* attacked human baits with a roughly similar trend both indoors and outdoors (Fig. 1). Outdoors, biting activity of *An. albimanus* increased sharply from sunset to the 2100–2200 h period and then declined gradually through the rest of the night, with a second but

minor peak at 0400–0500 h. Biting activity of this species peaked one hour earlier indoors than outdoors then fell sharply within 2 h to a very low level. *Anopheles vestitipennis* biting periodicity gradually increased to form a plateau and then declined. Very little biting occurred indoors by either species after 2400 h. Most biting occurred outdoors. Thus, in 10 nights of paired indoor/outdoor collections, 77.3% of 611 *An. albimanus* and 86.8% of 613 *An. vestitipennis* were caught biting outside. The difference between the numbers biting indoors and outdoors was highly significant for both *An. albimanus* ( $P < 0.01$ ) and *An. vestitipennis* ( $P = 0.01$ ) when compared using the Wilcoxon matched-pairs signed-ranks test (Siegel 1956). *Anopheles crucians* ( $n = 31$ ) and *An. grabhamii* ( $n = 2$ ) were collected in low numbers.

Seven all-night simultaneous indoor and outdoor biting collections were performed involving 15 man-nights inside and 15 outside. Considering only the samples collected between 1800 and 2200 h outside and between 2200 and 0600 h inside, of 236 *An. albimanus* 85.3% were captured outdoors and 14.7% indoors. In the case of *An. vestitipennis*, the proportion captured outside was 91.6% of 207 specimens. A person retiring indoors at 2200 h would thus have received 5.8 times as many bites by *An. albimanus* outdoors in 4 hours than indoors the rest of the night. For *An. vestitipennis* the outdoor/indoor ratio is much higher: 10.9. Indoor biting densities were invariably lower than outdoor densities (Table 4). The relative abundance of *An. albimanus* (outdoors  $n = 2,411$ ; indoors  $n = 145$ ) and *An. vestitipennis* (outdoors  $n = 1,366$ ; indoors  $n = 83$ ) in outdoor and indoor man-biting collections was not significantly different ( $\chi^2 = 0.151$ ,  $P = 0.697$ ).

**Light trap captures:** A total of 38,419 female *Anopheles* mosquitoes were collected indoors and outdoors using light traps (Table 5). The proportion of *An. vestitipennis* was considerably higher in animal-baited net trap and man-biting samples than in light trap samples (Table 6). The relative abundance of *An. albimanus* and

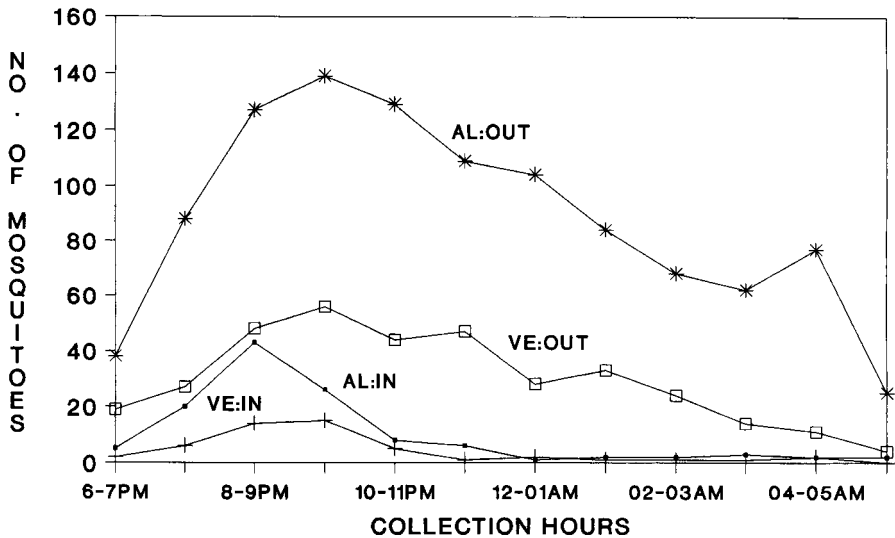


Fig. 1. Indoor (IN) and outdoor (OUT) biting cycles of *An. albimanus* (AL) and *An. vestitipennis* (VE) in Dajabon, Dominican Republic.

Table 4. Indoor (In) and outdoor (Out) man-biting densities of *Anopheles albimanus* and *An. vestitipennis* in Dajabon, Dominican Republic (1987-88).

Species	Coll. site	No. of man-nights	No. of specimens	Density/man-night*
<i>albimanus</i>	In	15	120	10.7
	Out	33	1,050	42.4
<i>vestitipennis</i>	In	15	50	4.4
	Out	33	355	14.3

\* For density determination the number of specimens was multiplied by 1.33 to make up for the 15 min of every hour not used for collection.

*An. vestitipennis* in paired man-biting and light trap samples was significantly different ( $\chi_1^2 = 208.82$ ,  $P < 0.001$ ). Indoor and outdoor densities (Table 7), compared using the Wilcoxon nonparametric test (SAS Institute 1985), were significantly different for both *An. albimanus* ( $\chi_1^2 = 26.89$ ,  $P < 0.001$ ) and *An. vestitipennis* ( $\chi_1^2 = 4.18$ ,  $P = 0.04$ ) in Calle Duarte. In Colonia Japonesa and La Bomba, however, such densities of *An. albimanus* were not significantly different ( $\chi_1^2 = 0.02$ ,  $P = 0.90$  and  $\chi_1^2 = 2.03$ ,  $P = 0.15$ , respectively) but were for *An. vestitipennis* ( $\chi_1^2 = 7.35$ ,  $P = 0.01$ ,  $\chi_1^2 = 4.70$ ,  $P = 0.03$ ).

## DISCUSSION

*Anopheles albimanus* was the most abundant of the 4 *Anopheles* species found in the Dajabon area. *Anopheles vestitipennis* was also found in relatively high numbers. *Anopheles crucians* and

*An. grabhamii* were relatively rare. *Anopheles (Ano.) pseudopunctipennis* (Theobald), not previously recorded from the Greater Antilles but recently reported from a coastal locality in Haiti by French workers (Molez et al. 1988), was not encountered.

*Anopheles albimanus* exploits a wide variety of man-made and natural aquatic habitats including fully or partially sunlit freshwaters and brackish waters of estuaries and lagoons (Horsfall 1972, Faran 1980). In the Greater Antilles, larvae of *An. albimanus* were found in many types of habitats (Belkin et al. 1970, Avila 1977), some of which are reminiscent of those described from Dajabon. The larvae of *An. vestitipennis* and *An. crucians* were found in association with *An. albimanus*.

Extensive rice cultivation in close proximity to human habitations provided important breeding sites for 3 of the 4 anopheline species. Not only does the abundance of irrigation water provide a suitable breeding medium, but it also renders the environment agreeable to adult mosquitoes by elevating atmospheric humidity and by promoting a luxurious growth of vegetation.

Attempts to collect *An. albimanus* populations from daytime resting sites has often been unsuccessful (Belkin et al. 1970, Hobbs et al. 1986). Breeland (1972) found *An. albimanus* under bridges, culverts and large rocks as well as in rock walls, ground holes, tree hollows and roots. Haitian populations of *An. albimanus* may use more exposed sites such as "ground vegetation" as diurnal harborages (Breeland 1974), thus raising the possibility that island strains may exhibit peculiar resting habits. In an area like Dajabon with diverse cultivated and wild vege-

Table 5. The number of anophelines collected by UV light traps from 3 types of habitats in Dajabon, Dominican Republic (1987-88).

Collection site	No. of trap-nights	No. anophelines collected*				
		AL	VE	CR	GR	Total
Corrals	5	405	1	0	0	406
Indoors	138	11,806	2,075	225	10	14,116
Yard/Out	268	22,510	963	405	19	23,897
Total	411	34,721	3,039	630	29	38,419

\*AL: *An. albimanus*; VE: *An. vestitipennis*; CR: *An. crucians*; GR: *An. grabhamii*.

Table 6. Relative abundance of *Anopheles albimanus* (AL) and *An. vestitipennis* (VE) in samples obtained using different collection methods from indoors (In) and outdoors (Out) in Dajabon, Dominican Republic (1987-88).

Coll. method <sup>1</sup>	Coll. site	No. of specimens			Proportions (%)	
		AL	VE	Total	AL	VE
Larva	Out	1,227	14	1,241	98.9	1.1
ABNT	Out	922	597	1,519	60.7	39.3
MBC	In	145	83	228	63.6	36.4
	Out	2,411	1,366	3,777	63.8	36.2
LT	In	11,613	2,072	13,685	84.9	15.1
	Out	20,914	863	21,777	96.0	4.0
MBC <sup>2</sup>	Out	725	364	1,089	66.6	33.4
LT <sup>2</sup>	Out	575	15	590	97.5	2.5

<sup>1</sup> Coll. method: Larva—dipping; ABNT—animal-baited trap; MBC—man-biting coll.; LT—light trap coll.

<sup>2</sup> These MBC and LT collections were done on the same nights and in the same localities. The data are included in the other MBC and LT data in this table.

Table 7. The density of *Anopheles albimanus* and *An. vestitipennis* at 3 locations in and around Dajabon Town, Dominican Republic, as determined using Fay-Prince UV light traps installed indoors (In) and outdoors (Out).

Locality	Coll. site	No. of trap-nights	Density/trap-night	
			<i>albimanus</i>	<i>vestitipennis</i>
Calle Duarte	In	34	11.2	0.5
	Out	89	58.4	1.3
Colonia Japonesa	In	46	157.2	16.0
	Out	51	137.2	2.0
La Bomba	In	33	60.5	9.2
	Out	61	68.3	4.3

tation, *Anopheles* mosquitoes can disperse widely and be difficult to find.

Animal-baited trap captures and nocturnal collections from corrals have been widely used for sampling *An. albimanus* (Lowe and Bailey 1981). In the present study, these methods were useful sources of live and blood-fed specimens that were used for bioassays and for breeding purposes.

Window traps have been found inefficient at capturing *An. albimanus* escaping from houses (Trapido 1952, Muirhead-Thomson and Mercier 1952). In Dajabon, the houses had too many openings in the walls and open spaces between the wall and roof so that window traps would have been unproductive even if the mosquitoes went inside the houses. However, indoor biting

activity by *An. albimanus* and *An. vestitipennis* was also very low.

The bimodal outdoor biting cycle of *An. albimanus* found in Dajabon is similar to that described from Jamaica (Muirhead-Thomson and Mercier 1952) and Central and South America (Elliott 1968, Bown et al. 1984). In Haiti, Hobbs et al. (1986) reported a biting cycle of *An. albimanus* wherein peak biting occurred during the first hour after sunset and then declined at a linear rate to sunrise. Biting activity with an evening and predawn peak is characteristic of many *Anopheles* species (World Health Organization 1975b).

As far as could be determined, only incidental observations of the biting activity of *An. vestitipennis* have been made (Foote and Cook 1959,

Belkin et al. 1970). This mosquito fed on man in relatively high numbers in Dajabon. It was found naturally infected with sporozoites in Belize (Kumm and Ram 1941) and was considered by early workers to play a major vectorial role in Jamaica (Boyd and Aris 1929).

Both *An. albimanus* and *An. vestitipennis* were found to be predominantly exophagic in Dajabon. Exophagy in *An. albimanus* has been demonstrated wherever it was studied, including Jamaica (Muirhead-Thomson and Mercier 1952) and Haiti (Hobbs et al. 1986). The proportions feeding outdoors in the 2 countries were similar to those found in Dajabon.

Although simultaneous indoor and outdoor biting collections provide an insight into the biting habits of the anophelines, to determine the significance of such habits in the transmission of malaria, one has to relate the biting activity of the mosquito to the behavior patterns of the people. The indoor retirement time of Dajabon residents was determined to be between 2200 and 2300 h. Residents generally spent the evening hours on the porch or in the yard where it is cooler than in the house. Given such human behavior and based on the exophagic habits of the anophelines, the chances of contracting malaria outdoors within the first 4 hours of the night are much higher than they are during the rest of the night indoors. The effect of exophagy in transmission and control of malaria has received much attention (Breeland 1974, Hobbs et al. 1986).

The value of UV-light traps in sampling *An. albimanus* populations, replacing or supplementing other widely used methods has been shown by Taylor et al. (1975) and Sexton et al. (1986). In the present study, light traps were the main source of anophelines collected. Light traps may not attract all species of mosquitoes to the same extent (World Health Organization 1975a). In Dajabon, much lower proportions of *An. vestitipennis* were captured by UV-light traps as compared with other methods of adult sampling. Since the proportion of *An. albimanus* and *An. vestitipennis* was virtually the same in samples taken by animal-baited trap and man-biting capture, the extent of their zoophily/anthropophily can be considered similar. Their relative abundance is thus probably best estimated by these methods of sampling.

One major reason for studying the bionomics of *Anopheles* mosquitoes is to obtain information useful in selecting appropriate vector control measures. Indoor application of insecticides remains the most widely used measure to combat malaria throughout the endemic world, including the Dominican Republic. However, in Dajabon all the findings suggest that indoor spray-

ing will not afford a level of protection from malaria that is commensurate with the operational complexities and expenses to be incurred. A sound case detection and treatment program combined with some source reduction and antilarval measures can be considered for routine control operations. These measures can be strengthened by space application of insecticides when high anopheline densities or malaria outbreaks occur.

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