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Mosquito Anthropophily: Implications on Malaria Transmission in the Northern Brazilian Amazon

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ABSTRACT - Seasonal variation of adults and larvae of anophelines was studied during 2003 and 2004 in Roraima State, located in the Northern region of Brazilian Amazon. Species diversity increased with distance of capture to human dwellings. Greater diversity was found in extradomiciliary collections than in peridomiciliary or intradomiciliary. A significant association between *Anopheles darlingi* Root and *An. albitarsis* (s.l.) Arribálzaga (Diptera: Culicidae) breeding sites and the proximity to human dwellings was observed. Malaria Sporozoite Antigen Panel Assay (Vectest^{TM} Malaria) indicated *An. albitarsis* s.l. as one of the local vectors in the studied area. In this study, an index to describe the anthropophilic behavior of each anopheline species is proposed.

KEY WORDS: *Anopheles darlingi, Anopheles albitarsis,* anthropophily, malaria, Brazil

In the Amazon Basin, *Anopheles darlingi* Root (Diptera: Culicidae) is believed to be the most important malaria vector (Tadei & Thatcher 2000), but other species of anophelines have also been implicated as vector of malaria in several other Amazonian states (de Arruda *et al* 1986, Póvoa *et al* 2006, Galardo *et al* 2007). Factors determining which species will act as regional vectors are important for control strategies, but are still poorly understood (Rubio-Palis & Zimmerman 1997). Anthropophily is an important factor in determining a vector capacity to transmit a disease (vectorial capacity), yet the term lacks precise definition and little information is available on its quantification (WHO 2002).

In here we correlate species diversity with the distance of capture to human dwellings as well as characteristics of anopheline breeding sites and key determinants of the presence of larvae, including distance to dwellings. A method for easily comparing the degree of anthropophily of each species of anopheline identified in this area is proposed in this study. Additionally, *Plasmodium* infectivity of adult mosquitoes was studied.

Material and Methods

Study sites. Two different ecosystems of Roraima State, in the Northern Amazonian region of Brazil were studied. One site was in the savanna area of Boa Vista (02°49'N, 60°40'W), bordering the gallery forest of the Branco River. The other site was in the rainforest, near a small temporary river (00°51'N, 60°21'W). These sites, as well as climates, have been described elsewhere (Barros *et al* 2007).

Adult mosquito surveys. Adult mosquitos were collected over six bimonthly periods from August 2003 to July 2004; comprising both dry (November to April) and rainy (May to October) seasons. Mosquitoes were collected when landing, following procedures approved by the Oswaldo Cruz Ethics Committee. Capturers were placed inside houses (intradomicilliary), 30 m away from the nearest house (peridomiciliary) and > 50 m away from the nearest house (extradomiciliary). Captures were performed over four days at each study site and always around the new moon. Mosquitoes were captured from 6PM to 6AM for two nights, then from 6PM to 10PM on the next two nights. In both study areas, captures were performed predominantly around the same house over the entire study period, but for comparison purposes several other houses were also systematically sampled when possible. Further details were previously described (Barros *et al* 2007).

Larval surveys. During the same collection periods, extensive larval surveys were conducted using a standard 500 ml dipper (Bioquip Co., Gardena). An effort was made to survey all anopheline breeding sites within 1 km from human settlements. Collections were made for 10 min to 40 min in the morning. The number of samples taken depended on the size of the breeding site. Based on the knowledge that *Nyssorhynchus* and

Stethomyia larvae are rarely found in open water, collections were made along the perimeter of the habitats in emerging vegetation, algae or surface debris. Larvae and pupae were collected and kept in flask containing breeding site water. Pupae and first/second instars larvae were reared for identification. Mosquitoes were identified following the key of Consoli & Lourenço-de-Oliveira (1994).

All large sites and most of the small water collections, with few exceptions, were sampled on every occasion. For each breeding site, the following physicochemical and environmental parameters were determined: soil type, bordering vegetation, pH, light incidence, and stream flow. The distance from the breeding site to the nearest house was also estimated by GPS. Millimetric rods measured the depth and size of the water bodies. The pH was determined by colored dipsticks (Merck™) and light incidence and stream flow were visually estimated and ranked.

Malaria sporozoite infection. To determine which species of *Anopheles* was transmitting malaria in this region an immunological assay (VecTest^{TM} Malaria) that identifies the presence or absence of specific peptide epitopes of circumsporozoite (CS) protein of *Plasmodium falciparum* and two strains of *P. vivax* (variants 210 and 247) was used (Ryan *et al*. 2001). This test has been extensively evaluated and presents an overall 92% sensitivity and 98.1% specificity as compared to standard CS enzyme-linked immunosorbent assay (ELISA) (Ryan *et al* 2002).

Data analysis. Analysis of variance (ANOVA) was used to compare the log-transformed number of adult mosquitoes captured during the year. Margalef´s diversity index (Margalef 1957) was used for determining species diversity in the different adult capturing points. This index was calculated as $D = (S – 1) / \ln N$, where S = number of species and N = number of individuals.

We propose an index to describe the degree of anthropophily of a given species, (Q), obtained by: $Q = P + I / 2*E$, where *P, I* and *E* represent the mean peridomiciliary, intradomiciliary and extradomiciliary biting rates of a given species over a period of time. The calculated anthropophilic indices for the species that occurred in our study areas were compared with data derived from published biting rates in Rondonia by Lourenço-de-Oliveira *et al*. (1989).

Multiple stepwise logistic regressions were performed for each species using dependent variables describing the presence or absence of a species in a given breeding site throughout the year. Parameters analyzed were soil type (soft sand = 0, hard sand = 1, mud = 2 and algae = 3), bordering vegetation (grass = 0, grass and algae = 0.5, algae = 1, leaves and algae 1.5 and leaves only 2.0), light incidence at midday (completely shaded = 0, partially shaded = 1 or totally sunlit = 2). Continuous parameters analyzed were the distance to the nearest inhabited house, pH, depth and the size of water bodies. The best regression subsets were analyzed and parameters were independently retested for significance.

Results

Adult mosquito surveys. A total of 13,046 specimens of anophelines were captured, belonging to eight species (Table 1). *Anopheles darlingi*, *An. albitarsis* s.l., *An. evansae* Brèthes, *An. triannulatus* s.l. Neiva & Pinto and *An. nuneztovari* Gabaldon were found in both savanna and forest ecosystems. *Anopheles braziliensis* Chagas and *An. peryassui* Dyar & Knab were recorded only in the savanna area and *An. oswaldoi* Peryassu was found only in the forest area. Adult densities of *An. triannulatus* s.l. fluctuated during seasons, reaching higher levels during the dry season. ANOVA of the log(n+1) transformed mean densities produced significant results ($F_{(5,594)} = 4.0$; $P < 0.01$). This was in sharp contrast with other species. In the savanna area, peak densities of *An. triannulatus* s.l. occurred in January (during dry season), and it was almost undetectable between May to August, which corresponds to the wet season. On the opposite, *An. darlingi, An. nuneztovari, An. albitarsis* s.l., *An. peryassui* and *An. braziliensis* had peak densities during the wet season (July to August) and reached their lowest levels in the dry season (November to May) (ANOVA, $P < 0.05$). In the forest area, due to the low number of captured specimens, seasonal trends were not observed except for *An. darlingi*.

A well defined crepuscular biting pattern was seen for *An. braziliensis*, *An. nuneztovari*, *An. peryassui* and *An.*

Table 1 Adult anophelines captured from August 2003 to July 2004 in the savanna area (*S*) and forest area (*F*).

Month	*dar*		*alb*		*bra*		*nun*		*per*		*tri*		*eva*		*osw*	
	S	*F*	*S*	*F*	*S*	*F*	*S*	*F*	*S*	*F*	*S*	*F*	*S*	*F*	*S*	*F*
Aug	974	179	1624	45	61	0	41	3	57	0	2	2	1	1	0	1
Nov	45	433	2217	102	72	0	0	8	25	0	7	8	0	1	0	2
Jan	24	460	338	85	18	0	0	0	6	0	10	4	0	0	0	0
Mar	19	397	231	18	3	0	0	2	1	0	1	2	0	1	0	1
May	428	470	851	263	8	0	24	92	1	0	0	19	0	0	0	0
July	424	254	2395	112	49	0	66	10	46	0	0	1	0	1	0	0
Total	1914	2193	7656	625	211	0	131	115	136	0	20	36	1	4	0	4

Abbreviations: dar = *An. darlingi*; alb = *An. albitarsis* s.l.; bra = *An. braziliensis*; nun = *An. nuneztovari*; per = *An. peryassui*; eva = *An. evansae*; tri = *An. triannulatus* s.l.; osw = *An. oswaldoi*.

triannulatus s.l., with highest densities soon after sunset (18:00h), dropping exponentially thereafter and reaching < 0.2 bites/man/hour before 22:00h (data not shown). The pattern was similar in both areas and was maintained throughout the year. There were no significant differences in the hourly biting indices between *An. darlingi* and *An. albitarsis* s.l. and the other species.

Margalef´s diversity index. *Anopheles darlingi* and *An. albitarsis* were commonly collected indoors in both study areas, whereas other species were almost absent (< 0.5 bites/man/night). When relative densities were used to obtain Margalef's diversity indices for mosquitoes captured in forest and savanna areas, values varied widely according to the distance from human dwellings (indoors, peridomestically and extradomiciliary). For the forest area, Margalef's indices were 1.81 indoors, 1.87 for the peridomestic and 5.83 for the extradomestic environment. For the savanna area, indices were 1.83 indoors, 1.87 for the peridomestic and 5.87 for the extradomestic environment.

Anthropophily index. Anthropophily indices were different among species collected in savanna and forest areas, with *An. darlingi* displaying the highest index. Nonetheless anthropophily indices did not vary significantly within species. Anthropophily indices for *An. darlingi* were 1.24 and 1.26 in the savanna and forest areas, respectively. Data from Rondonia (Lourenço-de-Oliveira *et al* 1989) indicated an index of 1.51. For *An. albitarsis* s.l., anthropophily indices were 0.47 and 0.56 in the savanna and forest areas (1.15 in Rondonia). For *An. nuneztovari* the anthropophily indices were 0.21 and 0.25 for the savanna and forest areas, respectively (0.11 in Rondonia). *Anopheles triannulatus* s.l. anthropophily indices were 0.07 in the savanna and 0.23 in the forest area (0.06 in Rondonia).

Malaria sporozoite infection. A total of 9,400 adults specimens were analyzed for malaria parasites. Only five *An. albitarsis* s.l. were found naturally infected with *P. vivax*. All specimens were captured in the savanna region, during July, 2004. Another 7,466 *An. albitarsis* s.l. specimens from the savanna and 380 specimens from the forest area tested negative. This corresponded to an infestation rate in the savanna, of 0.07% (5 out of 7466) or a total species infestation rate of 0.06% (5 out of 7846 specimens from the forest and savanna areas together). The entomological inoculation rate for *An. albitarsis* s.l. in July, when 2,494 mosquitoes were caught, was 0.2%, therefore there would be 0.39 infective bites/person/night and 11.60 bites/person during July.

The number of individuals of each anopheline species, captured from the forest and savanna areas, respectively, were, *An. darlingi*, 1841 and 1669; *An. nuneztovari,* 92 and 81; *An. triannulatus* s.l. 25 and 71; *An. peryassui* 0 and 61; *An. braziliensis* 0 and 100. All these tested negative for *P. falciparum* and *P. vivax*.

Larval surveys. The larval surveys retrieved 1,490 specimens, comprising all the species identified during adult surveys. The following zoophilic species were only identified in their larval stage: *An. mediopunctatus* Theoblad*, Chagasia bonneae* Root*, An. strodei* Root*,* and *An. mattogrossensis* Lutz & Neiva.

Multiple regression analysis indicated that the presence of shade and the proximity to human dwellings (P < 0.0001) were the best predictors for explaining the occurrence of *An. darlingi* breeding sites (Table 2). *Anopheles albitarsis* s.l. was associated with the proximity to dwellings, but not with other variables (P < 0.01) (Table 3). Meanwhile, *An. triannulatus* s.l., was associated with sunny breeding sites (P < 0.05), but also had an association with the proximity to dwellings (P < 0.05) (data not shown). Sunlit areas are usually those deforested by villagers living close by. *Chagasia bonneae* and *An. mediopunctatus* were both related to deep breeding sites and sandy bottoms (P < 0.01). No clear association was verified for *An. nuneztovari* or *An. peryassui*. Other species were not tested due to insufficient numbers.

Discussion

Both *An. albitarsis* s.l. and *An. darlingi* are well-documented anthropophilic mosquitoes (Consoli & Lourenço-

Table 2 Multiple stepwise logistic regression analysis of the presence or absence of *Anopheles darlingi* larvae as the dependant variable and breeding site parameters as independent predictors[1].

	β	Std. error β	t	P-level
Light incidence	-0.54	0.1294	5.2247 (18)	0.0001
Distance to nearest house	-0.45	0.1556	6.1598 (17)	< 0.0001
Soil type	0.22	0.2040	3.4705 (16)	0.0032
Depth	0.31	0.3001	0.9270 (15)	0.3686
Bordering vegetation	0.42	0.4083	-0.5972 (14)	0.5599
Size	0.35	0.3475	-1.289 (13)	0.2199
Current	-0.27	0.3594	-0.2158 (12)	0.8327
PH	-0.12	0.6898	-0.1820 (11)	0.8589

[1]Overall $R^2 = 0.8664$, $F_{(7,12)} = 11.1142$ $P = 0.0002$

Table 3 Multiple stepwise logistic regression analysis of the presence or absence of *Anopheles albitarsis* s.l. larvae as the dependant variable and breeding site parameters as independent predictors[1].

	β	Std. error β	t	P-level
Distance to nearest house	-0.44	0.1431	3.3437 (18)	0.0034
Light incidence	0.17	0.1628	2.5638 (17)	0.0195
Bordering vegetation	0.43	0.2945	0.2699 (16)	0.7905
Soil type	0.29	0.3847	0.6834 (15)	0.5042
Depth	0.14	0.4850	0.9171 (14)	0.3736
Current	0.13	0.5524	1.0310 (13)	0.3199
Size	-0.07	0.5717	-1.0041 (12)	0.3338
PH	-0.03	1.1787	0.4019 (11)	0.6948

[1] Overall $R^2 = 0.2208$, $F_{(1,19)} = 4.5268$, $P = 0.0466$

de-Oliveira 1994). Apart from the already mentioned behavior of *An. triannulatus* s.l., with peak numbers during the dry season, the biting activity of anthropophilic mosquitoes did not differ significantly from the zoophilic ones, with peak densities during the wet season.

The lowest values of Margalef's diversity index were found indoors, reflecting a decrease in the number of species due to adaptation to a specific feeding behavior. Interestingly, mosquito species retrieved near abandoned houses were similar to those in extradomiciliary sites, suggesting that the constructions *per se* were not influencing behavior, having human presence as a key factor.

The finding that anthropophilic species are commonly collected indoors, while zoophilic species were almost absent, suggests that anthropophily can be quantified by comparing mosquito captures at varying distances from habitations, i.e. intradomiciliary, peridomiciliary and extradomiciliary collection points. Zoophilic mosquitoes would tend to be more randomly dispersed, while anthropophilic ones would be recovered in greater numbers as capture sites were stationed closer to inhabited houses.

A reproducible quantitative measurement of the degree of anthropophily, i.e., the "anthropophilic index" (*Q*), could be easily obtained. Log transformations may be more appropriate when dealing with smaller samples, but were less robust with our data. There are advantages in using such an index as compared to performing simultaneous captures on human and animals, which usually require a large team, and cumbersome infrastructure. When the anthropophilic indices for the species that occurred in our study areas were compared with data from Rondonia, values were found to be relatively the same for each individual species and differed significantly among them by Friedman ANOVA ($F_{3,1} = 9.0$; $P < 0.05$). Species could be roughly ordered in decreasing degree of anthropophily as: *An. darlingi* > *An. albitarsis* s.l.> *An. nuneztovari* > *An. triannulatus* s.l. These results correlate well with the known behavior of such vectors (Rachou 1958).

A previous study using ELISA (Enzyme-linked Immunosorbent Assay) has implicated *An. albitarsis* s.l. as the most significant malaria vector in the contact area (Silva-Vasconcelos 2002), but *An. darlingi* also played a role. The predominance of *An. albitarsis* s.l. can be attributed to the nearby savanna vegetation, an environment to which this species is well adapted (Rozendaal 1990). Despite the sensitivity and specificity of the VecTest™, it remains notoriously difficult to detect infected mosquitoes in the field. One should be very cautious in implying that a species is not responsible for malaria transmission, even when it tests negative on serial studies. It is not uncommon for well-established vectors to test negative during malaria epidemics due to the small numbers of naturally infected mosquitoes captured in the field (Mercia Arruda, unpublished data). Adequate sample sizes in the field are unknown. In the forest area, probably due to the low hypoendemic malaria transmission rates, none of the species were found infected.

The results of multiple regressions on larval data showed a significant association between *An. albitarsis* s.l. and *An. darlingi* larvae and the distance to human dwellings. The occurrence of larvae of both anthropophilic species was associated with the distance to human dwellings, whereas non-anthropophilic species were related to other factors. Data suggested that determining key breeding site parameters could distinguish between anthropophilic and non-anthropophilic mosquitoes.

Through systematic comparison of biting and breeding behavior of mosquitoes, we have attempted to derive methods of quantifying the association of anopheline species with human presence. Further results are necessary for validating the observations presented here, refining the proposed index and defining its applicability in field studies. The degree of anthropophilic behavior could ultimately assist in determining which species are potential regional vectors, improving our knowledge of malaria prevalence models.

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