Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 11 - Biting activity and blood-seeking parity of *Anopheles (Kerteszia)* in South-Eastern Brazil*

Estudos sobre mosquitos (Diptera: Culicidae) e ambiente antrópico. 11 - Atividade hematófaga e paridade de Anopheles (Kerteszia) *na região Sudeste do Brasil*

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Abstract

An. (Ker.) cruzii and *An. (Ker.) bellator* were monitored by the use of human bait and Shannon trap collections during a one-year period in 1994. Indoor and outdoor collections were made on human bait and inside the forest environment a Shannon trap was used. Both were undertaken in the evening crepuscular period. Results showed a greater abundance of *An. cruzii* in the forest, where as *An. bellator* was more abundant in the domiciliary environment. Through the application of the Polovodova method an age grading was established. Computing the parous and nulliparous females with Christopher's Stage III and above, more than 30.0% of blood-seeking specimens of both anophelines had had a previous blood meal. The higher abundance of *An. bellator* as compared with that of *An. cruzii i*n the domiciliary environment may be explained by the influence of the neighboring low-lying coastal islands of the estuary system.

Anopheles, physiology. Ecology, vectors.

Resumo

Ao longo do período anual de 1994 foram realizadas observações sobre a atividade de An. cruzii *e* An. bellator *em isca humana e armadilha de Shannon. Aquela utilizada nos ambientes intra e peridomiciliar, enquanto esta o foi dentro da floresta natural pertencente ao sistema da Mata Atlântica. As coletas foram levadas a efeito concomitantemente, com ritmo quinzenal. A espécie* An. cruzii *manteve maior abundância no meio florestal mas não no domiciliar, onde* An. bellator *manteve freqüência de maior endofilia do que aquele. A paridade foi estimada mediante a adoção da técnica de Polovodova. Considerando*

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o conjunto das fêmeas oníparas e das nulíparas, que desenvolveram o estágio III ou além, de Christophers, pôde-se concluir que mais de 30.0% dos espécimens atraídos pela isca humana devem ter realizado previamente, pelo menos, um repasto sangüíneo. A maior abundância de An. bellator *pode ser explicada, ao menos em parte, pela influência da proximidade do sistema de ilhas estuarinas locais.*

Anopheles, fisiologia. Ecologia de vetores.

INTRODUCTION

Some data have already been published on the anophelines of the subgenus *Kerteszia* in the Ribeira Valley region of S. Paulo State, Brazil (Forattini et al.[4], 1993). These mosquitoes are regarded as local malaria vectors in a region which belongs to the Atlantic Rain Forest System. Nevertheless, the factors recognized as determinants of their vectorial capacity are not yet fully known. In the above-mentioned paper, synanthropic and blood-seeking parity patterns were described. The results there reported focused mainly on *Anopheles cruzii* and *An. bellator*, the former appearing as the dominant species, both in indoor and outdoor human bait catches. At the same time, information as to general parity conditions and endophagy parameters were obtained.

The length of the gonotrophic cycle, the time between blood meals, is recognized as a relevant transmission factor. As it is environmentally dependent, there is some reason for positing the hypothesis that mosquitoes might have different life spans in different places and/or seasons that would have a bearing on malaria transmission potential. Therefore, age-grading studies are appropriate in the attempt to measure the effects of these external factors (Clements[1], 1992). Also, by monitoring the age-composition of blood-seeking mosquitoes it should be possible to gather valuable data about the regional potential for malaria endemicity.

A further study, described below, focused on another area in the same region and allowed us to observe the influence of the local environmental on mosquito behaviour by a comparison with the previous study. Parity, seasonal abundance and biting cycle of these species were assessed and the results are presented here.

STUDY AREA

The present study was conducted on the "Sítio Gentil" (Gentil Farm), the localization of which is given in the map in Figure 1 (24°79' S and 47°66' W). It is in Cananéia County on the northern shore of the "Mar de Dentro" (Inner Sea) opposite Cananéia Island. The whole area is recognized as a natural undisturbed ecosystem (Hannah et al.[10], 1994). The environment is representative of the "Mata Atlântica" (Atlantic Rain Forest) covering the slopes of the regional Paratiu Mountain chain that in turn belongs to the "Serra do Mar" (Coastal Mountain Range) system. The anthropic environment is represented only by scattered dwellings along the Cananéia-Pariquera Açu road, usually located near the forest but without intensive agricultural activities. Facing the study area there is the large "Ilha Comprida" (Long Island) estuary system consisting of several channels, one of them being the "Mar de Dentro", where extensive mangrove vegetation (*Rhizophora, Laguncularia*) grows in salt marshes. In front of these there are extensive low-lying islands bordering the sea shore in the form of strips, largely covered by woody vegetation called "restinga" (wooded coastal lowland). Mosquito catches were undertaken in the local environment both in a house where the indoor and outdoor catches were made, as well as those which were carried out inside the nearby natural forest (Figs. 1 and 2).

MATERIAL AND METHOD

Biting activity was monitored fortnightly using two human bait collectors working simultaneously, one of them placed indoors and the other outdoors. Adult mosquitoes were caught as they landed on the collector, during a two-hour period starting at sunset. Due to seasonal variation, daily crepuscular data were obtained from the Nautical Almanac tables for local time. Mosquitoes were collected in this manner from January to December 1994.

For purposes of comparison, other adult collections were undertaken at the same time inside the natural forest environment between 17.00 and 20.00 hours by the use of a Shannon trap. It was necessary to establish this schedule, for operational reasons, so as always to include the sunset. Shannon trap collections were routinely performed from June 1993 to April 1995 thus prior and subsequent to the domiciliary collections.

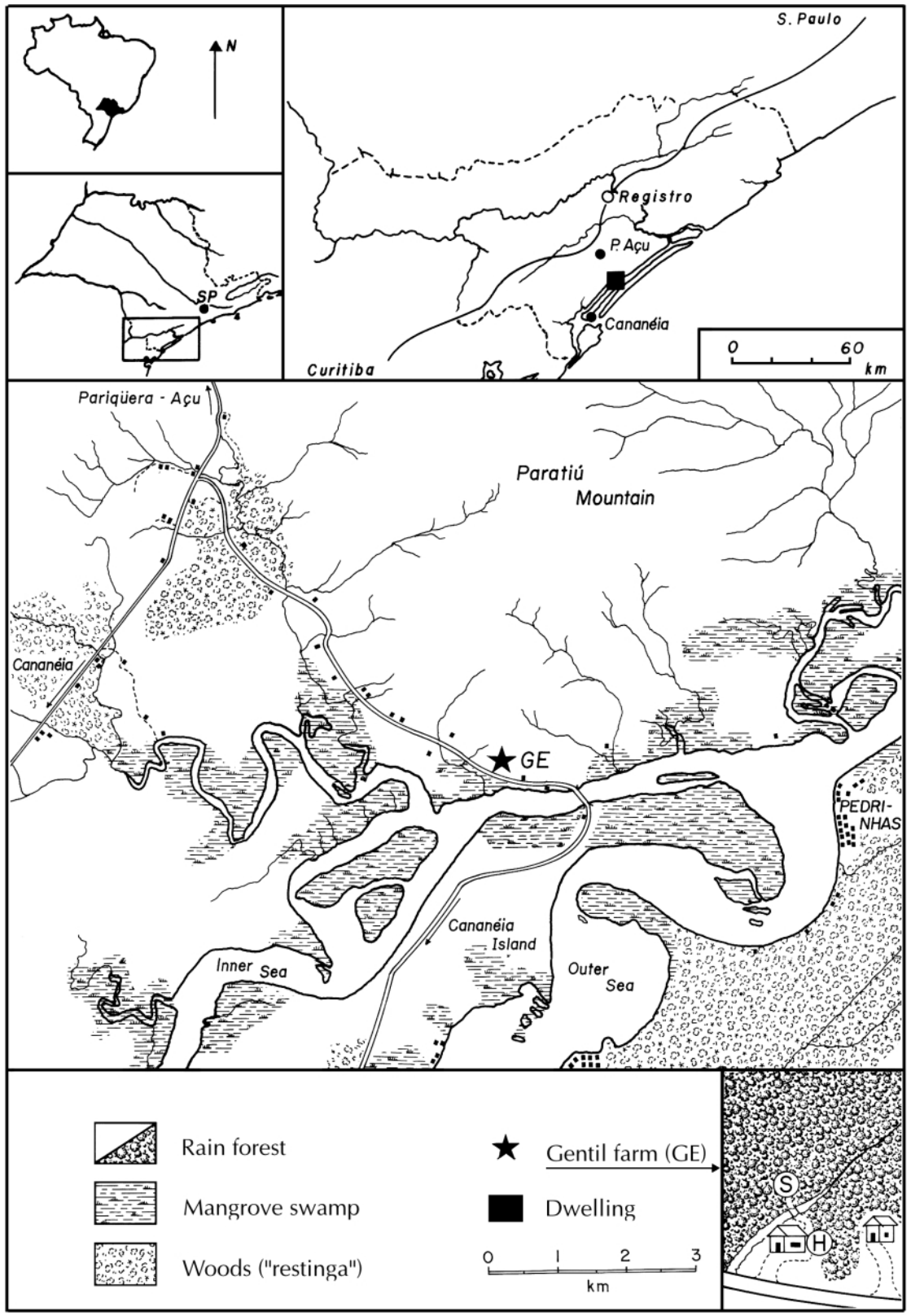

Figure 1 - Map of the study area showing the location of the Gentil Farm (Sítio Gentil) and the neighboring ecosystem. Cananéia County, S.Paulo State, Brazil.

Figure 2 - House of the Gentil Farm (Sítio Gentil) where indoor and outdoor catches were undertaken. The nearby natural rain forest may be seen in the background.

Randomly sampled adult females from the domicile collections were dissected to determine the state of the ovaries and of the follicular stalks. Parity conditions were determined and interpreted in accordance with the traditional technique (Detinova[2], 1962). Ovarian follicle stages were identified according to Christophers' classification (Clements[1], 1992). Due to gonotrophic discordance, nulliparous females reaching only stage II were considered as "pre-gravids"(Gillies[6,7], 1954, 1955). Besides these specimens, resting adults collected by the aspiration technique inside the forest environment were also dissected (Forattini et al.[5], 1993). Recently a new model of mosquito oogenesis has been suggested based on original morphological and functional observations (Sokolova[11], 1994). This model is still subject to discussion and not yet universally accepted.

To express the biting activity, Williams' mean ($\overline{X}_W$) was used as originally described (Haddow[8,9], 1954, 1960). For the collections with the Shannon trap the number of mosquitoes was expressed as an hourly arithmetic mean ($\overline{X}$). Macroclimatic data concerning rainfall and temperature levels were obtained from the records of the Cananéia Meteorologic Station belonging to the "Instituto Oceanográfico" (Oceanography Institute) of the University of S. Paulo (Fig. 3).

RESULTS

A total of 24,091 *Anopheles (Kerteszia)* females were obtained, 7,561 by means of the human bait collections and 16,530 through the use of the Shannon trap, as follows:

	Human bait		Shannon trap		Total	
An. cruzii	1,795	(23.7%)	9,492	(57.4%)	11,287	(46.9%)
An. bellator	5,766	(76.3%)	7,038	(42.6%)	12,804	(53.1%)
Total	7,561	(100.0%)	16,530	(100.0%)	24,091	(100.0%)

As a general yearly rainfall pattern, a wet and a dry period are evident. As shown in Figure 3, the heaviest rainfall occurs from October to April thus distinguishing the wet season while May to September correspond to the dry one. Both species were collected during throughout the period but, as was to be expected, with greatest abundance during the wet season, as shown by Figures 4 and 5.

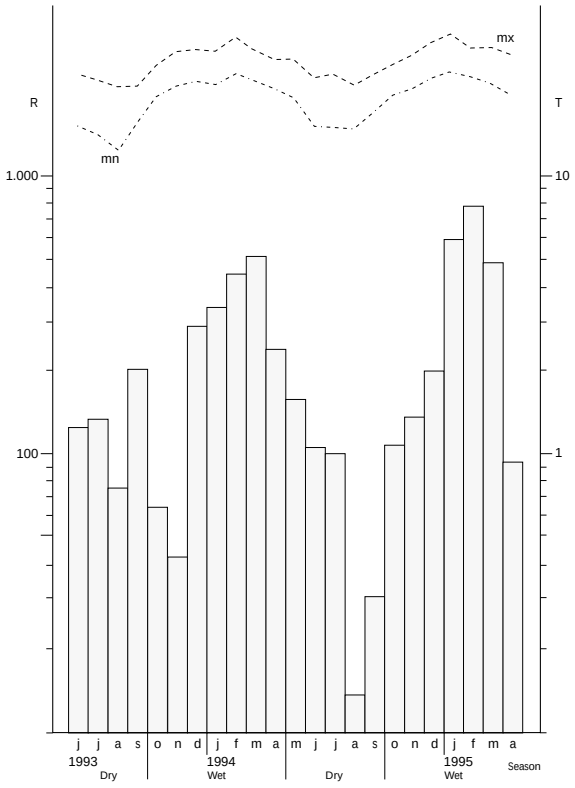

Figure 3 - Meteorological pattern of the study area. R - rainfall (mm) represented by bars. T - mean temperatures (ºC), maximal (mx) and minimal (mn).

From the human bait collections, *An. bellator*'s indoor presence (81.1%) was higher than that of *An. cruzii* (18.9%) as a proportion of the total number of specimens caught (Table 1). Comparing both the indoor and outdoor mosquitoes catches, they varied significantly ($x^2 = 33.39$; $P < 0.00000001$). Thus, *An. bellator* was at least four times more frequent than *An. cruzii* in the dwelling house environment.

Through hourly arithmetical means it was possible to compare the results obtained under domiciliary conditions, as a whole, with those of the primitive natural forest (Tables 1 and 4). The comparisons of the means showed *An. cruzii* as predominant in the natural environment, with an annual mean of 60.8 whereas that for *An. bellator* was 45.1. With respect to the domiciliary environment, *An. cruzii*'s annual mean was 17.3, thus differing significantly from bellator's ($x^2 = 197.97$; $P < 0.00000001$).

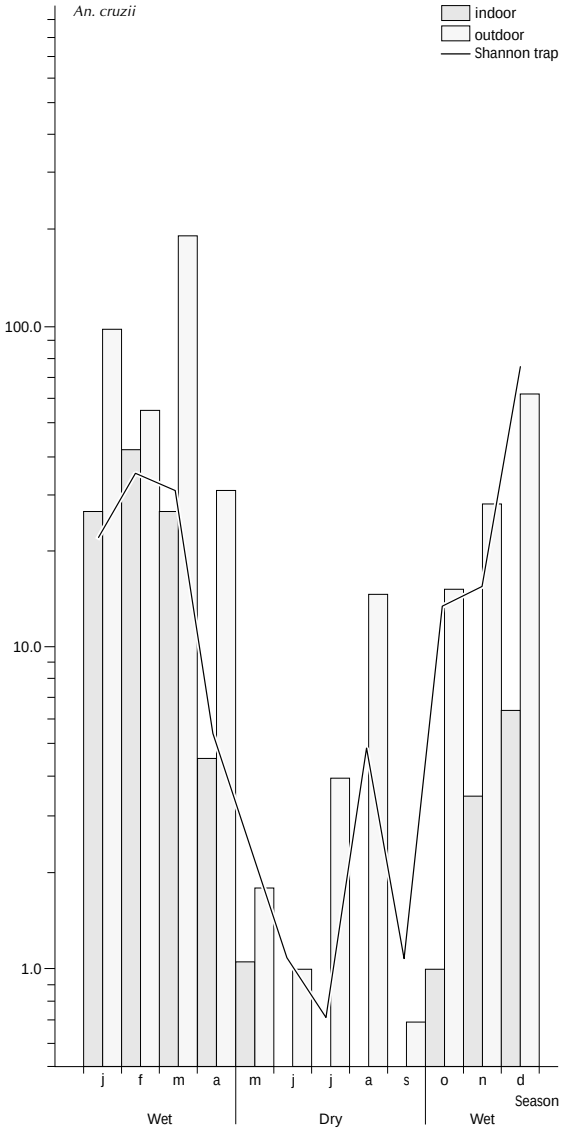

Figure 4 - Monthly captures of *An. cruzii* on human bait ($\bar{X}_w$) and by Shannon trap ($\bar{X}$) during 1994.

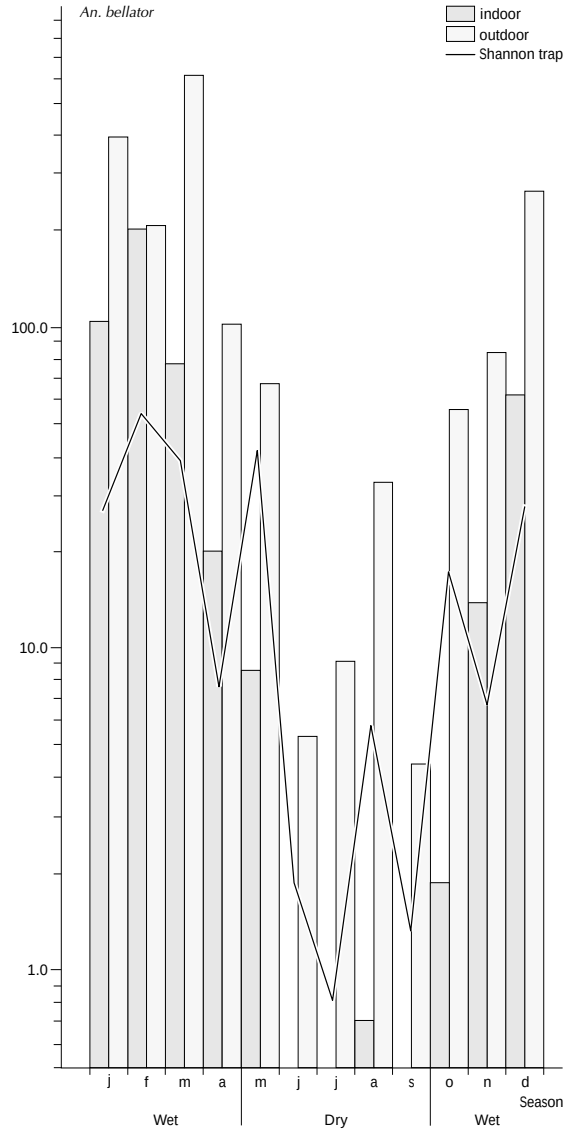

Figure 5 - Monthly captures of *An. bellator* on human bait ($\bar{X}$) and by Shannon trap ($\bar{X}$) during 1994.

Data about age composition were obtained through the dissection of 453 *An. cruzii* and 1,552 *An. bellator* females collected on human bait (Tables 2 and 3). The overall parous (with follicular stalk dilatation) rates were 31.6% for the former and 27.4% for the latter species, with no statistically significant difference between them. As regards the capture sites, indoor parity rates were 27.9% for *An. cruzii* and 22.9% for *An. bellator*, while the outdoor ones were 32.7% and 29.2%, respectively. That parous distribution was significant only for *An. bellator* ($x^2 = 6.20$; $P = 0.012772$).

Regarding the resting adults collected by aspiration inside the natural forest environment, 117 females were dissected with the following results (abbreviations as in Tables 2 and 3):

	PG	NP	1-P	Total
An. cruzii	68	5	15	88
An. bellator	16	1	12	29
Total	84	6	27	117

Thus the parity rates found were 17.0% for *An. cruzii* and 41.4% for *An. bellator*. Compared to human bait, both species showed significantly different rates ($x^2 = 7.52$; $P = 0.006116$ and $x^2 = 7.54$; $P = 0.006040$, respectively). However, the two techniques may not be comparable because of sample size and because the host seeking population subset might be different from the overall population.

Table 1 - Monthly numbers of *Anopheles (Kerteszia)* females caught landing on human bait at the Gentil Farm (January - December 1994).

Months	Nº colle-ctions	*An. cruzii*				T	*An. bellator*				T	Total				T
		Indoor		Outdoor			Indoor		Outdoor			Indoor		Outdoor		
		n	%	n	%		n	%	n	%		n	%	n	%	
January	2	59	18.7	256	81.3	315	213	20.4	829	79.5	1,042	272	20.0	1,085	80.0	1,357
February	2	82	36.6	142	63.4	224	869	52.7	781	47.3	1,650	951	50.7	923	49.3	1,874
March	2	182	28.4	458	71.6	640	180	16.2	932	83.8	1,112	362	20.7	1,390	79.3	1,752
April	2	11	8.7	116	91.3	127	60	21.3	222	78.7	282	71	17.4	338	82.6	409
May	3	8	8.4	87	91.6	95	73	18.6	319	81.4	392	81	16.6	406	83.4	487
June	2	-	-	2	...	2	-	-	15	...	15	-	-	17	...	17
July	2	-	-	4	...	4	-	-	28	...	28	-	-	32	...	32
August	2	-	-	54	...	54	2	2.2	89	97.8	91	2	1.4	143	98.6	145
September	2	-	-	2	...	2	-	-	9	...	9	-	-	11	...	11
October	3	4	3.9	99	96.1	103	12	4.6	247	95.4	259	16	4.4	346	95.6	362
November	2	7	9.9	64	90.1	71	30	14.5	177	85.5	207	37	13.3	241	86.7	278
December	2	13	8.2	145	91.8	158	129	19.0	550	81.0	679	142	17.0	695	83.0	837
Total	26	366	20.4	1,429	79.6	1,795	1,568	27.2	4,198	72.8	5,766	1,934	25.6	5,627	74.4	7,561

Table 2 - Age composition of *Anopheles cruzii* females collected on human bait at the Gentil Farm and dissected during 1994.*

Collection site	Season	Dissections				
		n	Nulliparous**		Parous	
			PG	NP	1-P	2-P
Indoor	Dry (May-Sept.)	8	5	2	1	-
	Wet (Oct.-April)	96	64	4	28	-
	Sub-total	104	69 (66.3)	6 (5.8)	29 (27.9)	-
Outdoor	Dry (May-Sept.)	82	55	5	22	-
	Wet (Oct.-April)	267	171	4	91	1
	Sub-total	349	226 (64.8)	9 (2.6)	113 (32.4)	1 (0.3)
Total		453	295 (65.1)	15 (3.3)	142 (31.3)	1 (0.2)

* NP - nulliparous at stage III
PG - pre-gravids (nulliparous at stage II)
I-P - one dilatation (uniparous)
2-P - two dilatations (biparous)
Percentages in parenthesis
**Without follicular stalk dilatations.

Table 3 - Age composition of *Anopheles bellator* females collected on human bait at the Gentil Farm and dissected during 1994.*

Collection site	Season	Dissections				
		n	Nulliparous**		Parous	
			PG	NP	1-P	2-P
Indoor	Dry (May-Sept.)	52	37	8	7	-
	Wet (Oct.-April)	384	263	28	93	-
	Sub-total	436	300 (68.8)	36 (8.3)	100 (22.9)	-
Outdoor	Dry (May-Sept.)	260	188	11	61	-
	Wet (Oct.-April)	856	579	12	263	2
	Sub-total	1,116	767 (68.7)	23 (2.1)	324 (29.0)	2 (0.2)
Total		1,552	1,067 (68.8)	59 (3.8)	424 (27.3)	2 (0.1)

* NP - nulliparous at stage III
PG - pre-gravids (nulliparous at stage II)
I-P - one dilatation (uniparous)
2-P - two dilatations (biparous)
Percentages in parenthesis
** Without follicular stalk dilatations.

Table 4 - Monthly collections of *Anopheles (Kerteszia)* females by the Shannon trap at the Gentil Farm (June 1993 April 1995).

	An. cruzii			*An. bellator*			Total		
Months	n	%	$\overline{X}$*	n	%	$\overline{X}$*	n	%	$\overline{X}$*
January	1,912	20.1	63.7	599	8.5	20.0	2,511	15.2	83.7
February	1,625	17.1	90.3	1,578	22.4	87.7	3,203	19.4	177.9
March	1,228	12.9	40.9	1,064	15.1	35.5	2,292	13.9	76.4
April	386	4.1	16.1	449	6.4	18.7	835	5.1	34.8
May	366	3.8	20.3	748	10.6	41.6	1,114	6.7	61.9
June	177	1.9	5.9	80	1.1	2.7	257	1.6	8.6
July	303	3.2	12.6	357	5.1	14.9	660	4.0	27.5
August	262	2.8	10.9	126	1.8	5.3	388	2.3	16.1
September	301	3.2	12.5	181	2.6	7.5	482	2.9	20.1
October	1,259	13.3	42.0	1,043	14.8	34.8	2,302	13.9	76.7
November	357	3.8	11.9	210	3.0	7.0	567	3.4	18.9
December	1,316	13.9	54.8	603	8.6	25.1	1,919	11.6	80.0
Total	9,492	100.1	31.0	7,038	100.0	23.0	16,530	100.0	54.0

* $\overline{X}$ - hourly arithmetical mean

As the numbers of females to be dissected were randomly chosen, it was not possible to reach any consistent conclusion about seasonal parity distribution. However, the data suggest a wet season parity increase for both species.

Parity was predominantly represented by the uniparous condition, i.e., the presence of only one follicular stalk dilatation. Nonetheless, about 5.0% of the females dissected showed nulliparous condition with ovaries developed at Christophers' Stage III. That total consisted of 20 *An. cruzii* and 60 *An. bellator*, thus showing that even without previous eggs laid, at least one blood meal had been taken. Females landing on human bait should be considered as blood-seeking. Thus, adding these Stage III nulliparous specimens to the parous ones, it was possible to establish that 34.9% of the *An. cruzii* and 31.1% of the *An. bellator* females dissected were taking at least a second blood meal.

DISCUSSION

It is recognized that *Kerteszia* anophelines are dominant in the primitive Atlantic Rain Forest ecosystem of Southern and South-Eastern Brazil. They are considered as regional malaria vectors, particularly *An. cruzii* and *An. bellator*. Observations made in the Ribeira Valley showed that even with exophilic behaviour their presence in human settlements is evident. Despite this, they have no tendency to rest inside dwellings (Forattini et al.[3], 1990). In earlier research carried out in a neighbouring area named "Sítio Andrade" (Andrade Farm), *An. cruzii* was the most abundant species caught on human bait. At the same time, *An. bellator* showed greater endophagy than *An. cruzii* (Forattini et al.[4], 1993). In the present research, a similar pattern was found, with *An. bellator* being four times more frequent in dwellings than *An. cruzii*. Nevertheless, the collections undertaken inside the forest with the Shannon trap, showed a predominance of *An. cruzii*, although *An. bellator*'s presence was more significant than that obtained at the "Sítio Andrade" (42.6 and 7.7%, respectively). Thus, judging from this evidence, it seems that *An. cruzii*, although predominant within the natural environment, do not have the same predominance in the anthropic one where *An. bellator* is more numerous. The higher adult density of this species, compared with that previously obtained at the "Sítio Andrade", may reflect, at least partially, the influence of the estuary system. This is mainly represented by the low-lying islands the covering vegetation of which consists mainly of scattered woods of stanted trees called "restinga". As is known, *An. bellator* prefers bromeliads that are more exposed to the sun to those that grow inside the woods on the coastal lowland (Veloso et al.[12], 1956).

The seasonal patterns followed what is already expected in the Neotropical region. The levels of greatest abundance were observed during the wet season, as the graphs in Figures 4 and 5 show, by the monthly distribution of Williams' means for human bait and the arithmetical hourly ones for Shannon trap collections. There was agreement between the variations, both in the domiciliary and natural environments.

The age compositions obtained through dissections of landing females are certainly valuable indications of previous blood meals. Therefore, the data on the

parity proportions may be considered as an assessment of the vector capacity. Regarding the indoor and outdoor collections, no significant difference was found for *An. cruzii*, differently from *An. bellator* which showed higher parity rates for the outdoor catches. The greater abundance of this species on the Gentil Farm as compared with the Andrade Farm (Forattini et al.[4], 1993) may explain the differences recorded between the two sites. Anyway, the presence of III and further of Christophers' Stages in nulliparous females shows that more than 30.0% of the blood-seeking specimens of both *An. cruzii* and *An. bellator* had taken at least one previous blood meal. Thus, this strongly suggests that there is the necessity for multiple blood feeding for a first clutch of eggs to be developed, i.e., as a consequence of a gonotrophic discordance, at least initially.

In conclusion, *An. bellator* keeps its higher endophily level as compared with that of *An. cruzii*. Otherwise, this latter mosquito mantains greater abundance within the natural environment, particularly in the rain forest covering the mountain slopes. In addition, there is evidence that no initial gonotrophic development occurs but that about 30.0% of blood-seeking females of both species had already taken at least one blood meal.

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