

Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 1- Parity of blood seeking *Anopheles* (*Kerteszia*) in South-Eastern Brazil*

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FORATTINI, O.P. et al. Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 1- Parity of blood seeking *Anopheles* (*Kerteszia*) in South-Eastern Brazil. *Rev. Saúde Pública*, 27: 1-8, 1993. Populations of *Anopheles* (*Kerteszia*) were sampled fortnightly over a one-year period (August 1991 to July 1992) at Ribeira Valley, S. Paulo State, Brazil. Indoor and outdoor collections were made on human bait at evening crepuscular period. The Polovodova technique for age grading was applied to 3,501 females of *Anopheles cruzii* and to 416 females of *An. bellator*. That sample represented 34.4% of the total number of mosquitoes collected. The most abundant species found was *An. cruzii*. However, *An. bellator* showed an endophagy that was almost three times greater than that of *An. cruzii*. The overall parous rate was 25.4% and uniparity was practically dominant one. A proportion of 26.9% of *An. cruzii* and 12.0% of *An. bellator* were found to be uniparous. Only three outdoor females of the former species (0.1%) showed biparity. Parity of *An. cruzii* was higher in females caught indoors than in those caught outdoors. Nevertheless, 497 nulliparous females examined (417 *cruzii* and 80 *bellator*) had ovaries that had advanced to Christophers and Mer stages III to V. These results imply that these females had already practised hematophagy. Relating these results to those from the parous females, a high statistical significance was found, leading to the conclusion that gonotrophic discordance is a common pattern among these anophelines. Further, these results obtained with human bait catches strongly suggest that nearly 38.0% of these host-seeking females had already taken at least one previous blood-meal. So it is possible that enough time could thus be available for the plasmodian development in the vectors.

Keywords: *Anopheles*, physiology. Parity. Endophagy. Domiciliation.

Introduction

Anopheline (*Kerteszia*) species are regarded as malaria vectors in the Atlantic Rain Forest System of southern and south-eastern Brazil, where that infection presents a low endemic level. Several interacting factors are recognized as determinants of the anopheline vectorial capacity. Combining parasitological and entomological parameters it is possible to attempt an estimation of the malaria reproductive ratio (Garrett-Jones^{11,12}, 1964; Dye⁵, 1986). As a critical parameter, vector survivorship may be mentioned because it determines the lon-

gevity, or expected duration, of vector infective life. The most commonly used measure is the parity, or parous rate, that is the age-composition of mosquitoes populations evaluated in terms of the female's gonotrophic cycles count. For that, the Polovodova technique represents the most widely used method (Detinova^{3,4}, 1962, 1968).

Indeed, just parity determination gives only a crude estimate of mosquito age-composition, mainly as regards the *Anopheles* species in which blood meals and eggs laying may occur soon after the emergence. However, parity knowledge allows the differentiation of the young female section of the population from the older one. In that sense it has been the most frequent index actually used for the determination of the vector's age-composition.

Through monitoring local anopheline populations, both in natural and anthropic environments, it should be possible to evaluate the regional receptivity to malaria endemicity. With this in view, the Ribeira Project was planned to carry out research in the so-called Valley region, in the south-eastern region of S. Paulo State, Brazil. In this paper, the results of observations on the age-composition of blood-seeking *Anopheles* (*Kerteszia*) *cruzii* and *An. (Ker.) bel-*

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lator, with indoor and outdoor human bait, are reported.

Study Site

Field works took place in an area near the vil-
lage of Itapitangui, Cananéia County, known as
the "Fonte" (Spring) region because it contains

(Andrade Farm), neighbouring on the "Sítio Ita-
poã" (Itapoan Farm), a former observation site, the
results from which have already been published,
including a more detailed regional description
(Fig.1) (Forattini et al.⁷, 1986).

The primitive natural environment is represent-
ed by the "Mata Atlântica" (Atlantic Rain Forest)
covering the slopes of the "Serra do Mar" (Coastal

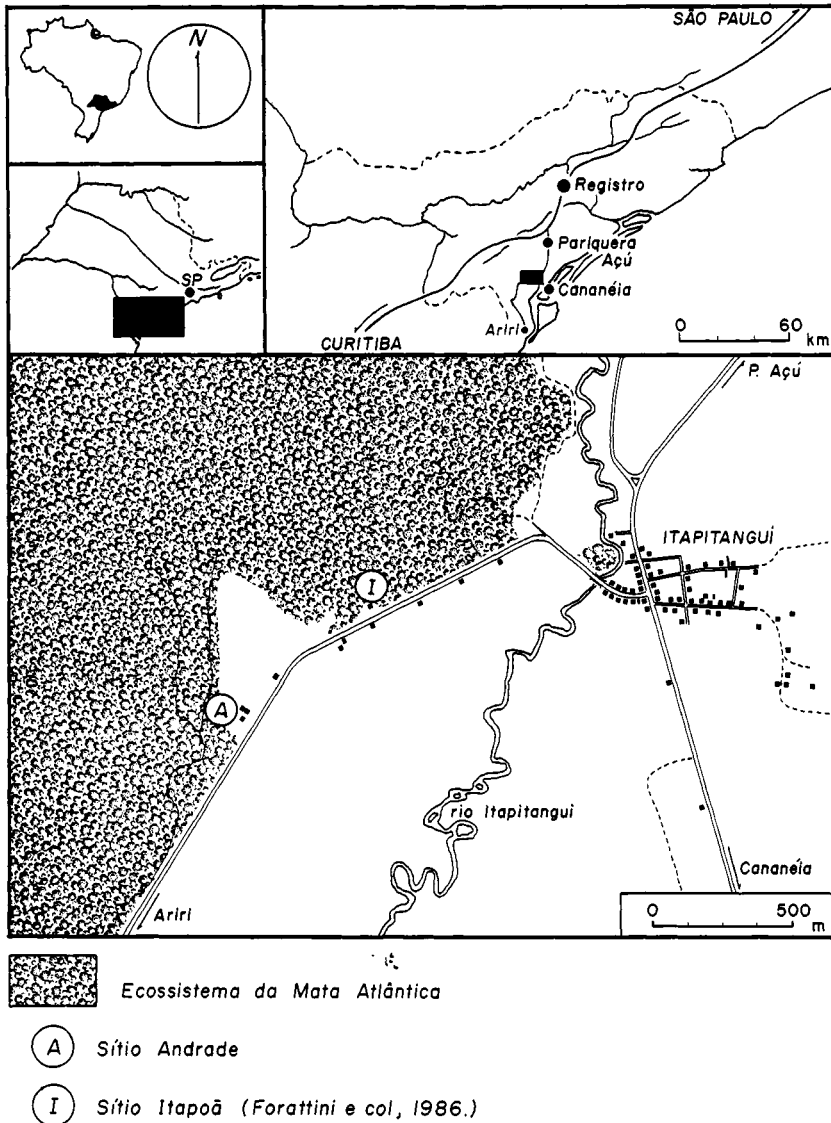


Figure 1. Map of study site at the Fonte region showing the Andrade Farm (Sítio Andrade) and the neighbouring Itapoan Farm (Sítio Itapoã). Cananéia County, S. Paulo State, Brasil.

reservoir for water supply. There are several local-
ities in that region, including the one that was chosen
for these observations, called "Sítio Andrade"

Mountain Range). Research took place at the local
anthropic environment, approximately one hundred
meters away, in a straight line, from that for-

est. Figures 2, 3 and 4 show landscape photographs of this study site.



Figure 2. Primitive rain forest on the Andrade Farm, belonging to the Atlantic Rain Forest ecosystem.

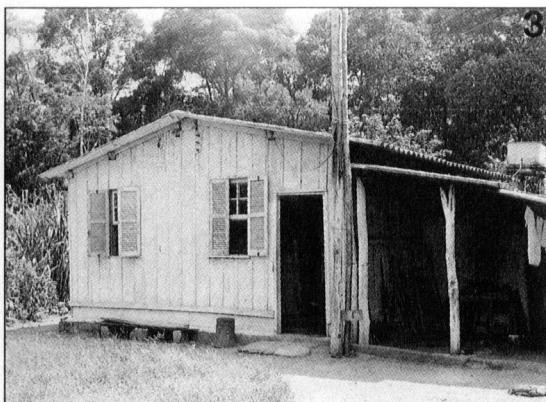


Figure 3. House where indoor catches were undertaken.

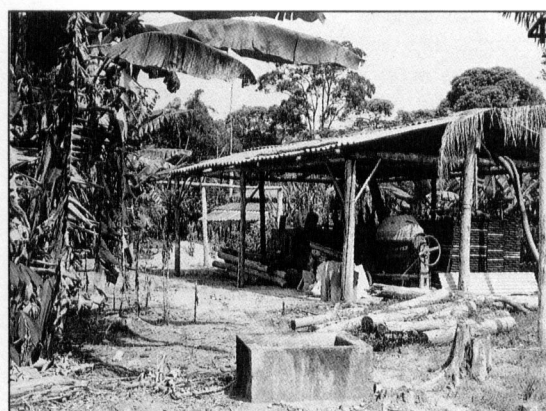


Figure 4. Peridomestic environment of the above house, where outdoor catches were undertaken.

Material and Method

To sample the biting mosquito population, the

collectors caught females as they came to feed from them on their exposed legs. The captures were performed indoors and outdoor during the evening crepuscular period (Forattini et al.⁷, 1986), for two hours uninterruptedly, with a regular fortnightly rhythm over one year, from August 1991 to July 1992.

After identification, the dissections were performed and the state of the ovaries and the condition of the follicular stalks were noted. The number of females dissected varied in accordance with the number of mosquitoes caught. As a whole, the percentage of specimens examined were 64.4 and 88.0 of the total *An. cruzii* and *An. bellator* indoor capture, while 30.8 and 40.0, respectively, were of each of those species caught outdoors. With the purpose of detecting blood present before egg-laying, ovarian follicle stages were identified according to the Christophers and Mer classification (Forattini⁶, 1962).

Results

Twenty-five sampling catches were made in all and 11,373 females of *Anopheles* (*Kerteszia*) were obtained (Table 1). Of that number, 92.3% were *An. cruzii* and 7.7% were *An. bellator*. Nevertheless, regarding the two collection sites, *An. bellator* indoor presence (22.6%) was higher than that of *An. cruzii* (7.4%) as a proportion of the total number of the anophelines obtained. Thus comparing the mean, both indoor and outdoor, of these mosquitoes, it varied significantly ($\chi^2=9.81$; $p=0.001737$). Thus, *An. bellator* has a probability of 3.71 higher than *An. cruzii* of being caught biting a man inside his house.

In view of the fact that in catches aimed at blood-seeking mosquitoes, the number caught corresponds, at least theoretically, to the number of bites that would be received by the catcher, that is, to the term *ma* of the equation of the malaria reproductive rate (Reisen¹⁶, 1989), it was possible to estimate the endophagy index (Ribeiro and Janz¹⁶, 1990) at 0.22 for *An. bellator* and 0.08 for *An. cruzii*.

Of the total obtained, 3,917 (34.4%) specimens were dissected, 3,501 (89.4%) of which were *An. cruzii* and 416 (10.6%) *An. bellator* (Tables 2 and 3). The overall parous rate was 25.4% which included 27.0% of the former and 12.0% of the latter species. With regard to the catching sites, indoor parity rates were 21.9% for *An. cruzii* and 11.6% for *An. bellator*, while the outdoor ones were 27.9% and 12.2%, respectively. That parous distribution was significant only for the former species ($\chi^2=7.44$; $p=0.0063678$). Regarding the seasonal distribution, it was not possible to reach any consistent conclusion, although data

Table 1. Monthly captures of *Anopheles (Kerteszia)*, on human bait (August 1991-July 1992) at the Andrade Farm.

Month	N ^o Collections	<i>Anopheles cruzii</i>				T	<i>Anopheles bellator</i>				T	Total				T
		indoor		outdoor			indoor		outdoor			indoor		outdoor		
		N ^o	%	N ^o	%		N ^o	%	N ^o	%		N ^o	%	N ^o	%	
1991 August	2	15	15.3	83	84.7	98	5	27.8	13	72.2	18	20	17.2	96	82.8	116
September	2	8	3.5	220	96.5	228	1	0.4	35	99.6	36	9	3.4	255	96.6	264
October	3	33	8.3	363	91.7	396	14	17.3	67	82.7	81	47	9.9	430	90.1	477
November	1	2	0.9	212	99.1	214	3	4.3	67	95.7	70	5	1.8	279	98.2	284
December	2	59	9.2	580	90.8	639	7	15.2	39	84.8	46	66	9.6	619	90.4	685
1992 January	2	35	50.7	34	49.3	69	4	11.1	32	88.9	36	39	37.1	66	62.9	105
February	2	61	4.1	1,434	95.9	1,495	16	12.6	111	87.4	127	77	4.7	1,545	95.3	1,622
March	2	30	2.7	1,061	97.3	1,091	12	36.4	21	63.6	33	42	3.7	1,082	96.3	1,124
April	2	137	7.3	1,731	92.7	1,868	58	30.9	130	69.1	188	195	9.5	1,861	90.5	2,056
May	2	266	17.5	1,253	82.5	1,519	36	43.9	46	56.1	82	302	18.9	1,299	81.1	1,601
June	3	113	6.6	1,612	93.4	1,725	33	28.0	85	72.0	118	146	7.9	1,697	92.1	1,843
July	2	18	1.6	1,137	98.4	1,155	9	22.0	32	78.0	41	27	2.3	1,169	97.7	1,196
Total	25	777	7.4	9,720	92.6	10,497	198	22.6	678	77.4	876	975	8.6	10,398	91.4	11,373

suggested some parity increase related to the February-April period.

The uniparous condition, corresponding to one follicular stalk dilation, was practically the only one found. Nevertheless, ovarian development stages, according to Christophers and Mer, were found, as shown by the Tables 4 and 5. Of the nulliparous females examined, 497 (17.0%) had ovaries that had reached stage III or above. Of that total, 417 (83.7%) were *An. cruzii* and 80 (16.1%) *An. bellator*. Of the parous females, 60 (6.0%) had ovaries at stage III, the greater number of them belonging to *An. cruzii*. Comparing those nulliparous and parous rates, highly significant differences were found, both for *An. cruzii* ($\chi^2=597.26$; $p<0.00000001$) and *An. bellator* ($\chi^2=11.04$; $p=0.0008933$).

Discussion

The dominance of the *Kerteszia* mosquito in the primitive Atlantic Rain Forest ecosystem of South and South-Eastern Brazil (Forattini et al.⁸, 1986) is well known. Despite the fact that these anophelines, particularly *An. cruzii* and *An. bellator*, are regular frequenters of the peridomiciliar environments situated near the forests, it seems that after their blood meal they have no tendency to rest inside human dwellings. For this reason they are considered as vectors with low domiciliary, at least in the Ribeira Valley and neighbouring regions (Consolim et al.², 1979; Forattini et al.⁹, 1990). Nevertheless, this exophilic behavior, commonly recorded for other species of the same subgenus, is a recognized feature assigned to these anophelines as malaria vectors (Forattini⁶, 1962; Quifones et al.¹⁵, 1984; Carvajal et

al.¹, 1989). In this case the indoor presence must be considered as characterizing endophagy, which seems to be higher for *An. bellator* than for *An. cruzii* (Table 1). Generally speaking, the endophagic habit of the former was about three times more frequent than that of the latter. To this behavior, it must be added to *An. bellator* very regular presence along the catching year, showing smaller seasonal variations at the higher density period (February-March) than was shown by *An. cruzii* over the same period. Thus it was possible to verify that the monthly catches of the latter species practically established the incidence profile (Fig.5). Therefore, it seems reasonable to suppose that, at least in the study area, *An. bellator* presented some level of domiciliar tendency, even if limited to some degree of endophagy.

Regarding parity of anopheline populations, there is general agreement that the most suggestive data are those obtained through the examination of blood-seeking female samples (Garrett-Jones and Grab¹¹, 1964). The obvious reason is that if they are females searching for hosts it would be interesting to investigate the possibility of previously-taken blood meals. Usually catches are simultaneously performed both indoors and outdoors. As for *Kerteszia* in the environment here studied data so far available concern *An. cruzii*. At Paraná State, biogeographically analogous to our region, indoor parous rates of 17.0% and outdoor ones of 15.0%, with as statistically significant difference, have been recorded (Luz et al.¹³, 1979). Nevertheless, the results here reported show outdoor parous rates of *An. cruzii* significantly higher than the indoor ones (Table 2). These data agree with the absolute values found and so point to the high exophily of this anopheline (Table 1). With

Table 2. Age composition of monthly captures of *Anopheles cruzii* at the Andrade Farm*.

Month	indoor						outdoor						Total														
	dissections		NP		1-P		dissections		NP		1-P		dissections		NP		1-P		dissections		NP		1-P		2-P		
	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	
1991 August	14	10	71.4	4	28.6	32	23	71.9	9	28.1	-	-	46	33	71.7	13	28.3	-	-	-	-	-	-	-	-	-	-
September	6	6	100.0	-	-	52	44	84.6	8	15.4	-	-	58	50	86.2	8	13.8	-	-	-	-	-	-	-	-	-	-
October	32	25	78.1	7	21.9	352	221	62.8	131	37.2	-	-	348	246	64.1	138	35.9	-	-	-	-	-	-	-	-	-	-
November	2	1	50.0	1	50.0	185	142	76.8	43	23.2	-	-	187	143	76.5	44	23.5	-	-	-	-	-	-	-	-	-	-
December	55	47	85.5	8	14.5	456	375	82.2	80	17.5	1	0.2	511	422	82.6	88	17.2	1	0.2	-	-	-	-	-	-	-	-
1992 January	32	29	90.6	3	9.4	115	85	73.9	30	26.1	-	-	147	114	77.6	33	22.4	-	-	-	-	-	-	-	-	-	-
February	60	43	71.7	17	28.3	369	270	73.2	99	26.8	-	-	429	313	73.0	116	27.0	-	-	-	-	-	-	-	-	-	-
March	30	24	80.0	6	20.0	277	173	62.5	104	37.5	-	-	307	197	64.2	110	35.8	-	-	-	-	-	-	-	-	-	-
April	89	67	75.3	22	24.7	302	215	71.2	87	28.8	-	-	391	282	72.1	109	27.9	-	-	-	-	-	-	-	-	-	-
May	63	48	76.2	15	23.8	163	131	80.4	32	19.6	-	-	226	179	79.2	47	20.8	-	-	-	-	-	-	-	-	-	-
June	85	65	76.5	20	23.5	442	314	71.0	128	29.0	-	-	527	379	71.9	148	28.1	-	-	-	-	-	-	-	-	-	-
July	17	14	82.4	3	17.6	271	183	67.5	86	31.7	2	0.7	288	197	68.4	89	31.0	2	0.7	-	-	-	-	-	-	-	-
Total	485	379	78.1	106	21.9	3,016	2,176	72.1	837	27.8	3	0.1	501	2,555	73.0	943	26.9	3	0.1	-	-	-	-	-	-	-	-

* NP - Without dilatations (nulliparous).
 1-P - One dilatation (uniparous).
 2-P - Two dilatations (biparous).

Table 3. Age composition of monthly captures of *Anopheles bellator* at the Andrade Farm.*

Monthly	indoor					outdoor					Total				
	N ^o dissections		NP		1-P	N ^o dissections		NP		1-P	N ^o dissections		NP		1-P
	N ^o	%	N ^o	%		N ^o	%	N ^o	%		N ^o	%	N ^o	%	
1991 August	1	100.0	1	100.0	—	1	100.0	1	100.0	—	2	100.0	2	100.0	—
September	1	100.0	1	100.0	—	7	85.7	6	85.7	1	8	87.5	7	87.5	1
October	14	92.9	13	92.9	1	66	87.9	58	87.9	8	80	88.8	71	88.8	9
November	3	100.0	3	100.0	—	59	93.2	55	93.2	4	62	93.5	58	93.5	4
December	7	100.0	7	100.0	—	29	96.6	28	96.6	1	36	97.2	35	97.2	1
1992 January	4	75.0	3	75.0	1	23	95.7	22	95.7	1	27	92.6	25	92.6	2
February	16	87.5	14	87.5	2	18	72.2	13	72.2	5	34	79.4	27	79.4	7
March	12	83.3	10	83.3	2	3	33.3	1	33.3	2	15	73.3	11	73.3	4
April	44	81.8	36	81.8	8	30	76.7	23	76.7	7	74	79.7	59	79.7	15
May	11	91.0	10	91.0	1	7	100.0	7	100.0	—	18	94.4	17	94.4	1
June	24	91.7	22	91.7	2	20	85.0	17	85.0	3	44	88.6	39	88.6	5
July	9	180.0	9	180.0	—	7	85.7	6	85.7	1	16	93.8	15	93.8	1
Total	146	88.4	129	88.4	17	270	87.8	237	87.8	33	416	88.0	366	88.0	50

* NP - Without dilatations (nulliparous).
 1-P - One dilatation (uniparous).
 2-P - Two dilatations (biparous).

Table 4. Ovarian development Stages (I-V) according to Christophers and Mer, in *Anopheles cruzii* at the Andrade Farm.

Parity	indoor					outdoor					Total				
	I/II		III a V		T	I/II		III a V		T	I/II		III a V		T
	N ^o	%	N ^o	%		N ^o	%	N ^o	%		N ^o	%	N ^o	%	
Nulliparous	272	71.8	107	28.2	379	1,866	85.8	310	14.2	2,176	2,138	83.7	417	16.3	2,555
Uniparous	99	93.4	7	6.6	106	785	93.8	52	6.2	837	884	93.7	59	6.3	943
Biparous	—	—	—	—	—	3	100.0	—	—	3	3	100.0	—	—	3
Total	371	76.5	114	23.5	485	2,654	88.0	362	12.0	3,016	3,025	86.4	476	13.6	3,501

Table 5. Ovarian development Stages (I-V) according to Christophers and Mer, in *Anopheles bellator* at the Andrade Farm.

Parity	indoor					outdoor					Total				
	I/II		III a V		T	I/II		III a V		T	I/II		III a V		T
	N ^o	%	N ^o	%		N ^o	%	N ^o	%		N ^o	%	N ^o	%	
Nulliparous	94	73.0	35	27.0	129	192	81.0	45	19.0	237	286	78.1	80	21.9	366
Uniparous	16	94.1	1	5.9	17	33	100.0	—	—	33	49	98.0	1	2.0	50
Total	110	75.3	36	24.7	146	225	83.3	45	16.7	270	335	80.5	81	19.5	416

respect to *An. bellator*, the similarity of the results obtained from the catches at the two sites can be interpreted as a result of the more uniform behaviour of this mosquito, in accordance with its endophagic habit.

As for age composition, parity was practically limited to the uniparous condition, that is, the find-

ing of only one dilation of the follicular stalk. The biparous condition appeared rarely, in no more than 0.1% of *An. cruzii* blood-seeking females, agreeing with the results, mentioned above, obtained in Paraná. These aspects contrasted notably with those reported in Colombia for *An. neivai*, a species of the same subgenus, which showed up to ten follicular

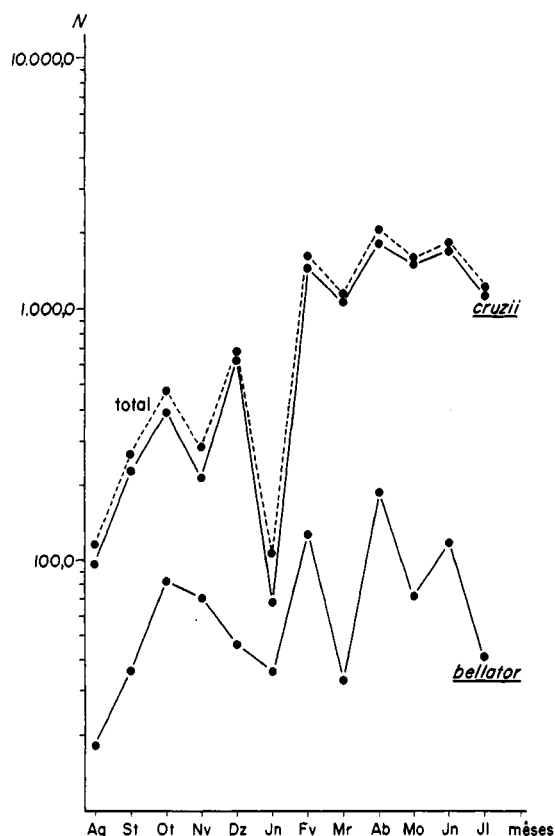


Figure 5. Monthly captures of *Anopheles (Kerteszia)* on human bait.

stalk dilations (Murillo et al.¹⁴, 1989). It may be possible to suppose a higher level of domiciliar activity for this anopheline due to more intense local human presence than at the site of the present studies, where *Kerteszia* mosquitoes seem to preserve a higher degree of sylvatic behaviour.

It is recognized that under normal circumstances nulliparous anophelines will not develop ovaries beyond the Christophers and Mer Stage II without a blood meal. So the discovery of Stages III or above in nulliparous individuals means that these females had already taken at least one blood meal. As shown in Tables 4 and 5, of the total nulliparous and parous females examined, 557 (14.2%) had ovaries that had advanced to Stage III or above. Of that total, 476 (12.1%) were *An. cruzii* and 81 (2.1%) *An. bellator*. The significant difference found between nulliparous and parous allows one to consider the presence of Stages III to V in nulliparous females elevated, higher than in the parous ones. Thus it seems quite consistent to admit the hypothesis that these mosquitoes present a significant proportion of females with gonotrophic discordance, starting hematophagy early, before their first egg-laying. As it is possible to infer from the

results obtained here, information has been obtained that might be useful in the assessment of south Brazilian *Kerteszia* vector capacity. Concerning longevity, the data yield knowledge of the age composition of the human-blood-seeking females. This initial phase of research only obtained information about general parity conditions and parameters of endophagy. Endophagic *An. bellator*, in spite of its relatively smaller density on human bait, shows evident endophilic behavior. Regarding the condition of completely dominant nulliparity, the non-negligible rates of nulliparous females with ovaries developed beyond Stage II of Christophers and Mer called our attention. So, the addition of these mosquitoes to the parous ones, leads to the conclusion that 1,493 (38.1%) of blood-seeking anophelines caught on human bait had taken at least one previous blood meal. By species, the rates were 34.8% for *An. cruzii* and 8.7% for *An. bellator*. These data strongly suggest that a significant part of that mosquito population need multiple blood feeding to develop a first clutch of eggs. This hypothesis leads to the supposition of an increased probability of malaria parasite infection and development in these vectors.

FORATTINI, O. P. et al. [Estudos sobre mosquitos (Diptera: Culicidae) e ambiente antrópico. 1- Paridade de *Anopheles (Kerteszia)* em atividade hematófaga, na região sudeste do Brasil.] *Rev. Saúde Pública*, 27: 1-8, 1993. Relata-se os resultados obtidos em coletas regulares de *Anopheles cruzii* e *An. bellator*, mediante o emprego de isca humana e por ocasião do crepúsculo vespertino. Objetivou-se, precipuamente, conhecer a paridade de populações dessas espécies, quando em plena tentativa hematófaga, tanto no ambiente intra como peridomiciliar. As coletas foram levadas a efeito no ecossistema primitivo da Mata Atlântica meridional do Brasil, em região do Vale do Ribeira, sudeste do Estado de São Paulo, Brasil, durante o período de agosto de 1991 a julho de 1992. Precedeu-se ao exame de amostra, correspondente a 34,4% do total de mosquitos coletados, mediante o emprego da técnica de Polovodova para a observação de dilatações ovariolares. Ao lado disso, o desenvolvimento dos ovários foi classificado de acordo com as fases de Christophers e Mer.

Os dados obtidos permitiram constatar a dominância de *An. cruzii*. No entanto, pôde-se detectar maior atividade endofágica por parte de *An. bellator*, a qual se mostrou três vezes maior do que a apresentada por aquele outro anofelíneo. No que concerne à paridade, houve franco predomínio da nuliparidade (74,6%), com o restante constituído, praticamente, por uniparidade (25,4%). A presença de maior número de dilatações ovariolares limitou-se a poucos casos de biparidade em *An. cruzii*. A paridade apresentada por este revelou-se maior no peridomicílio, reforçando a feição exófila de seu comportamento, ao passo que para *An. bellator* mostrou-se uniforme em relação dos dois locais de coleta. Observou-se

que 17,0% de fêmeas núlparas revelaram ovários cujo desenvolvimento tinha atingido as fases III a V de Christophers e Mer, o que evidenciou o exercício de hematofagia previamente à primeira oviposição. Dessa maneira, pôde-se considerar como sendo de 38,1% o percentual da amostra total examinada que, pelo menos, já realizara um repasto sanguíneo ao tentar repeti-lo na isca humana. Esses valores foram de 34,8% para *An. cruzii* e de 8,7% para *An. bellator*. Diante disso, conclui-se pela provável existência de discordância gonotrófica, o que permite levantar a hipótese de, entre outros fatores, haver viabilidade de desenvolvimento plasmodiano no organismo desses vetores.

Descritores: *Anopheles*, fisiologia. Paridade. Endofagia. Domiciliação.

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CORRIGENDA

REVISTA DE SAÚDE PÚBLICA (JOURNAL OF PUBLIC HEALTH)

27(1):1-8, 1993 - p. 1 (Summary) - line 11 and 12

Change the phrase to:

Parity of An. cruzzi was higher in females caught outdoors than in those caught indoors.