Population fluctuation of *Anopheles* (Diptera: Culicidae) in forest and forest edge habitats in Tucumán province, Argentina

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Received 11 June 2009; Accepted 28 January 2010

ABSTRACT: The aim of this work was to study the possible effects of forest and forest edge habitats on the population fluctuation of the *Anopheles* species in northwestern Argentina, taking into consideration the relationship between this fluctuation and climatic variables. This study is one of the first that involves the *Anopheles* fauna in the country and its dynamics in two different habitats. Sampling was carried out from October, 2002 to October, 2003, in the forest and on the forest edge. Both habitats were compared for species diversity and abundance, and multiple regression analyses were performed to analyze the effects of environmental variables on the population dynamics. Five hundred and sixteen adult specimens of *Anopheles* species were collected, the most numerous group being Arribalzaga (52.1%), followed by *Anopheles* (*Nyssorhynchus*) *strodei* (20.5%) and *Anopheles* (*Nyssorhynchus*) *evansae* (6.4%). Mosquito abundance was greatest in the forest, the most productive habitat. Samples were collected throughout the sampling period, with a smaller peak in summer. Small numbers of *Anopheles* (*Anopheles*) *pseudopunctipennis* were found throughout the year. Relative humidity, with a 15-day delay, was the factor that most strongly contributed to the temporal sample fluctuation. We conclude that the best season for anopheline development in the study area is from spring to fall, although the period with the greatest transmission risk is the fall, with the greatest *An. pseudopunctipennis* abundance. ***Journal of Vector Ecology* 35 (1): 28-34. 2010.**

Keyword Index: Population fluctuation, *Anopheles*, forest, forest edge.

INTRODUCTION

Thirty-one anopheline species have been found in Argentina (Darsie and Mitchell 1985, Darsie et al. 1991) and fifteen were reported in the northwest region including *Anopheles* (*Anopheles*) *pseudopunctipennis* Theobald, *Anopheles* (*Nyssorhynchus*) *strodei* Root, *Anopheles* (*Nyssorhynchus*) *rondoni* (Neiva & Pinto), *Anopheles* (*Nyssorhynchus*) *rangeli* Gabaldon, Cova Garcia & Lopez, and *Anopheles* (*Nyssorhynchus*) *argyritarsis* Robineau-Desvoidy (Darsie and Mitchell 1985).

Anopheles pseudopunctipennis is one of the main malaria vectors in Latin American countries such as Peru, Ecuador, Bolivia, and Argentina (Pan American Health Organization 1994). Mühlens et al. (1925) described the epidemiological situation with respect to malaria cases in northwestern (NW) Argentina and found *An. pseudopunctipennis* to be the most abundant species in the dwellings of sick individuals and the species most likely involved in malaria transmission. The studies of Duret (1950) and Bejarano (1951, 1956, 1965) also implicated this species as the vector in NW Argentina, connecting its presence to the physiographical and phytogeographical characteristics of the environment.

Until the 1990s, there was a void in the study of these mosquito vectors and in their relation to malaria transmission. Burgos et al. (1994) concluded that NW Argentina would be affected by a process of desertification so the distribution of *An. pseudopuctipennis* and, consequently, malaria outbreaks would extend further south. The opposite situation was proposed by Curto et al. (2003) who predicted that the distribution of malaria and *An. pseudopunctipennis* would be limited to the NW, with changes in the environment being one of the factors responsible.

In NW Argentina, urbanization and deforestation for agricultural development have recently altered the distribution of habitats for anopheline mosquitoes. This led us to hypothesize that a different fauna composition and spatial and temporal distribution, perhaps as a result of changes in temperature and rainfall, would be detected. Our aim was to determine the composition of *Anopheles* mosquitoes in two habitats (forest and forest edge) in Tucumán as well as the temporal fluctuation of the adult specimens and to relate this fluctuation to environmental variables.

MATERIALS AND METHODS

Sampling area

The study was conducted in the southern Yungas, in Sargento Moya (27° 11' S, 65° 38' W; 468 m.) (Figure 1). The area has high rainfall, temperature, and humidity (annual mean 1,200 mm, 18° to 25° C, and 82%, respectively) according to the records of Pueblo Viejo weather station (27° 12' S, 65° 37' W). Native vegetation, with the exception of areas near roads and stream banks where it becomes less dense, is closed arboreal vegetation (Meyer 1963,

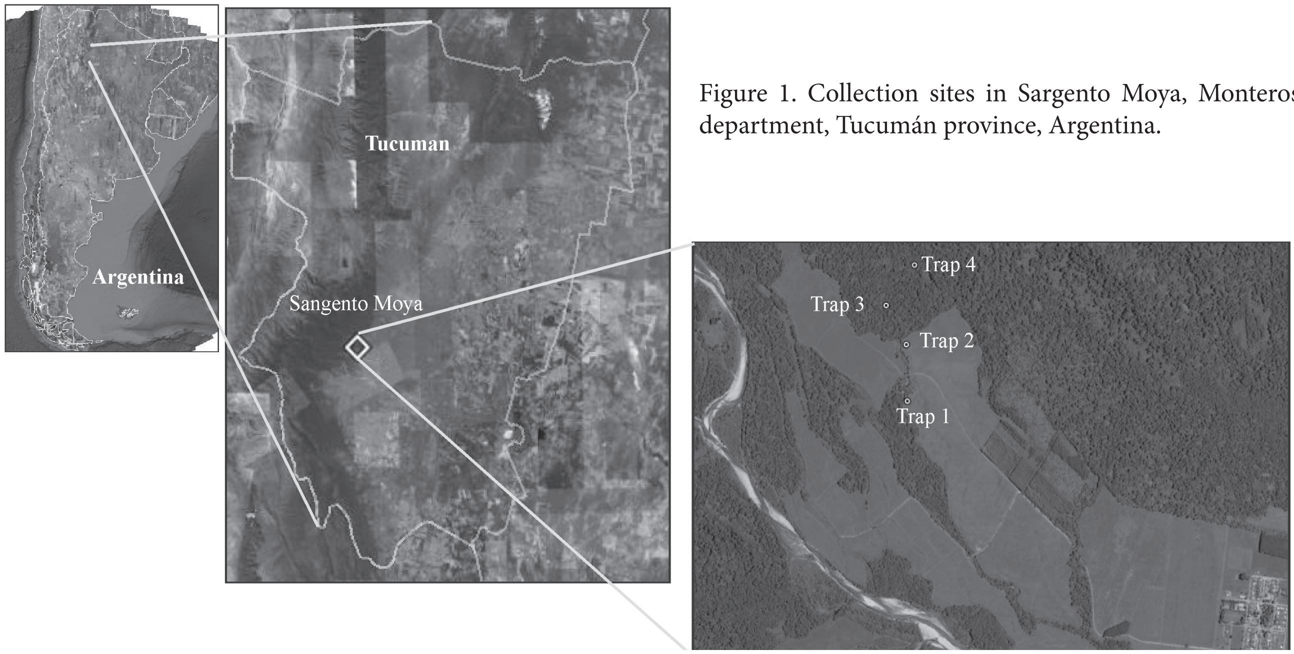

Figure 1. Collection sites in Sargento Moya, Monteros department, Tucumán province, Argentina.

Digilio and Legname 1966, Hueck 1972, Cabrera 1976). Canopy trees include *Blepharocalyx salicifolius* (H.B.K.) Berg., *Enterolobium contorsiliquum* (Vell.) Morong, and *Juglans australis* Griseb. Climber species belong to the Bignoniaceae, Ulmaceae, and Amarantaceae families. There are also vascular epiphyte plants such as Bromeliaceae. *Tipuana tipu* (Benth.) Kuntze, *Jacaranda mimosifolia* D. Don., *Tabebuia avellanedae* Lor. ex Griseb., *Tecoma stans* (L.) C. Juss. ex Kunth, and *Salix humboldtiana* Willd. found in clearings (Prado 1995). Human activity was present as large sugarcane and citrus fields.

After analyzing the area, the presence of two different habitats, forest and forest edge were determined. The forest was characterized by the presence of numerous trees above 25 m as well as shoots and was not related to any kind of environmental modifications. The forest edge habitat as a disturbed area was characterized by the presence of open vegetation, few trees above 25 m, many tree shoots, and few climbers, all of them related to human activity. The habitats where anopheline mosquitoes grow are related to forest areas, for example, habitats of *An. pseudopunctipennis* occur along the Andes valleys and foothills and agriculture, deforestation, and dam construction create new habitats that affect the mosquito population diversity and densities (Dantur Juri et al., unpublished data). Thus, in the present study, it was hypothesized that the conditions determined by the typical vegetation of these two habitats would be a potential factor in their relationship with the mosquito population.

Anopheline sampling

Sampling was carried out every 15 days for one year between October, 2002 and October, 2003. Two CDC light traps with carbon dioxide, active from 16:00 to 23:00, were placed in two habitats (forest and forest edge). Four traps were placed at least 100 m apart. In the forest, the traps were set approximately 100 m from the road edge. At the forest edge, traps were set 10 m away from the edge of a road or at 10 m from a stream border and 15 m away from the edge of a sugarcane crop (Figure 1).

Collected material was taken to the laboratory where the anophelines were mounted and identified using the morphological keys of Faran (1980) and Wilkerson and Strickman (1990).

Data analysis

The total abundance of mosquito species collected in both habitats was compared using a *t*-test. The abundance of the different species collected in the two habitats was analyzed using the Index of Species Abundance (Roberts and Hsi 1979).

Temporal variation observed between the different species was analyzed with multiple regression models using the different species as dependent variables. Regressive variables were quantitative environmental variables such as mean temperature, and maximum and minimum mean temperature, provided by the Pueblo Viejo weather station. In addition, the abundance of the anophelines collected was correlated with the environmental variables with a 15-day delay and, for the rainfall, with a 30-day delay, to verify whether the temporal accumulation of rainfall affected the fluctuation of anophelines (Table 1). The analysis also included relative ambient temperature and humidity recorded at the start of each sampling period using a digital thermo-hygrometer (Springfield Precision Instruments Inc.).

Several environmental variables measured at the sampling sites were taken into consideration, e.g., the state of the sky, the existence of wind or rain, and lunar phases (Table 1). Qualitative data were quantified. For instance, clear, partially cloudy, or cloudy sky was quantified as 0, 1, or 2, respectively, at the start of the sampling (sky code 1) and at its end (sky code 2); no wind, light, moderate, and strong wind were quantified as 0, 1, 2, and 3, respectively, and no rain, light, moderate, and strong rain as 0, 1, 2, and 3, respectively. Lunar phases were recorded during each sampling as new moon, waxing/waning, and full moon (0, 1, 2, respectively).

The general equation of the model developed was

$$y = a_0 + a_1 X_1 + \ldots + a_n X_n$$

where y represents each species of *Anopheles*, a_0 is the constant, and $a_1 \ldots a_n$ are the coefficients of the X_1 to X_n variables.

Multiple linear regressions were carried out using the Statistica 6.0 software (StatSoft Inc. 2001). Regressive variables were included in the multiple linear regressions, but the variables that contributed least were eliminated one by one taking into account the significance values ($P > 0.05$).

Table 1. List of environmental variables (X_1 a X_{21}) used to develop the models.

	Environmental variables
X_1	Mean temperature without delay
X_2	Mean temperature with a 15 day delay
X_3	Maximum mean temperature without delay
X_4	Maximum mean temperature with a 15 day delay
X_5	Minimum mean temperature without delay
X_6	Minimum mean temperature with a 15 day delay
X_7	Relative ambient humidity without delay
X_8	Relative ambient humidity with a 15 day delay
X_9	Wind velocity without delay
X_{10}	Wind velocity with a 15 day delay
X_{11}	Rainfall accumulated without delay
X_{12}	Rainfall accumulated with a 15 day delay
X_{13}	Rainfall accumulated with a 30 day delay
X_{14}	Temperature at the start of the sampling
X_{15}	Relative ambient humidity at the start of the sampling
X_{16}	State of the sky at the start of the sampling (sky code 1)
X_{17}	State of the sky at the end of the sampling (sky code 2)
X_{18}	Presence of wind at the start of the sampling (wind code 1)
X_{19}	Presence of wind at the end of the sampling (wind code 2)
X_{20}	Presence/Absence and abundance of the rain during sampling
X_{21}	Lunar phases during sampling

RESULTS

Five hundred and sixteen anopheline specimens were collected, the most abundant in the subgenus *Anopheles* of the Arribalzaga group (for example, *Anopheles* (*Anopheles*) *neomaculipalpus* Curry) (52.1%), followed by *An. strodei* (20.5%) and *An. evansae* (12.2%). *An. argyritarsis* (4.4%) and *An. pseudopunctipennis* (3.1%) were also collected. Only 6.6% of the anophelines captured could not be identified beyond the subgenus level (Table 2).

The Arribalzaga specimens were the most common in both habitats (standardized *ISA* = 1), followed in decreasing order by *An. strodei* (*ISA* = 0.88), *An. evansae* (*ISA* = 0.69), and *An. argyritarsis* (*ISA* = 0.44). The abundance of species estimated for *An. pseudopunctipennis* (*ISA* = 0.38), *An. rondoni* (*ISA* = 0.08), and *An. rangeli* (*ISA* = 0.02) reflect the small number collected per site.

All species were more abundant in the forest with 342 (66.3%) vs 174 individuals at the forest edge (33.7%) (Table 2). The *t*-test detected significant differences ($t = 0.3255$; $df = 14$; $P < 0.01$). All species were collected both in the forest and the forest edge except *An. rangeli* (forest edge only).

The greatest abundance for the specimens of the Arribalzaga group was in the fall with a smaller summer peak and a few specimens collected even in winter. *Anopheles strodei* showed a similar pattern, with a peak of abundance in the fall (Figure 2). *Anopheles argyritarsis* was the only species more abundant in the spring. *Anopheles*

Table 2. Absolute number (*N*) and percentage (%) of anopheline mosquitoes collected in two distinctive habitats in Sargento Moya, Tucumán, Argentina, from October, 2002 to October, 2003.

Species	Types of habitats: Forest Edge N	Forest Edge %	Forest N	Forest %	Total
An. (*Ano.*) *pseudopunctipennis*	8	4.6	8	2.3	16
An. (*Nys.*) *strodei*	44	25.3	62	18.1	106
An. (*Nys.*) *rangeli*	1	0.6	0	0	1
An. (*Nys.*) *argyritarsis*	4	2.3	19	5.5	23
An. (*Nys.*) *rondoni*	1	0.6	3	0.9	4
An. (*Nys.*) *evansae*	23	13.2	40	11.7	63
An. (*Ano.*) spp. (Arribalzaga group)	81	46.5	188	55.1	269
An. (*Nys.*) spp.	12	6.9	22	6.4	34
Total	174	100	342	100	516

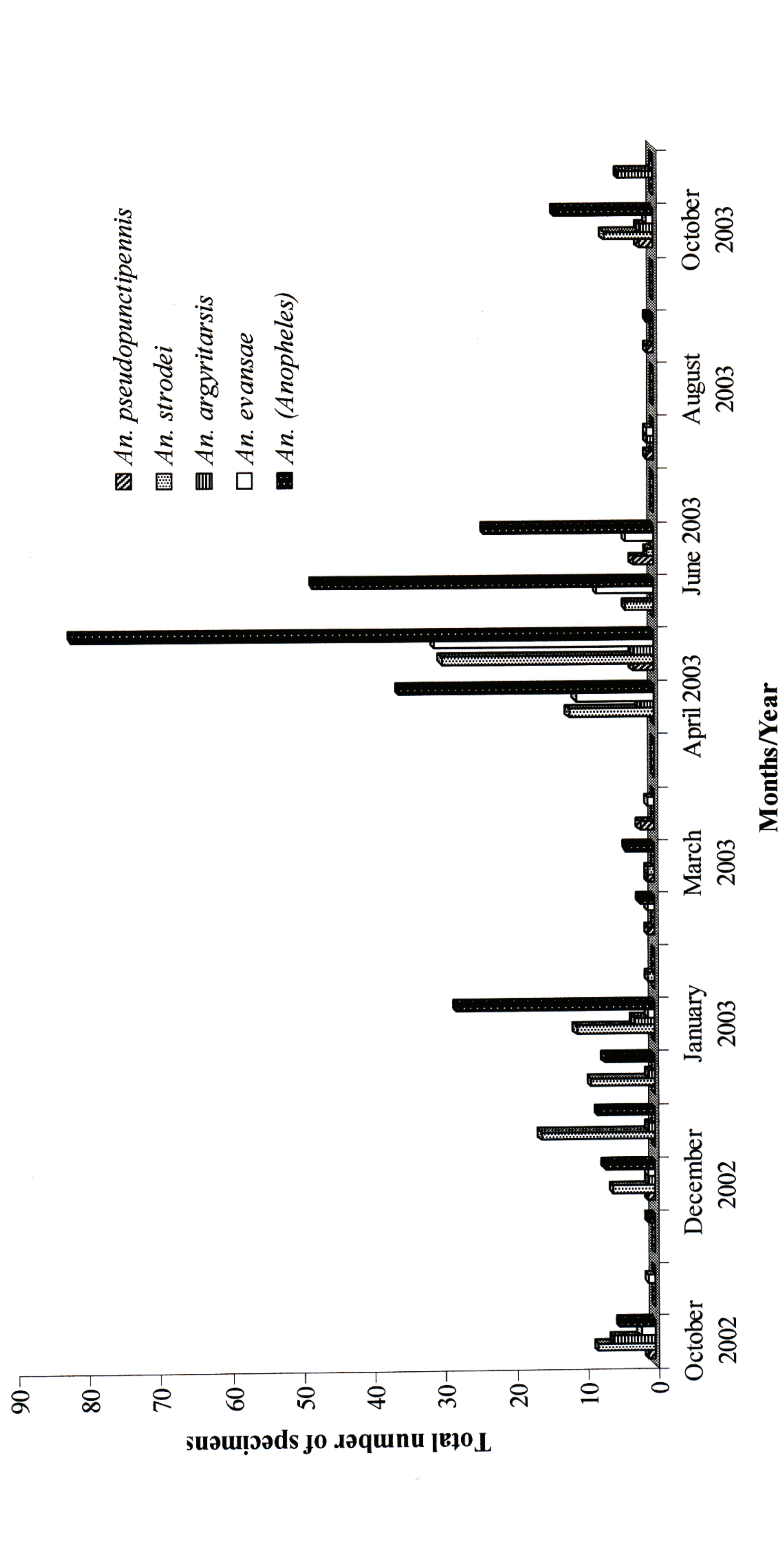

Figure 2. Specimens of *Anopheles* (*Anopheles*), *An. argyritarsis*, *An. strodei*, *An. evansae*, and *An. pseudopunctipennis* collected in Sargento Moya, Tucumán, Argentina, from October, 2002 to October, 2003.

pseudopunctipennis specimens, though few, were collected in all four seasons (Figure 2). The multiple regression analyses were significant for the specimens in the Arribalzaga group (R^2 adjust. = 0.48; $P < 0.001$) (Table 3), *An. strodei* (R^2 adjust. = 0.45; $P < 0.002$) (Table 4), *An. evansae* (R^2 adjust. = 0.32; $P < 0.001$) (Table 5) and *An. argyritarsis* (R^2 adjust. = 0.48; $P < 0.001$) (Table 6).

Relative ambient humidity with a 15-day delay (X_8) was closely related to the fluctuation of the specimens of the group Arribalzaga, *An. strodei* and *An. argyritarsis*. For *An. evansae*, wind speed without delay (X_9) was the variable most strongly connected with the temporal fluctuation.

DISCUSSION

The anopheline fauna of Argentina has been reported by several early authors including Paterson (1911), Petrocchi (1924), and Brèthes (1926). Juri et al. (2005) was the first to report the relationship between the temporal fluctuation of mosquito populations and environmental variability. According to the authors, the forest and the forest edge are on the altitudinal floor of the piedmont montane forest of the Yungas, mostly affected by different human activities, as described in the present paper.

Arribalzagia of the subgenus *Anopheles* were the most abundant in relation to the total number of specimens collected and to the habitat sampled, with the highest abundance of this anopheline in the forest. Despite their greater abundance and typical spatial distribution, it is not possible to determine whether or not they are malaria vectors. However, transmission could be favored by the continuing modifications of forest habitat resulting in the appearance of new habitats favorable to mosquito breeding. Thus, the growing deforestation occurring in Sargento Moya might favor the appearance of the forest edges where people are in closer contact with anophelines (Dantur Juri et al., unpublished data).

Anopheles strodei, *An. evansae,* and *An. argyritarsis* were also more abundant in the forest. *An. rondoni*, although not as abundant, were also more prevalent in the forest. *Anopheles pseudopunctipennis* was found in low numbers in both habitats. Although there were no differences in species diversity in the two habitats, the forest contained the largest number of anophelines. The forest edge is less productive, but the same potential vectors are present so it would not be free from the risk of transmission.

In El Oculto (Orán department, Salta province), in the northern Yungas, a study of the composition and temporal fluctuation of anophelines in three habitats (forest, forest edge, and around houses) (Dantur Juri et al. 2003) found, in order of abundance, *An. pseudopunctipennis, An. strodei, An. argyritarsis,* and *An. rangeli*. Although the forest was the most diverse, the analyses showed no significant differences between the three habitats, similar to the results of the present work. However, the greatest abundance of anophelines was detected in the forest border of the northern Yungas, while in this study the greatest abundance was in the forest.

Seasonally, the anophelines collected in Sargento Moya were more abundant in the fall, after the rainy season. The specimens of Arribalzaga were more abundant in the fall and to a lesser extent in the summer (January-April). The strong rains might flush out the larval habitats and reduce adult abundance. During the hot dry months, light rains and high temperatures would favor breeding places. In the fall and winter, the moderate temperatures and the rainfalls (as drizzle) would favor the growth of immatures and the later appearance of adults. Similar results were obtained by Dantur Juri et al. (2003) in El Oculto, where the Arribalzaga group was more abundant after the rainy season.

Anopheles strodei was also more abundant in the fall, decreasing in the summer and spring. In the northern Yungas, these anophelines appeared regularly throughout the year except in July, due to low temperatures, with the main abundance in the spring, when both temperature and rainfall are moderate (Dantur Juri et al. 2003). Furthermore, as in the northern area, *An. strodei* was the second most abundant species.

Table 3. Results of multiple regression analysis of the environmental variables related to the fluctuation of Arribalzaga group specimens in Sargento Moya, Tucumán, Argentina, from October, 2002 to October, 2003.

Variables/ Statistical coefficients	*Beta* ± *S.E.*	*B*	*P*
Intercept	-92.33 ± 22.45	-92.33	0.001
X_8	0.63 ± 0.16	0.94	0.005
X_{17}	0.47 ± 0.15	6.98	0.005
X_{14}	0.41 ± 0.16	0.99	0.05
X_{18}	-0.44 ± 0.18	-14.84	0.05

R^2 adjusted = 0.48; $P < 0.001$.

Table 4. Results of multiple regression analysis of the environmental variables related to the fluctuation of *An. strodei* specimens in Sargento Moya, Tucumán, Argentina, from October, 2002 to October, 2003.

Variables/ Statistical coefficients	*Beta* ± *S.E.*	*B*	*P*
Intercept	-34.76 ± 9.37	-34.76	0.001
X_8	1.08 ± 0.24	0.49	0.001
X_{17}	0.58 ± 0.16	2.70	0.005
X_{14}	-0.54 ± 0.15	-5.55	0.005
X_{15}	-0.50 ± 0.18	-0.24	0.05
X_{12}	-0.59 ± 0.21	-0.05	0.05
X_4	0.51 ± 0.24	0.46	0.05

R^2 adjusted = 0.45; $P < 0.002$.

Anopheles pseudopunctipennis was the only species collected in all four seasons, with few specimens, and an increase towards the spring (September-December). This distribution could have been favored by the moderate temperatures and by rainfall that was not heavy enough to eliminate the breeding places. In the northern Yungas, Dantur Juri et al. (2003) found a more regular temporal distribution of these anophelines, which were captured throughout the year in El Oculto, though maximum abundance occurred in the spring (September- December).

Anopheles argyritarsis was the only species more abundant in the spring in Sargento Moya, similar to that observed by Dantur Juri et al. (2003) in El Oculto. Perhaps spring and fall are similar, with moderate temperatures and rainfall that do not destroy the larval habitats and so favor these mosquitoes. Among the environmental variables analyzed, relative ambient humidity with a 15-day delay was the one that best explained such fluctuation for the specimens of Arribalzaga, *An. strodei,* and *An. argyritarsis*. In a study on *An. pseudopunctipennis* in the northern Yungas, maximum mean humidity was the variable that best accounted for the fluctuation (Dantur Juri et al. 2003). It can be inferred that in the Yungas the relative humidity is the variable most related to anopheline fluctuation.

In the case of *An. evansae*, wind speed, considered without delay in the analysis, as well as wind speed at the end of the sampling period, are the variables that best account for the fluctuation. This environmental variable would have to be further evaluated in the Yungas in relation to other variables such as vegetation covering, physiogeography, and availability of resting habitats.

Our results show that the Arribalzaga specimens are the most abundant anophelines and the only potential malaria vectors in the Yungas in Tucumán. The specimens of *An. strodei* were also abundant. This species is considered a secondary or potential vector and it was found naturally infected in malaria endemic areas in Amazonia (Consoli and de Oliveira 1994). Its presence in our study area and in the northern Yungas (Dantur Juri et al. 2003) suggests further studies to evaluate its role in malaria transmission. The risk of a malaria outbreak in the Yungas of Tucumán, due to the possible migration of infected people from endemic areas in Bolivia or Salta (Argentina), for example, could be increased by the presence of these potential vectors and by a series of favorable environmental conditions among which relative ambient humidity would be a key factor.

In the outbreak of 1996 in Salta, *An. pseudopunctipennis* was directly involved in the transmission of *Plasmodium vivax* (Boletín epidemiológico 1997). However, the role played by the other anopheline species in the region is unknown. Although the lowest number of anophelines was found in the forest edge in Sargento Moya, the diversity in both habitats was similar, so that in both, people can come in contact with potential *Plasmodium* vectors. The most propitious season for anopheline development in the area is from spring to fall, although the period with the greatest transmission risk would be the latter, when these mosquitoes are most abundant.

Table 5. Results of multiple regression analysis of the environmental variables related to the fluctuation of *An. evansae* specimens in Sargento Moya, Tucumán, Argentina, from October, 2002 to October, 2003.

Variables/ Statistical coefficients	*Beta* ± *S.E.*	*B*	*P*
Intercept	4.70 ± 1.29	4.70	0.001
X_{17}	-0.43 ± 0.13	-1.27	0.005
X_9	-0.45 ± 0.13	-0.93	0.005

R^2 adjusted = 0.32; $P < 0.001$.

Table 6. Results of multiple regression analysis of the environmental variables related to the fluctuation of *An. argyritarsis* specimens in Sargento Moya, Tucumán, Argentina, from October, 2002 to October, 2003.

Variables/ Statistical coefficients	*Beta* ± *S.E.*	*B*	*P*
Intercept	-10.31 ± 2.25	-10.31	0.001
X_{10}	1.07 ± 0.20	0.91	0.001
X_8	1.37 ± 0.27	0.14	0.001
X_{12}	-0.59 ± 0.16	-0.01	0.005
X_{15}	-0.51 ± 0.16	-0.05	0.005

R^2 adjusted = 0.48; $P < 0.001$.

Acknowledgments

We are grateful to Luis Oroño and Gabriela Quintana (INSUE, Facultad de Ciencias Naturales e IML, Universidad Nacional de Tucumán) and José Busnelli (Ingema, Facultad de Ciencias Naturales e IML, Universidad Nacional de Tucumán). We thank Francisco Ludueña Almeida (CIEC, Facultad de Ciencias Físicas, Exactas y Naturales, Universidad Nacional de Córdoba) for his assistance in the statistical analysis. We especially thank Jan E. Conn, Wadsworth Center, New York State Department of Health, for reviewing the manuscript. Also, we thank Ing. Lamelas (EEAOC, Tucumán) for providing meteorological data. This study was supported by the Agencia Nacional de Promoción Científica y Tecnológica (FONCyT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT).

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