Distribution of Phlebotomine Sand Flies (Diptera: Psychodidae) in a Primary Forest-Crop Interface, Salta, Argentina

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ABSTRACT Disordered urbanization and deforestation are the main activities proposed as causal factors of re-emergence of American cutaneous leishmaniasis caused by *Leishmania braziliensis*. The purpose of this work was to investigate, in the hyperendemic area of Argentina, the distribution of Phlebotomine sand flies at the modified primary vegetation-crop interface, as one of the potential sites where the effects of changing landscape on sand fly populations may be manifested. Twenty samplings were made between June 2004 and August 2005. The traps to catch sand flies were set on two consecutive nights every month (except in 5 mo, where it became every 15 d). The relationship between sand fly abundance and meteorological and landscape variables was analyzed using nonmetric multidimensional scaling and Kendall's correlation coefficients. *Lutzomyia neivai* (Pinto) was the most abundant species, followed by *Lutzomyia migonei* (França), *Lutzomyia cortelezzii* (Brèthes), *Lutzomyia shannoni* (Dyar), and *Lutzomyia punctigeniculata* (Floch and Abonnenc). Traps located close to modified areas collected the greatest numbers of sand flies, whereas traps located in the least modified area (adjacent to the primary vegetation) collected the fewest. There was a strong negative correlation between the abundance of sand flies and precipitation. This study shows that even small modifications in the landscape led to an increase in sand fly abundance, mainly *Lu. neivai*, a *Leishmania braziliensis* vector. This underscores the need for recommendations about the risk of American cutaneous leishmaniasis before any environmental intervention is done in an endemic area, as well as for the monitoring of sand fly population dynamics at the site of intervention, before, during, and after the process.

KEY WORDS ecosystems interface, *Lutzomyia neivai*, landscape modification, meteorological variables

The re-emergence of insect-transmitted diseases (e.g., leishmaniasis) worldwide, and the strong association of this phenomenon with environmental changes, have increased in the last two decades. Disordered urbanization and deforestation are the main anthropogenic activities proposed as causal factors (Lainson 1989, Mott et al. 1990, Walsh et al. 1993, Dedet 1999, Shaw 2007). Disordered urbanization implies the deforestation of secondary vegetation on the periphery of cities and the possible increase of human-vector contact as a result of alterations in the population dynamics of vectors (Salomón et al. 2008).

The fragmentation of forests is a potential cause of changes in microclimate, thus sand fly population structure, affecting both directly and indirectly the abundance, biomass, and diversity of species (Malcom 1997a, 1997b). The term "border effect" refers to a transitional zone between adjacent areas, in this work referred to as "interface." This concept has been extensively used to explain a wide variety of processes, such as changes in the origin, structure, and composition of borders and adjacent habitats at different scales of time and space (Fagan et al. 1999, Sarlov-Herlin 2001). De Luca et al. (2003), working in Mato Grosso State, southern Brazilian Amazonia, found changes in the abundance and species richness of Phlebotominae at the primary forest-crop interface, but did not find differences in abundance when analyzing the effects of the distance from primary forest to the forest edge.

The first records of American cutaneous leishmaniasis (ACL) in Argentina date back to 1916 (Bernasconi 1930). Salta province, located in northwestern Argentina, is one of the nine ACL-endemic provinces in the country (Salomón et al. 2004). The major vector species involved in the transmission of the causative agent, *Leishmania braziliensis*, in Argentina, and particularly in Salta province, is *Lutzomyia neivai* (Pinto

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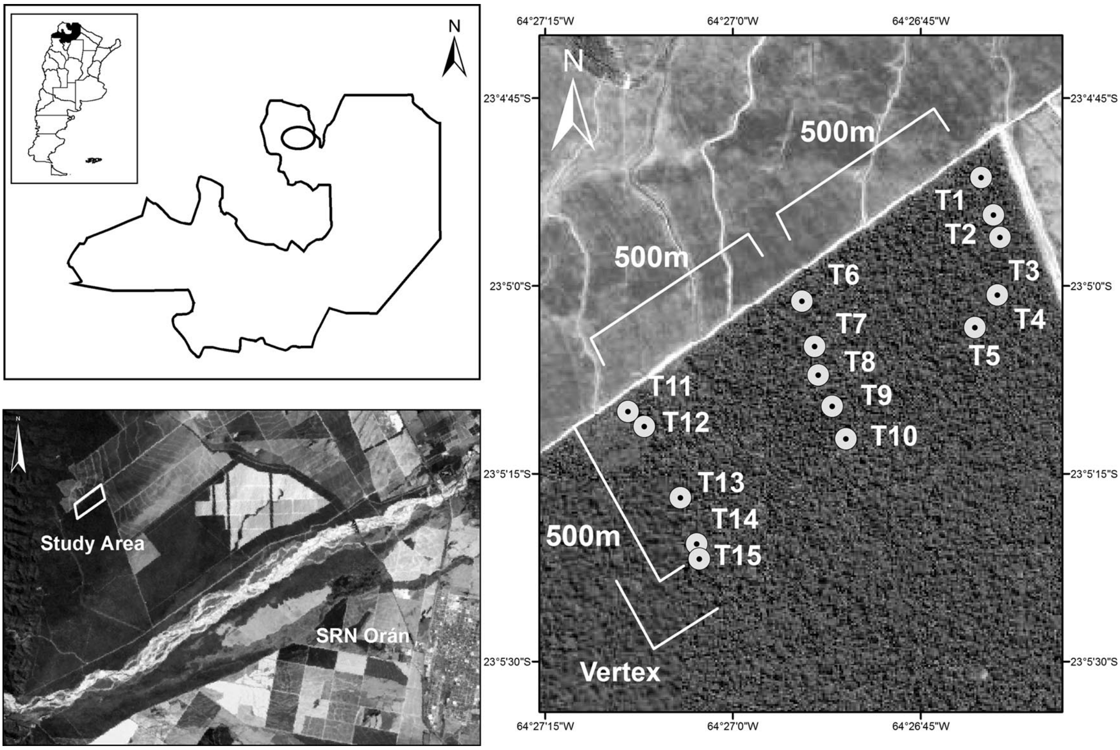

Fig. 1. Study area. Landsat 5TM satellite image (231/076, composition bands 752, 18/09/2006), provided by National Commission on Space Activities (CONAE), and image with diagram showing capture sites of adult Phlebotominae. Image was taken from Google Earth, version 5.1.3533.1731 (http://earth.google.com).

1926) (Diptera: Phlebotominae) (Córdoba-Lanús et al. 2006).

ACL has spread in Argentina, especially in the hyperendemic Argentine Northwest area (which includes northern Salta province), with increasingly frequent and more intense outbreaks since the first one occurred in 1985, concurrent with the advance of deforestation. However, the association between the risk of contracting ACL and deforestation is still speculative (Salomón 2002).

The study of *L. braziliensis* vectors has advanced since the 1950s, especially with respect to epidemic foci and peridomestic metapopulations. The relationship between the abundance of sand flies and meteorological variables in Argentine Northwest suggests bimodal or trimodal dynamics of sand fly population density associated with the rains and a higher proportion of gravid females during the highest peaks of transmission risk (Salomón 2002, Salomón et al. 2004).

The goals of this work were to explore the spatial and temporal relationships of the Phlebotomine sand fly community, analyzing their distribution with respect to the landscape and landscape disturbance, as well as the influence of meteorological variables. To achieve this, we studied the distribution of sand flies in the modified primary vegetation-crop interface as one of the potential sites where the effect of the landscape on the populations of Phlebotominae becomes manifest in the hyperendemic area of Argentina.

Materials and Methods

Study Area. This work was carried out in the Orán Department (23°08′S, 64°20′W), Salta province, at the Finca Los dos Ríos farming establishment, which comprises a 2.0 × 5.6-km area occupied by primary foothill forest modified by extraction of isolated trees, located at 400–450 m above sea level. The study quadrant is bounded by soy crops to the north and east in a continuum with primary vegetation to the west and south (Fig. 1). The foothill forest is part of the phytogeographic province of the Yungas or Tucuman-Oran cloud forest, with an annual mean temperature of 21.5°C and a well-marked dry season. Precipitation is concentrated in the summer season (November-May) and ranges between 800 and 1300 mm per year (data from Instituto Nacional de Tecnología Agropecuaria [National Institute of Agropecuary Technology]). This area has been subjected to intense deforestation for some years (Brown et al. 2001, Gasparri and Parmuchi 2003), mainly because of the expansion of soy crops. The study area, which encompasses modified primary vegetation in the municipality of Orán, a hyperendemic and epidemic area for ACL, was chosen on the basis of the history of anthropogenic alterations and epidemiological background information, with the aim of contributing to the development of surveillance and control strategies (Salomón 1999, Salomón et al. 2001, Alexander and Maroli 2003).

Sampling of Phlebotominae. Three 500-m-long transects, separated from each other by 500 m, were selected in the farming establishment. Transects were traced at a 107°43′ angle parallel to the edge of modified primary vegetation (Fig. 1). Centers for Disease Control and Prevention-type light minitraps (Sudia and Chamberlain 1962) were placed at 100-m intervals along each transect (15 traps). A total of 20 samplings was made between June 2004 and August 2005, with representation in all seasons. The traps were set on two consecutive nights each month (except 4 June, 4 August, 4 November, 5 February, and 5 July, where it became two consecutive nights every 15 d), from 1700 to 900 h, so that they were operational from sunset to sunrise.

Local meteorological data (minimum, maximum, and mean temperatures [°C]; total precipitation [mm]; mean relative humidity [%]; atmospheric pressure [mbar]; mean visibility [km]; mean wind speed [km/h]; maximum sustained wind speed [km/h]; total number of rainy days per month; total number of stormy days per month; and total number of foggy days per month) were obtained from two meteorological stations, one located in the Carmelitas plot, 4.3 km away from the study area, and another located at the Orán Aerodrome, 25 km away. Two secondary matrices were used, one with distance measurements (expressed as meters from each trap to the crop edge) and another with the meteorological variables (daily values).

Sand Fly Identification. The collected specimens were taken to the laboratory for subsequent processing and identification to species level according to the key of Young and Duncan (1994), including *Lu. neivai* following the redescription by Andrade et al. (2003). The insects were labeled by date and deposited in the collection of the Instituto-Fundación Miguel Lillo.

Analysis of the Results. To explore the spatial and temporal relationships of the sand fly species relative to trap locations and capture dates, we used nonmetric multidimensional scaling ordinations (NMDS) (Kruskal and Wish 1978), an ordination method that seeks the best position of *n* entities on *k* dimensions (axes) with lowest stress and displaying the strongest structure. Each of the new axes is a synthetic variable representing the primary gradients in species composition. Those axes are then related to measured environmental variables. The advantage of NMDS over other ordination methods is that no assumptions are made about how species are distributed along environmental gradients (Kenkel and Orlóci 1986).

NMDS is based on a Bray-Curtis distance matrix (Legendre and Legendre 1998), among the sampling units (traps) during the study period for spatial relationship, or the sample dates for the temporal relationship, both calculated from the abundance of sand fly species. Before performing the ordinations, the data were transformed using $\log_{10}(x + 1)$ to decrease the variation, and extreme values (those for which Bray-Curtis distance was ≥ 2 standard deviations) were discarded. To determine which species influenced the spatial segregation of traps, we calculated their average position on each axis using weighted averaging. This method accommodates for nonlinear, unimodal abundance curves along the axes (McCune and Grace 2002). The influence of the distance to the forest on trap segregation and species composition was estimated by Kendall ′s correlation coefficients (Sokal and Rohlf 1995) between the values of traps on the ordination axes and the distance of each trap to the edge of the nearest crop. The Kendall ′s correlation coefficient is commonly used to assess interjudge reliability, which expresses the simultaneous association (relatedness) between *k* sets of rankings (i.e., cases, variables). The range of Kendall ′s coefficient is from 0 to 1, with values close to 0 representing lack of agreement in the rankings of the variables among cases, whereas values close to 1 represent perfect agreement.

The same procedure was used to explore the influence of date of capture and distance to the edge on the spatial segregation of traps discriminated by sex, and to determine the relationship between species and spatial segregation by sampling day (S1 corresponds to the first day of sampling, and S1a to the second day of consecutive sampling). Kendall's correlation was calculated between the sample values on the ordination axes and the meteorological variables, to evaluate which of the meteorological variables influenced the segregation of samples and species composition.

For the ordinations, we used a two-dimensional configuration with final stress values of 5.12 (species composition of sand flies collected in traps), 16.83 (sand flies collected in traps discriminated by sex), and 6.88 (spatial segregation by sampling day), which were significantly different from random values (Monte Carlo: 50 runs with the randomized matrix, $P < 0.05$). The fit of these stress values was similar to or higher than most community ecology analyses, with stress values between 10 and 20 indicating a relatively good fit between the graphic form (perceptual map) and the matrix of Bray-Curtis similarity. A value <10 is good, and great when it is <5 (McCune and Grace 2002). The ordination graphs are biplots using weighted means. The multivariate analyses were made using PC-ORD 5.0 (McCune and Mefford 1999) and univariate analyses (STATISTICA 6.0, STATSOFT 2001). The ordination graphs were made with SIGMAPLOT 8.02 (SPSS 2002).

Results

A total of 4,673 Phlebotomine sand flies was collected belonging to five species: *Lu. neivai* (Pinto), *Lutzomyia migonei* (França), *Lutzomyia cortelezzii* (Brèthes), *Lutzomyia shannoni* (Dyar), and *Lutzomya punctigeniculata* (Floch and Abonnenc). *Lu. neivai* was the most abundant species at 94%. The sex ratio was 2.1:1 female:male (Table 1).

Table 1. Relative abundance of Phlebotominae species by samples, Orán Department, Salta, Argentina

Sample no.	*Lutzomyia neivai*	*Lutzomyia migonei*	*Lutzomyia cortelezzii*	*Lutzomyia shannoni*	*Lutzomyia punctigeniculata*	F:M	% GF *Lutzomyia neivai*	Total
S1	100	–	–	–	–	1.0	0.1	2
S2	97.95	1.37	–	–	0.68	16.8	10.0	146
S3	100	–	–	–	–	18.0	–	19
S4	96.91	2.64	0.45	–	–	8.5	8.8	227
S5	100	–	–	–	–	3.2	1.0	42
S6	75	25	–	–	–	3.0	–	4
S7	84.28	–	8.8	6.92	–	0.9	0.9	159
S8	94.16	–	5.11	0.73	–	1.0	0	137
S9	94.92	1.97	0.71	2.4	–	0.9	3.6	709
S10	28.57	28.57	42.86	–	–	2.0	–	7
S11	94.37	2.11	1.41	2.11	–	2.5	0.4	142
S12	94.76	0.94	2.28	2.02	–	1.5	18.8	744
S13	95	–	5	–	–	2.1	0.8	40
S14	91.9	2.7	2.7	2.7	–	2.0	0.2	37
S15	90.48	–	9.52	–	–	37.0	2.0	42
S16	98.23	0.59	–	1.18	–	13.5	20.0	340
S17	96.81	1.23	1.96	–	–	7.8	23.0	408
S18	100	–	–	–	–	6.1	5.8	114
S19	98	2	–	–	–	1.1	0.3	100
S20	90.11	9.33	0.16	0.4	–	2.4	3.0	1254
Total	93.88	3.47	1.41	1.22	0.02	–	35.04	4673

F:M, *Lutzomyia neivai* female/male ratio; GF, gravid females percent (>15 individuals).

The traps were arranged along two dimensions of the NMDS on the basis of sand fly species composition. The five species were located on the positive side of axis 1 (the first dominant pattern) (Fig. 2). *Lu. neivai*, *Lu. migonei*, *Lu. cortelezzii*, and *Lu. shannoni* were located in the center of the figure, whereas *Lu. punctigeniculata* was located away from the others four species, because only one specimen of this species was collected. The Kendall correlation analysis showed a significant negative strong association between the distance from each trap to the edge of the crop and axis 1 ($\tau = -0.48$, $N = 15$, $P = 0.01$). Axis 2 showed no relationship with the distance variable. The larger circles in Fig. 2 indicate greater distance to the primary forest-crop interface, whereas the smallest show proximity to the primary forest-crop interface, so the traps T1, T6, T7, T11, and T12 on the north side, and T2, T3, and T4 on the east side (smallest circles) showed the greatest abundance and were the closest to the edge of the crop. The traps with the lowest abundance were those located on the west side, at the vertex (the least modified part of the study area; see Fig. 1) adjacent to the primary vegetation, namely T5, T8, T9, T10, T14, and T15 (larger circles).

The particular case of T13 is noteworthy. Although this trap was not located near the crop edge (in Fig.

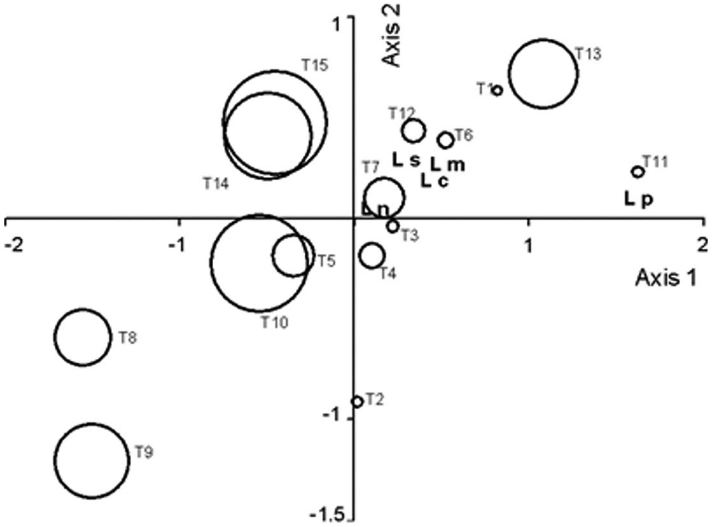

Fig. 2. Ordination diagram; NMDS of the location of traps on the basis of Phlebotominae species composition. Larger circles indicate greater distance to crop areas. The names of Phlebotominae are abbreviated to first letter of genus and species (Ln, *Lu. neivai*; Lm, *Lu. migonei*; Lc, *Lu. cortelezzii*; Ls, *Lu. shannoni*; Lp, *Lu. punctigeniculata*). T, traps.

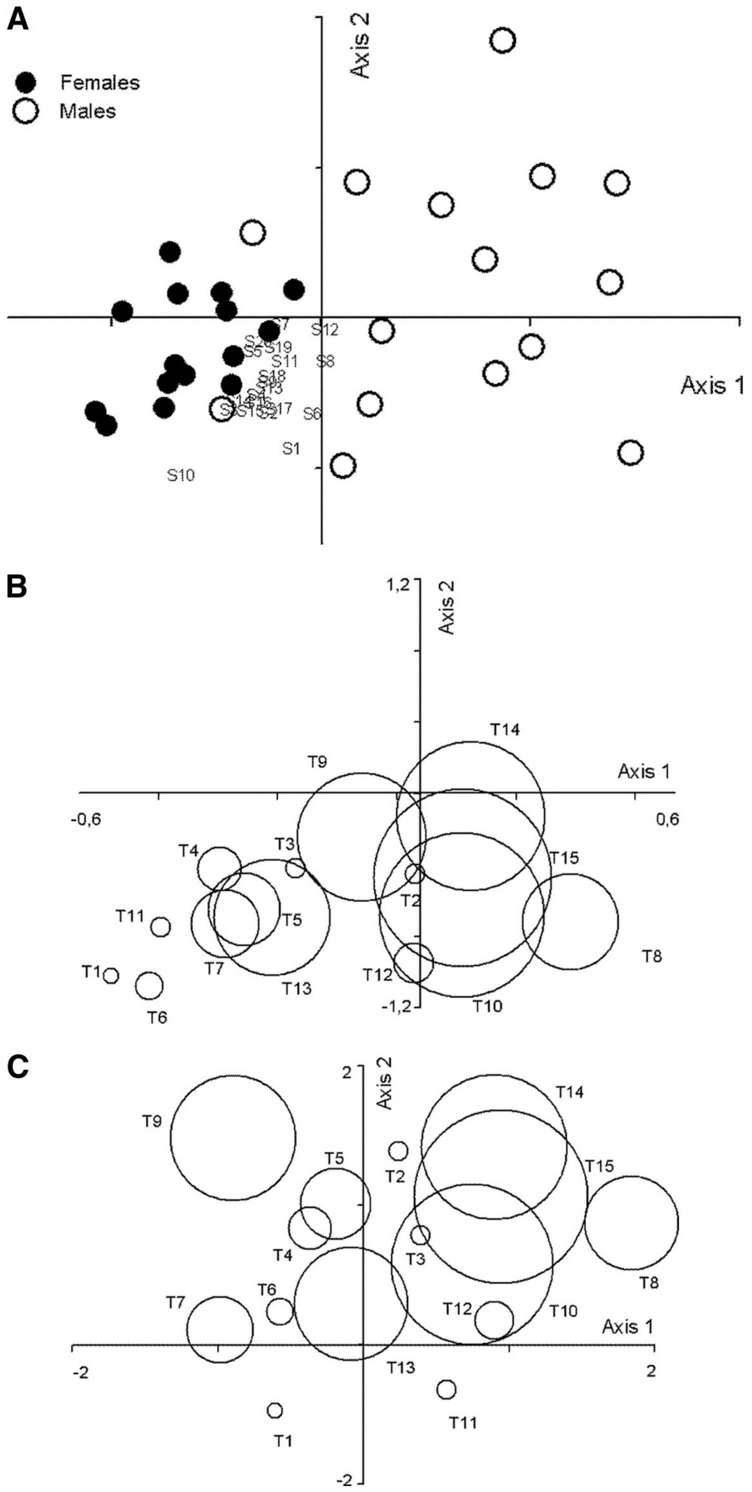

Fig. 3. Ordination diagram; NMDS of the location of traps discriminated by sex based on: (A) sampling days, and with the effect of distance on (B) females and (C) males. Larger circles indicate greater distance to crop areas. T, traps; S, samples.

2, with large circle), its surrounding landscape underwent intense modifications during the study period because of activities involving removal of wood, and consequently, this trap was clustered with those located near the crop edge.

The black dots in Fig. 3A represent traps 1–15 with

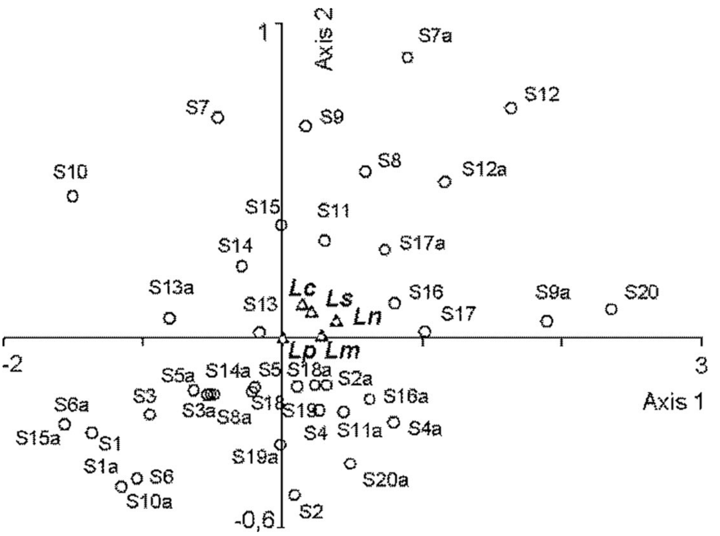

Fig. 4. Ordination diagram; NMDS of the location of sampling days on the basis of sand flies species composition. The names of Phlebotomine sand flies are abbreviated to first letter of genus and species. S, samples; a, second consecutive day of sampling.

total abundance of female, whereas empty dots represent traps 1–15 with total abundance of males, captured in each of them. The traps were spread along two dimensions of the NMDS on the basis of abundance of females and males by sampling days. The traps with females were clustered on the negative side of axis 1 with clustered samples, whereas the males were clustered on the positive side of the same axis. Most of the samples were concentrated on the negative side of axis 1 close to traps with females; this indicates that in the most samples, the females were more abundant than males in all traps, whereas S1, S6, S8, and S10 were placed toward the negative side of axis 2 (Fig. 3A) and samples S1, S6, and S10 had low abundance in total flies, and were only females; in S8, sex ratio did not differ. The effect of distance on males and females was positively (but weakly and not visibly obvious in the figure) associated with axis 1 ($\tau = 0.28$, $N = 30$, $P = 0.02$) (Fig. 3, B and C), and it was nonsignificantly associated with axis 2 ($\tau = 0.21$, $N = 30$, $P = 0.09$).

The sampling days were distributed along two dimensions of the NMDS on the basis of species composition. All the species were located in the center on the positive side of axis 1 (Fig. 4). The samples with similar species abundances are considered similar, and therefore, are closer in the multivariate space. The samples with similar species abundance were S9, S12, and S20 (highest abundances) positioned toward the positive side of axis 1; the samples S2, S4, S11, S16, S17, and S18 were located in the center (intermediate abundance values); whereas the remaining samples were placed toward the negative side of axis 1 (lowest abundance). The sampling dates showed four major peaks of species abundance, of which the highest corresponded to the spring of 2005 (S20), followed by the spring of 2004 (S9), then the summer of 2005 (S12), and lastly, the autumn of 2005 (S16 and S17). The Kendall correlation coefficients showed that precipitation, relative humidity, and occurrence of rain (total number of rainy days per month) were negatively associated with axis 1, whereas maximum temperature and mean visibility were positively associated with axes 1 and 2 (Table 2).

Discussion

The species of sand flies recorded in this study and their respective abundances are representative, given the previous knowledge of the area (Salomón 2002, Salomón et al. 2008). The traps located close to mod-

Table 2. Coefficients of Kendall correlations between the values of dates on the NMDS axes based on species composition and meteorological variables ($n = 40$)

Variable	Axis	Kendall τ
Maximum temp	1	0.281**
	2	0.309***
Minimum temp	1	0.045
	2	0.198
Precipitation	1	−0.377***
	2	−0.124
Atmospheric pressure	1	−0.081
	2	−0.191
Relative humidity	1	−0.256**
	2	−0.113
Mean visibility	1	0.242*
	2	0.321***
Mean wind speed	1	−0.109
	2	0.060
Maximum wind speed	1	0.025
	2	0.060
Rainy day	1	−0.280**
	2	0.035
Foggy day	1	−0.086
	2	−0.052

*, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

ified areas showed the greatest abundance; these results are similar to those obtained in studies of Diptera performed in northeastern and central-western Brazil (Malcom 1997b, De Luca et al. 2003). Thus, a small-scale change may trigger an increase in the abundance of sand flies, and these transition zones are possible hotspots for transmission (Salomón et al. 2006b, Ashford 2007).

In the case of those captures made in modified primary vegetation, the abundance of the different sand fly species was influenced by distance from or by the proximity to the crop areas or landscape changes. Few other studies have used this type of design. In Mato Grosso state (Brazil), the abundance of sand flies was also found to be lower in primary forest and higher in forests adjacent to crops, but that analysis showed a nonsignificant effect of distance (De Luca et al. 2003).

Abundance is shown as an increased gradient on a diagonal from continuous forest to the most altered zone (crop) of the study area. Thus, the capture of sand flies within the undisturbed forest seems to be more stable than captures made at the forest edge or peridomestic sites when they are related to extrinsic factors, such as landscape alterations and meteorological variables. Similar patterns were found in the city of Orán and surrounding areas (Salomón et al. 2008). During this study, great numbers of wild animals (mainly rodents) were observed leaving the primary vegetation patch boundary to feed on soybeans at the time of highest sand fly activity, in agreement with the results of Malcom (1997a) and Salomón (2002). The abundance of sand flies in the ecotone would be consistent with this available source of blood, despite the lack of a permanent source of blood in the areas of modified landscape. Females outnumbered males in all traps regardless of sampling dates. The effect of distance from the crop area on abundance was the same for both sexes.

The main peaks of abundance occurred in spring-summer and summer-autumn, which were separated by the rainy season; this agrees with previous studies (Salomón et al. 2004, Lemos and Lima 2005). However, sand flies occur in all seasons, with small as well as large abundance peaks. This contrasts with the situation found in Tucumán province (north-central Argentina), where seasonality is more pronounced, even though those studies (Córdoba Lanús and Salomón 2002, Salomón et al. 2004, 2006a) were performed in the same phytogeographical province as the present work.

In this work, species abundance was associated most frequently with precipitation, and therefore, with humidity and presence/absence of rain during the sampling dates. Although some association with maximum temperatures was found, this factor would not by itself be able to explain the changes in abundance. Other work carried out in the same phytogeographical area has evaluated similar relationships with meteorological variables, and the results agree with the current study (Salomón et al. 2004, 2006a). We conclude that the process of both large- and small-scale expansion of the agricultural frontier (mainly represented by soy plantations) resulted in a greater abundance of these vector insects, and potential reservoirs at boundary or interface areas, thus increasing the probability of human-vector contact. *Lutzomyia neivai,* the most abundant species found in this study, has been reported in high abundance in human-modified environments and has been incriminated as the vector during epidemics of *L. (V.) braziliensis* in several Argentinean and Brazilian foci of ACL (Salomón 2002, Rangel and Lainson 2003, Córdoba Lanús et al. 2006). Sand fly density is higher at the modified vegetation interface than inside the forest. This could be due not only to the adaptative capacity to changed environments shown by species such as *Lu. neivai,* but also to the concentrated feeding source provided by wild animals, plus human settlements with domestic animals close to the forest border usually seen in recent deforested scenarios (Salomón et al. 2008). Consequently, the effective contact between potential ACL-transmitting vectors and humans increases when new ecotones are created by deforestation, and even more so when the human settlements and domestic animals located near these edges provide a ready source of blood. Our results also show that even small modifications in the landscape generate an increase in the abundance of Phlebotominae; this reinforces the need for recommendations about the risk of ACL before any environmental intervention is made in an endemic area, as well as for the monitoring of the dynamics of sand fly populations at the site of intervention, both during and after the process.

Human activities associated with increased ACL risk include the deforestation of secondary vegetation to allow the expansion of cities by means of peripheral neighborhoods. As mentioned above, the relative abundance of sand flies inside the forest seems to be more stable than in modified areas, and therefore, those sites located at least 100 m away from the edge could be better indicators of natural dynamic population patterns of the Phlebotominae, compared with sites more prone to undergo man-made disturbances.

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