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ACTIVITY OF *LUTZOMYIA PSEUDOLONGIPALPIS* AND *L. LONGIPALPIS S.L.* (DIPTERA: PSYCHODIDAE) IN VENEZUELA

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Summary:

The nocturnal activity of the phlebotomine sandfly *Lutzomyia pseudolongipalpis* and two populations of *L. longipalpis s.l.* from different American visceral leishmaniasis foci in Venezuela was studied using collection bottle rotator traps. The activity of *L. pseudolongipalpis* from Lara State was continuous throughout the night, while that of *L. longipalpis s.l.* from El Layero, Guárico State and from Santa Ana del Valle, Margarita Island, was greatest before 23:00 h. The activity of sandflies of both populations and sexes steadily decreased thereafter. These different patterns seem to correlate with genetic data that indicate the presence in Venezuela of at least two sibling species in the *L. longipalpis* complex. The advantages of the bottle rotator trap for this type of study are discussed.

KEY WORDS : nocturnal activity, *Lutzomyia longipalpis s.l.*, *Lutzomyia pseudolongipalpis*.

Résumé : ACTIVITÉS DE *LUTZOMYIA PSEUDOLONGIPALPIS* ET *L. LONGIPALPIS S.L.* (DIPTERA: PSYCHODIDAE) AU VENEZUELA

L'activité nocturne du phlébotome Lutzomyia pseudolongipalpis *et de deux populations de* L. longipalpis s.l. *de différents foyers de leishmaniose viscérale américaine au Venezuela a été étudiée à l'aide de pièges à récipients tournants. L'activité de* L. pseudolongipalpis *de l'État de Lara est continue tout au long de la nuit, alors que celle de* L. longipalpis s.l. *d'El Layero, État de Guárico, et de Santa Ana del Valle, Île Margarita, est plus grande avant 23 h 00. L'activité des phlébotomes des deux populations et des deux sexes est franchement diminuée par la suite. Ces comportements différents semblent être corrélés aux données génétiques, avec la présence au Venezuela de deux espèces jumelles au sein du complex* L. longipalpis. *Les avantages du mode de piégeage retenu dans cette étude sont discutés.*

MOTS CLÉS : *activité nocturne*, Lutzomyia longipalpis s.l., Lutzomyia pseudolongipalpis.

INTRODUCTION

The phlebotomine sandfly *Lutzomyia longipalpis* (Lutz & Neiva, 1912) is the most widely distributed proven vector of the *Leishmania* spp. which cause the American visceral leishmaniasis (AVL) (Young & Duncan, 1994). Mangabeira (1962) was the first to suggest that this species might represent a species complex, based on differences in the number of pale spots on the male abdominal tergites. This hypothesis was later confirmed by crossbreeding studies (Ward *et al.*, 1983; Lanzaro *et al.*, 1993) and further supported by comparative analyses of pheromones (Ward *et al.*, 1988; Hamilton *et al.*, 1996a, b, c), and genetic profiles of different populations (Lanzaro *et al.*, 1993; Mutebi *et al.*, 1999; Arrivillaga *et al.*, 2000a). Many workers now accept that the *L. longipalpis* complex consists of at least four species (Uribe 1999; Arrivillaga *et al.*, 2003). Since not all of these have been formally described, the members are commonly referred to in the literature as *L. longipalpis sensu lato*.

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Isozyme analyses of Venezuelan populations have revealed the presence of two putative species distinguishable on the basis on hexokinase (HK), adenylate kinase (AK) and malic acid dehidrogenase-2 (MDH-2) loci (Arrivillaga, 1999; Arrivillaga *et al.*, 2000a, b). This has been confirmed by mtDNA (citocrome oxidase c unit 1) phylogenetic analyses of distinct geographical populations which also allowed two separate monophyletic lineages to be determined, one represented by the population from La Rinconada, Lara State (69° 55´ W, 9° 59´ N, 600 m a.s.l.) and the other including eight populations from separate AVL foci (Arrivillaga *et al.*, 1999). Further studies on the morphological characters of fourth larval instar (Arrivillaga & Feliciangeli, 2000) and adult female have allowed the population from La Rinconada to be described formally as *L. pseudolongipalpis* (Arrivillaga & Feliciangeli, 2001). Different epidemiological situations have been reported in Venezuelan AVL foci (Aguilar *et al.*, 1998; Delgado *et al.*, 1998; Feliciangeli *et al.*, 1999; Zerpa *et al.*, 2000). The population dynamics of *L. pseudolongipalpis* and other Venezuelan populations within the complex also show distinctive patterns (Feliciangeli *et al.*, 2001) suggesting that more bionomic studies are needed to provide a better understanding of AVL transmission dynamics. In this paper we give the results of observations on the nocturnal activity patterns of *L. pseudolongi-*

palpis and two populations of *L. longipalpis s.l.* from two geographical separate AVL Venezuelan foci.

MATERIALS AND METHODS

SANDFLY POPULATIONS

The nocturnal activity of *L. pseudolongipalpis* was studied during 10 nights in January and April 2002 at La Rinconada, Curarigua, Lara State (9° 59' N, 69° 55' W) the species type locality. The population of *L. longipalpis* from El Layero, Guárico State (9° 41' N, 67° 17' W) was collected in July and in September 2001. *L. longipalpis*, the vector of *Le. infantum* on Margarita Island, was caught in the village Santa Ana del Valle (11° 02´ N, 63° 54´ W) on 25 nights between October 2000 and January 2001.

RECORDING OF HOURLY ACTIVITY

Collection bottle rotators (CBR) with a platform containing removable pint jars (Model 1512, John W. Hock Comp.) and CDC miniature light traps (Model 512) were used to measure the hourly activity of sand flies. Each CBR had a timer circuit connected to a motor that rotated a new bottle under the light trap at one hour intervals. As each rotator has eight removable pint jars, two traps were programmed to complete 13 h from 18:00 p.m. to 07:00. Each jar contained 30 ml of absolute ethanol to trap and preserve the insects. The CBRs were always hung outdoors, in open animal sheds available at each locality, where, from previous collections, it was known that good numbers of sandflies would be caught, compared with traps hung around, e.g. in a goat shed in La Rinconada, a pigpen at El Layero and a chicken coop at Santa Ana del Valle.

STATISTICAL ANALYSIS

As the inspection of data by the Kolmogorov-Smirnov test showed that catches were not normally distributed in either La Rinconada, (Males: N = 130, Dmax = 0.2794, $p < 0.01$; Females: N = 130, Dmax = 0.3062, $p < 0.01$); El Layero (M: N = 117, Dmax = 0.3436, $p < 0.01$; F: N = 117, Dmax = 0.0358, $p < 0.01$) or Santa Ana del Valle (M: N = 325, Dmax = 0.4167, $p < 0.01$; F: N = 325, Dmax = 0.4616, $p < 0.01$), non-parametric tests were used to analyze the results. Sex ratios between species were compared by the χ^2 test. Because of the different situations in which the data were collected e.g.: different localities, years, months and animal shelters, the data in each locality were matched and the activity of the three populations throughout the night was analysed separately for each one and sex using the Kruskall-Wallis ANOVA, in order to identify intraspecific rather than interspecific variations.

RESULTS

The collection data for the three sandfly populations studied are presented in Table I, including the total of males and females collected, sex ratios and mean average numbers of sandflies collected per hour per night at each locality. The M:F ratio was significantly greater at La Rinconada than at El Layero ($\chi^2 = 140$, df = 1; $p = 0.0000$) and Santa Ana del Valle ($\chi^2 = 7.20$, df = 1; $p = 0.0073$). There was also a significant difference in the M:F ratio between El Layero and Santa Ana del Valle ($\chi^2 = 9.69$, df = 1; $p = 0.0019$) with more females in the former.

The mean number of sandfly counts per hour, transformed in Figs 1-3 as $\log_2$ (count +1) to normalize data, show the patterns of nocturnal hourly activity by species and sex. Activity of *L. pseudolongipalpis* was continuous in both females (H = 19.73, df = 12, $p = 0.0722$) and males (H = 19.95, df = 12, $p = 0.0680$). By contrast, the hourly activity patterns at El Layero was significantly different during the night in both, females (H = 53.53, df = 12, $p = 0.000$) and males (H = 39.27, df = 12, $p = 0.0001$), showing highest numbers of insects between 18:00-23:00 h. Activity then steadily declined during the

Species	Date	No. of nights	Total males	Mean males/hour/night (SD)	Total females	Mean females/hour/night (SD)	Sex ratio (m:f)
L. pseudolongipalpis Curarigua	25-31.I.02 17-19.IV.02	10	636	4.89 (8.37)	374	2.87 (5.37)	1.70:1
L. longipalpis s.l. El Layero	18-19.VII.01 25-27.VII.01 10-13.IX.01	9	1,242	10.61 (26.37)	1,752	14.97 (41.27)	0.71:1
L. longipalpis s.l. Isla Margarita	10-17.X.00 13.17.XI.00 12-15.XII.00 18-25.01.01	25	104	0.33 (0.71)	93	0.29 (0.68)	1.12:1

Table I. – Nocturnal collections of Venezuelan populations in the *L. longipalpis* complex.

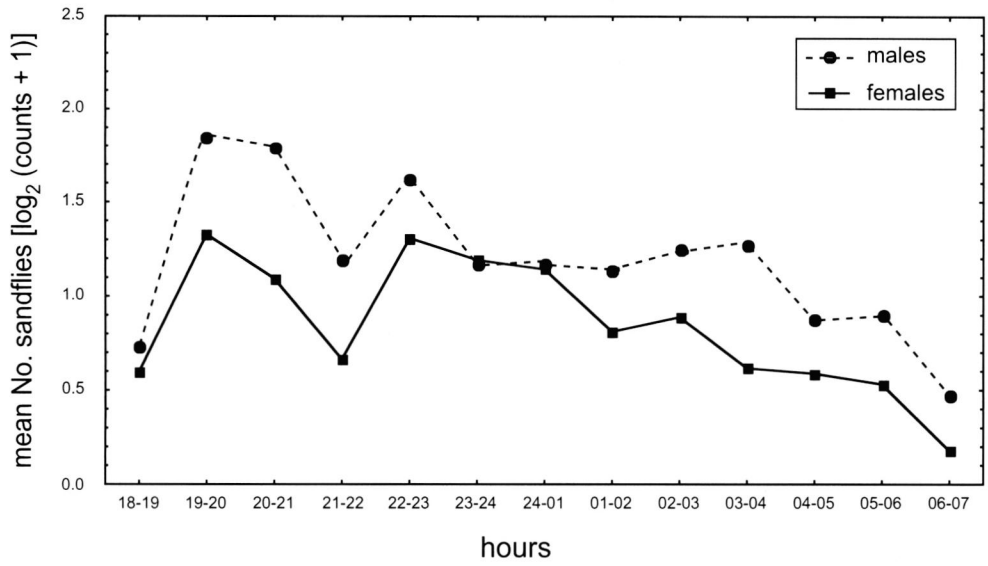

Fig. 1. – Hourly activity of *L. pseudolongipalpis* from La Rinconada, Curarigua, Lara State, Venezuela.

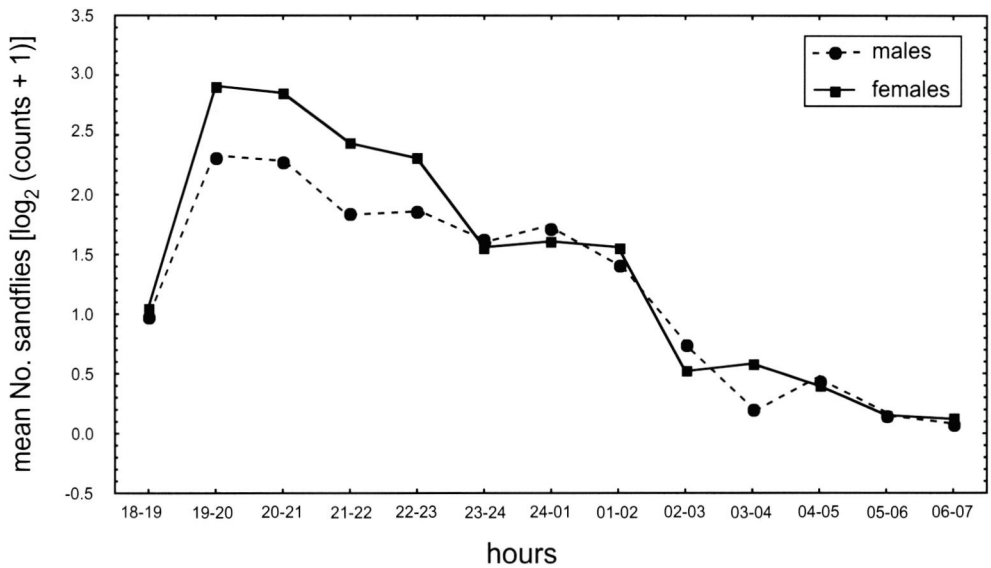

Fig. 2. – Hourly activity of *L. longipalpis s.l.* from El Layero, Guárico State, Venezuela.

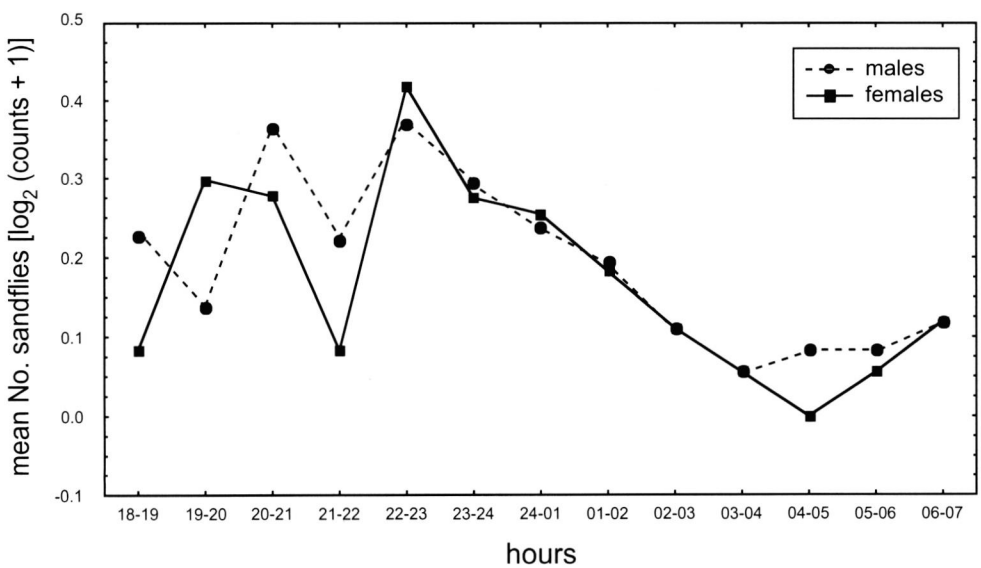

Fig. 3. – Hourly activity of *L. longipalpis s.l.* from the Island of Margarita, Nueva Esparta State, Venezuela.

rest of the night. Although more irregular, with a sudden fall of activity at 21:00, he *L. longipalpis* s.l. population in Santa Ana del Valle also showed a significant hourly variation in activity in both sexes: females (H = 34.56, df = 12, p = 0.005) and males (H = 26.20, df = 12, p = 0.0100) with most insects collected before 23:00.

DISCUSSION

Insect bionomics, which deals with the relationship of a given species and its environment, is affected by abiotic and biotic factors that vary over space and time. Thus, comparisons of the behaviour patterns of populations from different localities at the same and different times must be interpreted with caution. However, despite intraspecific variability due to climatological factors, different tropisms, food availability, etc., species-specific behaviour seems to be consistent and may reflect specific genetic features. Bruce-Chwatt (1980) felt that the biting cycles of many anophelines were so constant under different conditions that they reflected endogenous rhythms characteristic of particular species. The available evidences on specific biting activity patterns of members of the *Anopheles gambiae* complex, strongly supported this view (Braak *et al.,* 1994). Different hourly patterns *per se* could be a means of reducing competition between sympatric species, while it seems that there is no reason to suspect that different kind of hosts themselves would condition the activity pattern of a species, unless they would be available at different hours.

One of us (Feliciangeli, 1997) studied the activity patterns of *Lutzomyia ovallesi* (Ortiz, 1952) and *Lutzomyia gomezi* (Nitzulescu, 1931), sympatric proven vectors of *Leishmania* in an endemic focus of cutaneous leishmaniasis in Venezuela and observed that although there was some variability during different years and months, the shape of the curves clearly differed from each other.

In this study the populations were from three localities and collections were made in presence of different hosts and in different times of the year. Therefore, the analysis of the data, as referred above, was aimed to characterize the behavior of each population separately and not to make a comparison among them. The activity of both male and female *L. pseudolongipalpis,* was continuous throughout the night e.g.: no significant differences were found when comparing the numbers of sandflies caught in each hour. In El Layero it was observed most activity before 23:00 and a steady decrease in the numbers of both sexes until sunrise. In Santa Ana del Valle also the general trend for this population was similar to that of *L. longipalpis* s.l. at El Layero. To some extent, these results (e.g. two different patterns of activity) seem to correlate with genetic data that indicate the presence of at least two sibling species in Venezuela: *L. pseudolongipalpis* and *L. longipalpis* s.l. (Arrivillaga *et al.,* 1999; Lampo *et al.,* 1999; Arrivillaga *et al.,* 2003).

In Brazil a consistent pattern of activity has been reported in *L. longipalpis* s.l. from different AVL foci. Sherlock & Guitton (1969) in Jacobina, Bahia State, used the Castro's aspirator to sample *L. longipalpis* s.l. and observed that activity was greatest between 19:00 and 23:00 indoors and between 18:00 and 20:00 in nearby limestone caves. These authors also cited similar observations made by Deane in 1956 in Ceará State. Their findings were consistent with observations made by Quinnell & Dye (1994) on Marajó Island, Pará State, using CDC traps in chicken coops where the peak activity was registered between 20:00 and 22:00 h. Data on the nocturnal activity of *L. longipalpis* s.l. in Brazil correlate with isoenzyme and mitochondrial studies that revealed the existence of an unique Brazilian clade (Mutebi *et al.,* 2002; Arrivillaga *et al.*, 2003).

In El Callejón, Cundinamarca Department, Colombia, Morrison *et al.* (1995) studied the nocturnal activity of *L. longipalpis* s.l. during one year. Although they distinguished two activity patterns of *L. longipalpis* s.l., one with a peak at the early evening predominant in a pigsty and the other one being almost continuous throughout the night in a cattle corral, they reported that both patterns were observed in the two sites. Some years later in such locality, Mustermann *et al.*, (1998) found some substructuring in genetic variability of *L. longipalpis* s.l., possibly indicating the presence of more than one member of the complex in this area.

To conclude, studies on vector bionomics together with studies on vector genetics seem therefore expedient for the understanding of the transmission dynamics in epidemiological situations where sibling species are involved.

In relation with the method used to measure the hourly activity of the sandflies, it is worth to notice that this seems the first time that the CBR has been used for studies of sandfly activity. This method offers several advantages over protected human bait collections with manual aspirators or use of Shannon trap (Maroli *et al.*, 1997): *i)* ethical objections to the use of human volunteers in sandfly collections are circumvented; *ii)* labour requirements are reduced and costs lowered; *iii)* bias due to different levels of collecting expertise and dangers or stress involved with working at night are eliminated.

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