An experimental study of the peridomestic distribution of *Lutzomyia longipalpis* (Diptera: Psychodidae)

Rupert J. Quinnell and Christopher Dye

Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, and Instituto Evandro Chagas, Belém, Brazil

Abstract

Lutzomyia longipalpis (Lutz & Neiva), the vector of American visceral leishmaniasis (AVL), is much more abundant in animal sheds than in houses on Marajó Island, Pará State, Brazil. This difference in abundance is known not to reflect host preference. We show here that it also cannot be explained in terms of variable trapping efficiency, or insecticide application, and we exclude animal sheds as important daytime resting sites. In experimental sheds, the number of *L. longipalpis* increased markedly with the openness of the walls, though artificially large aggregations of flies could be generated in closed houses by using caged flies and hosts as attractants. We conclude that *L. longipalpis* tend to congregate at sites outdoors, including animal sheds, because these are the places where leks can most easily form on abundant, stationary (sleeping) and accessible hosts. These results help to explain why the seroprevalence of *Leishmania chagasi* infection is generally much higher among dogs than humans. They also indicate that human exposure to sandfly bites varies with the quality of house construction.

Introduction

The phlebotomine sandfly *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae) is the most important vector of American visceral leishmaniasis (AVL), caused by *Leishmania chagasi* (Lewis & Ward, 1987). AVL is a zoonosis: the main reservoir hosts are domestic dogs and the crab-eating fox *Cerdocyon thous* (Lainson *et al.*, 1983, 1990). Seroprevalence of AVL in canids is typically higher than in humans: on Marajó Island, Pará State, Brazil, 42% of dogs but only 2% of humans were found to be positive (Courtenay *et al.*, in press; Instituto Evandro Chagas, unpublished data). The role of entomological factors in explaining these different prevalences is unknown.

Lutzomyia longipalpis is much the commonest peridomestic sandfly on Marajó Island, being found in large numbers in animal pens, especially chicken sheds, and seemingly in much smaller numbers inside houses (Lainson *et al.*, 1983; Ryan *et al.*, 1984). The reason for this unequal distribution is unknown. It is not due to innate host preferences; *L. longipalpis* prefer people to dogs and chickens because they are larger (Quinnell *et al.*, 1992).

This paper examines other factors which may affect the numbers of *L. longipalpis* biting different hosts. We first quantified the abundance of sandflies in houses and in chicken sheds, taking into account the different trapping efficiencies and the use of insecticide. We then investigated whether accessibility to houses and the location of daytime resting sites were important determinants of the peridomestic distribution of these biting flies.

Correspondence: Christopher Dye, Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.

Materials and methods

Study site

The study was performed in a rural area in the municipality of Salvaterra, Marajó island, Pará state, Brazil (48°31'W, 004°S). This area is a patchwork of savanna, open woodland, cultivated land, secondary growth and remnant forest, with some flooded forest (varzea) along watercourses. The local inhabitants are mostly farmers; common domestic animals include chickens, ducks, dogs and pigs. Typical house construction is of dried mud on a wooden framework, or less commonly, all wood, with a

palm thatch or tiled roof. Poultry usually sleep in a shed made from wooden stakes with a thatched roof. Most houses in the study area are sprayed with DDT every few years by the Brazilian Ministry of Health (SUCAM).

Number of L. longipalpis *in houses and chicken sheds*

Seven houses which had never been sprayed with insecticide were chosen. Fifteen CDC miniature light-suction traps (hereafter 'CDC traps' or 'traps') were placed inside each house and a single CDC trap in its chicken shed on the same night. Fifteen traps was the maximum number that could be set inside a house without unduly inconveniencing the occupants. The dimensions of each house and chicken shed were also measured. In this and all following experiments traps were set from 18.30-06.30 h; traps were examined in the morning and all *L. longipalpis* counted and sexed. Sandflies were typically identified on external characters; doubtful females were identified by examination of spermathecal morphology.

Effect of accessibility

Experiments were carried out using 3 chicken sheds which differed only in their wall construction. Sheds were built on a wooden frame, $0.8 \times 0.8 \times 2.2$ m high, with plywood walls and door and a palm thatch roof. The height of the shed was chosen to equal the average height of houses in the area. One shed ('closed') had walls and door of sheet plywood, and access for sandflies was only possible through the roof and through small cracks around the door. The other sheds had walls and door constructed of six or seven plywood planks, each 10 cm wide, equally spaced, and were thus '25% open' or '12.5% open'.

In each experiment one shed was placed in the back yard of a house, 5-10 m from the resident chicken shed. At 18.30 h all chickens were removed from the resident shed and placed in the experimental shed, and a single CDC trap without a bulb was suspended above the chickens. Replicates (6-9) were carried out every 2-3 nights, and experimental sheds alternated between nights. Three experiments were carried out at two sites: comparing closed and 12.5% open sheds, closed and 25% open sheds (Site B) and 12.5% and 25% open sheds.

Effect of a bait on accessibility

This experiment was carried out in an unoccupied house, with no chicken shed nearby. The house was of typical construction, with earthen walls, palm thatch roof and wooden doors and windows. At night the only access points were through the roof or eaves, and through small cracks around the doors and windows. The experiment compared the ability of either two caged chickens or two chickens caged with *L. longipalpis* to attract sandflies into the house. Caged *L. longipalpis* had been trapped the previous night in a nearby chicken shed. When chickens and sandflies were used together, means of 300 males and 400 females were placed in a separate cage above the birds. Two CDC traps were set in the house each night.

Eight separate trials were each conducted over 2 nights. The first night served as a control with no chickens or sandflies; on the second night chickens were present with or without sandflies (4 occasions each). An interval of 2 or more nights separated each trial to prevent sandfly populations building up within the house or in the vicinity.

Number of L. longipalpis *resting in chicken sheds by day*

A single CDC trap was placed in each of two houses and their respective chicken sheds. On the following night one of the two sheds was covered in sandfly-proof netting at 18.00 h, and traps were again set in the sheds and houses A total of five pairs of sheds was used.

Hourly activity pattern of L. longipalpis

A single CDC trap was installed in each of 9 sheds over 4 nights (9 trap-nights). The cage was changed hourly between 18.00 h and 06.30 h, except for the period 23.00 h-04.30 h. During the same period, a single observer made hourly Shannon trap (essentially an illuminated sheet from which sandflies are aspirated) catches from 18.00-23.00 h, and from 04.30 h-06-30 h, on 4 nights in each of two yards in the vicinity of a house and a shed.

Results

Number of L. longipalpis *in houses and chicken sheds*

In most instances the quantity log (number of sandflies+1) was approximately normally distributed, and standard parametric tests were used. Where this was not the case, we used non-parametric tests.

Fifteen traps inside houses caught a total median number of 92 (range 10-118) *L. longipalpis*, compared to 835 (121-2786) caught by single traps in chicken sheds (Wilcoxon rank-sum test, $z=-2.37$, $n=7$, $P=0.018$). The mean volumes of houses and sheds were 100 m^3 and 3.6 m^3, respectively, a ratio of 28.

Effect of accessibility

In each experiment traps in the closed shed caught almost no *L. longipalpis*, and significantly fewer than the more open sheds (table 1). The 12.5% and 25% open sheds did not catch significantly different numbers of sandflies. In neither comparison between closed and open sheds were the numbers of individuals of other sandfly species (of which 80/83 were *L. trinidadensis* (Newstead)) significantly different.

Table 1. The total numbers of *Lutzomyia longipalpis* caught in experimental chicken sheds with more or less open walls

Shed	n	*L. longipalpis*			Other sandfly spp	Unidentified sandflies
		males	females	total		
25% open	6	125	77	203	0	0
closed	6	0**	1*	1**	8	1
12.5% open	6	27	5	32	13	1
closed	6	0**	0	0**	37	3
12.5% open	9	267	151	419	13	5
25% open	9	251	223	481	12	3

n=number of replicates; *$P < 0.05$, **$P < 0.01$, comparisons between pairs of sheds by Mann-Whitney U test.

Table 2. The geometric mean number of sandflies caught inside a house baited with 2 chickens ± male and female *Lutzomyia longipalpis*

Bait	n	*L. longipalpis*			Other sandfly spp.
		males	females	total (SD range)	
None	8	2.5	3.3	6.1 (3-12)	1.7
Chicken only	4	4.6	3.9	8.5 (2-30)	2.3
Chicken+*L. longipalpis*	4	26.9	40.2	68.3 (35-132)	2.5
$F_{2,15}$		10.4**	13.2***	12.0**	0.14

n=number of replicates; $^{**}P < 0.01$, $^{***}P < 0.001$, comparisons between rows by ANOVA.

Effect of a bait on accessibility

The number of sandflies attracted into the house by chickens only was very low, and not significantly greater than the number captured in the absence of a host (table 2). In contrast, chickens plus caged *L. longipalpis* attracted about six times as many males and ten times as many females as chickens alone.

Number of L. longipalpis *resting in chicken sheds*

Before the addition of sandfly-proof netting, similar numbers of sandflies were caught in control and experimental sheds (table 3). The effect of netting was to reduce numbers to 4% of the first night's catch, or to 5% of the catch taken in the control shed. Paired comparisons of untransformed proportions provided no evidence that the sex ratio of sandflies was affected by the netting (table 4). Catches inside adjacent houses were slightly, but not significantly, higher when access to the shed was prevented (table 3).

Hourly activity of L. longipalpis

Seventy-seven sandflies captured by aspiration in Shannon traps were caught throughout the night, but most were obtained between 19.00 h and 20.00 h (fig. 1). Hourly CDC trap catches of sandflies in sheds (3656 sandflies in total) were more homogeneous than the Shannon catches, though with a distinct and later peak of activity between 20.00 h and 22.00 h. Figure 1 plots the distributions for sandflies of both sexes; the patterns for males and females in animal sheds were essentially the same.

Table 3. Geometric mean number (and SD range) of *Lutzomyia longipalpis* captured in houses and chicken sheds where the shed was or was not covered with sandfly-proof netting.

Treatment	n	Number of *Lutzomyia longipalpis*	
		night 1 (−net)	night 2 (+net)
(a) Shed			
+Netting	5	432 (148-1259)	18** (5-63)
Control	5	504 (231-1099)	348 (105-1148)
(b) House			
+Netting	5	5 (1-16)	8 (4-16)
Control	5	6 (2-13)	4 (2-6)

n=number of replicates; $^{**}P < 0.01$, comparison between netted and control sheds by paired t-test.

Table 4. Mean sex ratio (males/total, ± SD) of *Lutzomyia longipalpis* captured in houses and chicken sheds where the shed was or was not covered with sandfly-proof netting.

Treatment	Sex ratio of *Lutzomyia longipalpis*	
	night 1 (−net)	night 2 (+net)
(a) Shed		
+Netting	0.683 ± 0.11	0.637 ± 0.12
Control	0.672 ± 0.13	0.613 ± 0.08
(b) House		
+Netting	0.563 ± 0.14	0.651 ± 0.26
Control	0.591 ± 0.26	0.467 ± 0.36

Discussion

Lutzomyia longipalpis is known to be very numerous in animal pens, both in the Amazon region and elsewhere in Brazil. However, comments regarding its relative abundance in animal pens and houses must be treated with caution, because of the differing catching efficiencies in these sites. A single CDC trap is likely to catch a smaller proportion of sandflies in a house than in a chicken shed: in sheds, hosts and trap are in closer proximity in a smaller total volume. Similar arguments apply to other methods of capture, such as aspirating sandflies off walls. In this study, we attempted to compensate for trapping efficiency by increasing the number of traps set in a house to 15. Whilst the fraction of total volume trapped in sheds was about twice (28/15) that in houses, traps in sheds caught nine times the number of sandflies.

There are several possible explanations for the disparity generally seen between houses and sheds: sandflies may have an inborn preference for chickens, they may be killed in houses or repelled from them by insecticide, or they may enter chicken sheds more readily. Previous experiments and observations on *L. longipalpis* discount the first of these. Sandflies do not prefer to feed on chickens rather than people; the most important determinant of preference is

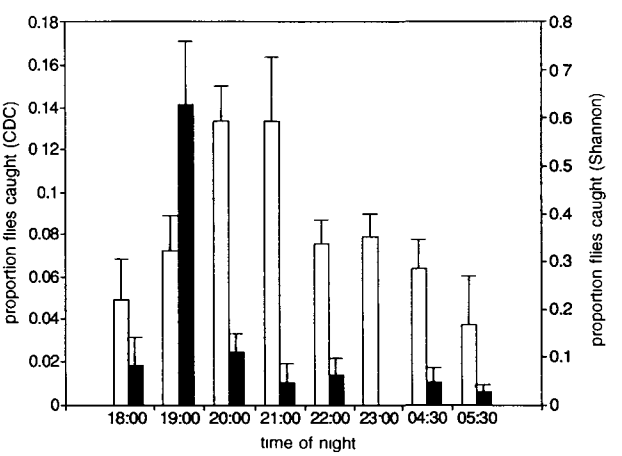

Fig. 1. Mean proportion (+SE) of a total night's catches of *Lutzomyia longipalpis* (both sexes) caught per hour in Shannon traps outdoors (solid bars, 6 nights), and in CDC traps in animal sheds (open bars, 9 nights). The x-axis gives the starting time of each trapping hour. No Shannon trap catches were made between 23.00 h and 04.30 h.

probably host size (Quinnell *et al.*, 1992). On this basis, more sandflies would be expected in houses. As for the second possibility, DDT is known to reduce the abundance of *L. longipalpis* in houses (Quinnell & Dye, in press), but insecticide is irrelevant to the results of the present study because we trapped in houses which had never been sprayed.

Experiments described here give most weight to the third explanation, that the interiors of houses are relatively inaccessible to *L. longipalpis*. Our closed experimental sheds, which could apparently be invaded by another species of sandfly, *L. trinidadensis*, attracted very few *L. longipalpis* in contrast to more open sheds. Even, when *L. longipalpis* were prevented from entering a chicken shed by sheathing it in netting, very few sandflies were apparently deflected to the nearest house.

Lutzomyia longipalpis are generally disinclined to feed in houses, but they can be drawn into them by large numbers of caged sandflies used as bait with vertebrate hosts. The males produce a pheromone which has been shown to attract females from a distance in the laboratory (Morton & Ward, 1989). Pheromones are presumably the means by which males can powerfully attract females, and to a lesser extent other males, to feeding and mating sites in the field (Dye *et al.*, 1991). Our results indicate that, under experimental conditions, the pheromone can attract other sandflies into a house with shuttered doors and windows, but hosts alone attracted few or no sandflies. High densities of sandflies are rarely found in houses; they tend to accumulate in chicken sheds instead. We suggest that, during the early evening, sandflies are more likely to begin assembling in the relatively open sheds. They are drawn there at first by host odour, but then a pheromone produced by the early males acts as an additional attractant to males and females, magnifying the difference in abundance between sheds and houses.

This difference is essentially re-established each night. Traps put in a shed which had been covered in sandfly-proof netting before the sandflies became active at dusk, caught a small percentage of the usual total number of sandflies, in the same sex ratio. This indicated that, whilst sandflies are abundant in sheds by night, most of them do not rest there by day. The comparisons between hourly Shannon trap catches made outdoors and CDC trap catches made in sheds imply that sandflies become active outside around 18.30 h but the majority find mating and feeding sites on roosting chickens and sleeping pigs in sheds by 20.00 h. Whilst aggregations of sandflies will form on stationary human baits outdoors (Quinnell *et al.*, 1992), a Shannon trap plus an active human collector is obviously not a suitable aggregation site.

If sandflies do not rest in sheds by day, they may rest nearby. When investigating the colonization of new chicken sheds placed within 10 m of resident sheds, Dye *et al.* (1991) observed a continuous change in sex ratio over a period of two weeks, implying that sandflies tend to return to the shed in which they were active the previous night.

The preference of *L. longipalpis* for aggregating at sites where stationary (sleeping) hosts are most accessible may help to explain the relatively low seroprevalence of AVL in humans on Marajó compared with dogs, though differences between hosts in their response to infection need to be considered too. In the study area people typically go indoors between 19.00 h and 20.00 h. They are unlikely to be bitten when active outdoors during the early evening, and are protected indoors when sleeping. Most dogs sleep outside houses and will therefore be more exposed to sandfly bites.

Highlighting a role for accessibility leads obviously to the proposition that people who live in poorly constructed houses are more likely to be bitten by *L. longipalpis*, and to be at greater risk of contracting *Leishmania chagasi* infection. A comparative survey of homesteads on Marajó Island found that houses which had more holes in walls and roofs did indeed have more sandflies (Quinnell & Dye, in press), although we have not yet confirmed that they harbour a larger fraction of the local infected sandfly population.

Acknowledgements

We thank N.F. Nazare, I.R. Barata, J.B.P. da Luz and A.F. Nascimento Filho for technical assistance and Dr J.J. Shaw & D. Kelly for advice. Dr J. Travassos da Rosa & Professor R. Lainson kindly provided facilities at the Insituto Evandro Chagas. The study was funded by the Wellcome Trust.

References

Courtenay, O., Macdonald, D.W., Lainson, R., Shaw, J.J. & Dye, C. (in press) Epidemiology of canine leishmaniasis: A comparative serological study of dogs and foxes in Amazon Brazil. *Parasitology*.

Dye, C., Davies, C.R. & Lainson, R. (1991) Communication among phlebotomine sandflies: a field study of domesticated *Lutzomyia longipalpis* populations in Amazonian Brazil. *Animal Behaviour* **42**, 183-192.

Lainson, R., Dye, C., Shaw, J.J., Macdonald, D., Courtenay, O., Souza, A.A. & Silveira, F-.T. (1990) Amazonian visceral leishmaniasis: distribution of the vector *Lutzomyia longipalpis* (Lutz & Neiva) in relation to the fox *Cerdocyon thous* (L.) and the efficiency of this reservoir host as a source of infection. *Memorias do Instituto Oswaldo Cruz* **85**, 135-137.

Lainson, R., Shaw, J.J., Silveira, F.T. & Fraiha, H. (1983) Leishmaniasis in Brazil: XIX. Visceral leishmaniasis in the Amazon region, and the presence of *Lutzomyia longipalpis* on the island of Marajó, Pará State. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **77**, 323-330.

Lewis, D.J. & Ward, R.D. (1987). Transmission and vectors. pp. 235-262 *in* Peters, W. & Killick-Kendrick, R. (*Eds*) *The Leishmaniases in Biology and Medicine*. London Academic Press.

Morton, I. & Ward, R.D. (1989) Laboratory responses of female *Lutzomyia longipalpis* sandflies to a host and male pheromone source over distance *Medical and Veterinary Entomology* **3**, 219-223.

Quinnell, R.J. & Dye, C. (in press) Correlates of the peridomestic abundance of *Lutzomyia longipalpis* (Diptera: Psychodidae) in Amazonian Brazil. *Medical and Veterinary Entomology*.

Quinnell, R.J., Dye, C. & Shaw, J.J. (1992) Host preferences of the phlebotomine sandfly *Lutzomyia longipalpis* in Amazonian Brazil. *Medical and Veterinary Entomology* **6**, 195-200

Ryan, L., Silveira, F.T., Lainson, R. & Shaw, J.J. (1984) *Leishmania* infections in *Lutzomyia longipalpis* and *Lu. antunesi* (Diptera: Psychodidae) on the island of Marajó, Pará State, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78**, 547-548.

(Accepted 5 January 1994)