

## DISPERSAL OF PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE) IN A COLOMBIAN FOCUS OF *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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*Dispersal of five species of phlebotomine sand flies was studied in a coffee plantation near Arboledas, Colombia, by mark-release-recapture studies using fluorescent powders. The estimated recapture rate for males of *Lutzomyia shannoni* marked and released during the day was 28.1%, significantly higher than that for all other species ( $p < 0.05$ ). Recapture rate of female *Lu. shannoni* was 9.5%, and no females of the other four species were recovered. This suggests either that *Lu. shannoni* is a more sedentary species than the others, or that the large trees on which these insects were captured and recaptured function as foci of lekking behaviour in males. The high recapture rates of females of this species may indicate that oviposition occurs in close proximity to the bases of these trees. Although most marked sand flies were recaptured within 200 m of their release point, a single female *Lu. gomezi* was recovered 960 m away 36 h after release. This suggests that the dispersal capacity of *Lutzomyia* species may be greater than has been thought, an important consideration in future control programs directed against these insects in Leishmania-endemic areas.*

Key words: *Leishmania (Viannia) braziliensis* – phlebotomine – coffee plantation – dispersal – mark-release – recapture – Colombia

Phlebotomine sand flies of the genus *Lutzomyia* include all known vectors of *Leishmania* in the New World (Killick-Kendrick, 1990). Their biology as it relates to human leishmaniasis differs somewhat from that of their Old World relatives of the genus *Phlebotomus*, particularly in their diurnal resting site preferences and degree of peridomestic and intradomestic activity. The latter are often found in urban areas where human habitations, shelters of domestic animals and rodent burrows offer microhabitats in which the insects can rest during the day as well as breed. By contrast, most *Lutzomyia* species are associated with a sylvatic habitat, so that man is only bitten when he enters or settles in forested areas (Lainson, 1983).

This dependence on some degree of forestation can be viewed both as advantageous and disadvantageous for leishmaniasis control programmes directed against the sand fly vectors. Insecticidal control cannot readily be im-

plemented unless intra- or peridomestic transmission of the disease occurs so that the walls of dwellings and animal shelters can be sprayed. Treatment of resting and breeding sites is generally impractical due to the difficulty in locating such areas and the nature of the substrate, which makes adequate spray coverage hard to achieve. Nevertheless attempts have been made (Chaniotis et al., 1982; Ready et al., 1985) with limited success. On the other hand, the diurnal resting and breeding site requirements of sand flies make the creation of vegetation-free "buffer zones", around human habitations in forested areas a potential alternative to chemical control (Esterre et al., 1986). Creation of such zones requires knowledge of the flight range and dispersal capability of vector species, in order to determine what width of cleared forest constitutes an effective barrier between forest and human dwellings.

It appears from the few studies of the dispersal of *Lutzomyia* species (Chaniotis et al., 1974; Alexander, 1987) that these insects generally limit their flights to 200 m or less, considerably less than the distances of up to 4 km recorded for *P. papatasi* (Scopoli) in Uzbekistan (Streklova & Kruglov, 1985). This apparent limit may reflect the concentration of

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all a sand fly's needs (host, sources of sugar, breeding sites and diurnal resting sites) rather than physiological limitations.

This article reports the results of mark-release-recapture experiments performed in a coffee plantation near the town of Arboledas, in the department of Norte de Santander, Colombia. The area lies in a focus of *Le. (V.) brasiliensis* (Young et al., 1987).

#### MATERIALS AND METHODS

Experiments were performed at Finca La Esperanza, approximately 3 km from Arboledas (7° 39'N, 72° 48'W), Norte de Santander, Colombia. The ecology of the study area has been described (Alexander et al., 1992). The sand fly fauna consists of 17 species, with *Lu. spinicrassa* Morales et al., *Lu. serrana* (Damasceno & Arouck), *Lu. shannoni* (Dyar), *Lu. ovallesi* (Ortiz) and *Lu. gomezi* (Nitzulescu) apparently far more numerous than the other species, based on resting site and protected human bait collections.

The procedure used in mark-release-recapture experiments has been described (Alexander, 1987). Adult flies were captured from diurnal resting sites, marked with coloured fluorescent powders and released at predetermined points within the plantation, all at least 5 m from the nearest large tree from which collections were made during the following two weeks. No attempt was made to anaesthetize the flies during the marking process, since reliable species identifications cannot be made on the basis of external characteristics alone, and no further information could be gained from immobilization of the insects. Anaesthetization is also undesirable due to the possibility of modified behaviour of the insects after release.

Marked flies were recaptured from large trees (including those from which the marked specimens had originally been collected), beginning 24 h after release. In all, six experiments of this type were carried out, one in November 1985, one each in June, July, October and November 1986 and one in March 1987.

A further three experiments were performed, at La Esperanza, in which sand flies caught between 18.00 and 21.00 on protected human bait (volunteers with all exposed areas of body protected by insect repellent) or in an illumi-

nated Shannon trap were marked and released within the plantations at the end of the collecting period, using the same procedure as before. Marked flies were recaptured by these methods as well as by direct aspiration from large trees. These experiments were performed in November 1985 and in June and July 1986.

The sections of the population sampled in daytime and night marking experiments are clearly different. In the former males and gravid females which had already fed on blood were marked, while in the latter a preponderance of younger, host-seeking females was collected.

#### RESULTS AND DISCUSSION

The relative positions of release and recapture points at La Esperanza are shown in Fig. 1. The exact numbers of each species marked and released could not be determined and estimates were therefore made based on the proportions of each species in resting site or human bait collections at the times of the experiments. The total numbers of sand flies marked and released in the daytime experiments varied between 91 (June 1986) and 639 (November 1985).

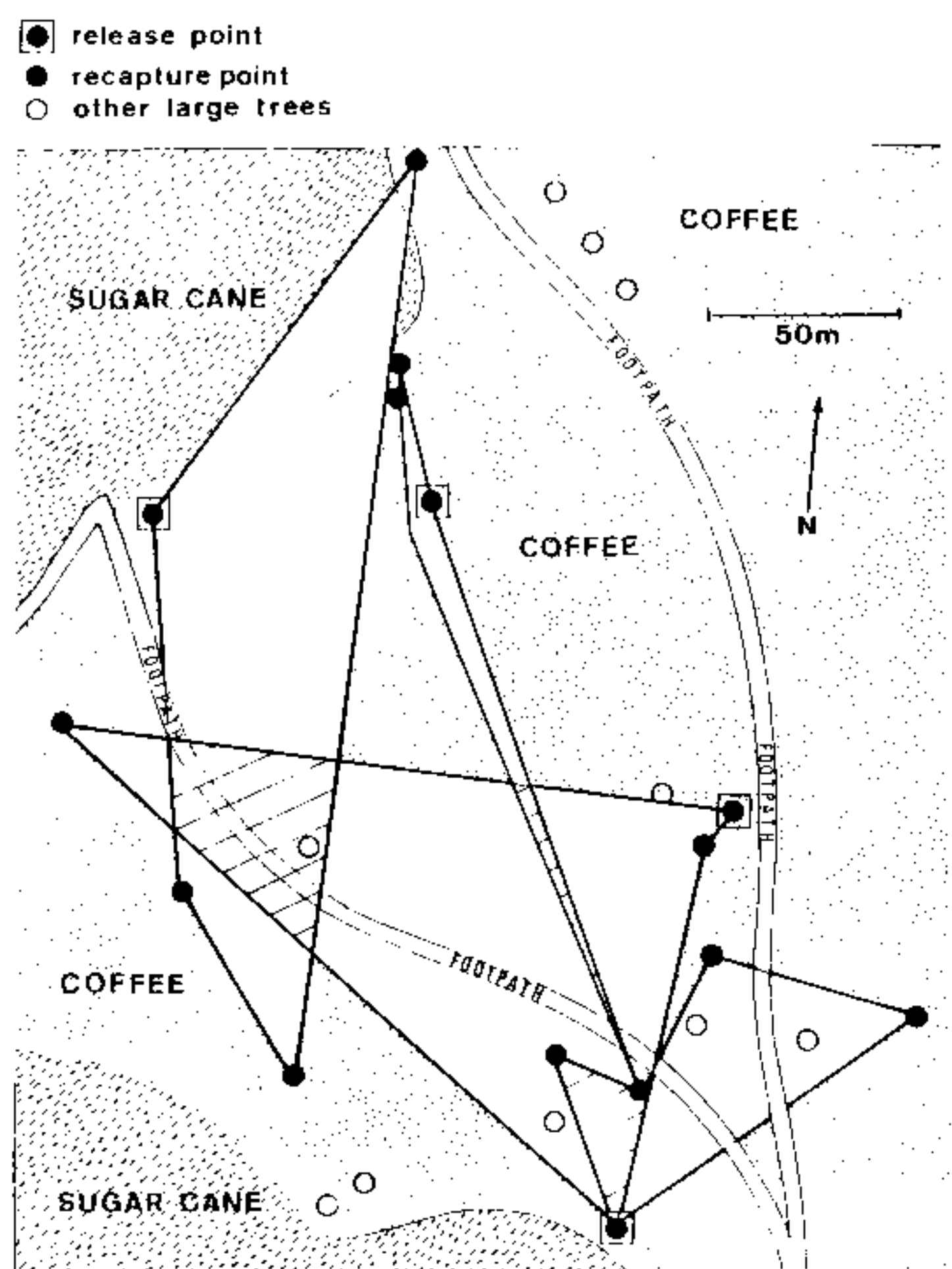


Fig. 1: diagram showing release and recapture points in mark-release-recapture experiments to investigate dispersal of five *Lutzomyia* species, Arboledas, Colombia November 1985 – March 1987.

TABLE I

Dispersal of sand flies collected on tree trunks, marked and released Arboledas, Colombia 1985-1987

Species	Sex	Est. No. Marked	Total No. Recaptured	Est. Rec. Rate	Max. Dist. Flown	Max period rel. -Rec	Mean distance flown/24 h
<i>Lu. spinicrassa</i>	♂	659	7	1.06%	120 m	72 h	38.7 m
<i>Lu. spinicrassa</i>	♀	148	0				
<i>Lu. serrana</i>	♂	185	8	4.32%	132 m	10 days	41.2 m
<i>Lu. serrana</i>	♀	62	0				
<i>Lu. shannoni</i>	♂	199	56	28.14%	157 m	11 days	20.9 m
<i>Lu. shannoni</i>	♀	84	8	9.52%	32 m	48 h	10.2 m
<i>Lu. ovallesi</i>	♂	94	1	1.06%	12 m	24 h	12 m
<i>Lu. ovallesi</i>	♀	43	0				
<i>Lu. gomezi</i>	♂	225	22	9.78%	15 m	9½ days	51.1 m
<i>Lu. gomezi</i>	♀	24	0				

TABLE II

Dispersal of sand flies collected on protected human bait/Shannon trap, marked and released. Arboledas, 1985-1987.

Species	Sex	Est. No. Marked	Total No. Recaptured	Est. Rec. Rate	Max. Dist. Flown	Max period rel. -Rec	Mean distance flown/24 h
<i>Lu. spinicrassa</i>	♂	6	0				
<i>Lu. spinicrassa</i>	♀	203	2	0.98%	153 m	36 h	76.5 m
<i>Lu. serrana</i>	♂	3	0				
<i>Lu. serrana</i>	♀	43	1	2.33%	132 m	12 h	132 m
<i>Lu. shannoni</i>	♂	1	6	600% <sup>a</sup>	87 m	60 h	16.5 m
<i>Lu. shannoni</i>	♀	37	2	5.40%	< 5 m	12 h	< 5 m
<i>Lu. ovallesi</i>	♂	0	0				
<i>Lu. ovallesi</i>	♀	25	0				
<i>Lu. gomezi</i>	♂	12	6	50.0%	140 m	10 days	42.8 m
<i>Lu. gomezi</i>	♀	123	3	2.43%	960 m	36 h	320 m

a: i.e. Number of marked sand flies captured exceeded the estimated number marked (based on relative proportions of species in parallel human bait/Shannon trap collections).

The numbers of flies marked and released in night-marking experiments were 137 (November 1985), 152 (June 1986) and 193 (July 1986), with males representing 0.7 and 6% of the collections respectively. The estimated recapture rate for each species, together with mean distances flown per 24 h period, are shown for day- and night-marked sand flies in Tables I and II respectively.

*Lutzomyia spinicrassa* – An estimated total of 807 *Lu. spinicrassa* (659 males, 148 females) were marked and released during the daytime experiments. Seven males (1.1% of the estimated number of males marked) and no females were recaptured. Three of the males were recaptured within 24 h of release, and two of these had remained at the nearest resting site, i.e., less than 5 m from the release point. The greatest distance flown by a male

*Lu. spinicrassa* was 120 m, and all marked specimens were recaptured within 72 h. This species was one of the most common at La Esperanza, and its numbers were particularly high in November 1985, when it constituted 73.7% of all sand flies collected off tree trunks (Alexander et al., 1992). Nevertheless, only one of an estimated 392 male flies (0.2%) was recaptured in a mark-release-recapture experiment made at that time (Alexander, 1987).

An estimated total of 6 males and 203 females of *Lu. spinicrassa* was captured as they came to protected human bait, were marked and released during the night-marking experiments. Only two (0.5%) were recaptured, both females, of which one was collected off a tree 36 h after being released, having travelled 153 m to the other side of the plantation during this interval.

*Lutzomyia serrana* – An estimated total of 247 *Lu. serrana* (185 males and 62 females) was captured during the daytime experiments. Eight males (4.3%) and no females were recaptured. Three of these were recovered within 24 h, and two males had not moved from the release point during this interval. One marked male was recaptured 10 days after release, having flown 132 m.

A single female *Lu. serrana* was recaptured of the estimated 3 males and 43 females released during the night-marking experiments. This represents a recovery rate of 2.3% overall, or 7.7% for that experiment, which was performed in June 1986. The recaptured female was collected off a tree trunk 132 m from the release point, 12 h after being marked.

*Lutzomyia shannoni* – In all, 56 males and 8 females of the estimated 283 (199 males, 84 females) marked were recaptured during the daytime experiments. These represent overall recapture rates of 28.1% and 9.5% respectively, values very much larger than those obtained for the other species. Of a total of 94 male sand flies marked and recaptured in these daytime experiments, 59.5% were *Lu. shannoni*, as were all recaptured females. Longest recorded distances travelled by males were 157 m in 11 days, 123 m in 24 h and 118 m in 96 h. No flights exceeding 32 m were recorded for females, and all those recaptured were caught within 48 h. Twenty-seven (48.2%) of the male *Lu. shannoni* recovered were recaptured less than 5 m from the release point, in one case 120 h after marking. Forty (71.4%) had flown less than 20 m when recaptured, and only 7 (12.5%) had flown further than 50 m.

According to estimates, only 1 male and 37 females should have been marked and released during the nighttime experiments. In fact, 6 males and 2 females were recovered, all but 2 of the former after 12 h on resting sites less than 5 m from the release point. The estimated recapture rate for female *Lu. shannoni* in this experiment was therefore 14.3%, again a remarkably high proportion when viewed alongside those obtained for other species.

*Lutzomyia ovallesi* – An estimated 94 males and 43 females of this species were captured on tree trunks, marked and released at La Esperanza, of which a single male (1.1% of

the estimated number of males marked) was recaptured in June 1986, only 12 m from the release point and after 24 h. No marked specimens were recaptured of those marked at night.

*Lutzomyia gomezi* – An estimated total of 225 males and 24 females of this species were captured on tree trunks, marked and released during the daytime experiments, of which 22 males (9.8%) and no females were recaptured. Longest recorded distances travelled were 157 m in 9 1/2 days, 153 m in 54 h and 125 m in 72 h. In all 10 (45.4%) were recovered 20 m or less from the release point, one male being recaptured at the release point after 120 h.

Of the estimated 12 males and 123 *Lu. gomezi* females caught on human bait, marked and released, 6 males (50%) and 3 females (2.4%) were recaptured. One male was recovered after 36 h 140 m from the release point and another at 105 m after 10 days. The remaining 4 males were all recaptured within 12 m of the release point, one of these after 10 days.

Two of the females were recaptured within approximately 20 m of the release point, but the third was retaken 960 m away on a tree trunk in the neighbouring plantation of La Quinta after 36 h. This represents the longest recorded distance travelled by any New World sand fly and exceeds all other records by a considerable margin (Chaniotis et al., 1974; Alexander, 1987). The recapture point lay on the other side of a small valley formed by the Arboledas river, with the intervening distance occupied by a small elementary school, several houses and a dirt road, on the other side of which 300 m of coffee plantation and secondary woodland must be crossed before the tree on which the fly was recaptured is reached. The marked fly was recaptured during a routine collection at La Quinta for the leishmaniasis study of which these experiments constituted a part.

Recaptures of marked sand flies in studies of this type are biased towards those insects that disperse least, so that calculations of the mean distance flown by these insects are largely meaningless. In the absence of data on mortality factors affecting sand fly populations, it is also difficult to determine whether the observed recapture rates represent a large or small proportion of the marked survivors. This is compounded in areas supporting a variety of

species by the difficulty in identifying marked flies to species at the time of marking and release.

Nevertheless, the differences among the recapture rates of the species marked after collection on diurnal resting sites in this study are noteworthy. Although the proportion of male *Lu. spinicrassa* in such collections averaged 37.8%, or 1.9 times that for *Lu. shannoni*, the latter outnumbered the former among recaptured sand flies by slightly more than 3 to 1. *Lu. spinicrassa* females outnumbered those of *Lu. shannoni* in resting site collections by 1.1 to 1, but eight of the latter species were recaptured and none of the former. Recapture rates were analyzed by Duncan's Multiple Range Test and males of *Lu. shannoni* shown to be recaptured at a significantly higher rate than those of the four other species ( $p < .05$ ). This suggests that males of *Lu. shannoni* are more sedentary than those of the other four species, or perhaps have more restricted resting site preferences. No attempt was made to look for marked sand flies in other microhabitats (such as leaf litter) within the sampling area and males of *Lu. spinicrassa*, *Lu. serrana*, *Lu. ovallesi* and *Lu. gomezi* may have flown to these after marking.

It is possible that *Lu. shannoni* males congregated at the bases of large tree trunks and mated with mature females, after which the latter laid eggs in the surrounding leaf litter or other microhabitat. The large preponderance of males in collections from tree trunks may indicate that lekking behaviour was involved, in which males competed for females by displaying in some way. Little is known of the courtship behaviour of sand flies, although pheromones are produced by males of *Lu. longipalpis* (Ward et al., 1988) and possibly *P. argentipes* (Lane et al., 1990). Differences in daily percentage mortality may also explain the dissimilar recapture rates between *Lu. shannoni* and the other species.

*Lutzomyia shannoni* was the only species of which females were recaptured (overall recapture rate 9.5%). Again, this might be explained by differences in dispersal capacity (although no significant differences were found between the five species with respect to the distances flown by marked and recaptured flies of either sex), resting site preference and mortality factors. It may also reflect differences in the age structures of populations

among the five species; a greater percentage of *Lu. shannoni* females might have been ready to lay eggs in the vicinity of the tree trunks on which they were captured, and be less inclined to move away from these oviposition sites even after marking and release.

High recapture rates were also seen for *Lu. shannoni* in an earlier study performed at the same site (Alexander, 1987). It is noteworthy that this species constituted only 7.8% of the 445 *Lutzomyia* specimens taken in Malaise traps at this site and was outnumbered by *Lu. davisi* (Root), a species that appeared to be somewhat rare in the habitat based on other types of collection (Alexander et al., 1992). The flight trap employs no attractive stimuli and catches are made by interception of flying insects and should give a relatively unbiased sample of the sand fly population. Since *Lu. shannoni* was undoubtedly abundant in the habitat in which these experiments were performed its comparatively low incidence in flight trap collections may be further evidence of sedentary behaviour.

*Lutzomyia spinicrassa* constituted a mean of 44.3% of female flies taken in protected human bait collections but only 2 (10.5%) of the 21 insects marked at night and recaptured belonged to this species. Significant differences were not found between the species with respect to the number of females recaptured however ( $p < .05$ ). The four male *Lu. shannoni* recaptured in the July 1986 experiment (when the estimated number marked was only one) illustrates the limitations of calculating recapture rates based on estimates of marked flies. This is particularly important in experiments such as these where it was logistically impossible to mark very large numbers of flies.

For the reasons mentioned earlier, calculation of mean distances travelled by sand flies per unit time are of little value. In studies of this type, the maximum distance attained between the release and recapture points are perhaps of more use. Thus while the female *Lu. gomezi* recaptured almost 1 km from the release point may not be typical of the dispersal of this species as a whole, it does illustrate its flight capacity and would have to be taken into consideration should leishmaniasis control by the clearing of "buffer zones" be attempted here or elsewhere in the range of this species.

Sand flies that return to seek a blood meal at or near the spot where they were marked one or a few nights previously may not be behaving naturally, since the marking process may have prevented them from feeding and necessitated a second attempt when conditions again become favourable. While day-marked sand flies marked and recovered at the same place are likely to have remained there in the interval, those marked as they came to bite need to pass the daylight hours in a shady, humid resting site. The distance between biting and resting sites in this study was determined for three female *Lu. spinicrassa*, one *Lu. serrana*, eight *Lu. shannoni* and seven *Lu. gomezi* (Table I). Although one female of this species was recaptured 960 m from the release point after 36 h, another was recovered on protected human bait only 12 m from the point where she had been marked while attempting to take a blood meal 10 days previously. It can be assumed that these two attempts were related to successive gonotrophic cycles rather than repeat attempts to obtain blood for a single batch of eggs, although this female was not dissected to determine parity.

Taylor (1978) pointed out that if dispersal of an insect is random in one direction then log recaptures should be linearly related to the square of distance. However Dye et al. (1991) found that dispersal of *Lu. longipalpis* (Lutz & Neiva) between chicken sheds was a variable rather than a constant, with proportion of male sand flies recaptured linearly related to the square root of distance between sheds. This is difficult to resolve for the results from the present study, in which the exact numbers marked of each species could not be determined and the sample size was very much smaller. Nevertheless the relationship between the log recapture rate and the square root of distance flown does appear to be linear for males of *Lu. shannoni* that were marked and recaptured on tree trunks (Fig. 2), and which constituted the largest group of recaptured flies. Assuming that these trees were the preferred diurnal resting sites of these species, then the probability of recapturing marked insects should be very high, given the relatively small numbers of such trees available in the plantation. Dispersal of *Lu. shannoni* males is therefore unlikely to be random.

In summary, this study provided further information of the flight ranges and longevity of several species of Colombian sand fly, includ-

ing several known and suspected vectors of *Leishmania* in the study area (Alexander et al., 1992). Such information could be used in a leishmaniasis control programme in this area to determine the width of buffer zones between human habitations and the coffee plantations in which the insects are present. These buffer zones need not consist of deforested strips around susceptible communities; alternatively areas could be delimited within which all *Lutzomyia* resting sites could be sprayed with residual insecticides or otherwise made unfavourable to the insects. It appears however that the flight capacity of New World sand flies is greater than thought previously.

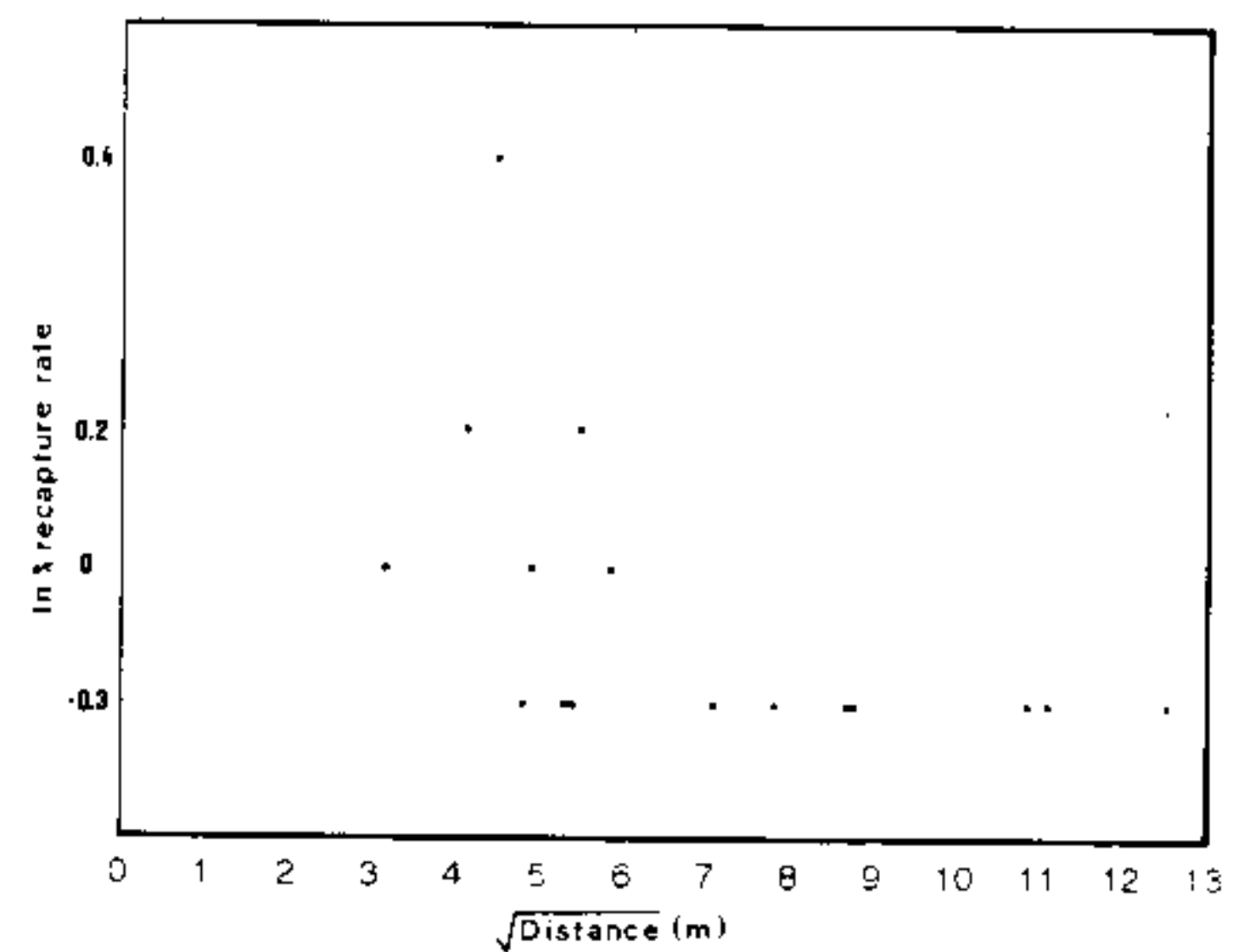


Fig. 2: log of percentage recapture rate for male *Lutzomyia shannoni* against square root of distance between release and recapture points.

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