walls of houses and animal shelters. (c) incrimination of the vector species: this will depend on isolating and identifying the parasite; finding a significant number of that species infected; demonstration of promastigotes in the anterior station of the gut; indicating an intimate reservoir-host/sandfly/man contact; and experimentally transmitting the parasite by the bite of the suspected vector species.

C. The Mammalian Population: Reservoir-hosts

(a) Anthroponoses

In India, parts of China and possibly Kenya, visceral leishmaniasis is thought to be an anthroponosis, with transmission directly from man to man.

(b) Zoonoses

Among domestic animals, the only proven and effective reservoir is the dog, which has become the "liaison" host of visceral leishmaniasis due to *L. infantum* and *L. chagasi*. The canine disease is usually fatal, but infection in wild canids (foxes, wolves and jackals) is mostly benign and inapparent. These animals offer, therefore, a constant threat of new outbreaks of visceral leishmaniasis when control measures are diminished. Entomological data on biting habits, preferential hosts and distribution will give clues as to wild mammalian hosts: analysis of fresh blood from sandflies is potentially useful, but the results must be interpreted with care because many sandflies have very catholic feeding habits. Finally, the screening of wild animals should always include the examination of both skin and viscera: the procedure (Lainson, 1982) is similar to that used for the isolation of *Leishmania* from man.

D. Identification, Description and Preservation of *Leishmania* Isolates

Correct identification of the parasite by recognised biological and biochemical criteria (Lainson & Shaw, 1986) is imperative in epidemiological studies – especially where a number of different leishmanias are circulating in nature. The original code of the laboratory in which an isolate is made should always be incorporated into the descriptive labelling of that parasite, using the coding method recommended by the World Health Organisation (Anon., 1984). Strains should be preserved in liquid N₂ as soon as possible after isolation, and deposited in one or more International Reference Centres.

Quantitative Studies

These should accompany the qualitative studies, to provide information on the prevalence and incidence of the human disease in a given area, ecology and population fluctuations of both sandfly vectors and mammalian reservoir-hosts, and the prevalence and incidence of canine visceral leishmaniasis – all of which forms a basic necessity in initiating control measures and measuring their efficacy.

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Part I.III Mate Recognition in a Sandfly (Diptera : Psychodidae)

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Introduction

The present paper summarizes preliminary studies on the sexual behaviour of the sandfly *Lutzomyia longipalpis* Lutz & Neiva. This phlebotomine is the vector of *Leishmania donovani chagasi* in South America, though it is now believed that *Lu. longipalpis* consists of at least two sibling species, which are as yet not clearly defined.

There is little information on the reproductive biology of psychodids including phlebotomine sandflies. Early observations by Fuerborn (1922) of psychodids described the presence of "scent glands" which it was thought emitted chemical secretions used in sexual signalling. An early description of sandfly sexual behaviour was from Malta, where Whittingham and Rook (1923) described the courtship of *Phlebotomus papatasi* Scopoli. To my knowledge there are no further descriptions of sexual behaviour in sandflies until Chaniotis (1967) described courtship in *Lu. vexator occidentis*. An earlier report from Brazil described the existence of an "odiferous gland" in male *Lu. longipalpis*, which it was thought was involved in stimulating the female before copulation (Barth, 1961). Other recent references to sexual behaviour in sandflies are those of Gemetchu (1976) on *P. longipipes* and Beach *et al.* (1983), who described the relationship between age in female *P. martini* and rates of insemination.

Morphological Variation and Crossing Relationships

Morphological variation in *Lu. longipalpis* was first noted by Mangabeira (1969). He observed that males captured in Pará state, north Brazil bore a single pair of pale tergal patches on segment four, in contrast to specimens he captured from the north-eastern state of Ceará which showed an additional pair of patches on segment 3. The different distributions of the two forms of the vector are of interest in view of the apparently uneven distribution of human kala-azar cases. For example, in the New World 97.5% of cases occur in Brazil and of these, 68% are recorded from Ceará. The suspicion that the distributions of the two-spot form in

the north-east of Brazil might be a factor in the very high numbers of cases seen in the area led to a study of the different forms of *Lu. longipalpis*. I found that the one-spot form of male occurs from Mexico to southern Brazil and extends west to Bolivia. In contrast the two-spot form has a smaller range, from Maranhão in north Brazil to Mato Grosso near to the Paraguayan border (Ward *et al.*, 1985).

Crossing experiments were carried out between a one-spot colony of *Lu. longipalpis* from Lapinha cave, near to Belo Horizonte in Minas Gerais, central Brazil, and a two-spot form from Morada Nova, Ceará. Hatch rates of egg batches laid from these crosses were reduced by 50-100% and it was discovered that insemination failure was the reproductive barrier despite apparent copulation (Ward *et al.*, 1983). The colonies used in this work were, however, over 8 years old and it was feared that they were genetically altered in some way which was influencing the results. In consequence, more collections were made in Brazil during 1982 and new colonies formed in Liverpool. New crosses carried out between 1982 and 1984 showed that a sympatric one- and two-spot colony were reproductively isolated and surprisingly, that there was significant isolation between

some allopatric one-spot populations (Ward *et al.*, 1983; Ward, 1986). At this stage it was concluded that there was a two-spot form of *Lu. longipalpis* which was cospecific over a range of 700km in the north-east of Brazil, from São Luiz, Maranhão to Morada Nova, Ceará. The partial isolation between allopatric one-spot populations separated by 1000-2000km was thought to perhaps indicate clinal variation, or the existence of semi-species as observed in some fruit flies, muscids and butterflies (Mayr, 1963).

One further crossing experiment and some investigations into male sandfly pheromones during 1985, however, showed that the number of pale tergal spots in *Lu. longipalpis* is not a valid species specific character. Flies from Marajo island (one-spot) were crossed with those from Morada Nova (two-spot) and shown to be reproductively compatible. The male progeny of this cross were either of the two-spot form, or an intermediate in which the 3rd tergal spot was reduced in size by 25-50%. Inter-hybrid crosses produced all three phenotypes. These results were of interest since I had observed 5 specimens of the intermediate form in Brazilian collections and had reports of their existence from Paraíba State in the

Figure 1. The crossing relationships between 6 populations of *Lutzomyia longipalpis* from Brazil. (---) = Reduced insemination, (—) = Normal insemination. ● = Male fly with one pair of pale spots on ergite 4, ●● = Male fly with similar pale spots on tergites 3 and 4.

north-east of Brazil (Marcondes, personal communication). The crossing results described above are summarized in Figure 1. These apparently confusing results are now explained by the fact that the males of *Lu. longipalpis* possess a pheromone gland beneath the pale spots and it is probably these semiochemicals that determine the reproductive compatibility of different siblings.

(b) Male Tergal Spots: Morphology and Histology

Examination of the pale tergal spots using scanning electron microscopy revealed the presence of numerous 3.0–3.5 μ cuticular papules each with a central pore and consistent with a site of pheromone emission (Lane & Ward, 1984). The tissue beneath the pale spots was subsequently sectioned and examined, using electron microscopy (Lane & Bernades, unpublished data). A gland was found with highly vacuolated cells each of which is equipped with an apparently secretory end apparatus. Boufana *et al.* (1986) later showed that the gland has a developmental cycle which is correlated with the onset of pheromone production.

Male Pheromones

The male glands of 6 populations of *Lu. longipalpis* from different areas of Brazil were examined by hexane extraction, followed by gas chromatography and mass spectral analysis. Four populations from the north and north-east including both male phenotypes, contained a hydrocarbon of molecular weight 272 ($C_{20}H_{32}$) and a mass spectrum which suggest the presence of a diterpenoid. Two other populations, of the one-spot type from the north-east and the south contained a hydrocarbon of molecular weight 218 ($C_{16}H_{26}$) with a mass spectrum similar to the pheromone homofarnesene (Lane *et al.*, 1985; Phillips *et al.*, 1986).

Pheromone Activity

To demonstrate some biological activity for the pheromones found in the gland an olfactometer was made in which female response to hosts was compared with their behaviour when male sandflies were present. In 6 preliminary experiments using a "diterpenoid colony" from Morada Nova, 261/384 females responded to hamsters. Of these, 40 were attached to a hamster alone and 221 to a hamster in the presence of male sandflies.

Auditory Communication

Another preliminary series of experiments in collaboration with Dr B. Burnett of Sheffield University has shown that male and female *Lu. longipalpis* produce distinct polycyclic sound pulses. These may prove to be species specific, but it is not yet known how much individual variation there is or if the pulses have a role to play in sexual communication (Ward *et al.*, 1986).

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Part I.IV The Transmission of Leishmaniasis by the Bite of the Sandfly

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Introduction

Perhaps the first published mention of leishmaniasis being transmitted by the bite of sandflies was by Beuno (1764) who noted that uta, a cutaneous form of leishmaniasis in Peru, was said to be contracted by the bites of a sandfly, also called uta. The first experimental evidence of transmission by bite did not arrive until 167 years later when Shortt *et al.* (1931) transmitted *Leishmania donovani* to hamsters by the bite of *Phlebotomus argentipes*. The transmission of oriental sore to man by bite was proven by Adler and Ber (1941) and of kala-azar to man by Swaminath *et al.* (1942).

Since the 1930's, much interest has focussed on the transmission of leishmaniasis. In particular, there are differences of opinion and contradictory observations on the forms of the parasite deposited in the skin of the vertebrate host when an infected sandfly bites. The