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Chemical analysis of compounds extracted from the tergal "spots" of *Lutzomyia longipalpis* from Brazil

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Summary

The chemical composition of the compounds contained in the tergal spots of *Lutzomyia longipalpis* was investigated. Four populations of *L. longipalpis* were examined, originating from: Sobral, Ceará, Brazil (one spot and two spot populations), Santarém, Pará, Brazil (one spot) and Marajó Island, Pará, Brazil (one spot). The tergal spots were dissected out, extracted in hexane and analysed on a gas chromatograph/mass spectrometer. Two compounds were found, identical to compounds found in earlier studies, but there was no correlation between number of tergal spots and type of compound present. It was suggested that the number of tergal spots could not be used as a marker for reproductively isolated populations, and that analysis of the compound present within the spots might be necessary to characterize potentially good vector populations.

Key words: sex pheromones; sandflies; *Lutzomyia;* Brazil, leishmaniasis.

Introduction

Recent studies on the epidemiology of visceral leishmaniasis in the Amazon Region of Brazil, and in Bolivia, have conclusively proved that the vector of the causative agent, *Leishmania donovani chagasi,* is the phlebotomine sandfly, *Lutzomyia longipalpis* (Lainson et al., 1977, 1984, 1985; Ryan et al., 1984; Le Pont and Desjeux, 1985). *L. longipalpis* has also been shown to be a species complex (Ward et al., 1983; Lane et al., 1985; Ward et al., 1985; in

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press). The latter findings were based on a dimorphism amongst the males in which some populations had one pale abdominal tergite ("one spot") and others two ("two spot") on abdominal segments 3 and 4 (Ward et al., 1983). In addition, the two forms have different geographical distributions with the "two spot" form confined to eastern and central Brazil, whilst the "one spot" form occurs from central America to south Brazil (Ward et al., 1985). Sympatric populations of one and two spot forms are found in some areas of north-eastern Brazil, Ceará, from which two populations in the present study were obtained (Sobral). Mating incompatibility between these forms from different areas, strengthened the view that *L. longipalpis* is a species complex.

Following a scanning electron microscope study of these abdominal segments (Lane and Ward, 1984; in press) which revealed the presence of structures consistent with a secretory role, the chemical nature of the substances contained therein was investigated (Lane et al., 1985). Lane et al. (1985) examined a "one spot" population from Laphina Cave and a "two spot" population from Morada Nova. The two populations were shown to have different compounds within these segments. The "one spot" Laphina Cave population contained a hydrocarbon of molecular weight (MW) 218 ($C_{16}H_{26}$) and a mass spectrum similar to structures known in other insect groups to act as pheromones (i.e. homofarnesenes). Such compounds have been described as trail pheromones in ants, *Myrmica rubra* (Attygalle and Morgan, 1982), in which the homofarnesenes are secreted by the Dufours gland. The "two spot" Morada Nova population, however, had a high concentration of hydrocarbon with a MW of 272 ($C_{20}H_{32}$) and a mass spectrum similar to the group of compounds known as diterpenoids. This structure is also consistent with the suggested function of these molecules as sex pheromones.

The present paper extends the work of Lane et al. (1985) to include four further laboratory colonies of *L. longipalpis* from different areas of Brazil to ascertain the chemical variation of the compounds contained in the relevant abdominal segments. Two of these colonies are derived from *L. longipalpis* populations incriminated as vectors in recent outbreaks of visceral leishmaniasis at the mouth of the Amazon, on Marajó Island, and at Santarém 500 km away, on the River Tapajos (Lainson et al., 1985).

Materials and Methods

The colonies of *L. longipalpis* used in this study were maintained by the methods of Ward (1977) and Killick-Kendrick et al. (1977). Extraction and analysis of the substance contained within dissected abdominal segments was carried out as described by Lane et al. (1985). Mass spectral analysis was also carried out using chemical ionization (CI). For this, methane was introduced into the mass spectrometer ion source at a pressure of 1.5 Torr. The resulting fragmentation is less severe than with electron impact, giving large amounts of the pseudomolecular ion and therefore allowing confirmation of molecular weight. Males from additional distinct colonies were examined and these were:

1. Sobral 1, Ceará, Brazil, "one spot",
2. Sobral 2, Ceará, Brazil, "two spot",
3. Marajó Island, Pará, Brazil, "one spot",
4. Santarém, Pará, Brazil, "one spot".

In addition, the effect of storing frozen segments and extracts was investigated. The relevant segments from the Laphina Cave and Marajó flies were either dissected and frozen, or extracted and the extracts frozen. Samples from Laphina Cave flies were stored for 2 weeks in a −70° C deep freeze of the Revco type, whilst Marajó flies were stored for 9 days at −10° C, in the freezer compartment of a refrigerator. Immediately before analysis the samples were thawed at room temperature for 30 min, and the extracted samples were resuspended with 10 μl of hexane.

Results

Chromatograms obtained from the dissected spots of the four different colonies gave clearly distinct peaks which correspond to either of the two compounds found in the Laphina Cave and Morada Nova populations. However, there is no correlation between the number of pale abdominal spots and the compounds present in these populations. Table 1 summarizes the findings. The sample spectra from Sobral 1 and Sobral 2 (Fig. 1) are identical to those substances described earlier by Lane et al. (1985) from the Laphina Cave and Morada Nova populations, respectively. Thus two pairs of populations possess the same compound in the one spot and the same in two spot populations. However, examination of the one spot flies from the Amazonian sites of Marajó and Santarém, indicate the presence of the compound of higher molecular weight (272) previously found in two spot populations of Morada Nova and Sobral 2.

The presence of two peaks (retention time c. 769 and 858 sec) in the Sobral 1 and Laphina Cave populations suggests either the presence of a product formed in the synthesis of the more abundant compound (peak r.t. 769) or an isomer of that compound. The difference in retention time suggests the former, whilst the similarity of the mass spectra suggests the latter. The concentrations of all these compounds was in excess of 50 ppm for at least 10 individuals. Control samples were run, containing the remaining abdominal segments, the rest of the fly, and

Table 1. A summary of the data concerning compounds associated with the tergal spots of different *Lutzomyia longipalpis* populations

Origin of colony	Number of spots on abdominal tergites	Retention time of major peak/s (sec)	MW of compound
Laphina Cave	1	769/858	218
Sobral 1, Ceará	1	769/858	218
Morada Nova	2	1228	272
Marajó, Pará	1	1232	272
Santarém, Pará	1	1227	272
Sobral 2, Ceará	2	1235	272

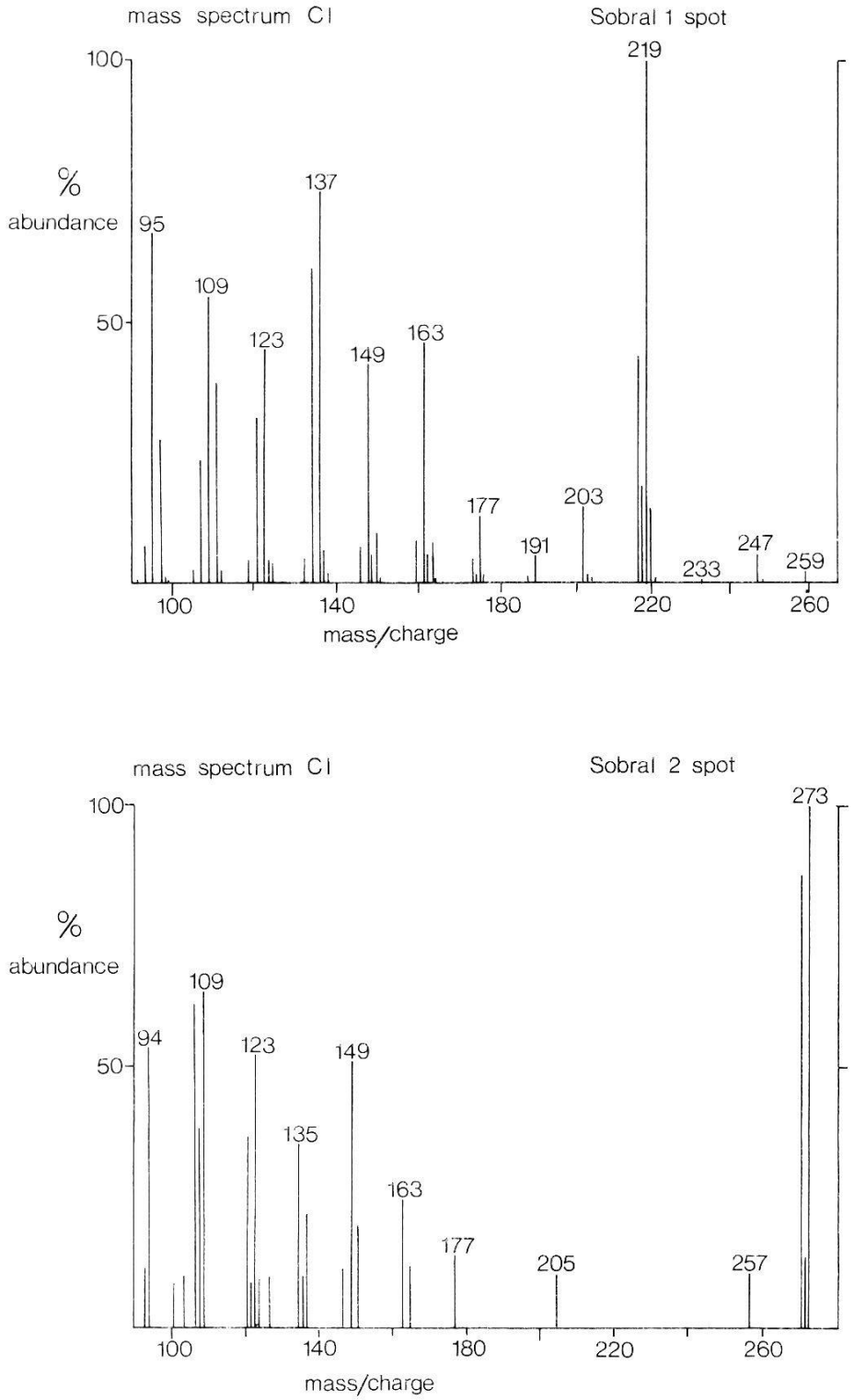

Fig. 1. Showing the chemical ionization spectra of the two compounds found. Each numbered peak represents the molecular weight of a fragment of the original compound and its abundance can be read from the Y axis. Peaks 219 (Sobral 1 spot) (Fig. 1 upper) and 273 (Sobral 2 spot) (Fig. 1 lower) are the pseudomolecular ions i.e. $M^{+}+H$.

solvent. Small quantities (5 ppm) of the compounds were occasionally found in association with the remaining segments. Both sets of stored frozen flies still contained enough of the compounds to enable a recognizable mass spectrum to be obtained. Whole segments stored (without extraction) at −70° C had c. 80% of the quantity expected from equivalent freshly dissected and extracted material, while those stored at −10° C had c. 10% of that expected. At both freezing temperatures (−70 and −10° C), the samples extracted before freezing contained less compound than segments stored and extracted after freezing. Therefore, storing the dissected spots, or whole abdomens of flies is possible at both −70° C and −10° C, without too great a loss of compound, and this has important implications in the transfer of material from the site of collection to the GC/MS facility.

Discussion

This paper has confirmed that compounds identical to those reported earlier by Lane et al. (1985) are found in other populations of male *L. longipalpis,* but our results demonstrate that there is no correlation between the numbers of pale tergal patches (spots) and the compound extracted from them. This applies in full to populations containing the higher molecular weight compound, but it is possible that two spot populations containing the lower molecular weight compound might be found in the future. Most significantly the Amazonian populations, which have a single spot, contain the high MW (272) compound described by Lane et al. (1985) from the "two spot" Morada Nova, Ceará populations. It is therefore clear that the number of tergal spots cannot, in future, be of use as an appropriate marker.

The studies of Lainson et al. (1985) on the epidemiology of visceral leishmaniasis in Marajó Island and in Santarém suggest that the *L. longipalpis* associated with the outbreak in Santarém was the one spot form. In the light of this and the findings presented in this paper, a reappraisal of Ward's hypothesis (Ward et al., 1983), that two spot flies are more effective vectors due to their domestic behaviour and greater degree of anthropophily, is perhaps needed. The differences between the behaviour of one and two spot flies noted by Ward, may in fact reflect differences between the two "chemo-forms" present, but clearly not between all one and two spot flies. There is at present, no clear association between vectorial capacity in endemic visceral leishmaniasis foci and behaviour or morphological types of flies. In the future, it will be necessary therefore to consider chemical analysis of *L. longipalpis* tergal "spots", as a possible factor in characterizing potentially good vector populations.

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