

SEPARATION OF
PSYCHODOPYGUS CARRERAI
CARRERAI AND *P. YUCUMENSIS*
(DIPTERA : PSYCHODIDAE) BY
GAS CHROMATOGRAPHY OF
CUTICULAR HYDROCARBONS.

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SUMMARY

Specimens of two species of sandfly, *Psychodopygus carrerai carrerai* and *P. yucumensis*, vectors of *Leishmania braziliensis braziliensis* in the subandean lowlands of Bolivia were subjected to cuticular hydrocarbon analysis to ascertain if the technique could separate the isomorphic females. A high degree of separation (87%) was obtained based on significant differences in the cuticular hydrocarbon composition of these two species. Statistical treatment of the data shows that these species, initially identified by the colour of the mesonotum, can also be distinguished by their hydrocarbons.

Key-Words : *Psychodopygus carrerai carrerai* ; *P. yucumensis* ; sandflies; leishmaniasis; Bolivia; cuticular hydrocarbons.

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Introduction

Sandflies of the genus *Psychodopygus* are proven vectors of *Leishmania (Viannia) braziliensis braziliensis* in South America. *Psychodopygus wellcomei* is recognised as the vector of leishmaniasis in the Amazon region of Brasil (LAINSON *et al.*, 1973; SHAW and LAINSON, 1987) while in Bolivia three species : *P. yucumensis*, *P. carrerai carrerai* and *P. llanosmartinsi* have been shown to be vectors of *L. b. braziliensis* using techniques such as isoenzyme typing and monoclonal antibody characterisation (LE PONT and DESJEUX, 1986; LE PONT *et al.*, 1986, 1988).

The difficulty of distinguishing females of certain related taxa of *Psychodopygus* species, particularly those involved in the transmission of leishmaniasis, currently hinders the interpretation of epidemiological data. This identification problem applies specifically to members of the *P. squamiventris* series including the sympatric species *P. wellcomei* and *P. complexus* (READY *et al.*, 1982) and to the species incriminated as vectors in Bolivia *P. yucumensis* and *P. c. carrerai* (CAILLARD *et al.*, 1986; LE PONT *et al.*, 1988). Isoenzyme analysis of *P. wellcomei* and *P. complexus* females did not reveal any diagnostic enzyme systems which could be used in identification (READY and DA SILVA, 1984). However, RYAN *et al.* (1986) were able to distinguish females of these species on the basis of significant differences in their cuticular hydrocarbons. PHILLIPS *et al.* (1988) has also described the presence of three hydrocarbons in *P. squamiventris* s. str. females which were not found in either *P. wellcomei* or *P. complexus*.

The description of the species *P. yucumensis* was based on the dichotomy of specimens caught on human bait in the Alto Beni region of Bolivia. These samples could be separated into two groups based on their general pigmentation and in particular the colour of the mesonotum. *P. c. carrerai* typically has a pale mesonotum while those with a light brown mesonotum were classified as the new species *P. yucumensis*. Isoenzyme analysis has also revealed two diagnostic enzyme systems; peptidase and xanthine dehydrogenase which indicate a degree of reproductive isolation between these species (LE PONT *et al.*, 1986; CAILLARD *et al.*, 1986).

This paper describes the results obtained from the chromatographic analysis of the cuticular hydrocarbons of *P. c. carrerai* and *P. yucumensis* isomorphic females. The specimens were derived from flies collected in Bolivia where three species of *Psychodopygus* are sympatric in the subandean tropical lowlands and where cutaneous leishmaniasis caused by *L. b. braziliensis* is prevalent (DESJEUX *et al.*, 1986 and 1987). This study follows the earlier use of cuticular hydrocarbon analysis in the investigation of other cryptic sandfly species (refer to PHILLIPS *et al.*, 1988).

Materials and methods.

The study area where sandflies were collected by human landing catches was the Alto Beni region of Bolivia which has been described in detail by LE PONT and DESJEUX (1986) and DESJEUX *et al.* (1987). The region is endemic for cutaneous leishmaniasis caused by *L. b. braziliensis*. Female sandflies were separated for analysis on the basis of the colour of the mesonotum and other minor morphological features (see LE PONT *et al.*, 1986). The cuticular hydrocarbons were extracted by immersion in solvent and analysed by on-column capillary gas chromatography (GC). Full details of the method are given in PHILLIPS *et al.* (1988). The cuticular wax layer is efficiently removed by solvent extraction and clear separation and quantitation of all components is possible using capillary GC. Standard GC protocol was employed but no attempt was made to separate different classes of compounds in the extract. Over thirty compounds are contained in the cuticular extract of a typical sandfly and statistical treatment of the data enabled us to determine the significance of variations in quantity or type of hydrocarbon present on these specimens. Furthermore, calculations were made of the predicted success of identifying unknown specimens of these two species by this method. A step-wise discriminant analysis using the peak areas was employed for this purpose (specifically the BMDP85 discriminant analysis program with jackknifing).

Results

Fifty three sandflies were analysed; 27 females of *P.c. carrerai* and 26 females of *P. yucumensis*. Examples of the hydrocarbon profiles obtained are shown in Figure 1 and these profiles have been chosen to highlight the five peaks which account for most of the separation. The classification table (table 1) shows a high degree of separation of the two species and predicts that 87% of unknown flies would be correctly identified using the discriminant function derived from this data.

Discussion

The public health importance of these anthropophilic vectors in relation to their distribution and abundance in the area of settlement is clear from earlier studies on disease prevalence (DESJEUX *et al.*, 1987). Both *P. c. carrerai* and *P. yucumensis* have been identified as vectors of cutaneous leishmaniasis in the subandean lowlands of Bolivia - an area which has been selected for the settlement of people from the high altitude plateau. Currently, there is massive immigration of non-immune populations into this endemic region; with activities such as road building, forest exploitation for timber, housing construction close to primary forest and hunting, all resulting in high rates of transmission. The continuing geographical spread and the growing incidence of leishmaniasis in this region thus have important socio-economic consequences. For the future implementation of control measures it is important therefore, to identify the different elements of the transmission cycle in this area.

P. yucumensis and *P. c. carrerai* are sympatric only in the lowest altitudes of *P. c. carrerai* distribution, that is the subandean corridor and the foothills of the first range of the Andean cordillera, alongside the Beni plain up to an altitude of 500m. However, they are both important vector species with *P. c. carrerai* the most abundant while *P. yucumensis* is the most aggressively anthropophilic. The studies described here together with the electrophoretic and morphological information already available provide an accurate description of these species. Also, the results support the theory that differentiation of cuticular hydrocarbons is associated with reproductive isolation and may, in fact, be an intrinsic part of the isolating mechanism (see PHILLIPS *et al.*, 1988).

The distinction of isomorphic female sandflies (RYAN *et al.*, 1986) and the determination of differences between sandfly populations (KAMHAWI *et al.*, 1987; PHILLIPS *et al.*, in press) can be revealed by examination of the cuticular hydrocarbons. The specificity of hydrocarbons suggests that they have a role in pre-mating recognition and thus in the processes of incipient speciation, although the genetic control of hydrocarbon expression is not yet understood. However, this study confirms the value of cuticular hydrocarbon analysis in the study of sandfly biology

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Fig.1 : Examples of the chromatograms obtained from the extracted cuticular wax of individual female *Psychodopygus carrerai carrerai* and *P. yucumensis*. The x-axis represents increasing time, temperature and molecular weight of compound, while the height and area under the peak represent the concentration. Compounds other than hydrocarbons are also extracted (e.g. fatty acids, esters etc.). The hydrocarbon peaks are indicated (as determined by gas chromatography/mass spectrometry) and those accounting for most of the separation (i.e. > 77%) are denoted by an asterisk (*).

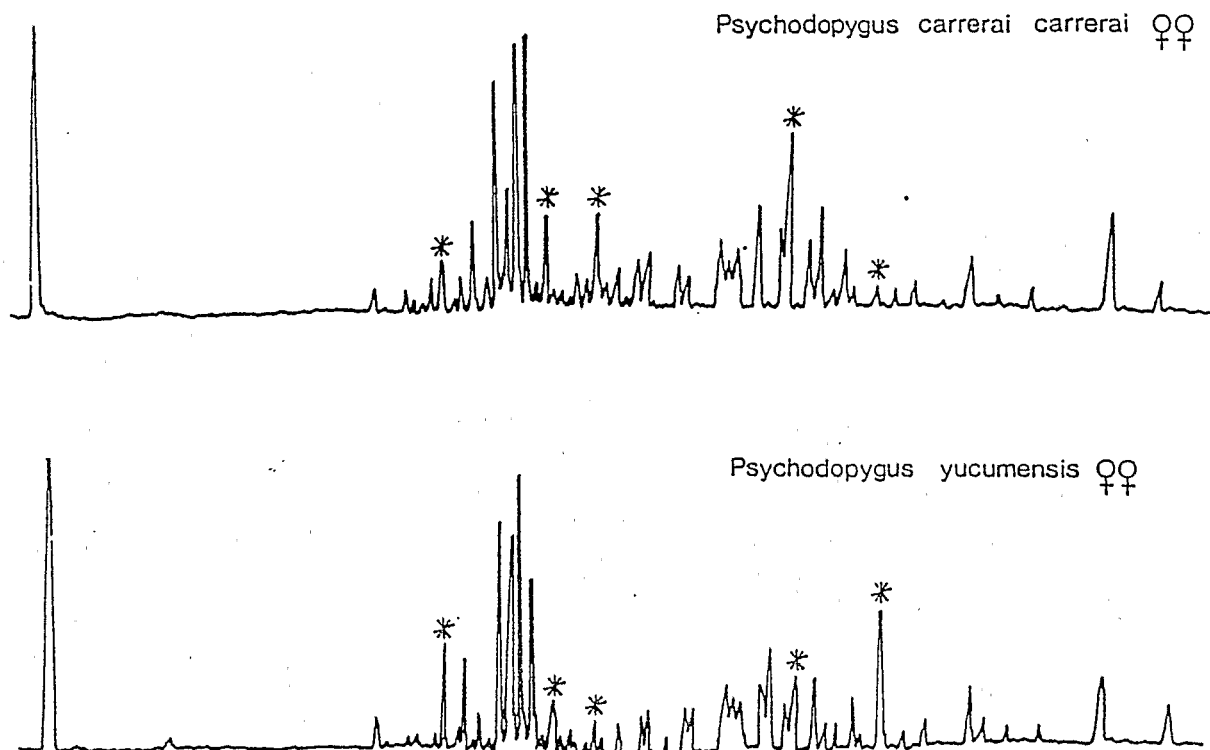


Table 1.

Table showing the predicted classification success using the discriminant function calculated from the data.

Actual group	Predicted group	
	<i>P.c. carrerai</i>	<i>P. yucumensis</i>
<i>Psychodopygus c. carrerai</i>	24	4
<i>Psychodopygus yucumensis</i>	3	22
TOTAL	27	26

Average correct classification rate = 87%