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## Separation of female *Psychodopygus wellcomei* and *P. complexus* (Diptera: Psychodidae) by cuticular hydrocarbon analysis

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### Summary

*Psychodopygus wellcomei* (Diptera: Psychodidae), vector of *Leishmania braziliensis braziliensis* – causative agent of cutaneous leishmaniasis in Brazil, cannot be distinguished from sympatric *P. complexus* either by morphometrics or isoenzyme profiles. We report here the use of cuticular hydrocarbon analysis in the successful separation of individual female *P. wellcomei* and *P. complexus*. This technique involves the use of gas liquid chromatography and discriminant function analysis.

**Key words:** sandflies; *Psychodopygus wellcomei*; *P. complexus*; *P. squamiventris*; cuticular hydrocarbon analysis; taxonomy; leishmaniasis.

### Introduction

Cuticular hydrocarbon analysis has recently been used in the separation of members of sibling species complexes. Medically important species complexes studied to date include adults and larvae of the *Anopheles gambiae* complex and adults of the *Simulium damnosum* complex (Carlson and Service, 1979, 1980; Hamilton and Service, 1983; Carlson and Walsh, 1981; Phillips et al., 1985). Successful separation of members of these complexes was possible on the basis of quantitative differences in their cuticular hydrocarbons, as detected by capillary gas chromatography. This technique had not been applied to taxonomic problems of the species groups of Phlebotominae, although Lane et al. (1985)

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have described differences in the compounds produced by the tergal spots of *Lutzomyia longipalpis* (authorities for all sandflies may be found in Martins et al., 1978) males, using combined gas chromatography/mass spectrometry. This latter finding gives further support to the view that *L. longipalpis* is a species complex (Ward et al., 1983).

*Psychodopygus wellcomei* and *P. complexus* (members of the series *squamiventris*) are sympatric in the Serra dos Carajás, Pará State, Brazil, where *P. wellcomei* has been shown to be the vector of *Leishmania braziliensis braziliensis*, the causative organism of cutaneous and muco-cutaneous leishmaniasis in man (Lainson et al., 1973; Ready et al., 1984; Ryan et al., 1986). The males of *P. wellcomei* and *P. complexus*, and indeed of all the species in this series, are readily distinguished, but the females cannot be satisfactorily separated on morphology alone (Lane and Ready, 1985) or isoenzyme techniques (Ready and da Silva, 1984).

This paper reports the first use of cuticular hydrocarbon analysis in the identification of species groups of sandflies, and shows that the technique can distinguish females of *P. wellcomei* and *P. complexus*.

## Materials and Methods

Captures of *P. wellcomei*/*P. complexus* were made in the Serra dos Carajás study area (Ward et al., 1973) during October 1983 and May 1984. Over 2000 females were caught in Shannon traps or with human bait, and rapidly transferred from the aspirators to a net cage. At the end of each capture an anaesthetized hamster, restrained by a wire mesh covering, was introduced into the cage and the flies left to feed for 6–8 h. Fed flies were removed within 4 h to holding tubes and the eggs oviposited were reared to adulthood as isofemale broods (refer to Ward, 1977, for details). Adult males were used to identify the brood, and the females placed into paper envelopes using hexane washed forceps and maintained over silica gel.

Extraction of the cuticular wax from individual flies was achieved by immersing each fly in 10 µl distilled hexane for 10 min. Some extracts were then treated to preparative column chromatography on silica gel contained in Pasteur pipettes (mesh size 70–230; 5 cm depth). Hexane, with increasing quantities of ether was used as the elutant, so that the polar and nonpolar constituents could be separated. Fractions containing hydrocarbons only were thus obtained. Further separation of the saturated and unsaturated hydrocarbons was deemed unnecessary as the degree of saturation and in some cases complete identification of compounds was possible using the gas chromatograph/mass spectrometer (GC/MS). A few samples were run on the GC/MS (a Perkin-Elmer Sigma 3B gas chromatograph plus a Finnigan Mat 1020 ova quadropole mass analyser linked by a Data General Nova 4 computerized data system). The GC/MS had both electron impact and chemical ionisation capability. All samples for analysis were evaporated to dryness and resuspended with 1 µl hexane containing an internal standard. Sample size for analysis was 1 µl, injected on-column. The gas chromatograph used was a Hewlett-Packard 790A linked to a recording integrator, with a 10 m CPsil5 CB capillary column (i.d. 0.32 mm and 0.15 µm phase thickness). Helium was the carrier gas at a flow rate of 1 ml/min. Sample runs began at 120°C followed by 7°C/min rise to 310°C. Integrated peak areas were standardised by comparison with the internal standard, to eliminate variations in injection volume and machine response. The standardised areas were then compared using discriminant analysis as described by Phillips et al. (1985).

## Results and Discussion

A total of 45 adult female flies were analysed by GLC. A further 8 flies (4 *P. wellcomei*, 4 *P. complexus*) were analysed by GC/MS for tentative peak identification. Fig. 1 compares the profiles obtained from individual female *P. wellcomei* and *P. complexus*. Peaks numbered 5, 12, 19 and 26 were the most important in discriminating between the groups and they are  $C_{17}$ ,  $C_{24}$ ,  $C_{27}$  and  $C_{35}$  compounds, respectively. Peak 26 is the larger peak in a doublet which occurs in both species. A more positive identification of these compounds requires intensive mass spectral analysis and chemical ionisation spectrometry, which are currently in progress. In the final analysis 15 out of 50 peaks were used, and the percentage of correct classification was 97.7%. Fig. 2 shows the histogram derived from analysis on 15 peaks. Cuticular extracts were shown to have a range of predominantly saturated branched and straight chain hydrocarbons with carbon numbers from  $C_{20}$  to  $C_{38}$ . Fatty acids and their esters were also present, originating, most probably, from internal lipid. These are numbered peaks 1–3 in Fig. 1 and are  $C_{16}$  to  $C_{19}$  compounds. Initial observations suggest there is no consistent qualitative difference between the two groups although more detailed GC/MS work may prove this. This work is also continuing in order to increase the numbers of individuals analysed, and to create a bank of data with which unknown female flies can be compared.

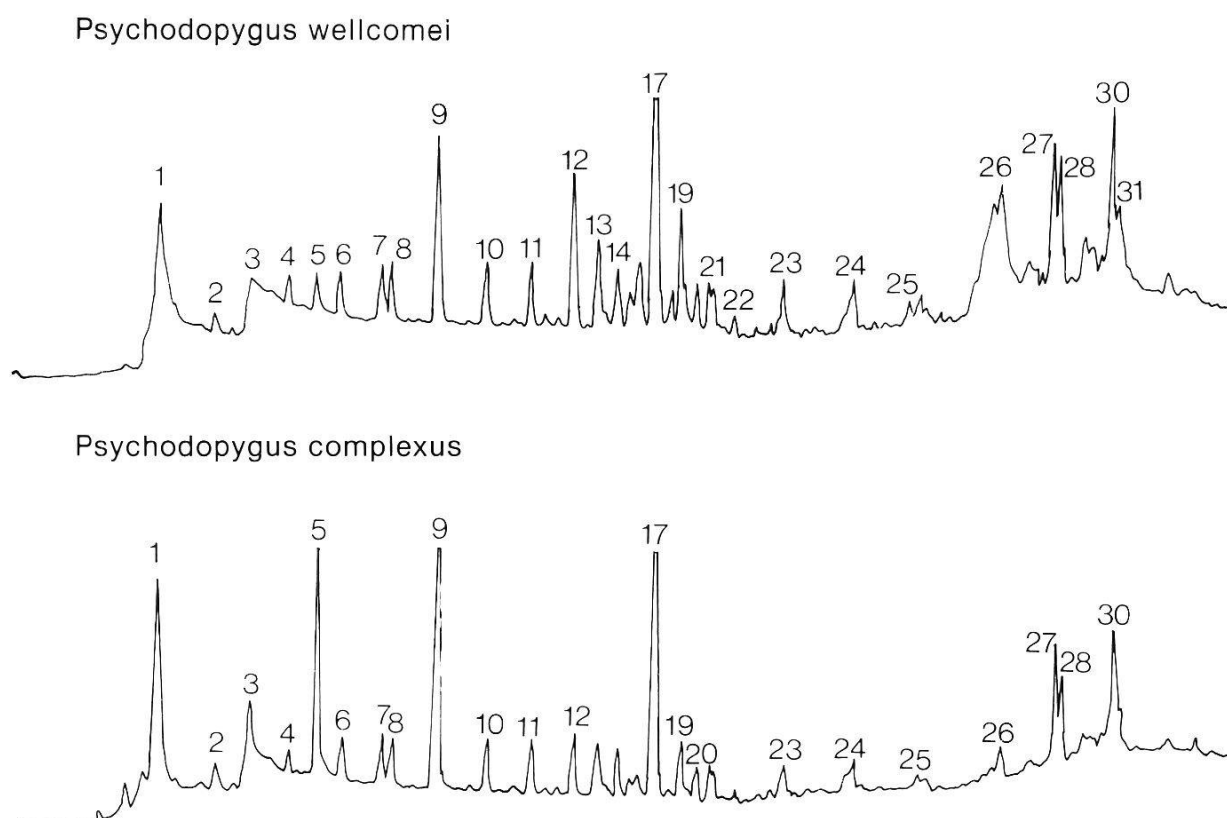


Fig. 1. Showing gas liquid chromatograms (profiles) of hexane extracted cuticular waxes from individual females. The peaks 1–3 are fatty acids, peak 4 onwards, are hydrocarbons. Differences can be seen between the profiles, e.g. in peaks 5, 12, 19, 26, etc.

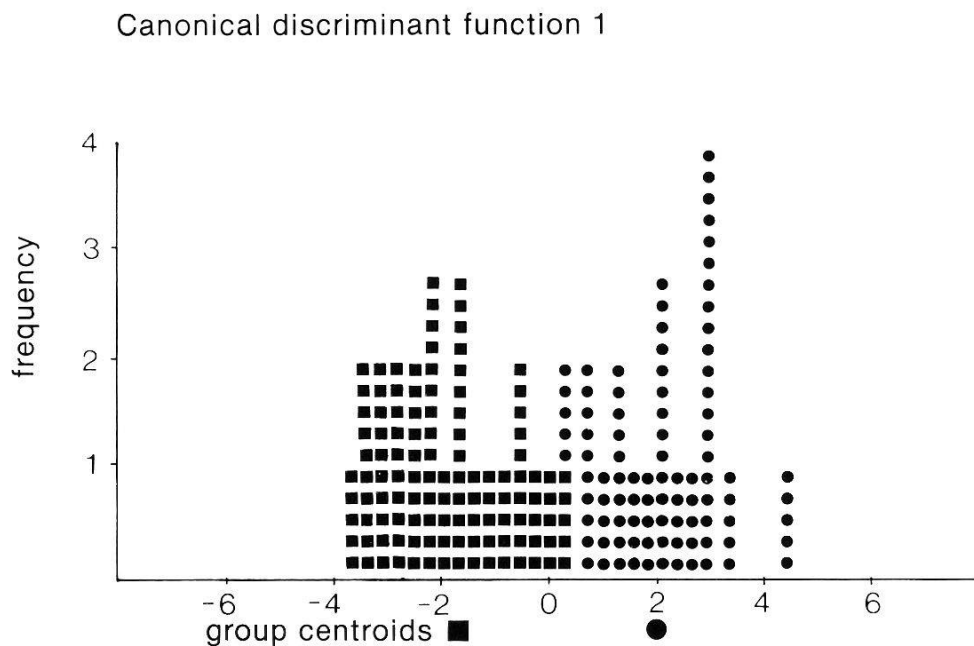


Fig. 2. Frequency histogram of discriminant function scores of individual ♀♀ *P. wellcomei* (■) and *P. complexus* (●), showing group centroids.

The ability to separate *P. wellcomei* and *P. complexus* using cuticular hydrocarbon analysis will provide a useful tool in the study of the epidemiology of *L. b. braziliensis*. Prior to this study the identification of females required rearing of isofemale broods which, if the eggs diapause, could take in excess of 4 months (Ryan et al., 1986). To date, the only member of the *squamiventris* group found to be infected with *Leishmania* is *P. wellcomei*, although suggestions that other members of the group may serve as vectors have been made on the basis of their anthropophily (Forattini, 1973). The recent detection of *P. squamiventris squamiventris* and *P. chagasi* infected with a *Leishmania* of the *braziliensis* complex (Ryan, Lainson and Shaw, unpublished observations, 1985) emphasises the importance of this group and of these studies which will be continued with other members of the *squamiventris* series. Cuticular hydrocarbon analysis will therefore be of use in identifying infected flies, and work is underway to use the remains of dissected individual females.

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