

ELECTROANTENNOGRAPHY IN THE STUDY OF TWO STINGLESS BEE SPECIES (HYMENOPTERA: MELIPONINI)*

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(With 1 figure)

ABSTRACT

The first recorded electroantennographic preliminary studies on stingless bees have been performed using two species of *Frieseomelitta* from Brazil. Experiments with *F. silvestrii* and *F. varia* showed that antennae respond to hexane extracts of heads and abdomens of both species and posterior tibia of *F. silvestrii* (which carry plant resin), as well as to the pure compounds 2-heptanol and 2-nonanol, which occur in the mandibular glands of both species, and to the terpenes α -cubebene, humulene, and β -caryophyllene found on their tibia and in the cerumen of their nests.

Key words: *Frieseomelitta*, antennal response, exocrine glands, propolis, odor.

RESUMO

Eletoantenografia no estudo de abelhas sem ferrão (Hymenoptera: Meliponini)

Relatam-se aqui os resultados preliminares de estudos eletroantegráficos realizados com duas espécies de *Frieseomelitta* brasileira. Os experimentos com *F. silvestrii* e *F. varia* mostraram que as antenas respondem aos extratos obtidos com hexano das cabeças e dos abdômens das duas espécies e da tibia posterior de *F. silvestrii* (que transporta resinas coletadas de plantas). Respondem também aos compostos puros, 2-heptanol e 2-nonanol, e aos terpenos, α -cubebeno, humuleno e β -cariofileno, encontrados nas túbias e no cerume de seus próprios ninhos.

Palavras-chave: *Frieseomelitta*, resposta antenal, glândulas exócrinas, própolis, odor.

INTRODUCTION

Stingless bees of the hymenopteran group Meliponini display complex communication patterns, and much of that communication must stem from glandular secretions. The study of pheromones in stingless bees is still in its infancy. The most important exocrine glands in stingless bees that might be implicated in pheromone production include, in the head, the mandibular glands; in the thorax, the thoracic salivary glands; and in the abdomen, the Dufour gland and epidermal glands.

Mandibular gland secretion of stingless bees has been more extensively studied because, among them, it appears to play an important part in chemical communication (Kerr *et al.*, 1963). Engels and Francke have together analyzed the cephalic secretions of a large number of species and made relations to age and development (Engels *et al.*, 1993, 1997; Francke *et al.*, 2000; and earlier papers). We have only recently made the first chemical studies of the Dufour glands of stingless bees (Cruz-López *et al.*, 2001; Patricio *et al.*, 2003). Some early studies with pure chemicals were made to study their effects on

the behaviour of stingless bees (Weaver *et al.*, 1975; Smith & Roubik, 1983), but the subject has progressed slowly.

The electroantennograph technique (EAG) measures the summation of receptor potentials from responding cells in insect antennae (Kaissling, 1971). It has been much used to identify active pheromone substances in a mixture of volatile compounds extracted from insects, and has played an important part in the identification of lepidopteran pheromones. As for honeybees, it is known that they detect odours by using olfactory receptor cells in their antennae (Lacher, 1964; Vareschi, 1971; Williams *et al.*, 1982), and no doubt stingless bees do too.

The recognition of active compounds in EAG studies can be a short-cut in identifying compounds recognized by antennal receptors that may be used in pheromone communication. Until our present work there have been no EAG studies on any stingless bees. We have analyzed the mandibular glands (Cruz-López *et al.*, 2002), the hind tibia that carry plant resin and nest propolis (Patricio *et al.*, 2002), and the Dufour glands (Patricio *et al.*, 2003) of three species of *Frieseomelitta*. They therefore provided a good starting point from which to initiate EAG studies. In our experience, while EAG with Lepidoptera is relatively easy and yields large potential responses, with ants it is very difficult and gives much lower potentials (Wadhams & Morgan, unpublished). We give here a full account of the first studies by EAG with stingless bees, using two species of *Frieseomelitta* from Brazil, that show that this approach to studying communication chemicals is practical and promising. A brief report on our study of the chemicals from the heads of *Frieseomelitta* has already been published (Cruz-López *et al.*, 2002).

METHODS AND MATERIALS

Insects. Workers of *F. varia* Lepelletier 1836 and *F. silvestrii* Ihering 1912 were provided from the collection of Professor Paulo Nogueira Neto and from the Laboratório de Abelhas, Instituto de Biociências, Universidade de São Paulo, Brazil. *F. varia* were collected at Ribeirão Preto in northern São Paulo State. *F. silvestrii* came originally from Luiziania in Goiás State, near Brasília. Groups of about 50 worker bees with brood cells, honey, and pollen pots were used, and supplied with water.

Extracts were prepared by placing the body parts of worker bees (heads, abdomens, and hind legs), and of pieces of cerumen taken from their nest, in small glass tubes containing hexane (0.5 ml), and allowing them to stand for 30 min. Pure compounds (geranylarnesol, α -cubebene, humulene, and β -caryophyllene, 10 mg each) were dissolved in hexane (10 ml).

Electroantennography was performed using excised antennae of *F. silvestrii* and *F. varia* workers. Electrodes were drawn from 1 mm i.d. borosilicate tubes to produced capillaries with a taper length of 0.5 cm with the tips filled with saline solution prepared according to Maddrell (1969) but omitting the sucrose. An antenna was placed between two electrodes, one inserted at the base of the antenna and serving as the reference electrode, and another, the recording electrode, placed at the tip of the antenna. In all, 20 antennae were used for *F. silvestrii* and five for *F. varia*.

For the electroantennogram, hexane solution (10 ml) was impregnated onto a strip of filter paper, 15 x 1.5 mm, which was then placed in a disposable glass pipette. While air was continuously blown over the antenna, the pipette tip containing the strip of filter paper was inserted in a hole at the end of the tube carrying the air stream, thus delivering vapor from an unknown quantity (equivalent to one worker head, abdomen, or tibia) of extract or from 10 mg of one of the pure compounds. The vapor was puffed from the filter paper with a solenoid-controlled nitrogen stream (3 ml/sec). The signals, amplified with an AC coupled amplifier, were displayed on an oscilloscope (Devices 3131, Dynamic Electronics Limited). A permanent trace was obtained with a Devices M2 pen recorder. Only two replicates of the experiment were made with *F. varia* because only a limited number of live bees were available. Results of *F. silvestrii* were analyzed using student's *t* test. No statistical test was performed with the *F. varia* results because of the small number of replicates.

RESULTS

Satisfactory electroantennography experiments were conducted with *F. silvestrii*, but by the time the experiments were performed with *F. varia*, only two good replicates were obtained. Table 1 shows the EAG responses of *F. silvestrii* to abdominal, head, and hind

leg extracts in hexane. A typical electroantennogram is shown in Fig. 1. Also tested were two important components of the mandibular secretion of *F. silvestrii*: 2-nonanol and 2-undecanol; three volatile terpenes from the posterior tibia: α -cubebene, humulene, and β -caryophyllene; and geranylarnesol, a component of the Dufour gland (Patricio *et al.*, 2003). All the extracts and synthetic compounds except geranylarnesol gave significant EAG responses. One of the sesquiterpenes from the tibia, α -Cubebene, (Patricio *et al.*, 2002) gave a particularly strong response (Table 1), although there was less of this compound found on the tibia of *F. silvestrii* (Patricio *et al.*, 2002) than β -caryophyllene (which gave a weaker response). However, from the analytical conditions used in the analysis of resin on the tibia (Patricio *et al.*, 2002) it was not possible to know which enantiomer of β -caryophyllene was present on the tibia, since commercially available (-)- β -caryophyllene was used. The limited experiment on *F. varia* nevertheless gave strong responses to solvent extracts of abdomen, head, and cerumen (Table 1). Nonanal and 2-undecanol were the important mandibular compounds to test with *F. varia*. Shortage of live bees limited the experiment on *F. varia*; nevertheless, responses to solvent extracts of head, abdomen, cerumen, and the

pure compounds nonanal and 2-nonanol were all strong, and only that to 2-undecanol was comparatively weak (Table 1).

DISCUSSION

The electroantennographic (EAG) technique has been used to determine which of the compounds present in a mixture of volatiles can stimulate olfactory receptors. No physiological information of this type was available for stingless bees, therefore preliminary EAG studies were undertaken using the antennae of *F. varia* and *F. silvestrii*. Head, abdominal, and tibial extracts of *F. silvestrii* all gave equally strong antennal responses. The responses of *F. varia* antennae to body extracts were very similar to those obtained with *F. silvestrii*. Strongest responses of the pure compounds were given by α -Cubebene and 2-nonanol. It is noteworthy that in both species 2-nonanol gave stronger responses than 2-undecanol. Further planned experiments that included extracts of individual dissected glands were cut short by the decline of the colonies. Geranylarnesol is only a trace component in the Dufour glands of *F. silvestrii* but is the major substance in the closely related *F. silvestrii languida* (Patricio *et al.*, 2003).

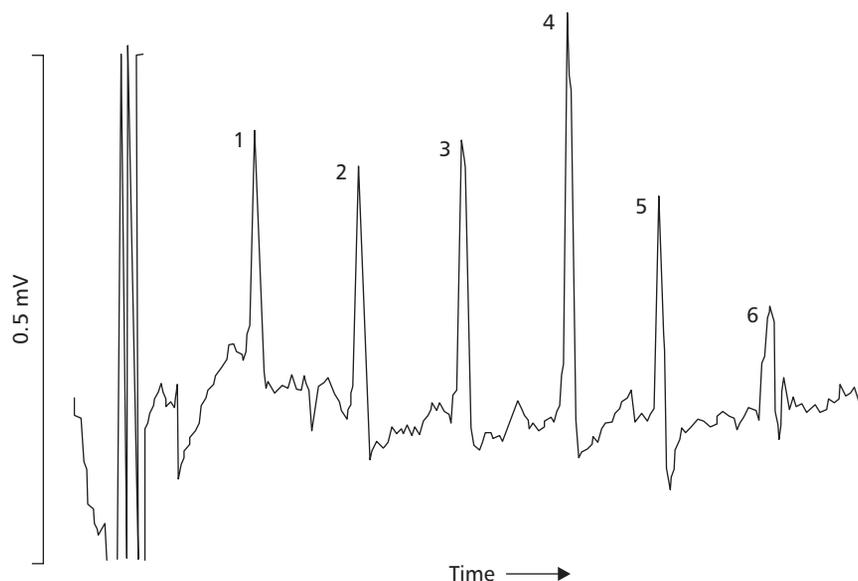


Fig. 1 — An example of an electroantennogram taken from the antenna of a worker of *Frieseomelitta silvestrii*. Peaks were obtained after applying the following extracts or pure chemicals: 1, head extract; 2, tibial extract; 3, abdominal extract; 4, cerum extract; 5, 2-nonanol; 6, 2-undecanol.

TABLE 1

Mean and standard deviation of the EAG responses of the *Frieseomelitta silvestrii* and *F. varia* antennae to some body extracts and synthetic samples of compounds identified in their exocrine glands.

Stimulus**	<i>F. silvestrii</i>		<i>F. varia</i>	
	Response (mV ± SD)	Replicates	Response (mV ± SD)	Replicates
Head extract	0.095 ± 0.013*	15	0.105 ± 0.035	2
Hind legs extract	0.101 ± 0.020*	8	–	–
Abdominal extract	0.12 ± 0.025*	5	0.100 ± 0.010	2
Cerumen extract	–	–	0.055 ± 0.055	2
Nonanal	–	–	0.027 ± 0.000	2
2-Nonanol	0.109 ± 0.016*	7	0.025 ± 0.005	2
2-Undecanol	0.063 ± 0.011*	7	0.010 ± 0.010	2
Geranylarnesol	0.012 ± 0.004 NS	5	–	–
α-Cubebene	0.16 ± 0.014*	16	–	–
Humulene	0.069 ± 0.011*	9	–	–
(-)-β-Caryophyllene†	0.062 ± 0.009*	8	–	–

* Significantly different from control at $p = 0.001$, NS = not significantly different from control at $p = 0.001$ (control hexane, 10 μ l).

**Applying 10 μ l of the hexane extract (see Methods and Materials) and 10 μ l of a solution (1 g L⁻¹) of pure compounds.

† The absolute configuration of the β-caryophyllene on the tibia (Patricio *et al.*, 2002) has not been determined.

It was available for tests, but its poor response, not significantly different from that of the hexane control, could be due to low volatility because of its relatively high molecular mass (358 mass units). We have reported earlier on the plant resin substances found on the hind tibia of these species (Patricio *et al.*, 2002) and we have tested three of the substances found there both on *F. silvestrii* and an extract of the cerumen from the nest of *F. varia*. The results show that individual substances and mixtures of compounds present in the abdomen, cerumen, and on the tibia of *Frieseomelitta* bees can elicit olfactory responses in bee antennae. This is consistent with the hypothesis that such volatiles can elicit behavioral responses. What kind of behavior these materials induce has yet to be discovered.

Our brief study shows that EAG can provide a route to learning more about chemical communication in stingless bees. From the mixtures of chemicals found in glands, the EAG can select substances that show a positive response and deserve further study in behavioral experiments. The relatively

simple mixture of substances in *F. silvestrii* makes it a suitable candidate for behavioral studies with synthetic compounds.

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*This paper is published in honor of Father J. S. Moure, D. D., a professor at the Federal University of Paraná. An entomologist, he has dedicated his life to the study of biology, particularly bee systematics. It was he who identified the specimens studied here. At over 90 years of age, he continues on as a tireless researcher, readily responding to requests for guidance and suggestions from fellow scientists working within his professional area. For beginning and veteran scientists alike, his example will endure. With gratitude, we salute him.

*Este artigo é publicado em homenagem ao cientista Pe. J. S. Moure, D. D. Entomólogo, Professor da Universidade Federal do Paraná, que tem dedicado sua vida à pesquisa da biologia e sistemática de abelhas. Devemos a ele a identificação dos exemplares estudados. O Pe. Moure, embora com mais de 90 anos de idade, é um incansável pesquisador, pronto a atender a solicitações e a ajudar a solucionar problemas que lhe são apresentados. É um exemplo para novos (e velhos) cientistas. A ele, nossa homenagem e gratidão.