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ODOR COMPOUND DETECTION IN MALE EUGLOSSINE BEES

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Abstract—Male euglossine bees collect fragrances from various sources, which they store and use for as yet unknown purposes. They are attracted, often specifically, to single odor compounds and blends thereof. We used gas chromatography with electroantennographic detection (GC-EAD) and electroantennography (EAG) to investigate the response to 8 odor compounds by males of two euglossine species, *Euglossa cybelia* Moure and *Eulaema polychroma* (Mocsàry). In *E. cybelia*, we recorded EAD reactions in response to 1,8-cineole, methyl benzoate, benzyl actetate, methyl salicylate, eugenbl, and methyl cinnamate. *E. polychroma* responded to the same compounds in EAG experiments, while (ls)(-)α-pinene and β-pinene failed to trigger EAD or EAG responses in the bees. Blends of two compounds triggered larger responses than single compounds in EAG experiments with E. *polychroma*, however, when α-pinene was added, reactions decreased. In the light of existing data on the bees' behavior towards these odor compounds, our work indicates that both peripheral and central nervous processes influence the attraction of euglossine bees to odors.

Key Words—Euglossine bees, GC-EAD, EAG, odor compound detection.

INTRODUCTION

Male euglossine bees are attracted by the scent of certain orchid flowers where they collect odor substances and thereby pollinate flowers (Vogel, 1966; Dodson et al., 1969). Although this behavior has attracted considerable research interest, the purpose of the odor collection behavior remains elusive (Eltz et al., 1999). Synthetics of chemicals found in orchid flowers have been used to perform tests

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that show bees are attracted selectively to certain compounds and/or specific mixtures of volatiles (Dodson et al., 1969; Williams and Dodson, 1972; Ackerman, 1989). Orchid flowers pollinated by euglossine bees may produce specific floral odor bouquets that are thought to be responsible for attraction of their pollinators (Hills et al., 1972; Williams and Whitten, 1983; summary in Roubik and Hanson, in press). The olfactory mechanisms underlying selective behavioral attraction towards odor compounds and mixtures thereof have not previously been investigated.

Odor detection by olfactory neurons may have a major influence on the behavior of euglossine bees, while compounds undetected on a receptor level may have little effect. In a blend of two or more compounds, however, undetected substances may inhibit responses of olfactory receptors to active compounds (Cromarty and Derby, 1998). On the other hand, if more than one compound in a blend is detected individually, then interactions of the signals in the central nervous system may govern behavior (Smith and Getz, 1994). Our study investigates for the first time the detection of odor compounds and mixtures thereof in two species of euglossine bees, *Euglossa cybelia* and *Eulaema polychroma*, by using gas chromatography with electroantennographic detection (GC-EAD) and electroantennography (EAG).

METHODS AND MATERIALS

Male *Euglossa cybelia* Moure and *Eulaema polychroma* (Mocsàry) were collected at 800 m elevation on 8 April, 2002 in central Panama. The bees were transferred to Zürich, Switzerland, where the electrophysiological investigations were conducted.

Gas Chromatography with Electroantennographic Detection (GC-EAD). We used 8 synthetic compounds for our recordings [(ls)(-)α-pinene, β-pinene, 1,8-cineole, methyl benzoate, benzyl acetate, methyl salicylate, eugenol, and methyl cinnamate], which are common odor constituents of euglossine-pollinated orchids (Hills et al., 1972). Many such odors are also known as general attractants for euglossine bees (Dodson et al., 1969; Ackerman, 1989). One μl of a mixture of all compounds (100 μg/ml each) was injected splitless at 50°C (1 min) into a gas chromatograph (HP 6890N) followed by opening the split valve and programming to 300°C at a rate of 10°C/min. The GC was equipped with an HP-5 column (30 m $\times$ 0.32 mm $\times$ 0.25 μm); helium was used as carrier gas. A GC effluent splitter (SGE Australia; split ratio 1:1) was used, and the outlet was placed in a purified and humidified airstream. This air was directed over a male bee's antenna prepared as follows: The tip of the excised antenna was cut off and the antenna mounted between an electrode holder using electrode gel. One electrode was grounded, the other was connected via interface box (Syntech, Hilversum) to a PC. EAD signals and flame ionization detector (FID) responses were simultaneously recorded. GC-EAD analyses of two males of *Euglossa cybelia* were obtained.

Electroantennography (EAG). One antenna of *Eulaema polychroma* was mounted on an electrode holder, and signals were recorded as described in the previous paragraph. 10 μg of a synthetic compound in 10 μl solvent, and mixtures of synthetic compounds with 10 μg each were applied on a filter paper in a pipette tip. A control filter paper was treated with 10 μl solvent only. Enough time was allowed for the solvent to evaporate. During stimulation, 2.5 ml of air were puffed over the filter paper and into an airstream directed over the antenna. Two stimulations of each test compound were applied with minimum 40 set intervals. To normalize and compensate for mechanoreceptive artifacts, EAG responses (−mV) were expressed as percent response of the control stimulant. From these values, mean responses to each compound and compound mixture were calculated.

RESULTS AND DISCUSSION

In *Euglossa cybelia,* consistent EAD reactions were recorded from all tested compounds except α- and β-pinene (Figure 1). Responses to most compounds were of similar intensity; however, the reactions to 1,8-cineole and methyl cinnamate were weaker than to other compounds. Similarly, *Eulaema polychroma* responded

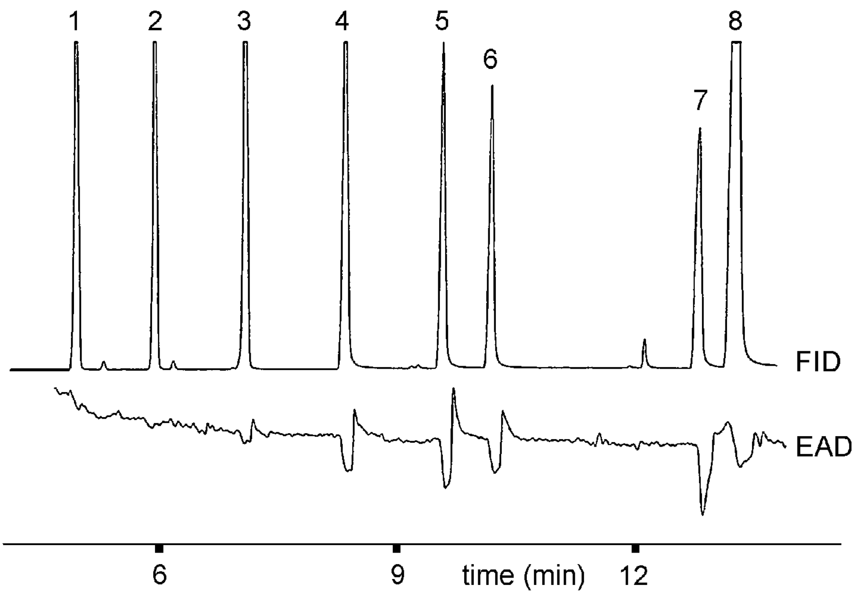

FIG. 1. Gas chromatographic analysis of a mixture of synthetic compounds with flame ionization detector (FID) and electroantennographic detection (EAD) recorded simultaneously using an antenna of a *Euglossa cybelia* male. For names of compounds see Table 1.

TABLE 1. RELATIVE EAG RESPONSES OF A *Eulaema Polychroma* MALE TO VARIOUS ODOR COMPOUNDS AND MIXTURES THEREOF (CONTROL = 100%)

Compound/mixture[a]	Mean relative EAG response (in %) ± s.e.m.
1) (1s)(-)α-pinene	85.71 ± 0.00
2) β-pinene	85.71 ± 0.00
3) 1,8-cineole	192.86 ± 21.43
4) methyl benzoate	350.00 ± 78.57
5) benzyl acetate	600.00 ± 14.29
6) methyl salicylate	571.43 ± 100.00
7) eugenol	300.00 ± 0.00
8) methyl cinnamate	200.00 ± 14.29
methyl salicylate + benzyl acetate	864.29 ± 21.43
1,8-cineole + benzyl acetate	871.43 ± 0.00
α-pinene + 1,8-cineole + benzyl acetate	664.29 ± 35.71

[a] Each compound/blend was applied two times on the antenna.

to all of the tested compounds except α- and β-pinene in the EAG experiments (Table 1). Mixtures of 1,8-cineole + benzyl acetate and benzyl acetate + methyl salicylate resulted in stronger reactions than application of single compounds. However, the addition of α-pinene to the mixture of 1,8-cineole + benzyl acetetae lead to a smaller reaction than with 1,8-cineole + benzyl acetetae alone.

It is now well established that odor compounds may attract euglossine bees selectively. Certain compounds attract certain species, and mixtures of compounds often attract the bees more selectively than single compounds (Dodson et al., 1969; Williams and Dodson, 1972; Ackerman, 1989). Ackerman (1989) showed that the compounds used in our investigation are attractive for a range of *Euglossa* and *Eulaema* species. *E. cybelia* is, however, attracted only to 1,8-cineole, benzyl acetate, and methyl salicylate, but not to eugenol, methyl benzoate, or methyl cinnamate. The chemicals α- and β-pinene were not tested by Ackerman (1989), but are generally known as poor attractants (Dodson et al., 1969). *E. polychroma* is attracted to 1,8-cineole, but not by the other compounds tested here. Because both bee species could detect all the tested compounds except α- and β-pinene, we conclude that a lack of attraction does not necessarily imply a lack of detection ability. The attractiveness of a certain chemical seems, however, to be influenced by processes in the central nervous system, which mediate behavioral patterns of the bees. Electrophysiologically active floral fragrances may in general not only attract, but also deter flower visitors.

In our EAG experiments, it was evident that the addition of α-pinene to a blend reduced the EAG response. Myrcene and α-, β-pinene were shown to reduce the attractiveness of 1,8-cineole, in field assays, by attracting fewer species and each species in smaller numbers than 1,8-cineole alone (Williams and Dodson, 1972). Our results indicate that such a reduced attractiveness may, at least in part, be due to inhibition of receptor responses by certain compounds, which may influence the detection of molecules by the bee. Such inhibition mechanisms may include binding interactions, in which compounds mutually inhibit binding to receptor sites (Cromarty and Derby, 1998). Since α-pinene appears not to be detected as a single compound, an interaction with signals triggered by other compounds on the central nervous system level seems unlikely.

In summary, we present evidence that both detection mechanisms at the peripheral and central nervous system processes may be important for the selective attraction of euglossine bees to odor compounds and mixtures thereof. Our study has further shown that electrophysiological methods such as EAG and GC-EAD are tools that may help to better understand complex interactions of euglossine bees and floral odors.

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