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# Fragrances, male display and mating behaviour of *Euglossa hemichlora:* a flight cage experiment

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**Abstract.** Male euglossine bees (Apidae: Euglossini) collect volatile substances (fragrances) from floral and nonfloral sources and store them in hair-filled cavities in their hind tibiae. Over time, males accumulate large quantities of complex and species-specific blends of fragrances. Various hypotheses have been put forward to explain this behaviour, including the idea that fragrance stores reflect the genetic quality of individual males and have evolved through sexual selection and female choice. Clear support of this hypothesis is lacking, largely because male–female interactions are both rare and difficult to observe in nature. Here, we report a flight cage experiment performed in Panama that permitted mating between virgin females (raised from brood cells) and males captured in the forest at fragrance baits. In the cage, eight individually marked males defended small territories around vertical perch sites and showed a characteristic display, which included a previously unreported 'leg-crossing' movement, possibly related to fragrance release. A total of six copulations and three copulatory attempts by *Euglossa hemichlora* were observed and partly recorded on video. The copulations, all of which were initiated by the female landing on a male perch, were short (4–10 s) and showed no signs of the transfer of chemical substances from male to female. In some cases, the male hovered directly over the female before descending to mount her, possibly facilitating fragrance evaluation by the female. After the experiment, the contents of the males' hind legs were analysed by gas chromatography-mass spectroscopy, which detected complex mixtures of terpenoids and aromatics (totalling 70 different compounds) dominated by hexahydrofarnesyl acetone, farnesene epoxide, ocimene and *p*-dimethoxy benzene. Individual total amounts of fragrances were neither related to display activity or perch occupancy by given males, nor to the frequency of matings achieved. Display activity was the only positive correlate of mating frequency. Generally, individuals had uniformly large amounts of stored fragrances in comparison to a previous study of three other species of Panamanian *Euglossa*.

**Key words.** Copulatory behaviour, female choice, fragrance exposure, mating, GC-MS, orchid bees, sexual selection, territorial behaviour, velvet area.

## Introduction

The neotropical orchid bees (Apidae, Euglossini; five genera, >200 species) are well known for the specialized pollination services that fragrance-seeking males provide for orchids and other neotropical plants (Williams, 1982). Male euglossines have complex behavioural and morphological

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adaptations for collecting volatile chemicals. They are attracted to diverse fragrant objects (e.g. flowers, rotting wood, or sap exposed on plant wounds), on which they land and apply a mixture of lipids produced by their labial glands. Then, the nonpolar fragrance compounds (mostly terpenoids and aromatics) are dissolved in the lipids and quickly adsorbed on dense brushes of setae situated on the male fore-tarsi. In a concerted movement involving all three pairs of legs, the mixture is then transferred into sponge-like tissue in cavities filling most of the enlarged hind tibiae (Vogel, 1966; Kimsey, 1984; Whitten *et al*., 1989, 1993).

After storage in the male hind tibiae, what happens next to the fragrances remains speculative, as is the ultimate cause of fragrance collection. It has been suggested that the substances are somehow taken up into the males' haemolymph, modified chemically, and reused as sex pheromones (Williams & Whitten, 1983). However, gas chromatography-mass spectroscopy (GC-MS) studies of male *Euglossa imperialis* kept in a flight cage for up to 15 days found no decrease in the total amount of fragrances stored over time, nor any evidence for qualitative changes in composition (Eltz *et al*., 1999). In conjunction with the finding that the amount of fragrances stored was correlated with age-related wing wear in *Euglossa cognata* (Eltz *et al*., 1999), these results strengthen the view that the males' tibial cavities are primarily storage devices.

How did male fragrance accumulation evolve? Although no direct evidence exists, the fragrances very likely play a part in euglossine courtship. Matings are very rarely observed, but reportedly occur in or around the small territories that males defend on vertical perches on tree trunks or stems in the forest understory (Dodson, 1966; Kimsey, 1980). At these perches, the males perform a characteristic display during which they may buzz their wings (*Eulaema meriana*) or show brief hovering flights (*E. imperialis*) (Kimsey, 1980; Stern, 1991). The potential release of fragrances during the display may lure in receptive females, ostensibly over some distance (Vogel, 1966). Analysing tibial contents of three Panamanian species of *Euglossa*, Eltz *et al.* (1999) found specific differences in chemical composition, suggesting that the fragrances could be used as a means of species recognition. However, the idea of long-range sexual attraction suffers from the fact that female bees are not attracted during fragrance bioassays; neither to artificial pure compounds nor to the complex bouquets evaporating from crushed hind legs of conspecific males. Instead, only males are attracted (Ackerman, 1989; Eberhard, 1997; Roubik, 1998). This finding stimulated the hypothesis that males use the fragrances in order to congregate and form 'leks' (Dodson *et al*., 1969). Although this may hold true in some situations, the lek-hypothesis is weakened by the fact that most males display solitarily, and by the general impression that males show overt aggression at the appearance of other males. Yet another hypothesis suggests that the fragrances are transferred as nuptial gifts to the females during copulation and are later used during nest construction because of their antimicrobial effect (Roubik, 1989).

At present, most researchers of euglossine biology favour the view that the fragrances are somehow exposed during the brief premating encounters between the sexes and serve as an indicator of male genetic quality (Whitten *et al*., 1989; Eltz *et al*., 1999; Roubik & Hanson, in press). This idea assumes that fragrances are either energetically expensive to collect, costly to retain, or difficult to detoxify. If this is the case, and if females prefer males with a certain fragrance phenotype, fragrance collection could have evolved through sexual selection. As indicated, all current explanations suffer from a lack of detailed observation of euglossine mating behaviour that might permit evaluation of the hypotheses. Here, data are presented from a cage experiment performed in Panama, in which encounters between field-caught males and virgin females of *Euglossa hemichlora* Cockerell were studied. The primary objective was to test one prediction of the female choice/sexual-selection hypothesis, namely that male mating success is positively related to the quantity or complexity of fragrances stored in their hind tibiae. In the course of the experiment, the first close-up video recordings of euglossine copulations were obtained and previously unreported components of male display behaviour were observed.

## Materials and methods

### *Nests and virgin females*

Nests of *E. hemichlora* were obtained by placing 50 wooden trap-nests in small shelters provided by farmers living along the 'El Llano-Carti' Road (Carti road), 80 km east of Panama City. The trap-nests, small wooden boxes (15 × 10 × 5 cm) with a drilled 5-mm entrance hole, were provided with a small lump of wax/resin mixture of 'cerumen' nesting material obtained from the nests of *Melipona panamica*, a large local, stingless bee, as an inducement to nest. The nests were placed in the field between June and July 1995, and almost half were colonized by females of *E. hemichlora*. At the end of July, 14 of the oldest nests (with from one to 11 brood cells) were transported to Panama City where the bees eclosed (see below). These nests contained a total of 51 females, three males (sex ration 17 : 1), and numerous parasitoids: an unidentified chalcidoid wasp and some cleptoparasites [*Coelioxys* sp. and *Hoplostelis* sp. (Megachilidae)]. The egg-to-adult development of *E. hemichlora* lasted approximately 8 weeks.

The nests and boxes were placed on a shelf in one compartment (2.3 × 3.5 × 2 m) of a large nylon flight cage (7 × 3.5 × 2 m) set up in a shaded backyard in Curundu, Panama City. Emerging females learned quickly to drink from artificial flowers (1.5 mL Eppendorf tubes with a plastic corolla) provided daily with a fresh 50% honey-water solution, as well as from cut natural flowers placed in the cage. During the actual experiment (28 August to 8 September), approximately 30 unmated females of different ages were active at any given time.

### *Males and behavioural observations*

On 13 and 15 August, males of *E. hemichlora* were captured on concealed fragrance baits near Carti road (a mixture of *p*-dimethoxybenzene, methylsalicylate, benzylbenzoate and eugenol in equal parts) and transported to Curundu. Eight of these learned to forage for honey-water at artificial flowers, were individually marked on the second tergite with enamel paint, and released into a separate compartment of the flight cage (also 2.3 × 3.5 × 2 m, adjacent to the female compartment). The hind legs of four other individuals were directly sampled for GC-MS analysis (see below). During the next 2 weeks, the males adjusted well to the cage situation and, finally, on day 10 of their captivity, started to perch and display on the stems of potted treelets present in the cage. During captivity, the males did not have access to obvious sources of fragrances and no fragrance collecting behaviour was observed.

On the mornings of 26, 28, 29 and 31 August, as well as on 2–4, 6 and 8 September, the nylon screens between female and male compartments were removed temporarily (between 08.00 and 14.00 h, depending on weather), encouraging encounters between the sexes. At the same time, territorial activity of the males was monitored. All displaying males were identified every 10 min and the perch site noted. From these data, individual display activity (display rate averaged over all 10-min intervals for each male), the number of different perches used by a male, and perch constancy (the percentage of observations at each male's preferred perch) were calculated. During the intervening time interval, all perch sites were observed to avoid missing possible matings. For detailed observations at greater distance, binoculars (8 × 22, close focus to 1.5 m) were used. Video recordings were made with a Canon EX-Hi equipped with a 15x-VL-Zoom lens (courtesy of J. Nieh), facilitating format-filling shots of bees from relatively great distance (approximately 1 m). At the end of the daily observations, both cage parts were closed again and the males and females separated by hand net.

On 8 September, the males were captured, killed by freezing, and their hind legs were stored in 0.5 mL *n*-hexane (with internal standard) for later GC-MS. Chemical analysis of extracts used a Hewlett-Packard 5995 GC-MS with a 30-m DB-5 column at the Department of Natural Sciences at the Florida Museum of Natural History. The oven temperature was programmed from 25–290 °C at 3° per min. Most fragrance compounds were identified by comparison with authentic standards, and the spectra of unidentified compounds were searched against the NIST/EPA/NIH spectral library (Ausloos *et al.*, 1992).

## Results

### *Male territorial behaviour*

The territorial behaviour of male *E. hemichlora* was similar to that observed in wild *E. imperialis* (Kimsey, 1980). Males perched on a variety of vertical structures present in the cage, including stems, branches and petioles of live plants (small shrubs and treelets with 0.5–1 cm in diameter), as well as some of the larger dead stems used as cage support (up to 5 cm in diameter). When at the perch, the males performed repeated series (10–30 s) of display behaviour, interrupted by patrolling flights into the immediate vicinity of the perch (radius 0.5–1 m). During the actual display, 'standing' on the perch (2–3 s) alternated with brief hovering flights (1–2 s) approximately 1 cm off the perch. When 'standing' on the perch, the mid-legs were normally held close to the thorax and did not touch the perch. No form of 'wing buzzing' was observed when the male was stationary. In approximately 30% of the brief hovering flights, a peculiar, rather stereotype 'leg-crossing' movement was observed that had not been reported previously in other studies. Once noticed, the behaviour was recognized easily by an observer. Single frame analysis of close-ups of 15 complete movements (filmed from different angles and on several individuals) led to the following description: shortly after take-off from the perch, one of the hindlegs (e.g. the right one as indicated in Fig. 1) was swung widely forward underneath the body. In this way, the inner side of the hindtibia was facing the body, its outer side (along with the majority of the hairy groove) was turned downwards and away from the body. When the hindleg had reached its maximal position (Fig. 1, frames 3 + 4; the metatarsus was somewhat underneath the tegulae), an oppositely directed thrust of the contralateral midleg was initiated (e.g. the left one as indicated in Fig. 1). During this fast movement, which was not clearly resolved at 25 frames/s, the mid-leg was stretched completely between the underside of the bee's body and the forward-bent hindleg (Fig. 1, frames 5–7), giving the impression of a brushing movement involving the mid-tibial 'velvet area' (see Discussion). At its maximal position, the tip of the midleg was almost beyond the tip of the abdomen (frame 7). Subsequently, both legs returned to their normal position (frames 8 and 9). The entire sequence was completed within 5–10 frames (0.2–0.4 s). The 'leg-crossing' is clearly different from any component of the regular grooming behaviour of *E. hemichlora* (T. Eltz, unpublished observations).

When males were undisturbed, they sometimes performed long sequences of territorial behaviour at a given perch (up to 20 min) before finally flying to the feeders. Frequently, however, other males intruded and were immediately confronted by the resident male. The opponents circled each other briefly or flew zig-zag patterns when facing each other or the perch. Then one of them, in most cases the intruder, left the area. Body contact or grappling were never observed. Very rarely did two males display synchronously at the same perch. Individual males showed considerable variation in their display rate, preferred perch, and in the extent to which they confined their display to certain perches (Table 1).

In addition to the described territorial behaviour, males were sometimes observed appearing to 'mark' small selected areas on elevated cage poles, once they had gained access to the female compartment. The males walked slowly on the

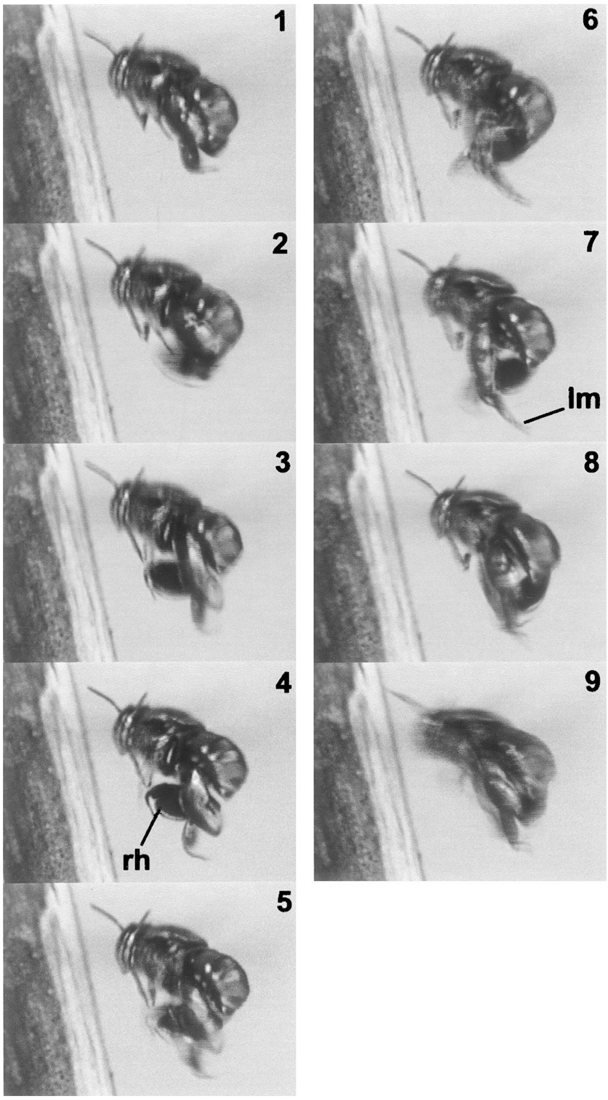

**Fig. 1.** Single-frame video sequence of a 'leg crossing' taking place during the hovering phase of territorial display in male *Euglossa hemichlora*. rh, right hind tibia; lm, left mid leg. Further details are provided in the text.

bark of the poles and repeatedly moved their heads towards and away from the bark. When doing so, they often held their mandibles open and extended the antennae towards the pole, giving the impression that cephalic secretions were applied to the substrate. However, GC-MS analysis of hexane extracts of collected bark samples did not reveal any soluble contents above the detection threshold.

### *Mating*

A total of six copulations and three copulatory attempts were observed. In all cases, the participating female flew close to the ground into a territory occupied by a single male and initiated all further action. Six of the interactions (four copulations, two copulatory attempts) were observed at close distance from beginning to end, and three of them were filmed on video. These interactions could be assigned to either of two patterns: (i) The female entered the territory. The male noticed the female below him, took flight and moved slightly away from the perch. The female landed without hesitation close to the display site. The male immediately flew in from behind, landed on her back and copulated ($n = 2$ copulations). (ii) The female entered the territory. The male noticed the female below, took flight and flew slightly away from the perch. The female hovered in front of the trunk approximately 10–15 cm below the male. After briefly hovering (2–3 s) above the female, the male began to fly down towards the female. When descending, he stretched the hindlegs forcibly far below (Fig. 2a). The female landed on the trunk close to the display site. The male landed on her back and either copulated ($n = 2$ copulations) or tried to copulate but was thrown off her back by the female ($n = 2$ copulatory attempts).

The copulations were short (4–10 s) but, in all cases, the male had clearly inserted its sexual organs and carried out thrusting and pumping movements. Sperm transfer remains hypothetical. Two of the videotaped copulations allowed a more detailed description on the relative position of body parts. In both cases, the male clung with its forelegs to the thorax and with its midlegs to the female's hindlegs which were lifted off the perch. The hindlegs of the male were stretched widely and pushed against the perch (Fig. 2b).

**Table 1.** Summary of territorial behaviour, fragrance contents, and mating frequency of eight caged males of *Euglossa hemichlora*.

| Male | Display rate (44 screens) | No. of perches used | Preferred perch (% observations here) | Amount of volatiles stored[a] | No. of different compounds | No. of copulations | No. of copulatory attempts |
|---|---|---|---|---|---|---|---|
| 1 | 0.41 | 7 | 12 (42) | 113.1 | 37 | – | – |
| 2 | 0.11 | 2 | 3 (–) | 87.6 | 30 | – | – |
| 3 | 0.25 | 6 | 5 (25) | 125.2 | 44 | – | 1 |
| 4 | 0.43 | 5 | 5 (76) | 127.2 | 34 | – | – |
| 5 | 0.64 | 3 | 12 (95) | 86.6 | 28 | 4 | 2 |
| 6 | 0.57 | 6 | 2 (60) | 158.7 | 50 | 2 | – |
| 7 | 0.43 | 5 | 2, 5 (37) | 125.8 | 37 | – | – |
| 8 | 0.36 | 5 | 13 (44) | 72.0 | 21 | – | – |

[a] Units are sums of integrated ion currents × $10^6$.

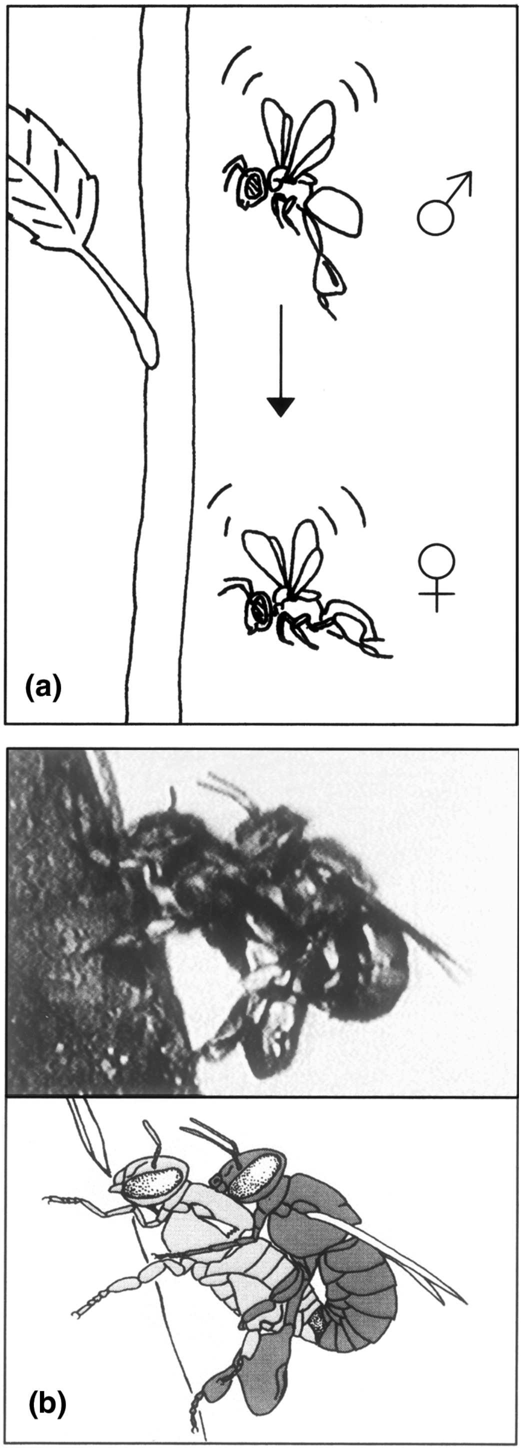

**Fig. 2.** Mating behaviour of *Euglossa hemichlora*. (a) Schematic drawing of precopulatory behaviour observed before copulations or copulatory attempts took place. Male and female are in hovering flight facing the perch. The male stretches his hind legs downwards when descending to the female. (b) Single frame of video sequence of a copulation (top) and a schematic drawing based on the same sequence (bottom). Note the position of the male hind tibia and associated groove. Left wings of female are omitted in the schematic drawing.

At no time did the hair-covered groove on the male hind tibia, which communicates with the fragrance container (Vogel, 1966), come into physical contact with any part of the female. Furthermore, the 'velvet area' and associated tufts on the male mid tibia did not touch the female. In general, there was no sign of direct transfer of substances during copulation. Copulations were terminated unambiguously by the female, when she slid backwards or to the side and eventually freed herself of the male.

### *Stored fragrances, display activity and mating success*

GC-MS analysis of tibial contents of 12 individual extracts (eight from the experimental males, four from males extracted directly after capture) revealed two sets of compounds. First, the analysis detected a series of high molecular weight alkanes, alkenes, esters, acetates and diacetates found in the labial glands of male *Euglossa* (Whitten *et al*., 1993; Eltz, 1997), which were hypothesized to function as a nonpolar carrier during fragrance collection (Whitten *et al*., 1989). All had retention times above 60 min, and no further details are given here. Second, extracts contained complex mixtures of terpenoids, mostly mono- and sesquiterpenes and aromatic compounds (Table 2), with retention times between 9 and 63 min. A total of 70 different substances were found, including some that are known to be collected by male *E. hemichlora* in pure form (e.g. *p*-dimethoxy benzene, methyl salicylate, benzyl benzoate, and eugenol; T. Eltz, unpublished observations). Cineole, which was present in small quantities in almost all individuals, is the most potent and universal attractant for male euglossines (Ackerman, 1989). Other identified compounds, including ocimene, limonene, α-pinene, β-pinene, and α-bergamotene, are well known from neotropical orchid fragrances (Williams & Whitten, 1983; Gerlach & Schill, 1991). Approximately two-thirds of the compounds, most of them small peaks with predominantly longer retention times (40–60 min) could not be identified. However, they were of clear terpenoid or aromatic character (based on mass spectra), and it is therefore likely that they were also exogenous. All 12 males had substantial stored fragrances, similar in amount among experimental males and males that had been extracted directly upon capturing in the field (Table 2). Also, no consistent differences in composition were observed between the two subsets, with the possible exception that many experimental males were missing some highly volatile, early eluting compounds such as α- and β-pinene, and limonene.

The individual total amounts of fragrances stored at the end of the experiment were not correlated with display rate (Pearson's $R = 0.32$; $P > 0.4$; $n = 8$) or the number of perches used for display ($R = 0.43$; $P > 0.3$; $n = 8$). The small number of observed copulations clearly limits powerful statistical tests on factors influencing male mating success. However, no association with the total amount of fragrances was found ($R = 0.03$; $P > 0.9$; $n = 8$). The sexual interactions were distributed unequally among males, with two

**Table 2.** Relative quantities of terpenoid and aromatic compounds found in *n*-hexane extracts of hind legs of 12 individual male *Euglossa hemichlora*. Eight of the males (1–8) were held captive in a flight cage and subject to behavioural observations. Four (9–12) were directly extracted upon capture.

| | | Cage experiment | | | | | | | | Directly extracted | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RT (min) | Compound | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Mean |
| 9.28 | α-Pinene | – | – | 0.1 | – | – | 0.1 | – | – | 0.1 | 0.1 | 0.1 | 0.1 | 0.05 |
| 10.72 | β-Pinene | – | – | – | – | – | 0.1 | – | – | 0.1 | 0.1 | 0.1 | 0.1 | 0.04 |
| 11.15 | ? | – | – | – | – | 0.1 | 0.1 | 0.1 | – | 0.1 | 0.1 | 0.1 | 0.1 | 0.07 |
| 12.62 | Limonene | – | – | – | – | – | 0.1 | – | – | 0.1 | 0.1 | 0.1 | 0.1 | 0.04 |
| 12.72 | Cineole | 0.2 | – | 0.4 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0.23 |
| 12.93 | Ocimene, Z | 0.5 | 0.1 | 0.5 | 0.7 | 0.6 | 0.6 | 0.7 | – | 1.0 | 0.9 | 0.5 | 1.0 | 0.64 |
| 12.97 | ? | – | – | – | – | – | – | – | – | – | – | – | 0.1 | 0.01 |
| 13.5 | Ocimene, E | 6.8 | 3.8 | 6.7 | 9.3 | 8.4 | 6.8 | 8.7 | 3.7 | 11.3 | 10.6 | 6.4 | 12.4 | 8.19 |
| 16.82 | 2,4,6-Octatriene, 2,6-dimethyl, isomer I | 0.3 | 0.3 | 0.3 | 0.4 | 0.2 | 0.2 | 0.3 | – | 0.4 | 0.3 | 0.2 | 0.6 | 0.31 |
| 17.35 | 2,4,6-Octatriene, 2,6-dimethyl, isomer II | 0.2 | – | 0.2 | 0.2 | – | 0.2 | 0.2 | – | 0.4 | 0.2 | 0.2 | 0.4 | 0.20 |
| 18.38 | *p*-Dimethoxy benzene | 4.7 | 5.4 | 4.6 | 6.7 | 5.3 | 5.1 | 5.8 | 3.1 | 7.3 | 6.3 | 6.1 | 7.0 | 5.76 |
| 19.72 | Methyl salicylate | – | 0.7 | – | – | – | – | 0.6 | – | – | – | – | 0.7 | 0.17 |
| 27.17 | Eugenole | 0.2 | 1.0 | 0.3 | 0.2 | – | – | 1.0 | – | 0.1 | – | – | 0.9 | 0.31 |
| 27.73 | 1,2,4-Trimethoxy benzene | – | – | – | – | – | – | – | – | 0.2 | – | 0.1 | 0.1 | 0.04 |
| 28.92 | Germancrene A (?) | 0.4 | – | – | – | – | – | – | – | – | – | – | – | 0.03 |
| 30.78 | α-Bergamotene, Z trans | – | – | – | – | – | – | – | – | – | – | – | 0.1 | 0.01 |
| 32.88 | ? | 0.2 | – | 0.2 | – | – | – | 0.2 | – | – | – | – | 0.1 | 0.05 |
| 33.2 | Z, E α-Farnesene | 0.1 | 0.2 | 0.2 | – | – | 0.2 | 0.1 | – | 0.1 | 0.1 | 0.2 | – | 012 |
| 33.82 | E, E α-Farnesene | 3.0 | 3.6 | 2.0 | 2.2 | 2.4 | 3.9 | 2.5 | 3.7 | 3.3 | 3.0 | 4.8 | 2.7 | 3.14 |
| 34.78 | ? | 1.0 | 0.3 | – | 0.4 | 0.7 | 0.6 | 0.8 | 0.3 | 0.4 | 1.0 | 0.4 | 0.5 | 0.53 |
| 35.07 | ? | 9.3 | 3.3 | 0.6 | 4.6 | 7.1 | 5.6 | 7.9 | 3.3 | 3.7 | 9.0 | 3.8 | 5.0 | 5.23 |
| 35.55 | ? | 1.1 | 0.4 | – | 0.7 | 1.1 | 1.0 | 1.2 | 0.3 | 1.2 | 1.3 | 0.3 | 0.6 | 0.76 |
| 35.68 | ? | – | 0.2 | – | – | – | – | – | – | – | – | 0.1 | 0.2 | 0.05 |
| 36.05 | ? | 1.4 | – | 0.3 | 1.4 | 1.4 | – | 0.9 | 0.5 | 0.8 | 0.3 | – | – | 0.51 |
| 36.13 | 3-Methoxycinnamaldehyde | – | 0.7 | 0.9 | – | – | 1.0 | – | – | – | – | 0.6 | – | 0.30 |
| 36.18 | ? | – | – | – | – | – | – | – | – | 0.4 | 0.2 | – | – | 0.05 |
| 36.25 | ? | – | – | – | – | – | 0.8 | – | – | – | – | 0.7 | – | 0.17 |
| 36.63 | ? | – | – | – | – | – | 0.1 | – | – | 0.2 | – | 0.1 | – | 0.04 |
| 37.02 | Methyl-*p*-methoxy cimmamate, cis + vanillin | 1.1 | 0.8 | 1.1 | 1.1 | 1.0 | 1.0 | 1.1 | 0.5 | 1.1 | 0.7 | 1.1 | 0.6 | 0.96 |
| 37.65 | Farnesen epoxide isomer | 0.5 | 0.5 | 0.5 | 0.5 | 0.4 | 0.6 | 0.4 | 0.2 | 0.6 | 0.6 | 0.6 | 0.5 | 0.52 |
| 38.4 | Farnesen epoxide, trans | 10.7 | 12.9 | 8.8 | 10.8 | 9.4 | 11.0 | 9.5 | 7.8 | 11.7 | 9.1 | 11.8 | 11.4 | 10.54 |
| 38.9 | ? | – | – | – | – | – | – | – | – | – | – | 0.2 | 0.5 | 0.08 |
| 39.58 | ? | – | – | – | – | – | – | 0.3 | – | – | 0.3 | 0.2 | – | 0.07 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 39.78 | ? | – | – | 0.1 | – | – | 0.2 | – | – | – | – | 0.1 | – | 0.05 |
| 39.95 | ? | 0.9 | – | 0.2 | 0.5 | 1.2 | 0.7 | 0.6 | – | 0.5 | 2.5 | 0.5 | – | 0.63 |
| 40.18 | ? | – | – | – | – | – | 0.3 | – | – | – | – | – | – | 0.03 |
| 40.35 | Methyl-*p*-meocinnamate, trans | 0.8 | 1.6 | 0.6 | 3.1 | 3.0 | 4.1 | 2.2 | 2.3 | 3.3 | 1.1 | 3.7 | 2.5 | 2.47 |
| 40.62 | ? | 6.1 | 2.2 | 0.4 | 3.2 | 6.5 | 5.5 | 6.7 | 1.9 | 2.8 | 9.0 | 2.7 | 2.9 | 4.15 |
| 40.78 | ? | – | – | – | 0.3 | – | 0.1 | – | – | 0.4 | 0.2 | 0.2 | 0.1 | 0.13 |
| 41.27 | ? | – | – | 0.2 | – | – | – | – | – | – | – | – | – | 0.01 |
| 41.58 | ? | – | – | – | – | – | – | – | – | – | – | 0.1 | – | 0.01 |
| 41.75 | ? | – | – | – | – | – | – | – | – | – | – | 0.1 | – | 0.01 |
| 42.45 | ? | 0.2 | – | 0.2 | 0.3 | – | 0.2 | 0.2 | – | 0.3 | 0.1 | 0.3 | 0.3 | 0.20 |
| 42.8 | ? | 0.3 | 0.3 | 0.5 | 0.2 | 0.2 | 0.1 | 0.2 | – | 0.1 | – | 0.2 | 0.2 | 0.20 |
| 43.67 | Benzyl benzoate | 2.4 | 2.3 | 1.5 | 0.4 | 8.3 | 5.3 | 4.2 | 0.5 | 0.3 | – | 6.5 | 1.5 | 2.87 |
| 45.13 | ? | 0.2 | – | 0.3 | – | – | 0.1 | 0.1 | – | – | – | 0.1 | – | 0.07 |
| 46.85 | Hexahydrofarnesyl acetone | 34.1 | 45.2 | 41.1 | 36.5 | 27.6 | 28.7 | 33.5 | 37.6 | 36.6 | 37.1 | 30.9 | 33.1 | 34.67 |
| 47.72 | ? | 0.3 | 0.3 | 0.4 | 0.2 | 0.2 | 0.2 | – | 0.3 | 0.4 | 0.3 | 0.3 | 0.4 | 0.28 |
| 48.5 | ? | – | 0.3 | 0.3 | 0.2 | – | 0.1 | 0.2 | – | 0.3 | 0.2 | 0.2 | 0.5 | 0.21 |
| 48.73 | ? | 0.4 | 0.3 | 0.7 | 0.2 | 0.2 | 0.2 | 0.3 | – | 0.1 | 0.2 | 0.2 | 0.2 | 0.25 |
| 49.15 | ? | 2.0 | 1.8 | 3.7 | 1.4 | 1.6 | 1.4 | 1.6 | 0.9 | 0.8 | 0.8 | 1.1 | 1.0 | 1.50 |
| 49.53 | ? | – | – | 0.5 | – | – | 0.2 | – | – | – | – | – | – | 0.06 |
| 49.68 | ? | – | – | 0.3 | – | – | 0.1 | – | – | – | – | – | – | 0.04 |
| 49.88 | ? | – | – | 0.6 | – | – | 0.3 | – | – | – | – | – | – | 0.08 |
| 50.43 | ? | 5.7 | 4.8 | 9.8 | 3.9 | 4.0 | 3.8 | 4.2 | 2.4 | 2.6 | 2.4 | 3.3 | 3.1 | 4.17 |
| 50.73 | ? | 0.4 | – | 0.8 | 0.3 | – | 0.4 | 0.3 | – | 0.2 | – | 0.3 | – | 0.23 |
| 51.13 | ? | – | – | 0.9 | – | – | – | – | – | – | – | – | – | 0.08 |
| 51.35 | ? | – | – | 0.2 | – | – | 0.3 | – | – | – | – | – | – | 0.05 |
| 51.38 | ? | – | – | 0.2 | – | – | – | – | – | – | – | – | – | 0.01 |
| 51.55 | ? | 0.5 | – | 1.0 | 0.3 | – | 0.3 | 0.3 | – | – | – | 0.3 | – | 0.24 |
| 51.88 | ? | 0.6 | 0.5 | 1.3 | 0.6 | 0.4 | 0.9 | 0.5 | – | 0.5 | 0.3 | 0.4 | 0.3 | 0.55 |
| 52.08 | ? | – | – | – | 0.3 | – | 0.5 | – | – | – | – | – | – | 0.08 |
| 52.32 | ? | 3.0 | 2.4 | 5.3 | 1.7 | 2.1 | 1.6 | 2.3 | 1.0 | 1.4 | 1.0 | 1.8 | 1.5 | 2.09 |
| 53.08 | ? | – | – | – | – | – | 0.2 | – | – | 0.2 | 0.2 | 0.2 | 0.2 | 0.10 |
| 53.52 | ? | 0.2 | – | 0.4 | – | – | – | – | – | – | – | 0.2 | – | 0.06 |
| 53.85 | ? | – | – | – | – | 0.2 | 0.2 | – | – | – | – | 0.1 | – | 0.04 |
| 55.97 | ? | 0.2 | 0.3 | 0.6 | – | – | 0.3 | 0.2 | 0.5 | 0.4 | 0.3 | 0.3 | 0.6 | 0.32 |
| 56.68 | ? | – | – | – | – | – | – | – | – | – | – | 0.1 | – | 0.01 |
| 57.57 | ? | – | 3.5 | – | 6.9 | 6.2 | 4.6 | – | 28.9 | 3.9 | – | 6.6 | 5.3 | 4.77 |
| 62.62 | ? | 0.4 | – | – | – | – | – | – | – | – | – | – | – | 0.03 |
| | Total amount of volatiles stored | 113.1 | 87.6 | 125.2 | 127.7 | 86.6 | 158.7 | 125.8 | 72.0 | 122.0 | 115.8 | 168.7 | 161.6 | |

Units are sums of integrated ion currents × $10^6$. Percentages were calculated from relative peak areas (integrated ion current/peak).

individuals being responsible for all six copulations and two of the three copulatory attempts (Table 1). The only factor positively associated with the number of copulations was display rate ($R = 0.77$; $P < 0.05$; $n = 8$). The two copulating males were also characterized by their strong and lasting attachment to their preferred perches, where all their respective encounters with females took place (Table 1).

## Discussion

It was not demonstrated unequivocally that mating success of male *E. hemichlora* is related to the quantity or complexity of their stored fragrances. The attempt to do so was hampered by two problems. First, the number of observed copulations was too low for thorough statistical testing, but perhaps, more importantly, the amount of natural variation in fragrance quantity among experimental males was unexpectedly low. Although the two *E. hemichlora* males that copulated successfully were at opposite ends of the observed range, they both had outstandingly rich contents (in total amount and complexity) when compared with previous findings in other species. When investigating tibial extracts of a total of 140 male *E. cognata*, *E. imperialis* and *Euglossa tridentata* from Barro Colorado Island, Panama, Eltz *et al.* (1999) found noteworthy variation in the total amounts of fragrances stored by individuals. They varied by over two orders of magnitude, with a strong skew in favour of males with only tiny amounts (Eltz *et al.*, 1999). Although even smaller in body size, and in size of their hind tibiae, all 12 *E. hemichlora* were among the top 20 males in a cross-species comparison. The reasons for such differences are likely to include regional and seasonal differences in fragrance supply and male age.

The present study more than doubles the number of carefully observed euglossine matings (see below) and, based on close-up video documentation, provides more details on euglossine copulatory behaviour. Although any conclusions must be drawn cautiously because of the artificial setting in a large flight cage, the observations should apply in general to wild bees. First, the present observations confirm the role of male perch sites as non-resource-based mating territories. In all observed cases, the female approached a displaying male actively and finally signalled her willingness to mate by landing on the perch. This is consistent with observations of copulations of wild *Euglossa ignita* ($n = 1$) (Dodson, 1966), *E. imperialis* ($n = 1$) and *E. meriana* ($n = 2$) (Kimsey, 1980). In the only reported exception, a female of *Eulaema cingulata* clung with her mandibles to a leaf neighbouring the perch site and was mounted there (Dodson, 1966). The fact that male display is a likely prerequesite for mating is underlined by the evident association between copulation frequency and display rate in our cage experiment. Earlier research using a flight cage demonstrated a positive correlation between fragrance collection and display intensity of *E. imperialis* (Schemske & Lande, 1984). Second, the sequence of precopulatory behaviour suggests females evaluate the males' fragrance bouquets (female choice). Half of the matings encompassed a phase of mutual taxation during which the male hovered right above the female and his hindlegs were stretched down towards her. The relative positions, as well as the air movement created by the hovering male's wing stroke, seem perfectly suited for the postulated fragrance communication. This view does not preclude chemical communication before this or continued communication during the copulation itself. Third, during the copulations, there was no evidence of a transfer of substances from the male hind tibiae to any part of the female. Thus, the use of the fragrances as a nuptial gift seems unlikely.

Speculations about fragrance-based communication are hampered by a lack of knowledge of the mechanisms that could lead to an exposure of the collected fragrances, whenever it might happen. The tibial fragrance container of *Euglossa* is a cuticular invagination that is populated densely by branched and interwoven setae (Vogel, 1966; Eltz, 1997). This sponge-like cavity is connected to the outside by a short canal leading to a hair-filled groove on the surface of the tibia. Non-polar liquids can be applied to the posterior end of the groove and are drawn inside by what appear to be capillary forces (Vogel, 1966). Vogel (1966) speculated that the fragrances are also released passively from the anterior end of the tibial groove, which is covered by broad and scale-like hairs. However, in contradiction to this idea, caged males of *E. imperialis* did not detectably diminish their fragrance stores over more than 2 weeks of captivity, making continuous passive exposure very unlikely (Eltz *et al.*, 1999). Instead, it seems that an active mechanism is required that allows the male to trigger fragrance release voluntarily at certain times. The 'leg crossing' movement observed in displaying males of *E. hemichlora* could be important in this regard. To interpret the movement, it is first necessary to consider a morphological oddity of the male midtibia: a large part of the outer surface of the male (but not the female) midtibia of all euglossine species is covered in a thick carpet of short, spiralled, hook-shaped hairs (Figs 3a.b). In the genus Euglossa, at the basal end of this 'velvet area', there are an additional one to three tufts of longer hairs, whose arrangement, shape and size are important characters in species identification (Dressler, 1978; Kimsey, 1987). The function of these surface structures is unknown. They play no active part in the grooming behaviour of *E. hemichlora* (T. Eltz, unpublished observations) or during the copulations described above. Instead, it is likely that their function is in connection with the described leg-crossing, during which the midtibia appears to move against the inner side of the hindtibia. The 'leg crossing' gives the impression of a brushing movement which takes place in the direction shown in Fig. 3(c). The inner side of the hind tibia of male *E. hemichlora* is covered in short hairs of two different types that are all directed more or less against the pushing direction. The first type is spatulate, 80–100 µm long, covers almost the entire surface, and is known to be used for grooming the wing surfaces (Kimsey, 1984). The second type is somewhat longer, pointed like a thorn, and forms a thick seam along

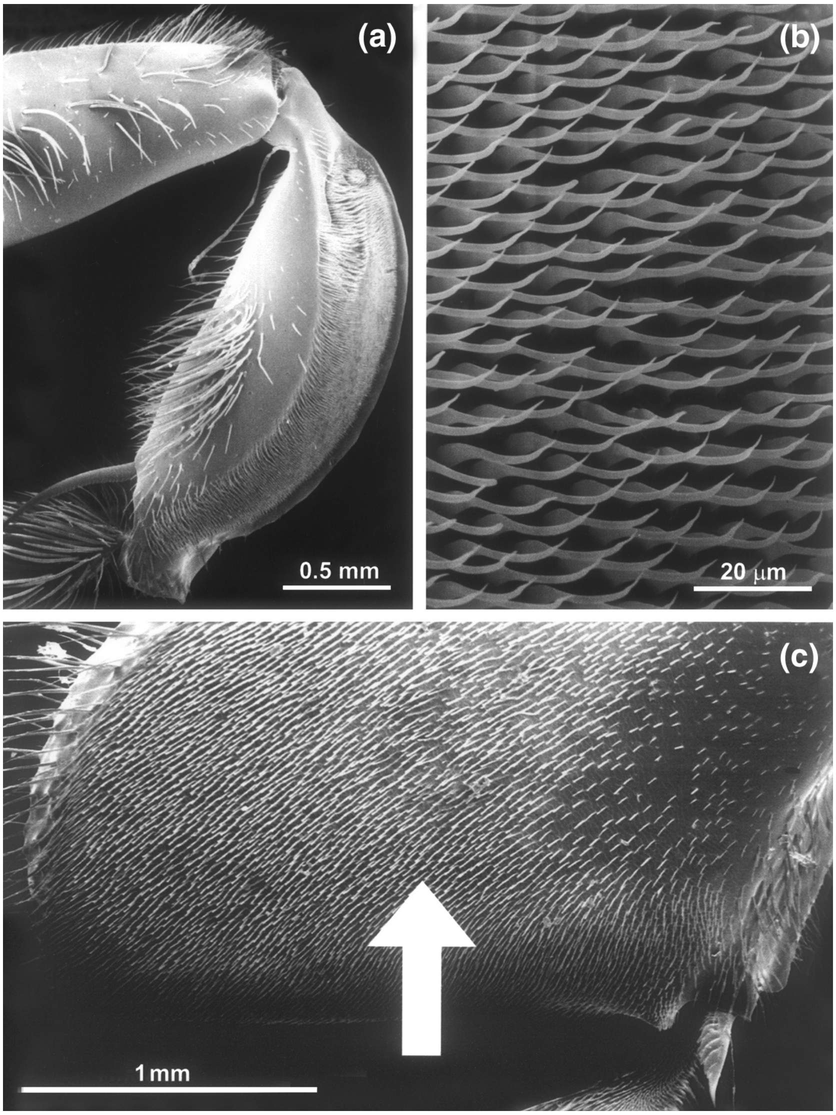

**Fig. 3.** (a) Mid tibia of *Euglossa tridentata* with 'velvet area', covering much of the dorsal surface of the tibia. (b) The smooth and 'velvety' appearance arises from dense fields of short and twisted setae. (c) During the 'leg crossing', the 'velvet area' appears to push against the inner surface of the hind tibia (in the direction indicated by the arrow), which is also covered by setae. Further details are provided in the text.

the distal end of the inner side of the tibia (Fig. 3c). The encounter of the described surfaces of mid- and hind leg might lead to deformations of the leg exoskeleton that could be transferred to the hindtibial container and result in a mobilization of its contents. Vibrations of high frequency (including resonance effects) may lead to fragrance release in a yet unspecified way.

If the 'leg-crossing' is truly responsible for fragrance release, the quantities released must be small. Experimental males of *E. hemichlora* still had considerable stores of fragrances after up to 10 days of frequent display (certainly involving hundreds of individual 'leg-crossings'), and without access to fragrances. Perhaps the leg-crossing does not so much affect fragrance release itself but merely 'charges' the hind tibia for later release (e.g. by relocating the drops of fragrant liquid within the container and moving them close to the point of exit). In this context, it is interesting to note that 'leg-crossings' took place in rapid succession briefly before males stretched their hind legs and descended downwards to the females before copulating (videotaped sequences). The activity of tarsal musculature during this movement may be responsible for fragrance exposure. The tarsal muscles are in intimate contact with the fragrance container almost over its entire length (T. Eltz, unpublished observations) and are therefore well-placed for for squeezing or vibrating the container and effecting fragrance release.

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