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ORIGINAL ARTICLE

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Experience-dependent choices ensure species-specific fragrance accumulation in male orchid bees

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Abstract Male neotropical orchid bees (Euglossini) collect volatile chemicals from orchid flowers and other sources and store them in hind leg cavities. The accumulated fragrance bouquets are later emitted at mating sites. Although most other insects synthesize pheromone blends de novo, specific euglossine perfumes are derived from active choices in a changing fragrance market. Male bees of three species of *Euglossa* possessed distinctive fragrance phenotypes showing little variation by locality and habitat in mainland Central America. In cage experiments, fragrance choice by male *Euglossa imperialis* was influenced markedly by a bee's collection history. Collection of a given chemical strongly reduced its attractiveness on subsequent occasions, an effect that was retained over days. Experimentally adding the chemicals directly to bee hind legs produced no effect. We conclude that bees learn and remember chemicals they collect. Innate odor preferences, memory and the avoidance of overcollecting by negative feedback may be the primary mechanisms that ensure unique blends of pheromone analogs in these tropical forest bees.

Introduction

Species-specificity is generally regarded as an important aspect in the evolution of insect semiochemicals, especially in the context of courtship (Linn and Roelofs 1995; Roelofs 1995; White et al. 1995). Species-specificity permits long-range attraction of mates in a noisy chemical background or helps members of the signalling sex to congregate at mating sites (Cardé and Baker 1984; Minckley et al. 1991; Bergman and Bergstrom 1997; Ayasse et al. 2001). Specificity in the signal may be conveyed by a single type of chemical that is so complex in structure that it is not duplicated in nature. In many cases, however, the involved chemical substances are blends of relatively simple and common components (Bjostad et al. 1987). Here, specificity is frequently communicated by the relative quantities of components in the mix, which are controlled by highly conserved and precisely tuned biochemical machinery present in exocrine glands. Among the best studied examples are the fatty acid derived long-range sex pheromones of female lepidopterans (Bjostad et al. 1987; Morse and Meighen 1987; Roelofs et al. 2002; Roelofs and Rooney 2003). In contrast to exocrine pheromones, the attainment of specificity may be a formidable task if the blends must accumulate from varied exogenous sources in a heterogeneous environment, as in the case of euglossine orchid bees (Dressler 1982; Roubik and Hanson 2004). Males of these neotropical bees are famous for collecting volatile chemicals (fragrances) from flowers and other sources (Vogel 1966; Dodson et al. 1969; Williams and Whitten 1983; Whitten et al. 1993). The behavior evolved more than 20 million years ago (Engel 1999; Cameron 2004) and has given rise to an entire pollination syndrome, including more than 700 species of orchids and many other plants (Dressler 1968; Janzen 1971; Williams and Dodson 1972; Dodson 1975; Williams 1982; Ackerman 1983). The bees collect the terpenoid and aromatic volatiles by a process known as fatty extraction, analogous to "enfleurage" used in the perfume industry. Small quantities of lipoid cephalic gland secretions are placed on the fragrant surface and used to transfer the essential oils to hind leg pouches (Whitten et al. 1989), where diverse blends accumulate over the male's life (Eltz et al. 1999; up to several months (Ackerman and Montalvo 1985)). Recent evidence demonstrates that the fragrances are

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actively exposed by displaying males at perch trees (Eltz et al. 2005), where conspecific males interact and matings take place (Kimsey 1980; Stern 1991; Eltz et al. 2003).

Male bee collection of fragrances has been studied by baiting assays, where synthetic chemicals are presented on filter paper. To date, approximately 50 different compounds are known to attract male euglossines in pure form (Williams and Whitten 1983; Ackerman 1989; Roubik and Hanson 2004). Species-specific preferences for certain chemicals have been found, but at the same time there is an astonishingly broad overlap in the range of compounds sought by different species (Janzen et al. 1982; Pearson and Dressler 1985; Ackerman 1989), as well as pronounced geographical and seasonal shifts in preferences (Ackerman 1989). Although the spectrum of chemicals exploited by a given species is likely to have a strong genetic basis, these shifts raise the question of context-dependent refinements of fragrance choice.

The present study had two parts. First, we investigated fragrance phenotypes of selected species and asked whether individual bouquets are species-specific among the males from distant and ecologically divergent localities. Second, we explored the mechanisms that might lead to species-specificity given a variable fragrance market in natural habitats. Specifically, we asked how overcollecting of abundant components/blends is avoided. Two alternative hypotheses were tested experimentally:

1. Odor compensation: Individuals smell the odor of their own hind-tibial pouches, leading to a reduction in responsiveness toward dominant components, e.g. by sensory adaptation. This would reduce further collection of abundant fragrances.
2. Collection experience: Individuals learn odors during discrete bouts of collection, memorize their collection history, and avoid collecting abundant fragrances on subsequent occasions.

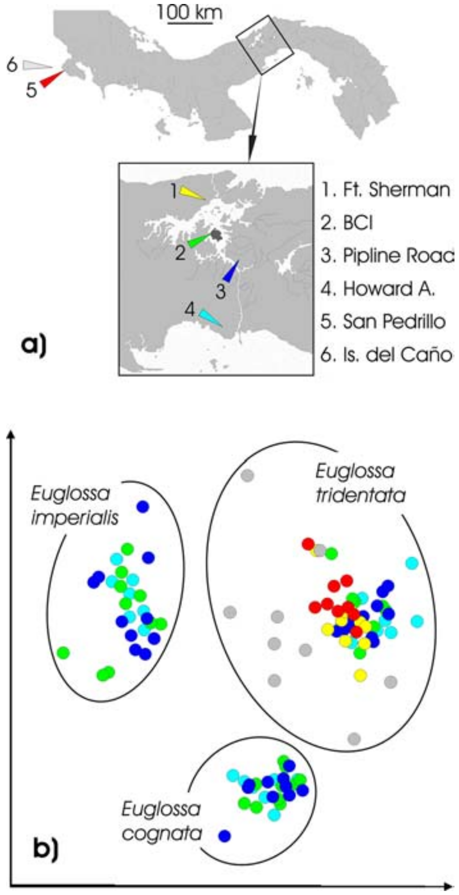

Fig. 1 Similarity of fragrance phenotypes of three *Euglossa* species from six localities in Central America. **a** Map of Panama and eastern Costa Rica showing the sampling localities, with magnified insert of the Panama Canal Zone. **b** Two-dimensional MDS plot based on Bray–Curtis similarities (stress = 0.14). Colors of circles correspond to the six localities in a); species affiliation is indicated by the black outlines

Materials and methods

Geographic versus species effects on fragrance composition

Localities

From March 2 to 27, 2003, fragrance loads of three *Euglossa* species were sampled at Atlantic, central, and Pacific localities within the Panama Canal Zone, as well as at two localities in Costa Rica (Fig. 1). Localities included varied forest area, seasonal rain fall, flowering phenology, and local flora. These variables influence type and abundance of chemicals available for bees to collect. The localities Fort Sherman (1, STRI canopy crane site), Barro Colorado Island (2, radio tower clearing), Pipeline Road (3, next to Rio Limbo), and San Pedrillo (5, P.N. Corcovado, Osa Peninsula) are situated within large tracts of old growth lowland forest. Howard Air Force Base (4, a forested ridge near the Pacific ocean) is characterised by secondary forest surrounded by settlements. Isla del Caño (6, island midpoint) is a 320 ha island 17 km from Osa peninsula, of pristine forest with very low plant species richness. It has only two resident euglossine bee species, versus >30 on mainland sites (Janzen 1981; Krehbiel and Nagel 1998).

Bee species and sampling

Euglossa tridentata (11–12 mm body length; subgenus *Euglossa*) was sampled at all six localities. Two additional species, *Euglossa cognata* (13 mm; *Euglossa*) and *E. imperialis* (15 mm; *Glossura*), were sampled at three localities in Panama (localities 2, 3, and 4; Fig. 1a). All three species are common throughout Central America (Roubik and Hanson 2004). The bees were collected during standardised baiting assays using 10 different chemicals in mesh-covered baits that allow only minimal collection of substances. For each species and locality, the first 10 males at baits were collected. *E. tridentata* was taken on 1,8-cineole, eugenol, 1,4-dimethoxy benzene, *t*-methyl cinnamate, vanillin, and benzyl benzoate, *E. cognata* on *p*-dimethoxy benzene, methyl salicylate, and 1,8-cineole, and *E. imperialis* on 1,8-cineole and methyl salicylate. Fragrance loads were sampled by extracting individual pairs of hindlegs in 0.5 ml of hexane containing 1 mg/ml 2-undecanone as an internal standard.

Chemical analysis

Gas Chromatography/Mass Spectrometry (GC/MS) was done at the Department of Neurobiology, Düsseldorf, using a HP 5890 II GC fitted with a 30 m non-polar DB-5 column and a HP 5972 mass selective detector. Injection was splitless, the oven programmed from 60 to 300°C at 3°C/min with automatic pressure programming. Retention times were 15–25 s shorter than those given by Adams (2001) for essential oil components.

Mass spectra and associated retention times of integrated peaks were compared and cross-referenced with entries in the local user library (T. Eltz, unpublished). New components were added over the course of the study. Chemical characterisation of components was done by comparison with authentic standards or by matching spectra *and* retention times with those in Adams (2001). Mass spectral characteristics alone were not considered sufficient for compound identifications, but were used for an assignment to broader substance classes. Additional identifications were made by R. Kaiser (Givaudan, Switzerland), who analyzed representative samples of all three species. Excluded from the analysis of fragrance composition were all straight chain lipids (alkanes, alkenes, alcohols, acetates, diacetates, and esters), products of the bees' labial glands (Whitten et al. 1989; Whitten et al. 1993) and prominent in head extracts of the study species (T. Eltz, unpublished).

Similarity analysis

Fragrance composition was analyzed with non-metric Multidimensional Scaling (MDS) and associated techniques (Clarke and Green 1988; Clarke 1993; Legendre and Legendre 1998; Clarke 1999). MDS is conceptually simpler than other ordination techniques, makes almost no assumptions about the form of the data, and is flexible concerning the similarity measure employed (Clarke and Warwick 2001). Absolute peak areas (integrated ion currents) were square-root transformed and standardized in order to represent relative peak contributions to individual samples (in percentage). From the data matrix we derived a triangular similarity matrix based on the Bray–Curtis index (Legendre and Legendre 1998). Similarities were ordinated in two dimensions using the non-metric MDS algorithm in PRIMER v5 (Clarke and Gorley 2001). Ideally, MDS plots have inter-point distances that exactly match the rank order of dissimilarities between samples in the underlying similarity matrix. Deviations from this match are expressed in terms of "stress," with stress values <0.15 indicating a good fit concerning the overall structure of the plot (Clarke and Warwick 2001).

We tested the null hypothesis that the factors *species* and *locality* had no effects on the rank order of between-individual similarities using ANOSIM permutation tests with one-way or two-way crossed design (Clarke and Green 1988). Finally, we identified the fragrance compounds that were primarily responsible for creating the observed similarity patterns using the PRIMER SIMPER algorithm. This routine calculates percent contributions of each compound to the overall *similarity within species*. The index weighs both the extent and consistency (in inter-comparisons of sample pairs) by which each compounds contributes to overall similarity (Clarke and Warwick 2001).

Mechanisms of fragrance shopping

In December 2003 two experiments were conducted simultaneously at the Smithsonian Tropical Research Institute outdoor facilities in Gamboa, Panama. For both, males of *Euglossa imperialis* were captured at 1,8-cineole baits in nearby forest, marked individually with numbered plastic tags, and introduced into 2.20 m × 2.00 m × 2.00 m flight cages, where they learned to forage on honey–water feeders.

Odor compensation experiment

A total of 41 males were kept jointly in one flight cage. To test whether the amount of a fragrance component present in an individuals' pouch affects the likelihood of collecting this compound, we artificially supplemented individual loads with 1,8-cineole (N=19) or methyl salicylate-d_3 (N=22). Individuals were cooled on blue ice, and 0.5 µl droplets of pure compound were pipetted onto hind-tibial slits, allowed to be passively drawn into the pouches for 2 min, then swabbed with paper tissue. Using a deuterium-labeled methyl salicylate-d_3 allowed an assessment of the supplementation effect after the experiment by GC/MS: extracts of males supplemented with methyl salicylate-d_3 contained on average 154.52 ± 51.35 µg of this compound, 350 times more than they contained of the natural, non-deuterated equivalent. In all such individuals, methyl salicylate (incl. -d_3) was the dominant fragrance compound, which is in sharp contrast to wild-caught *Euglossa imperialis* (Eltz et al. 1999). Three days after the treatment we identified individually marked bees collecting at bait dishes holding saturated filter pads with 1,8-cineole or methyl salicylate-d_3. The dishes were covered by fine nylon mesh and prevented bees from accessing the chemicals directly, but elicited normal collecting behavior on the screen surface. An individual was registered as collecting fragrances once it performed brushing movements on the screen. The two chemicals were offered for intervals of 30 min (interchangeably, twice per day) over four consecutive days, between 800 and 1400. For statistics and Fig. 3 we pooled records of all intervals.

Collection experience experiment

To test whether *E. imperialis* males adjust their fragrance choice in response to previous collecting experience, we conducted a cross-treatment experiment with two groups of bees in separate cages. Group A (N=20) was allowed to collect 1,8-cineole twice per day for 30 min from mesh-covered dishes, whereas group B (N=19) was offered methyl salicylate-d_3. This was continued over four consecutive days. On the fifth day both groups were offered both chemicals, each twice for 30 min. Individual arrival at the baits as well as the time each individual spent at the baits were recorded. To compare the effect of active collection vs. artificial supplementation (odor compensation experiment, s. a.) on hindleg contents we performed GC-MS after the experiment. Males accumulated 0.49 µg methyl salicylate-d_3 each minute spent at the bait (Linear Regression: R^2=0.16; $F_{1,37}$=9.81; p<0.01), amounting to 4.72±2.82 µg in group B individuals. This is far less than achieved by artificial supplementation (s. a.), providing sufficient contrast for assessing the effect of collection experience.

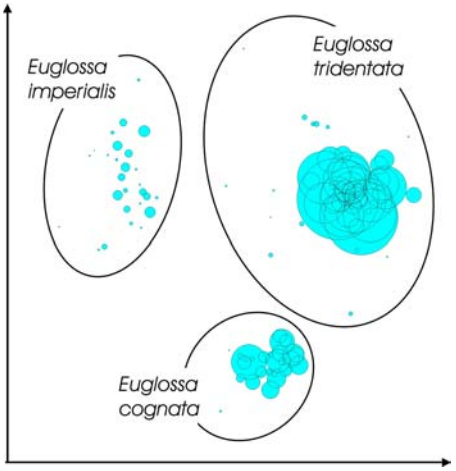

Fig. 2 Two-dimensional MDS bubble plot based on the same individuals and similarity matrix as in Fig. 1b. The circles represent individual male bees, with circle area corresponding linearly to the total amount of fragrances collected by individuals (sums of fragrance peak ion currents). Note minute amounts detected in peripheral individuals, especially those from the off-shore Isla del Caño

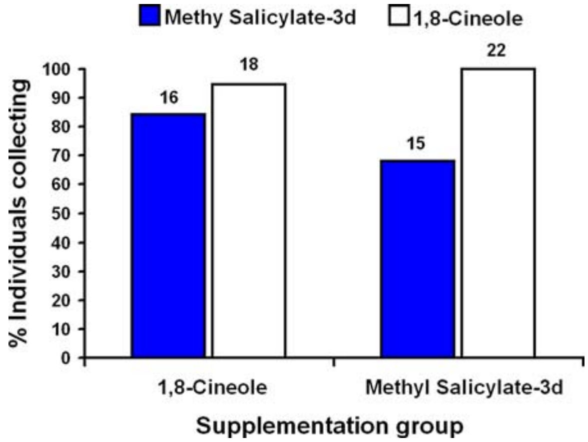

Fig. 3 Percentage of individuals collecting methyl salicylate-d_3 and 1,8-cineole depending on whether their pouches were artificially supplemented with one of the two compounds. See text for statistics

Results

Fragrance composition

Individual bouquets contained highly variable fragrance quantities, composed of 2–50 from a total of 220 different components. There were significant effects of species affiliation on the total amount of fragrances (sums of integrated ion currents; Kruskal–Wallis H=35.21, p<0.001, N=90) and the number of components (H=27.37. p<0.001, N=90). The total amount and the number of fragrance components per sample were highly correlated across species (Spearman R=0.92, p<0.0001, N=90). *E. tridentata* had both the largest amounts (see Fig. 2) as well as the most diverse bouquets (Median: 27 components), followed by *E. cognata* (14 components) and *E. imperialis* (10 components). These tests included only individuals from the mainland localities BCI, Pipeline Road, and Howard AFB, from which all three species had been sampled. Among those, locality had an effect on the number of compounds in extracts (H=8.97, p=0.02, N=90), but no effect on total amount of fragrance (H=5.94, p=0.06, N=90). For similarity analysis of fragrance composition we included only individuals with at least six different fragrance compounds above the detection threshold. These compounds were distributed non-randomly among individuals, both qualitatively and quantitatively. MDS produced distinct clusters for each of the three species, with little substructure related to locality (Fig. 1b). An ANOSIM two-way crossed permutation test including individuals from BCI, Pipeline Road, and Howard AFB showed significant effects of both factors, with an overwhelmingly larger magnitude for the species effect (Species: R=0.989, p<0.01; Locality: R=0.132, p<0.01; N=90). Table 1 lists components that contributed most strongly to the *similarity* within species (for a complete list including all compounds, compound incidences, and average relative amounts see S1). In each species 90% of within-species similarity was derived from a limited set of *typical* compounds (11, 12, and 17 compounds in *E. imperialis*, *E. cognata*, and *E. tridentata*). Most compounds were shared by two or even three species, but dominated in only one. Exceptions were 1,8-cineole, which was characteristic for both *E. imperialis* and *E. tridentata* (although in different relative quantities), an unsaturated alcohol (3,7,11,15-tetramethyl hexadeca-2,6,10,14-tetraen-1-ol ?), which was characteristic for both *E. tridentata* and *E. cognata*, and (E)-nerolidol, which was consistently abundant in all three (Table 1).

For *E. tridentata*, there was a significant effect of locality on fragrance composition across all six localities (one-way ANOSIM: R=0.379, p<0.01, N=60), almost entirely due to high variability and distinctness of individuals

Table 1 Percent contribution of major compounds to within-species similarity of the three *Euglossa* species calculated using the SIMPER algorithm in PRIMERv5 (Clarke and Warwick 2001). Calculations include only individuals from localitites 2, 3, and 4. Bold numerals add up to 90+% cumulative contribution to similarity within each of the species. A detailed table showing similarity contributions, average relative amounts, and incidences in individual bees for all detected compounds is provided as S1 in the electronic version

RT [min]	Name	Contribution to within-species similarity (%)		
		E. cognata	*E. imperialis*	*E. tridentata*
5.52	Pinene, alpha-	–	**10.28**	0.34
6.56	Sabinene	–	**2.83**	–
6.83	Pinene, beta-	–	**5.10**	0.03
8.58	Cineole, 1,8-	–	**21.74**	**2.59**
12.86	Ipsdienol	**3.37**	–	0.11
13.72	Dimethoxy benzene, 1,4-	**8.16**	–	0.98
14.95	Methyl salicylate	**2.73**	0.35	–
21.34	Epoxygeraniol, 2,3-	–	–	**3.62**
22.22	Eugenol	0.06	–	**2.01**
26.55	Farnesene, (E)-beta-	**2.47**	0.01	0.12
26.92	Geranyl acetate, 2,3-epoxy-	–	–	**7.36**
27.72	Germacrene, D	**2.97**	**2.50**	0.03
28.78	Farnesen, (E,E)-	**7.00**	–	0.03
28.92	Bisabolene, beta-	0.10	–	**4.92**
29.39	Acetoxynonanal, 9- ?	–	**2.87**	–
29.99	Sesquiterpene alcohol, similar elemol	–	–	**3.70**
31.10	Nerolidol, (E)-	**9.73**	**5.74**	**3.34**
31.30	*p*-Methoxy cinnamaldehyde	–	–	**10.08**
31.66	*p*-Methoxy cinnamic alcohol	–	–	**10.96**
33.25	Farnesene epoxide, (E)-alpha-	**4.52**	0.05	0.14
34.78	Sesquiterpene ketone, mw 218	**20.84**	–	–
35.69	Hedycaryol	–	–	**7.67**
36.53	Methoxy eugenol ?	–	–	**3.50**
37.30	*p*-Methoxy cinnamyl acetate, (E)-	–	–	**8.43**
37.33	Sesquiterpene alcohol, mw 220	0.63	**4.13**	0.08
37.43	?	–	**6.90**	0.03
39.07	Benzyl benzoate	–	–	**8.86**
39.60	?	–	**11.14**	–
40.18	?	**11.23**	–	–
49.55	?	–	–	**3.16**
50.29	Benzyl cinnamate	**7.03**	–	**1.07**
52.92	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,15-tetramethyl- ?	**11.15**	–	**6.44**
53.83	?	–	**18.33**	–
54.39	Diterpene alcohol	–	–	**2.72**

S1 Incidence, average relative abundance, and contribution to within-species similarity of fragrance components in hind-leg extracts of three species of *Euglossa* in mainland Panama (pooled across localities 2, 3, and 4)

S2 Incidence, average relative abundance, and contribution to within-species similarity of fragrance components in hind-leg extracts of *Euglossa tridentata* at six localities in Panama and Costa Rica

from Isla del Caño (Fig. 1b). A summary of the findings is provided in S2 in the electronic version. The island individuals contained extremely small amounts of fragrances (Fig. 2; Isla del Caño vs. San Pedrillo: $H=8.69$, $p<0.01$, $N=20$). Across all six localities the mean similarity of an individuals' fragrance bouquet to that of all conspecifics increased asymptotically with increasing amount of fragrances ($y=47.97*(1-e^{-11.59x})$), with a maximum near 48% similarity.

Odor compensation experiment

Almost all individuals collected fragrances at both compounds, regardless of whether their legs had been artificially filled with 1,8-cineole or methyl salicylate-d_3 (Fig. 3). The proportion of individuals collecting 1,8-cineole did not significantly differ between treatment groups (Fisher Exact test: $N=41$, $p=0.46$). Equally, there was no difference in the proportion of individuals collecting methyl-salicylate-d_3 ($N=41$, $p=0.29$).

Collection experience experiment

All but one individual in each cage collected the available compound (either 1,8-cineole or methyl salicylate-d_3) during the first 4 days of the experiment. Collecting activity was high on the first day and decreased abruptly (Cage B, methyl salicylate-d_3) or gradually (Cage A, 1,8-cineole) on the following days (Fig. 4). When individuals were given a choice of both compounds on the fifth day, there was a

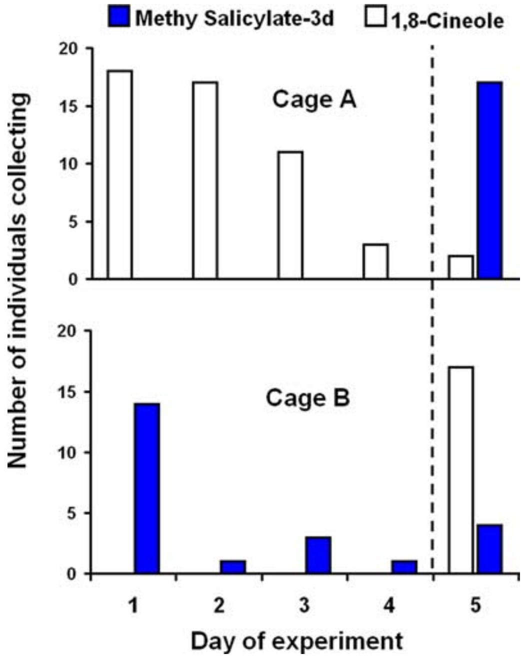

Fig. 4 Number of individuals collecting methyl salicylate-d_3 or 1,8-cineole during the operant learning experiment. During the first 4 days only one of the compounds was offered in each cage, on day 5 both compounds were offered alternately in both cages. See text for statistics

clear effect of cage/treatment affiliation, with individuals concentrating on the previously unavailable compound: A much greater proportion of cage A individuals collected methyl salicylate-d_3 than did cage B individuals (Fisher Exact test: N=39, p<0.0001). Conversely, a much larger proportion of cage B individuals collected 1,8-cineole (N=39, p<0.0001).

Discussion

Male *Euglossa* fragrance bouquets were found to be broadly species-specific at a range of ecologically divergent mainland sites in Panama and Costa Rica. The aberrant perfumes of *E. tridentata* from isolated Isla del Caño do not generally discredit this finding: bees on this tiny island were clearly excluded from suitable fragrance sources, as evidenced by their extremely small fragrance quantities.

The species-specificity of euglossine perfumes strengthens the view that they have evolved as an intraspecific chemical signal analogous to endogenous pheromones (Vogel 1966). Recently, this view has obtained direct support from field experiments, which confirmed that hindleg chemicals mediate remote attraction of conspecific males to display sites (Roubik, Zimmermann & Eltz, unpublished data). The chemical attraction of conspecific females remains to be demonstrated.

When considering the behavioral mechanisms involved in attaining chemical species-specificity, it is first important to visualize the euglossine fragrance market. The majority of fragrance compounds identified by the present study are known from published euglossine sources. The floral scents of euglossophilous flowers are normally chemical mixtures, although frequently of low complexity and characterized by pronounced dominance of one or two components (Williams and Whitten 1983; Whitten et al. 1986; Gerlach and Schill 1989; Gerlach and Schill 1991; Whitten and Williams 1992; Kaiser 1993). Single component scents occur in decaying wood visited by male euglossines (Whitten et al. 1993; Eltz et al. 1999). The fragrance phenotypes found in *Euglossa* cannot be attributed to any known single source, but are clearly assembled from several to many. The complexity of the mixtures lends emphasis to novel perceptual qualitites that are unique to the complex blends, while lacking in individual sources (see Smith and Getz 1994; Cromarty and Derby 1997; Chandra and Smith 1998; Hosler and Smith 2000). In euglossine bouquets these essential qualities will only emerge slowly during the process of fragrance accumulation and after many collection events. In this process, an individual fragrance source, e.g. a given species of orchid, may bear as little resemblance to the final bouquet as does an individual instrument to the listeners perception of Beethovens' Ninth Symphony. This is illustrated by *Euglossa tridentata*, known to collect fragrances at up to 30 different sources (Ramirez et al. 2002; Roubik and Hanson 2004), including some with strikingly different scent compositions (Williams and Whitten 1983; Gerlach and Schill 1991; Whitten and Williams 1992).

The shopping-basket acquisition of species-specific mixtures seems no trivial task for an insect and requires an adequate explanation. We suggest that species-specificity is attained by (i) an innate preference for certain chemical/perceptual qualities, coupled with (ii) experience-dependent refinement of these preferences through learning and negative feedback. In this scenario innate preferences broadly define the spectrum of attractive odors, whereas learning refines quantitative aspects, e.g. avoids overcollecting from abundant sources. Dosage-dependent avoidance was present only in bees that had actively collected the critical compound, which suggests that odor learning has an operant component. The neural mechanism that mediates negative feedback is clearly stimulus-specific, active upon short-term exposure, and seems to remain activated without repeated collection over lengthy periods of time (at least 4 days in case of methyl salicylate-d_3). Neither peripheral sensory adaptation nor habituation are likely to create such lasting effects (but see Carew et al. 1972; Stopfer et al. 1996). More likely, the observed loss of interest in a fragrance stimulus arises from the same neural mechanisms that underlie the CS pre-exposure effect or "latent inhibition," which has also been demonstrated in odor-learning honeybees (Chandra et al. 2000). Although operationally similar to habituation, CS pre-exposure does not require frequent re-stimulation in order to remain effective (Wasserman and Miller 1997). Yet another explanation, although perhaps a less parsimonious one, would be that avoidance is mediated by associative learning of mild fragrance poisoning. During collection substantial parts of the males' body have intimate contact with the collected chemicals. Uptake of trace amounts of a fragrance into the bee hemolymph over the digestive tract, tracheae, or intersegmental membranes might lead to nauseous effects that bees associate with the perceived fragrance. Similar to diet mixing, which results from the learned avoidance of secondary plant chemicals in herbivores (Freeland and Janzen 1974; Provenza 1996), the negative feedback would lead to a more even abundance distribution of chemicals in individual loads.

Is learned avoidance modified or lost after subsequent collection of other attractive odor qualities? In the simplest case, bee memory capacity may limit the number of learned associations, which might lead to progressive loss of odor-specific avoidance and result in dynamic cycling of fragrance preferences. Thus, males may return to collect fragrance A, but only after its learned avoidance is replaced by avoidance of substances B and C. In fact, dynamic avoidance seems a necessary prerequesite for maintaining quantitative proportions of components in accumulating fragrance loads. These ideas await experimental assays with caged bees.

Learned avoidance of odors in bees also has consequences for euglossine-pollinated plants. Many euglossophilous orchids produce copious quantities of fragrances in order to lure male bees (Gerlach and Schill 1991). Although this will increase the number of arriving pollinators, it may also lead to fragrance-saturation in individual bees and suppress or delay pollen transport to conspecific plants. Life history traits of orchids have evolved in part to counteract this adverse effect, e.g. scent production decreases dramatically after pollinarium removal in male *Catasetum* and *Cycnoches* (Williams 1982). This may prevent bees from becoming satiated on scents of one plant individual, while increasing the chance of successive visits to fragrant conspecifics. Also, if learned odor avoidance is reversible, the longevity of orchid pollinaria becomes critical. Although pollinarium retention on live bees has not been field tested, laboratory measurements suggest pollinia can remain viable for weeks or even months (Murren 2002). Thus, orchid pollen may be able to survive until bees regain their interest in previously collected odors.

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