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FRAGRANCE COLLECTION, STORAGE, AND ACCUMULATION BY INDIVIDUAL MALE ORCHID BEES

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Abstract—Individually marked males of two species of *Euglossa* were sighted repeatedly and over considerable periods of time (up to 44 days) at artificial fragrance baits exposed on Barro Colorado Island (BCI), Panama. Individuals switched between different bait chemicals that are attractive for the respective species, and no bait preferences or individual bait constancy was observed. GC-MS analyses of 153 males of three species showed that individual hind tibiae contain highly variable quantities of a complex and species-specific blend of fragrance compounds, mainly terpenoids and aromatics. In all three species, frequency distributions of individual quantities were strongly skewed towards individuals with small amounts, and individual amount and complexity were positively correlated. Tibial contents of male *Euglossa imperialis* that were kept alive in a flight cage for 0, 5, 10, or 15 days showed no qualitative or quantitative change over time, suggesting that the fragrances are very efficiently stored in the hind legs. In *Euglossa cognata* wing wear, an established age correlate of the species, was positively correlated with individual fragrance quantity. Our results suggest that male euglossines forage continuously for a variety of volatiles, store them, and finally acquire large quantities of a complex and specific fragrance bouquet. Both qualitative and quantitative aspects of individual contents are likely to contain information on male phenotypic and genotypic quality.

Key Words—Euglossine bees, fragrance collection, sexual selection, species recognition, GC-MS.

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INTRODUCTION

The males of all neotropical orchid bees (Apidae: Euglossini) share the peculiar characteristic of collecting exogenous volatile substances (fragrances) from varied floral and nonfloral sources (Dressler, 1982). The fragrances are absorbed with modified foretarsal brushes and transferred into spongelike, hair-filled cavities in the grossly enlarged male hind tibiae (Vogel, 1963, 1966; Kimsey, 1984). During fragrance collection, the males also apply a mixture of certain nonpolar labial gland lipids to the fragrant surface. This seems to help them to retain the volatiles and therefore increases the efficiency of fragrance uptake, analogous to enfleurage, a process of scent extraction widely used by the perfume industry (Whitten et al., 1989). It is still not known what the males do with the collected fragrances, and the ultimate causes of the phenomenon are obscure. One view is that male bees accumulate large quantities and varieties of fragrances over time and that the individual fragrance bouquet serves as an indicator of male genotypic and phenotypic quality (e.g., reflecting foraging skills and/or survival abilities) to female bees (Kimsey, 1982; Whitten et al., 1989). If females prefer males with certain fragrance phenotypes, fragrance collecting behavior could have evolved by sexual selection. So far no direct evidence exists to support this hypothesis.

Males of some species of orchid bees have been observed to establish small mating territories in the vicinity of treefalls in dense forest. Within these territories the males perch and display on vertical tree trunks (Dodson, 1966; Kimsey, 1980; Stern, 1991). Matings are known to take place in these territories (Dodson, 1966; Kimsey, 1980), but so far it is unclear whether the fragrances play a role in courtship behavior or, more generally, whether they are exposed at all during male display.

As has been known for some time, male euglossines can be attracted to a number of pure artificial fragrance compounds that are presented in the bees' natural habitat (Dodson et al., 1969). In an extensive one-year study on Barro Colorado Island (BCI), Panama, Ackerman (1989) found that the males of a given species were attracted to up to 10 of the 16 compounds that were exposed. There was considerable overlap in fragrance choice between species, but each showed attraction to a characteristic set of compounds.

We studied qualitative and quantitative aspects of fragrance collection and storage of male orchid bees in the Barro Colorado Natural Monument (BCNM) in lowland Panama. To evaluate the potential for sexual selection in promoting male fragrance collecting behavior, we were particularly interested in the temporal patterns of fragrance accumulation and asked whether fragrance quality or quantity are likely to reflect individual foraging history and survival of male bees. Using various methods we attempted to answer the following questions:

1. Do male Euglossini forage for fragrances over long periods of their lifetime?

2. Are individual males interested in collecting a variety of compounds as expected from observations at the species level (Ackerman, 1989)?

3. What quantities and qualities of fragrances are found in the hind tibiae of individual males captured at fragrance baits?

4. What happens to the fragrances once they have been transferred into the hind tibiae? Are males able to retain the volatiles over time, or are the fragrances lost, chemically modified or exposed during display?

5. Is there a relationship between the age of individual bees and the quantity of fragrances in their hind tibiae?

METHODS AND MATERIALS

Mark–Recapture at Artificial Fragrance Baits. To assess individual fragrance choice and the temporal patterns of fragrance collecting behavior, we individually marked and released male euglossines captured at artificial fragrance baits on BCI. A total of eight compounds was used: 1,8-cineole (c), benzyl acetate (ba), methyl salicylate (ms), *p*-dimethoxybenzene (p-db), vanillin (v), *p*-cresyl acetate (p-ca), terpinene-4ol (t-ol), and *t*-methyl cinnamate (t-mc). These compounds were previously known to be good attractants for a range of euglossine bee species (Ackerman, 1989; Whitten, unpublished observations). Fragrances were simultaneously exposed on herbarium blotter pads in the radio tower clearing on BCI on 25 days (for 2–4 hr between 9:00 and 14:00 hr) between May 31 and July 23 in 1994 (every second day, except on rainy days). The baits were concealed in a screen of fine nylon mesh and not directly accessible to the bees. However, attracted bees brushed extensively on the mesh as if they were collecting fragrances. Males of two species [*Euglossa cognata* (small species) and *Eulaema meriana* (large)] were caught with hand nets and marked individually with colored numbered bee tags that were glued to the thorax with Super Glue. A total of 321 *Eg. cognata* and 120 *El. meriana* were marked. In case of repeated visits to the baits, the markings could normally be read without disturbing the bees. For each visit of marked bees, we recorded the bait, date, and time. To obtain information on the quality of wing wear as an age correlate, we repeatedly captured and remeasured a subset of the marked *Eg. cognata*. As individuals showed only marginal wing damage, we refrained from establishing damage classes based on area (as, e.g., Stern, 1991; Mueller and Wolf-Mueller, 1993) but rather measured wing wear as the number of nicks in fore- and hind wings on both sides of the body. Readings were made using a standard hand lens (magnification: 9×).

Fragrance Analysis and Samples. We analyzed the tibial contents of a total of 153 individual orchid bees belonging to three species of the genus *Euglossa* using gas chromatography-mass spectrometry (GC-MS). The bees were captured at concealed fragrance baits (see above) in the Barro Colorado Natural Monument (BCNM) between June 27 and July 27 in 1994. If not stated otherwise below, the individuals were placed in Eppendorf caps, cooled to immobility using a hand-held cooler (ice chest), and transferred to the laboratory within 2 hr following capture. There we immediately removed both hind legs of the individuals and placed them pairwise in glass vials (1 ml crimp-top vials with viton or butyl septum) that were filled with 0.5 ml *n*-hexane. The *n*-hexane contained 1 mg/liter 2-undecanone as internal standard (ISTD). GC-MS analysis was done using a Hewlett-Packard 5995 GC-MS with a 30-m DB-5 column at the Department of Natural Sciences at Florida Museum of Natural History. The oven temperature was programmed from 25 to 290°C at 3°C/min. Most fragrance compounds were identified by comparison with authentic standards, and spectra of unidentified compounds were searched against the NIST/EPA/NIH spectral library (Ausloos et al., 1992). When exact identification proved impossible, compounds were classified as fragrance compounds if they were of clear terpenoid or aromatic character (based on mass spectra). Thus, in almost all cases a clear separation between fragrances and labial gland lipids (mostly acetates and diacetates) was possible (see Whitten et al., 1989). The following samples were analyzed: 54 *Euglossa cognata*, captured at *p*-dimethoxybenzene, methyl salicylate, and vanillin baits [41 captured on Barro Colorado Island (BCI), 13 at the nearby mainland (Pipeline Road)]; 50 *Euglossa tridentata*, captured at cineole and vanillin baits on BCI; and 49 *Euglossa imperialis*, captured at cineole baits on BCI, subjects of the fragrance withdrawal experiment described below.

Data Analysis. Retention times and standardized peak areas (integrated ion current, measure of quantity) of identified fragrance compounds were recorded. Total individual amounts of fragrances were calculated by summing peak areas of all compounds present in a sample. Thus, within- and between-species comparisons of individual fragrance quantities were possible. To assess intra- versus interspecific variation of individual fragrance composition (quality), we calculated cluster analyses using NTSYS 1.8 (Rohlf, 1993). For that purpose a subset of individuals of the three species was selected that were similar in terms of total fragrance amount and complexity. A minimum of seven identified compounds and an upper limit of total fragrance amount of 15×10^6 units of total integrated ion current served as numeric criteria. The quantitative fragrance data of the subset were then transformed into qualitative presence/absence data. The resulting 0/1 matrix served as the basis for calculating distances using the Simple Matching Coefficient and Dice Coefficient in the SIMQUAL module of NTSYS 1.8. Clustering was done with UPGMA in the SAHN module.

Fragrance Withdrawal Experiment. To test for an effect of time on quantity and quality of individual fragrance contents, we conducted a cage experiment on *Euglossa imperialis*, a common euglossine bee in Central Panama. Forty-nine males of this species were captured at concealed cineole baits. Fifteen of these were killed at once and their hind legs were sampled for GC-MS as described above. The other individuals were kept alive in a large (7 × 3.5 × 2 m) nylon mesh flight cage that was set up in the BCI laboratory clearing. During the first days of captivity, the bees learned to feed at artificial flowers (Eppendorf tubes with a yellow plastic corolla) holding a honey–water solution (50%) that was renewed daily. No obvious fragrance sources of any kind were present in the cage, nor were any males ever observed to collect exogenous substances during their captivity. On day 5 of the experiment some males started to perch and display on several of the 15 dead vertical tree tunks (3-6 cm in diameter) present in the cage. The observed behavior was found to be very similar to the territorial behavior of *Eg. imperialis* in its natural habitat as described by Kimsey (1980). Territorial activity in the cage continued to increase towards the end of the experiment until a substantial (but unknown) fraction of the males present were engaged in this behavior. After 5, 10, and 15 days of captivity random samples of 10, 10, and 12 individuals were captured and their hind legs were extracted in *n*-hexane. GC-MS analysis and data processing was carried out as described above.

RESULTS

Mark–Recapture. Table 1 summarizes the results of the study. The proportion of resighted individuals was high (around 20%) in both species of euglossine bees. Some individuals visited the baits repeatedly (up to three times in *El. meriana* and up to six times in *Eg. cognata*), partly covering substantial periods of time (up to a maximum of 44 days in both species; Figure 1). Resighted individuals of *Eg. cognata* were more likely to be resighted again than was expected from the average probability of resightings in that species ($\chi^2 = 16.2$; $N = 386$; $P < 0.001$). This is reflected by the fact that 84 of the 120 resighting events were due to only 29 individuals that were resighted repeatedly (more than once).

Male bees of both species did not exhibit obvious individual preferences for certain bait chemicals but switched between baits in a way that seemed to reflect the fragrance preferences that are characteristic of the species as a whole. Individual bees visited up to three different bait chemicals. In *Eg. cognata*, for which we had a sufficient sample size (number of resightings), we were able to test for individual bait constancy. Of 59 males that were first sighted at *p*-dimethoxybenzene (p-db) baits, 53 were resighted at p-db and six at methyl salicylate baits. These frequencies do not significantly differ from the frequencies found for first visits at the species level ($\chi^2 = 2.07$; $N = 376$; NS).

Individual Fragrance Contents—General Remarks. Only individuals of the three species that were collected at fragrance baits on Barro Colorado Island were included in the analysis. Besides *Euglossa cognata* ($N = 41$) and *Euglossa triden-*

TABLE 1. SUMMARY OF RESULTS OF MARK–RECAPTURE STUDY AT ARTIFICAL FRAGRANCE BAITS ON BARRO COLORADO ISLAND

	Marked individuals (*N*)	Compound visited when captured for first time (%)	Resightings (*N*)	Number of Resighted individuals [N(%)]	Resightings/ individual [N, mean (range)]	Time between first and last sighting [days, mean (range)]	Compounds visited/ resighted ind. [N, mean (range)]
Euglossa cognata	321	p-db: 80 ms: 18.7 v: 1.3	120	65 (20.2)	1.85 (1–6)	8.98 (0–44)	1.3 (1–3)
Eulaema meriana	120	ms: 47.5 c: 20.1 ba: 16.7 t-ol: 5.8 p-ca: 5.8 v: 0.8	37	24 (20.0)	1.5 (1–3)	12.0 (0–44)	1.6 (1–2)

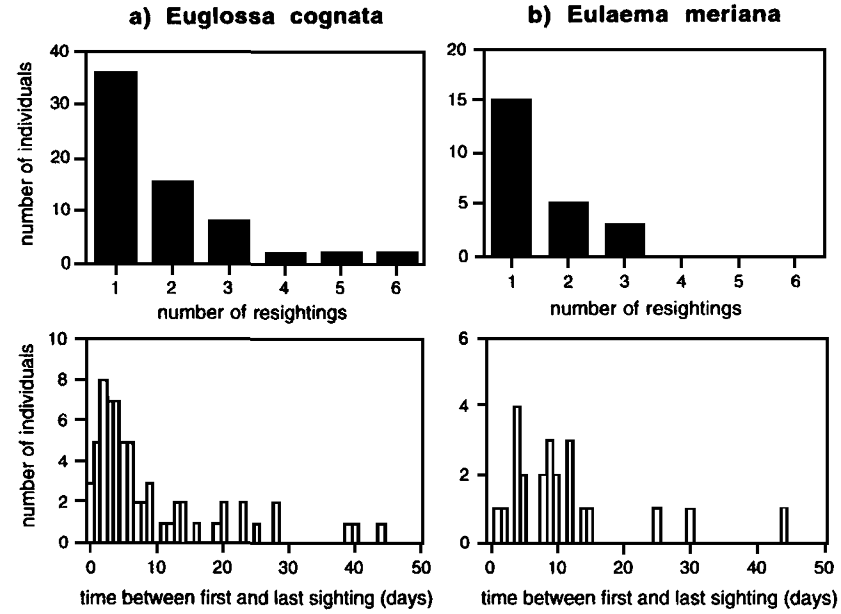

FIG. 1. Frequency distributions of the number of resightings/individual (above) and of the time between first and lasting sighting (below) of *Euglossa cognata* (a) and *Eulaema meriana* (b). Only individuals that were resighted at least once are included.

tata (N = 50), we also decided to include the 49 individuals of *Euglossa imperialis* that were subject to the fragrance-withdrawal experiment. The inclusion is based on the assumption that fragrance quantity and quality did not change over time during their captivity. This is confirmed by the results of the experiment (see below); therefore, a comparison with the two other species seems justified. Hind tibial extracts of individual males contained variable quantities of partly complex mixtures of fragrance compounds, consisting of open monoterpenes, bicyclic monoterpenes, sesquiterpenes, and diverse aromatics. A total of 143 different fragrance compounds were found in the three species. Retention times and, if available, identifications of the compounds as well as their distribution among species are given in the Appendix.

Quantity and Complexity of Individual Fragrance Contents. Table 2 summarizes the results of the GC-MS analysis for the three species. Quantity and complexity of the fragrance contents varied considerably between species and individuals. On average individuals of *Euglossa cognata* contained the lowest quantities, surpassed by *Euglossa imperialis* (~2× cognata) and finally *Euglossa tridentata* (~5× cognata). The average complexity of the individual contents as

TABLE 2. SUMMARY OF GC-MS ANALYSES OF HIND TIBIAE OF 141 INDIVIDUAL MALES OF THREE SPECIES OF *Euglossa* FROM BCI[a]

	Eg. cognata ($N = 41$)	*Eg. imperialis* ($N = 49$)	*Eg. tridentata* ($N = 50$)
Fragrance quantity			
Median ($\times 10^{-6}$)	3.52	6.37	17.7
Quartiles ($\times 10^{-6}$)	2.36/5.95	2.71/9.84	6.54/38.50
Skewness of distribution	2.03	1.59	1.57
Fragrance complexity			
Total number of fragrance compounds	34	51	105
Median of number of compounds/individual	6	8	16
Quartiles	4/7	4/12	10/28

[a] Values of fragrance quantity are sums of integrated ion currents × 10^{-6}.

well as the total number of compounds found per species followed the same pattern.

A large amount of variation, however, was also found among individuals within species. Figure 2a shows the frequency distributions of individual fragrance quantities. In all three species the distribution is highly skewed towards individuals with comparatively small amounts of fragrances. Few males had large fragrance stores. Although emphasis must be given to between species differences (note different scales on the *x* axes), this general pattern was remarkably similar among species. In all three species there was a strong positive correlation between individual fragrance quantity and complexity (Figure 2b). Individual mixtures extracted consisted of up to 47 different fragrance compounds.

Composition and Specificity of Individual Fragrance Contents. As emphasized by the data given in the Appendix, we found considerable overlap in the fragrance composition among species. Of the 143 compounds found, 12 were present in all three species and another 23 occurred in two of the three species. However, in spite of this partial overlap, cluster analyses revealed greater similarity of fragrance composition within than between species (Figure 3). Both similarity indices used in combination with UPGMA proved to be consistent and produced exclusively species specific clusters. The observed differentiation between species was mainly caused by differences in major compounds that were present in a large fraction of the individuals of a given species (see Appendix).

Fragrance Storage Over Time. Figure 4 shows the medians and quartile ranges of individual fragrance quantities of 47 male *Euglossa imperialis* grouped by the duration of their captivity. Statistical analysis did not reveal any decrease

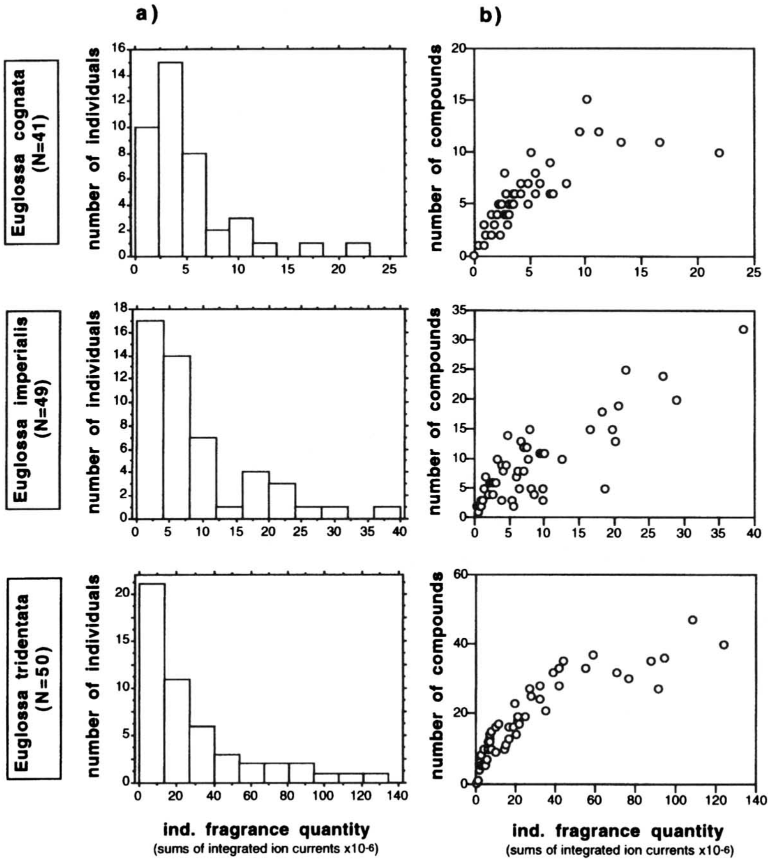

FIG. 2. (a) Frequency distributions of individual fragrance quantities of three species of *Euglossa* on BCI revealing considerable variation and high levels of skewness towards individuals with only little amounts. (b) Relationship between individual fragrance quantity and complexity (number of fragrance compounds).

or increase in the individual amounts of fragrances over time (Kruskall-Wallis ANOVA: $H = 2.50$; $N = 47$; $df = 3$; $P > 0.48$). To test for changes in fragrance quality, we selected the eight major compounds found in the *imperialis* extracts (hexahydrofarnesyl acetone, cineole, methyl salicylate, α-pinene, nerolidol, sabinene, germacren D, and β-pinene) and, for each compound, tested for changes over time using Kruskal-Wallis ANOVA. Two of the compounds (nerolidol and germacren D) significantly increased ($H = 14.06$, $P < 0.01$; and $H = 10.30$; $P < 0.05$), whereas the other compounds exhibited no change over time.

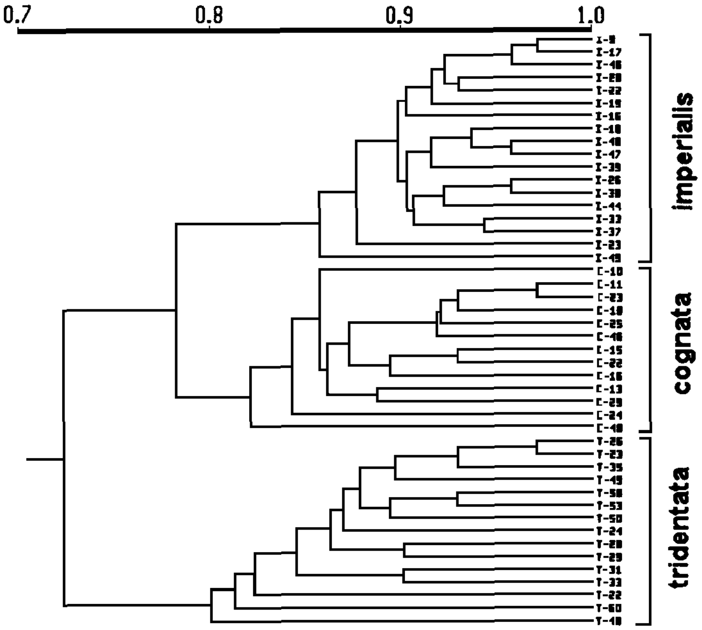

FIG. 3. Dendrogram of individual males of three sympatric species of *Euglossa* from BCI, based on qualitative similarity of fragrance composition extracted from the hind tibiae. Distances are simple matching coefficient, clustering was done with UPGMA in NTSYS 1.80 (Rohlf, 1993). Individuals cluster in three groups according to their species.

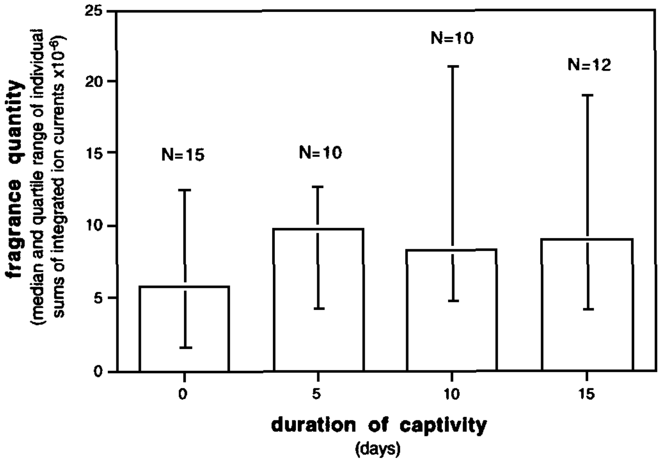

FIG. 4. Median and quartile range of the fragrance quantities of male *Euglossa imperialis* plotted against the duration of their captivity. No change over time was observed.

Fragrance Quantity and Individual Age. We tested the applicability of wing wear, measured as the number of notches in the edges of fore- and hind wings, as an age predictor for male *Eg. cognata* during the mark–recapture study (see above). The increase in wing wear of individual males was dependent on time between first and last measurement at the fragrance baits (Regression analysis; $y = 0.423x - 0.609$; $r^2 = 0.76$; $F = 105.92$; $N = 35$; $df = 1$; $P < 0.001$; Figure 5a). However, individual rates of accumulation showed considerable variation,

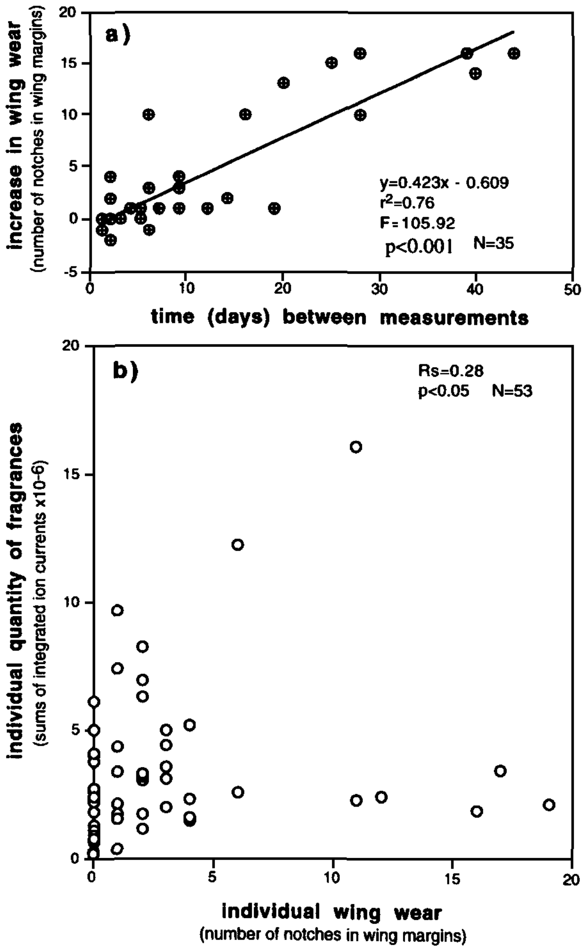

FIG. 5. (a) Dependency of the amount of increase of wing wear on the time between measurements in *Euglossa cognata*. (b) Correlation between the individual quantity of fragrances stored and individual wing wear.

limiting the value of wing damage as a precise age indicator. Nonetheless, there was a weak but significant positive correlation between individual wing wear and the amount of fragrances stored by male *Eg. cognata* (Spearman $R_S = 0.28$; $N = 53$; $p < 0.05$; Figure 5b).

DISCUSSION

Fragrance Quality and Specificity. The results of our GC-MS analyses showed that the hind tibiae of individual males contain variable quantities of complex blends of terpenoid and aromatic fragrance compounds. Male euglossines have been observed collecting volatiles from a variety of sources including flowers of Orchidaceae, Solanaceae, Bignoniaceae, and Euphorbiaceae, as well as nonfloral sources such as decaying wood, rotting fruits, feces, and tree wounds (Zucchi et al., 1969; Dressler, 1982; Williams, 1982; Whitten et al., 1993). The scents produced by attractive sources, if analyzed, vary considerably in their complexity and chemical composition. The bouquets of euglossophilous orchids typically consist of 3–10 different compounds, of which one or two are dominant (Williams and Whitten, 1983; Gerlach and Schill, 1991, Kaiser, 1993), whereas scents of other plant species visited by euglossines can be even more complex (e.g., Sazima et al., 1993). Pure single-compound scents may be rare in the bees' natural habitat, but have been shown to occur in decaying wood visited by euglossines (Whitten et al., 1993; see below). The majority of the identified compounds found in our *Euglossa* samples are known from floral and/or nonfloral sources, but, due to the seemingly endless number of potential combinations, it is presently impossible to link certain sets of compounds to specific fragrance sources.

The tibial fragrance contents of each respective *Euglossa* species were dominated by a certain number of major compounds present in the majority of the individuals. Apart from these, the extracts contained a varying number of minor compounds (usually in low quantities) only found in a small number of individuals. One might conclude that it is the major compounds that the bees are actually seeking, whereas the minor ones are unintentionally accumulated. However, the truth is likely to be more complicated, as some of the minor components prove to be highly attractive to the respective species when exposed in pure form as fragrance bait. Vanillin, e.g., was present in the extracts of only three individuals of *Eg. tridentata*, but is known to be one of the strongest attractants of this species in Central Panama (Ackerman, 1989; T. Eltz, unpublished observation). Generally, there is no simple relationship, positive or negative, between the predominance of certain compounds in a species' extracts and their attractivity in bioassay. Of the two most dominant compounds found in samples of *Euglossa imperialis*, one, cineole, is its strongest known attractant (e.g., Ackerman, 1989), whereas the other, hexahydrofarnesyl acetone, completely failed to attract any bees (T. Eltz, unpublished observation). The reasons for this are entirely unknown. Many fragrance compounds are chiral, and bees might be attracted only to certain enantiomers and neglect others. In addition, some of the fragrance compounds may only be perceptible to the bees when present in combination with modifying components. Detailed studies of male chemoreception in combination with adequate bioassays are required to resolve this issue.

In spite of considerable intraspecific variation in fragrance composition, the results of our cluster analyses show that the bouquets of the three *Euglossa* species are sufficiently different from each other to allow the individuals to be assigned to their respective species. Thus, we have demonstrated for the first time that the fragrances of male euglossines have the potential of being used as a means of species recognition. Whether they are actually used in that process remains an open question.

Fragrance Accumulation. During our mark–recapture study, several males of both species periodically visited the artificial fragrance baits over considerable periods of time (up to 44 days), suggesting that the bees do engage in some fragrance foraging during most, if not all, of their lifetime. The findings certainly underestimate the true extent of time and energy allocated to the collection of volatiles, as the marked bees most likely have visited natural fragrance sources as well. So far no information has been published on the quantity of volatiles that a collecting bee can extract from natural fragrance sources. Preliminary data from a study on Cerro Campana in Central Panama suggests that quantities can be small indeed. Tibial extracts of 15 male *Euglossa deceptrix* that had collected fragrances at a decaying log for 1–10 min did not contain any detectable traces of the single volatile compound produced by the log (*p*-dimethoxybenzene) (Eltz and Whitten, unpublished data). Although it is unknown whether the log from Cerro Campana can be considered an ordinary euglossine fragrance source, it is likely that fragrances are generally scarce and that the bees must allocate considerable time and effort to the acquisition of large fragrance quantities.

Although the evidence is circumstantial, our results do suggest that the males accumulate the fragrances continuously over considerable periods of time, perhaps throughout their lifetimes. The following findings support this hypothesis of long-term fragrance accumulation:

1. The fragrances can be stored very efficiently in the hind tibiae over long periods of time, as evidenced by the absence of any change in fragrance quantity during the fragrance-withdrawal experiment. This superior storage capability is probably achieved through the combined effects of the retaining properties of the nonpolar carrier lipids secreted from the labial glands (Whitten et al., 1989) and the strong capillary forces imposed by the sponge-like morphology of the invaginated cuticle inside the hind tibia that serves as a storage container (see Vogel, 1963, 1966; Cruz-Landim et al., 1965).

2. There is a positive relationship between the individual fragrance quantity and the established age correlate wing wear in *Euglossa cognata*. The weakness of the relationship may partly result from the low accuracy of wing wear as an absolute age predictor (variable individual rates of acquiring wing wear), as well as from the influence of confounding variables that lead to individual differences in the rate of fragrance acquisition (e.g., foraging skills and activity, see below).

3. The frequency distributions of the individual fragrance quantities are skewed towards individuals with only small amounts in all three species. Assuming long-term fragrance accumulation, such a distribution would be expected from a population that is numerically dominated by young individuals. Wing wear data from baiting studies in Panama suggest that euglossine populations do indeed frequently show age distributions skewed towards young individuals (Ackerman, 1983; Zimmerman and Madrinan, 1988; D. W. Roubik, unpublished data).

4. In all three species there was a strong positive correlation between quantity and complexity of individual fragrance stores. Although this relationship may be partly an artifact of the GC-MS analysis (more small peaks may remain underneath the detection threshold in low-quantity extracts), there is certainly some biological substance to it. During foraging from diverse and ephemeral fragrance sources, the males are likely to add new compounds continuously to their acquired fragrance bouquets. Thus, a correlated increase in quantity and complexity is in general agreement with the hypothesis of long-term fragrance accumulation.

Any evaluation of the hypothesis is of course hampered by the complete lack of indications on the actual use of the fragrances by the males. The results of the fragrance-withdrawal experiment suggest that the volatiles are neither absorbed into the hemolymph for further processing and translocation to exocrine organs as hypothesized by Williams (1982), nor chemically modified within the tibial cavities. Furthermore, as fragrance quantities remained unaltered over time, there was no indication that the bees actually exposed substantial amounts of the fragrances during territorial display, as was suggested by Vogel (1966). However, it can hardly be doubted that the fragrances are used at some point in the males' lives. At present, the most likely scenario is that the volatiles are only exposed during the brief and rarely observed encounters of courtship between the sexes at the males' territories.

If the hypothesis of long-term fragrance accumulation is correct, then the accumulated bouquet of males will contain information on the individuals' foraging history and survival. This intrinsic information on male performance may be the basic prerequisite for sexual selection to act on and shape male fragrance collecting behavior. If the ability of individual males to find and exploit fragrance sources and to survive at the same time, varies, and if this variation has a genetic background, the fragrance bouquet will in some way reflect male genotypic quality. If females have inherited preferences for certain high-quality fragrance phenotypes, then fragrance collection could have evolved through female choice and sexual selection. The collection of fragrances most probably does inflict the high, survival-reducing costs on male bees that are required by genetic handicap or indicator models (Zahavi, 1975, 1977; Maynard Smith, 1985; Pomiankowsky, 1987), e.g., an increased risk of predation or the costly detoxification of certain toxic fragrance compounds. In addition to indirect genetic benefits (high-quality offspring), female euglossines may also gain direct benefit from choosiness. Female preference for males that closely match a specific optimum fragrance phenotype may reduce the risk of wasteful and potentially fatal hybrid matings. As outlined above, individual fragrance bouquets do indeed provide clues for species recognition.

So far it is unclear what exact aspect of the individual fragrance bouquet (quantity, complexity, specificity, or a combination of these) is the true target of selection. Detailed studies on the sexual behavior and sensory physiology of orchid bees are required to obtain further insights into the evolution of fragrance collection.

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APPENDIX. FRAGRANCE COMPOUNDS FOUND IN HIND TIBIAE OF THREE SPECIES OF *EUGLOSSA* FROM BCI[a]

RT (min)	Compound	*Eg. cognata* (*N* = 41)	*Eg. imperialis* (*N* = 49)	*Eg. tridentata* (*N* = 50)
9.25	α-thujene		6	
9.48	α-pinene	2	**31**	16
9.72	benzaldehyde			12
10.38	camphene		6	
10.80	sabinene		**27**	9
10.93	β-pinene	1	**25**	11
11.20	myrcene	1	14	19
11.81	*p*-menth-2-*ene*, trans		1	
11.92	α-phellandrene		9	4
12.22	3-carene		13	3
12.40	α-terpinene		3	
12.68	*p*-cymene		1	
12.78	limonene	4	5	4
12.80	β-phellandrene		2	
12.95	cineole		**42**	**37**
13.18	(*Z*)-ocimene	1	4	11
13.88	(*E*)-ocimene		1	**29**

APPENDIX. CONTINUED

RT (min)	Compound	*Eg. cognata* ($N = 41$)	*Eg. imperialis* ($N = 49$)	*Eg. tridentata* ($N = 50$)
14.08	γ-terpinene		4	
	?		4	
15.12	terpinolene	4	6	4
	?		2	
15.47	linalool	1	4	2
16.03	phenylethyl alcohol			4
16.85	2,6-dimethyl-2,4,6-octatriene			3
17.50	ipsdienol	11		13
18.25	*p*-dimethoxybenzene	**23**		21
18.68	benzoic acid			8
18.78	menthol		1	
18.98	terpinen-4-ol		2	
19.58	α-terpineol		4	2
19.73	methyl salicylate	10	**32**	2
20.95	2,3-dihydrobenzofuran			13
22.38	*p*-anisaldehyde			1
22.58	phenylethyl acetate			1
23.67	anis alcohol isomer			1
23.93	anis alcohol			2
23.97	anethole			4
24.88	*trans*-cinnamyl alcohol			1
26.23	?			19
27.20	eugenol			**37**
27.78	1,2,4-trimethoxybenzene			1
27.87	decanoic acid			8
28.28	α-copaene		8	1
28.35	*trans*-methyl cinnamate			6
28.42	geranyl acetate			2
28.85	β-cubebene		3	
28.93	vanillin			4
28.93	germacrene A	4	4	23
29.27	methyl eugenol			1
29.88	anisyl acetate			1
30.10	?	9		
30.17	caryophyllene	2	18	9
30.52	cinnamic acid	1		
30.53	?	2		
30.77	?		2	1
30.82	(*Z*)-*trans*-α-bergamotene	10		1
30.82	paeonol		1	
31.15	?			1
31.18	*trans*-isoeugenol			4
31.22	?			1
31.38	geranyl acetone		1	

APPENDIX. CONTINUED

RT (min)	Compound	*Eg. cognata* (*N* = 41)	*Eg. imperialis* (*N* = 49)	*Eg. tridentata* (*N* = 50)
31.60	*cis*-β-farnesene	**28**		5
31.66	α-humulene		4	1
31.83	?			18
31.90	ethyl cinnamate			1
32.00	NIST 27977		1	
32.60	β-himanchalene ?	1		
32.65	γ-muurolene		2	
32.82	β-cubebene		1	
32.87	germacrene D	**33**	**27**	6
33.23	bergamotene?	1		
33.25	α-zingiberene	1		
33.27	*trans*-methyl isoeugenol		1	
	α-selinene		1	
33.50	germacrene B?	2		
33.50	α-selinene			1
33.62	?			5
33.85	(*E*,*E*)-α-farnesene	**24**	7	1
33.68	β-bisabolene			**35**
34.23	γ-cadinene		6	
34.52	δ-cadinene		6	
34.53	*trans*-β-farnesene	3		9
34.83	?			17
35.03	?			**29**
35.03	?			1
35.28	?			1
35.52	?			18
35.58	?			2
35.67	elemicin	7		1
36.02	nerolidol	**22**	**31**	4
36.08	dodecanoic acid			5
36.15	methoxycinamaldehyde?			**30**
36.22	?			**29**
36.73	?			1
36.85	?			1
37.03	cis-methyl *p*-methoxycinnamate			4
37.55	methoxyeugenol	1		3
37.68	farnesene oxide I	1		
38.23	farnesene oxide?		1	9
38.27	?		2	3
38.35	farnesene oxide II	16		
39.27	zingiberone		8	
39.38	isoelemicin	13		
39.67	NIST 11930		1	

APPENDIX. CONTINUED

RT (min)	Compound	*Eg. cognata* (*N* = 41)	*Eg. imperialis* (*N* = 49)	*Eg. tridentata* (*N* = 50)
39.87	?	1		
40.25	trans-methyl-*p*-methoxycinnamate			8
40.55	elemol?			6
40.58	?			**34**
40.62	?			1
40.90	α-bisabolol	1		11
41.37	?			20
41.73	?			11
41.85	?	2		
42.12	spathulenol?		12	
42.17	farnesol?			2
43.77	benzyl benzoate			**38**
45.57	?			3
45.93	hexahydro farnesyl alcohol?		1	4
46.55	hexahydrofarnesyl acetone		**44**	2
47.28	benzyl salicylate			5
50.13	decanoic acid, phenylmethyl ester			3
51.37	?			2
51.88	aff. hexahydrofarnesyl alcohol		4	
52.57	geranyllinalool			2
53.45	?			7
54.15	?			**36**
54.58	benzyl cinnamate	1		10
56.00	?			2
56.02	dodecanoic acid, phenylmethyl ester			1
57.53	?			**40**
58.43	?			2
58.95	?			18
59.97	?			**33**
60.18	?			6
60.42	?			3
62.10	?			2
62.58	?			6
65.35	?			14
67.05	?			1
68.93	?			8
70.17	?			4
70.45	?			1

[a] (*Eg. cognata*, *N* = 41; *Eg. imperialis*, *N* = 49; *Eg. tridentata*, *N* = 50). Counts refer to the number individuals that contained the respective compound. Counts of dominant compounds (found in more than 50% of individuals of a given species) are given in bold numerals.

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