Variation in Cephalic Volatile Substances in Relation to Worker Age and Behavior in the Stingless Bee, *Scaptotrigona postica* **

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Head extracts of adult workers of the Brazilian stingless bee *Scaptotrigona postica* (Trigonini, Meliponinae: Apidae) were analysed for volatile substances by gas chromatography/mass spectroscopy. Individual worker bees performing clearly defined tasks and representing five age groups were collected. A total of 36 compounds was identified, 22 of which had not been previously described for a *Trigona* species.

The major components are 2-heptanol, 2-tridecanone, 2-pentadecanone, *Z*-5-tetradecenylbutanoate and *Z*-7-hexadecenylbutanoate. 11 of the 36 compounds increase in concentration with age, but only 2 decrease. The other substances are present in more or less constant concentrations. These data are discussed assuming more complicated pheromone-mediated interactions within an age-dependent polyethism between old workers acting inside and outside the nest than between young house bees.

Introduction

The honeybee queen substance was one of the first pheromones extensively studied [1, 2]. However, only in recent years have other volatile substances been investigated in different social bee species [3–6]. The more than 300 species of stingless bees (Meliponinae: Apidae) represent an enormous diversity of highly eusocial life and ecological adaptations [7–12]. A study of their chemical languages seems promising, not only through a comparative approach, but also assuming that any complexity of social organisation must depend on an elaborate communication system.

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Materials and Methods

The stingless bee colonies used were originally obtained from Ribeirão Preto, Sao Paulo, Brazil.

Free flying bees from colonies maintained in our laboratory at Tübingen were collected immediately when a particular behavior was observed. Heads from individuals were extracted in pentane for later analysis. The following 5 functional worker stages were defined: 1. newly emerged adults with brownish colour taken from Kindergarden assemblies on the involucrum. 2. Nursing workers with brown scutellum taken from the brood nest area. 3. Garbage carrying bees caught when flying out. 4. Guard bees taken from the entrance funnel. 5. Forages with full pollen baskets returning to the entrance. According to previous observations [12, 18, 19], worker bees of these 5 stages are approximately 1–5, 10–25, 18–35, 30–40, resp. 30–60 days old.

Gaschromatographic/mass spectroscopic analyses were carried out on a Varian MAT 311 A coupling system using a 50 m glass capillary column with WG 11 as a stationary phase. Confirmation of the structures of natural occurring compounds is based on comparison of mass spectra and gaschromatographic retention times with pure synthetic reference samples.

Results

By gaschromatographic/mass spectroscopic analyses of the pentane extracts of heads of adult *Scaptotrigona postica* workers 36 volatile compounds were identified (Fig. 1; Table I). Eight of these are absent in the newly emerged bees; five are absent in nurse bees. The concentration of eleven compounds increases in the workers removing waste, guarding the entrance, or foraging pollen. Two unsaturated ketones, however, were found to be relatively more concentrated in the young house bees.

The most abundant in *Scaptotrigona postica* head extracts are 2-heptanol, 2-tridecanone, 2-pentadecanone, *Z*-5-tetradecenylbutanoate and *Z*-7-hexadecenylbutanoate.

Discussion

Most of the volatile worker head compounds described here for *Scaptotrigona postica* show unbranched carbon skeletons and thus certainly

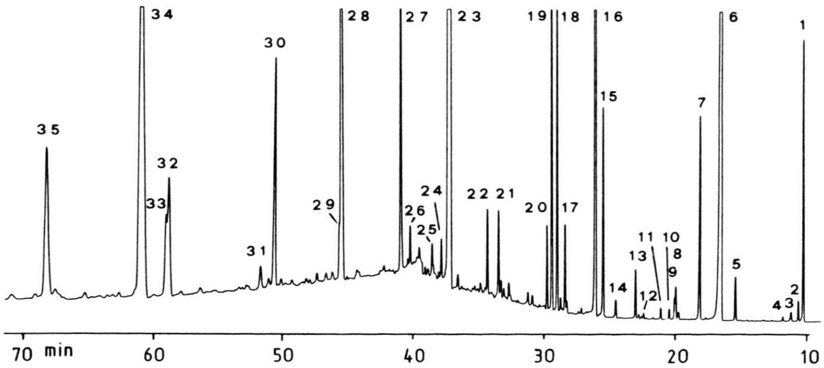

Fig. 1. *Scaptotrigona postica* worker head extract gas chromatogram. 50 m glas capillary with WG 11 as a stationary phase, temperature program 50–200 °C at a rate of 3 °C/min.

Table I. Volatile compounds from pentane head extracts of *Scaptotrigona postica* workers as identified by GC/MS. Indicated concentration of the components: × = trace, ×× = medium, ××× = main. – Previously investigated stingless bee species according to the references: A *Trigona postica*, B *Trigona depilis*, C *Trigona tubiba*, D *Trigona xanthotricha*, E *Trigona mexicana*, F *Trigona pectoralis*, G *Trigona bipunctata*, H *Trigona spinipes*, I *Trigona benjoim*, J *Trigona carbonaria*, K *Trigona gribodoi*.

No.	Compounds	newly emerged	nurse	garbage carrier	guard	pollen forager	Insect spp. (Literature)
1	2-Heptanone	×	×	××	××	××	A, B, C, D (14); E, F (15); K (13)
2	Dodecane	×	×	×	×	×	
3	3-Methyl-1-butanol	×	×		×	×	
4	2-Hexanol	×		×	×	×	
5	Tridecane	×	×	×	×	×	
6	2-Heptanol	×	××	××	××	×××	A, B, C, D, G (14); E, F (15); H (16)
7	Hexanol	×	×	×	×	×	
8	2-Nonanone	×	×	×	×	×	A, B, C, D (14); E, F (15)
9	Tetradecane	×	×	×	×	×	
10	2-Pentylhexanoate	×	×	×	×	×	
11	2-Octanol, 1-Hexylbutanoate				×	×	
12	Hexyl-3-methylbutanoate			×	×	×	
13	3-Methylbutylhexanoate		×	×	×	×	
14	Pentandecane	×	×	×	×	×	
15	2-Nonanol	×	×	××	××	××	I (14); E, F (15); H (16); K (13)
16	Benzaldehyd		×	××	××	××	A, B, C, D (14); E, F (15)
17	2-Heptylhexanoate		×	×	×	××	
18	2-Undecanone	××	××	××	××	××	A, B, C, D (14); E, F (15)
19	Hexylhexanoate		×	××	××	××	
20	Undecenone	×	×	×	×	×	
21	2-Undecanol	×	××	××	××	×	A, B (14); F, G (15)
22	Hexyl-E-2-hexenoate			×	×	×	
23	2-Tridecanone	×××	×××	×××	×××	×××	A, B, C, D (14); E, F (15); G (5)
24	2-Tridecenone (a)	××	×	×	×	×	
25	2-Tridecenone (b)	××	×	×	×	×	
26	2-Phenylethanol			×	×	×	
27	2-Tridecanol	××	××	××	××	××	B, D (14); E, F (15); H (16)
28	2-Pentadecanone	×××	×××	×××	×××	×××	A, B, C, D (14); E, F (15)
29	Dodecylbutanoate	×	×	×	××	×	
30	2-Pentadecanol	×	××	××	××	××	A, B (14); E, F (15)
31	γ-Decalactone	×	×				J (17)
32	2-Heptadecanone	××	××	××	××	××	A, B, C, D (14); E, F (15)
33	Tetradecylbutanoate	××	×	××	××	××	
34	*Z*-5-Tetradecenylbutanoate	×	××	××	×××	×××	
35	2-Heptadecanol	××	××	×	××	××	A, B, G (14); E, F (15)
36	*Z*-7-Hexadecenylbutanoate	×	×	××	×××	×××	

origin from the acetate pool [20]. The number of the components identified is much higher than in previous works [13–17] on stingless bees pheromones, particularly in the Trigonini. Only 14 of the 36 components we found in *Scaptotrigona postica* head extracts (Table I) have previously been reported for this or related *Trigona* species [5, 13–17].

In general, in workers older than two weeks and engaged in activities inside and outside the nest the cephalic secretion is more complex and the concentration of volatile compounds is much higher. Especially the amount of esters increases significantly with age; esters, which are very widespread among bees [21] may play an important role in intraspecific communication. Butanoic acid esters are reported as components of defensive secretions of different Hemiptera species [4] and volatile constituents of the scent mark of a South American primate [22]. Octylcaproate was previously identified from *Trigona fulviventris* [23] while octyloctanoate and octyldecanoate have been found in *Trigona spinipes* [16]. In addition to the unbranched compounds, *Trigona fulviventris* contains nerol [23], while *Trigona subterranea* uses geranial and neral as trail pheromones [6]. However, up to now we obtained no indications for the presence of terpenes in *Scaptotrigona postica*. We also did not find acetates which have been identified from other *Trigona* species [5, 14]. The esters of *Scaptotrigona postica* carry the functional group more towards the middle of the molecule, like those of the *Trigona* species mentioned above, and similar to the less volatile honey bee wax esters [21].

Our results confirm the only data presently available on compositional changes with age in a South African species, *Trigona gribodoi*, of which two developmental stages of adults workers, namely nurse bees and foragers, were analysed [13]. Of the eight compounds identified for *Trigona gribodoi*, 2-heptanone was predominat in the mandibular gland secretion of foragers, but in *Scaptotrigona postica* it is present only in medium concentrations (Table I).

The age-dependent polyethism described for stingless bees [12, 19] seems to include more complicated pheromone interactions especially in communications between older workers engaged in tasks inside as well as outside the nest. Biotests are planned to analyse this presently nearly unknown chemical language. Caste and sex specific bouquets of volatiles and their function in the communication system have to be studied. Determination of double bond position in some of the compounds described here is under investigation.

Acknowledgements

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