Short Communication

Marking Pheromones of *Megabombus* Bumble Bee Males

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Bumble bee males patrol along flight-path circuits, and mark objects i.e. trees, bushes and tufts of grass, with a pheromone secretion to attract virgin females for mating.[1] We have previously analysed this secretion, which emanates from the labial gland in males belonging to the genera *Bombus, Pyrobombus, Alpinobombus* and *Psithyrus*.[2–5] Each species has a characteristic blend of straight-chain fatty acid derivatives, especially esters, and acyclic mono-, sesqui- and di-terpenes, which are alcohols, aldehydes and acetates. This study reports the chemical analysis of marking pheromone secretions from male bumble bees belonging to species of the genus *Megabombus* (Hymenoptera, Apidae). The chemical analyses were carried out as a prerequisite for studies on the behavioural, species isolating, and evolutionary role of the pheromones used in flight-path marking. In the studies reported herein, we have analysed most of the ca. 30 species of bumble bees occurring in Scandinavia.

Seven species were investigated: three in the subgenus *Thoracobombus,* two in *Subterraneobombus* and two in *Megabombus*.[6] Four are reported on here for the first time, namely *M.(T.) veteranus, M.(T.) humulis, M.(S.) distinguendus* and *M.(M.) consobrinus*. Complementary studies, carried out on *M.(T.) muscorum, M.(S.) subterraneus* and *M.(M.) hortorum,* have been briefly reported earlier.[2] In addition, previous results from three *Thoracobombus* species, *M.(T.) sylvarum, M.(T.) pascuorum* and *M.(T.) ruderarius,*[7,8] are included here for comparison.

Table 1. Analysed material of *Megabombus* male bumble bees.

Species	No	Country: locality
M.(T.) veteranus	1	Finland: Uukuniemi
M.(T.) humulis	4	Sweden: Fiskebäckskil
M.(T.) muscorum	3	Sweden: Fiskebäckskil, Segerstad
M.(S.) subterraneus	15	Sweden: Uppsala
M.(S.) distinguendus	2	Sweden: Mora
	2	Austria: Seefeld
M.(M.) hortorum	12	Sweden: Mora, Torslunda, Uppsala
M.(M.) consobrinus	2	Sweden: Tärnaby
	10	Norway: Enger

Materials and methods

Biological material. The collection sites and number of specimens used for each species are shown in Table 1. The nomenclature follows that of Reinig.[6] Reference specimens are deposited in B. G. Svenssons collections.

Chemical analysis. Volatile material from the bumble bees was isolated by extracting single whole heads or labial glands dissected from single individuals in 0.2 ml distilled hexane. No appreciable differences in chemical composition were found between the two extraction methods.[9,10] Heads and glands were extracted for one day and the extracts kept at −20 °C for later analysis. The chemical analyses were made by combined gas chromatography–mass spectrometry (GC–MS). The fused silica capillary columns (25 m × 0.2 mm ID) were coated with either OV-351 or Superox FA. A column coated with Silar 88 (25 m × 0.22 mm ID) was used to determine the geometrical (*E,Z*) isomerism, especially when the unsaturation was located in the middle of the hydrocarbon chain. Volatile compounds were identified by comparison of mass spectral data and gas chromatographic retention values with those of reference compounds; double bond positions were determined, after fractionation by GC,[11] by ozonolysis[2,7,12] and by epoxidation.[2,7,13]

Results

The major components of the male labial gland volatiles in the ten species of the genus *Megabombus* are given in Table 2. The characteristic marking compounds are sesqui- and di-terpenes, straight-chain monounsaturated aldehydes, alcohols and acetates, and one C_{19}-hydrocarbon. All of the secretions also contained substantial amounts of the odd-numbered straight-chain saturated and monounsat-

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Table 2. Relative amounts (%) of the major compounds in the labial glands of male bumble bees belonging to the genus *Megabombus.*

Mol. wt.	Compound	Species									
		M.(M.) hortorum	*M.(M.) consobrinus*	*M.(T.) pascuorum*[a]	*M.(T.) humulis*	*M.(T.) muscorum*	*M.(T.) ruderarius*[b]	*M.(T.) sylvarum*[b]	*M.(T.) veteranus*	*M.(S.) subterraneus*	*M.(S.) distinguendus*
222	all-*trans*-Farnesol	17									
288	Geranylgeranial	21	3								96
290	Geranylcitronellal									100	
290	Geranyllinalool										4
238	Hexadecenal			40[c]	4[e]						
266	Octadecenal				32[f]						
294	Icosenal				3						
240	Hexadecenol			60[c]	44[d]	48[d]	88[d]	29[d]	65		
268	Octadecenol	7[d]	1		8[f]		12[d]	2	15		
296	Icosenol				6						
282	Hexadecenyl acetate				3			69[c]	15		
310	Octadecenyl acetate					52[d]			5		
266	Nonadecene	55[d]	96[d]								

[a]Data from Ref. 7. [b]Data from Ref. 2. [c]*Z*-7. [d]*Z*-9. [e]Δ-9. [f]Δ-11.

urated hydrocarbons, C_{21}–C_{27}, which commonly occur in the insect cuticle. They are not included in Table 2 since they were present in all species and are probably not behaviourally significant.

With respect to the species studied for the first time, the following results were obtained. The major volatile compounds of one individual *M.(T.) veteranus* was found to be monounsaturated alcohols and acetates, the predominant one being a hexadecenol. *M.(T.) humulis* secretions had monounsaturated alcohols, aldehydes and one acetate, including predominantly (*Z*)-9-hexadecenol. In *M.(S.) distinguendus* the major compound was geranylgeranial, detected together with smaller amounts of a geranyllinalool isomer. Analyses of *M.(M.) consobrinus,* showed the major component to be (*Z*)-9-nonadecene, also present in *M.(M.) hortorum.*[2] Geranylgeranial was the major component in *M.(S.) distinguendus.*

The complementary work on *M.(T.) muscorum, M.(S.) subterraneus* and *M.(M.) hortorum,* performed in order to confirm the presence of the major components and to determine the double bond positions in the unsaturated compounds, yielded the following results. *M.(T.) muscorum* contains (*Z*)-9-octadecenol and (*Z*)-9-octadecenyl acetate. In *M.(S.) subterraneus* geranylcitronellal is the major component. In *M.(M.) hortorum* the four main components are (*Z*)-9-nonadecene, geranylgeranial, all-*trans*-farnesol and (*Z*)-9-octadecenol.

Chemical comparison within and between Megabombus *subgenera.* Each of the three subgenera *Thoracobombus, Subterraneobombus* and *Megabombus* are characterized by, respectively, isoprenoids (sesqui- and di-terpenes), fatty acid derivatives (straight-chain monounsaturated aldehydes, alcohols and acetates with 16, 18 and 20 carbon atoms) and (*Z*)-9-nonadecene, see Table 2. Within *Thoracobombus,* the six species differ in relative amounts of isomers of the straight-chain monounsaturated aldehydes, alcohols and acetates. This situation is similar to findings for sex pheromones in Lepidoptera[14] and in many other insects.[15] All six *Thoracobombus* species have one or two major components (44–88 %); no isoprenoids were detected. Large amounts of the two diterpenes geranylgeranial and geranylcitronellal are characteristic of the two *Subterraneobombus* species, and in contrast with *Thoracobombus,* no fatty acid derivatives are present. The dominant volatile compound in the two species of the subgenus *Megabombus* is (*Z*)-9-nonadecene. This compound has not been found in any other of the labial gland secretions so far studied.[3] In addition to (*Z*)-9-nonadecene, *M.(M.) hortorum* is characterized by substantial amounts of all-*trans*-farnesol, geranylgeranial and an octadecenol. The three subgenera are thus distinctly different and, in fact, each of them shows larger similarities with species belonging to subgenera other than the genus *Megabombus (Bombus, Pyrobombus, Alpinobombus).*[3–5]

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