Are there pheromonal dominance signals in the bumblebee *Bombus hypnorum* L (Hymenoptera, Apidae)?

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Summary — In the bumblebee *Bombus hypnorum* (Apidae) 132 chemical compounds could be identified by GC/MS and coinjection. The behavior of individual workers was observed to characterize them as dominant, subordinate or foragers. Queens, dominance groups of workers and newly emerged workers showed significant differences in both the amounts and proportions of volatiles, in the status of the ovaries, in the size of the fat body, and in the body size.

bumblebee / *Bombus hypnorum* / dominance / volatile bouquets / chemical analysis

INTRODUCTION

In all eusocial Hymenoptera queen control over workers is probably inseparable from the mechanism of queen recognition (Fletcher and Ross, 1985; Keller and Nonacs, 1993). The recognition / inhibition system hypothesized for highly eusocial Hymenoptera may be derived from a simpler recognition system of primitively eusocial species where each individual is recognized by a specific composition of the odor components (Michener, 1977). In primitively eusocial halictine bees, pheromonal queen

recognition has been demonstrated (Buckle, 1982), and in primitively eusocial *Polistes* wasps, chemical cues may function to maintain dominance hierarchies in conjunction with direct aggression (West-Eberhard, 1981). The evolution of specific queen pheromones might coincide with the development of a larger worker population inside the nest (Velthuis, 1976). In such cases, agonistic behavior by a queen towards all individuals is impossible and the single queen needs to develop a warning or recognition system to extend her influence presence beyond the reach of her mandibles

and sting. In honey bees, the queen's pheromone is transmitted within the colony mainly by retinue workers (Naumann *et al*, 1991).

Bumblebees, like many other primitively eusocial bees, have 2 castes: queens and workers (Michener, 1974). Queens are larger than workers, live longer and mate. Only the mated queens lay fertilized eggs. Therefore, they alone are responsible for the production of female offspring. Among the workers, a division of labor exists. There are foragers, guard bees and nest bees. Furthermore, a dominance hierarchy is established among them which is partly connected to the different tasks performed by the workers. Some of them show queen-like behavior; they become egg-layers when the queen loses her dominance at the end of colonial cycle. Dominant workers show aggressive behavior towards other workers (van Doorn, 1988; Röseler and van Honk, 1990).

The fertility of workers is not only controlled by the presence or absence of a dominant queen but also by other dominant workers (Röseler and van Honk, 1990). In *Bombus terrestris*, the queen retards the development of workers' ovaries by means of pheromones (van Honk *et al*, 1980; Röseler *et al*, 1981) and she inhibits oviposition when workers have fully developed ovaries (van Honk *et al*, 1980, 1981). The queen's inhibitory pheromone is produced in her mandibular glands and spread over her body surface (Röseler *et al*, 1981). If the foundress queen is lost or experimentally removed, ovaries start to develop and eggs are produced in one or more of her orphaned workers. Whether some workers retard the ovarian development of others and stabilize the dominance hierarchy by pheromonal control (Katayama, 1974; Röseler and Röseler, 1977) or by specific dominance behavior is as yet unknown. Furthermore, no chemical analyses have been performed to identify the queen pheromone.

In contrast to *B. terrestris*, no evidence was found in *B. impatiens* for a queen-derived pheromonal dominance signal (Pomeroy, 1981); queens with their mandibles removed were reported to retain their dominant position by physical interactions with the workers, which might suggest that the mechanism or existence of pheromonal control may differ from species to species. In more primitive species with a relatively small number of workers, a poorly developed use of pheromones and a greater reliance upon agonistic behavior may lead to a queen's dominance.

B. hypnorum is presumably a bumblebee species representing a more primitive state of social evolution in comparison to *B. terrestris*, having smaller colonies and a weaker caste polymorphism. Furthermore, similar to honeybees, in *B. terrestris* larvae are determined to become workers or queens during their first 3.5 d, while in *B. hypnorum* the amount of food a larva receives throughout its whole period of development influences the caste determination (Röseler, 1970). In a study analyzing the Dufour's gland secretion of *B. hypnorum* (Hefetz *et al*, 1993), intertidal and intranidal variation in the secretions of workers was found. Workers within a nest clustered into defined odor groups according to their glandular composition. In addition, there was a correlation between the odor patterns and the size of the bees that might reflect different dominance groups of workers.

The objectives of our present study were to investigate and compare the odor bouquets of queens and different functional worker groups of *B. hypnorum*, with the intention of evaluating the evolution of caste- and sub-caste-specific pheromonal recognition or dominance signals, which has not yet been studied in bumblebees. Volatiles from different sources and individual bees were analyzed by capillary GC and GC/MS.

MATERIALS AND METHODS

Rearing bumblebees

In May 1993, established colonies of *B (Pyrobombus) hypnorum* (L.) were collected from various locations surrounding the Ecological Research Station of Uppsala University at Öland, SE Sweden. Prior to transferring the bees into artificial nesting boxes, each female was individually marked with a number on her scutum. The artificial nesting boxes allowed workers to forage in the field.

Behavioral observations

From mid-June to mid-July 1993, 3 laboratory nests were observed at least 3 times a day under red light. The interactions between the workers were recorded during periods of 20 min. Seventeen different behavioral patterns were scored (see *Results*). Based on their behavior, 3 different groups ('dominant workers', 'subordinate workers' and 'foragers') were distinguished.

Sample collection

Workers belonging to the 3 behavioral groups, plus newly emerged workers (0–2 h old), and egg-laying queens were collected to obtain headspace samples. Individuals of the worker groups and queens were killed by freezing and stored at -70°C until dissection.

Individual headspace samples were obtained by placing 1 bee in a glass vial (volume: 30 ml) for 30 min. After careful removal of the bee, the vial was stored at -20°C for at least 40 min to condense the odor compounds onto the inner glass surface. Subsequently, the glass surface was rinsed with 3 ml pentane (Merck, Uvasol), and the sample was concentrated in a capillary flask to a final volume of 20–40 μl . Cuticular washings were obtained by rinsing an individual abdomen for 30 s in pentane (worker: 0.5 ml; queen: 1.0 ml). Heads were cut off from the thorax, and the mandibles were gently opened to allow solvent penetration and extracted in 0.2 ml pentane for 24 h. Individual Dufour's glands were

extracted in 0.1 ml pentane (Uvasol, Merck) for 24 h.

Size and reproductive status

Prior to dissection, the size of the bees was determined by measuring the head width and 1 distance of the right forewing (distance from the proximal tip of the discoidal cell to distal tip of the radial cell). The size of the fat body was recorded as large (1), intermediate (2), small (3), or very small (4) and the status of the ovaries was examined. Six ovarian stages were distinguished: 1) ovarioles small, all follicles beyond the vitellogenic phase; 2) vitellogenesis started; 3) vitellogenesis in a progressive phase; 4) ovarioles enlarged, no matured eggs; 5) ovarioles large, with only a few matured eggs; and 6) ovarioles large, with several matured eggs (see Michener *et al.*, 1971).

Chemical analysis

A Hewlett-Packard 5890 Series II gas chromatograph, equipped with a DB-5 capillary column (30 m $\times$ 0.32 μm) was used, operating at 120°C for 30 s, followed by programming to 290°C at $4^{\circ}\text{C}/\text{min}$ (constant flow, 1.6 ml/min). For analyses of absolute quantities *n*-octadecane was added as an internal standard to all samples. GC/MS analyses were carried out with an HP 5890 coupled to a VG 70/250 SE.

Statistical analysis

Relative and total amounts of chemical classes of compounds were tested for significance of differences between queens and worker groups with an analysis of variances (Anova) followed by a multiple comparison test (LSD-test). Single compounds were used for a stepwise discriminant function analysis (Backhaus *et al.*, 1987; Norusis, 1993a,b). The contribution of each variable to the discriminant functions was assessed by its standardized canonical discriminant function coefficient.

The Spearman rank correlation coefficient was used to test the significance of the female

relationships between size of the females, size of their fat bodies, ovarian status and the total amounts of volatiles in their extracts. The size of the females of different groups was compared using the unpaired *t*-test, and the size of the fat bodies and the status of the ovaries with the Mann–Whitney *U*-test. Computations were performed using SPSS for Windows (Norusis, 1993a, b).

RESULTS

Behavioral observations, size and reproductive status

Three worker groups could be identified based on the tasks they performed and on dominance behavior. We observed significant differences between dominant, sub-

ordinate workers and foragers in 11 of 17 behavioral patterns (fig 1). Compared to subordinate workers and foragers, dominant workers were most aggressive in interactions with other bees. Dominant behavior included bumping and biting but also egg laying. Furthermore, dominant workers had significantly larger ovaries (fig 2) compared to the other worker groups (Mann–Whitney, $n = 48$, $p < 0.001$) and tended to have a large fat body (not significantly different, $p > 0.05$); 17% showed a large and 60% an intermediate fat body. Subordinate workers characteristically walked around within the nest and fed the larvae. Workers' eggs were recorded in the ovarioles of only one of the 14 subordinates, while 8 of the 12 dominant ones had at least a few to several eggs. The fat body in 7% of the subordinate workers was large, and was inter-

Fig 1. Comparison of the frequency (%) of intranidal behavioral patterns of 3 worker groups. In interactions with other workers, dominant bees showed aggressive behavior such as biting and pushing away other bees with the head. * Significant difference to the other 2 groups; ° significant difference between dominant workers and foragers; + significant difference between dominant and subordinate workers; analysis of variance, $p < 0.05$. Behavioral patterns: A: entering the nest with pollen; B: leaving the nest; C: entering the nest without pollen; D: standing at the nest entrance; E: standing close to the brood cells (not warming the brood); F: standing far away from the brood; G: walking within the nest; H: warming the brood; I: feeding larvae; J: putting the head into a brood cell filled with pollen or honey; K: building brood cells; L: building the nest periphery; M: ventilating longer than 4 s; N: ventilating shorter than 4 s; O: grasping the abdomen of another bee, biting; P: push away another bee with the head, bumping; Q: egg-laying.

Fig 2. Comparison of the status of the ovaries in 3 behavioral worker groups and newly emerged workers. Dominant workers had the largest ovaries (58% had eggs ready for oviposition). In 50% of the foragers the ovarioles were enlarged and vitellogenic follicle growth was initiated. In all newly emerged workers, the ovarioles were small and the follicles were beyond the vitellogenic stage.

mediate in 57%. Foragers showed the smallest ovaries and a small fat body as well (0% large, 42% intermediate). Foragers characteristically foraged, warmed the brood, and fed larvae (fig 1). All of the newly emerged workers had small ovarioles. Interestingly, they were similar to the subordinate and dominant workers in the size of the fat body (10% large, 60% intermediate).

Queens were found to be significantly larger than workers (*t*-test, head-width (HFW): $t = 7.21$, $n = 55$, $p < 0.001$; wing length (WL): $t = 10.94$, $n = 55$, $p < 0.001$, fig 3). Within the workers, dominant bees were larger than subordinates (HFW: $t = 2.43$, $n = 26$, $p < 0.05$; WL: $t = 2.365$, $n = 26$, $p < 0.05$) and foragers (HFW: $t = 2.54$, $n = 24$, $p < 0.05$; WL: not significant, $t = 1.86$, $n = 24$, $p = 0.08$). The size of the last 2

groups was not significantly different ($n = 26$, $p > 0.05$).

Chemical analysis

In Dufour's gland (DG) and head extracts (HE), cuticular washings (CWS) and headspace samples (HS), 132 compounds could be identified by GC/MS analyses and coinjection (table I). In accordance with Tengö *et al* (1991), we found straight chain saturated and unsaturated hydrocarbons, branched hydrocarbons, carboxylic acids, ethyl and methyl esters of fatty acids, unsaturated and saturated acetates, terpenes and terpenoid esters. In all sample types, queens and the worker groups showed striking differences in both total amounts (fig 4) and relative proportions of volatiles (tables II–V).

Fig 3. Size of queens and different worker groups. We measured the head width (HFW, □) and the distance of the right forewing (WL, ○). Queens were significantly larger than workers (*t*-test, $n = 55$, $p < 0.001$, HFW, WL). Among the different worker groups dominant workers were larger than subordinate ones (*t*-test, $n = 25$, $p < 0.05$, HFW, WL) or foragers (*t*-test, $n = 24$, $p < 0.05$, HFW, WL).

Qualitative differences between sample types

With a few exceptions almost no qualitative differences were found between the different sample types. Geranyl citronellol was only identified in the cuticular washings, head extracts and headspace samples, but not in the Dufour's glands. The corresponding wax-type esters (geranyl citronellyl hexadecenoate and octadecenoate) could be found in all sample types. While their relative amounts were below 1% in the Dufour's gland and below 0.5% in the cuticular washings and the headspace samples, they represent the main compounds in the head extracts, with a relative amount of 20% in workers and more than 30% in queens.

n-Alkanes and alkenes were found to be the major chemical classes of compounds in the Dufour's glands and in the cuticular washings and headspace samples.

Quantitative differences—absolute amounts

The absolute amounts of volatiles were found to be highest in breeding queens (3–7-fold the amount in comparison to the workers, fig 4). Furthermore, they were higher in dominant than in subordinate workers. Smaller amounts of volatiles could be detected in foragers, and the smallest in newly emerged workers. The mean amount of volatile material in the head extracts and the cuticular washings was similar in differ-

Table I. Volatile compounds identified in *Bombus hypnorum*.

<i>Alkanes</i>	(Z)-13-Hexacosene	Geranyl citronellyl tetradecanoate
Tetradecane	(Z)-11-Hexacosene	Geranyl citronellyl tetradecenoate*
Nonadecane	(Z)-10-Hexacosene	Geranyl citronellyl hexadecenoate*
Eicosane	(Z)-9-Hexacosene	Geranyl citronellyl octadecenoate*
Heneicosane	(Z)-7-Hexacosene	Geranyl citronellyl eicosanoate
Docosane	(Z)-13-Heptacosene	Geranyl citronellyl eicosenoate*
Tricosane	(Z)-12-Heptacosene	
Tetracosane	(Z)-11-Heptacosene	<i>Carboxylic acids</i>
Pentacosane	(Z)-9-Heptacosene	Decanoic acid
Hexacosane	(Z)-7-Heptacosene	Dodecanoic acid
Heptacosane	(Z)-14-Octacosane	Tetradecanoic acid
Octacosane	(Z)-13-Octacosane	Hexadecanoic acid
Nonacosane	(Z)-12-Octacosane	Octadecanoic acid
Triacontane	(Z)-11-Octacosane	9-Hexadecenoic acid
Hentriacontane	(Z)-10-Octacosane	9-Octadecenoic acid
	(Z)-14-Nonacosene	9,12-Octadecadienoic acid
<i>Branched alkanes</i>	(Z)-13-Nonacosene	Octadecatrienoic acid*
11-Methyl tricosane	(Z)-12-Nonacosene	
9-Methyl tricosane	(Z)-11-Nonacosene	<i>Acetates</i>
7-Methyl tricosane	(Z)-10-Nonacosene	Hexadecyl acetate
5-Methyl tricosane	(Z)-9-Nonacosene	Octadecyl acetate
3-Methyl tricosane	(Z)-7-Nonacosene	Eicosyl acetate
13-Methyl pentacosane	(Z)-9-Triacontene	Docosyl acetate
11-Methyl pentacosane	(Z)-15-Hentriacontene	Tetracosyl acetate
9-Methyl pentacosane	(Z)-14-Hentriacontene	(Z)-7-Hexadecenyl acetate
7-Methyl pentacosane	(Z)-12-Hentriacontene	(Z)-9-Octadecenyl acetate
5-Methyl pentacosane	(Z)-11-Hentriacontene	(Z)-11-Eicosenyl acetate
3-Methyl pentacosane	(Z)-9-Hentriacontene	Docosenyl acetate*
13-Methyl heptacosane	(Z)-7-Hentriacontene	
11-Methyl heptacosane		<i>Ethyl esters</i>
9-Methyl heptacosane	<i>Alkadienes</i>	Ethyl hexadecanoate
3-Methyl heptacosane	Tricosadiene*	Ethyl octadecanoate
15-Methyl nonacosane	Pentacosadiene*	Ethyl (Z)-9-octadecenoate
13-Methyl nonacosane	Hexacosadiene*	Ethyl octadecadienoate*
11-Methyl nonacosane	9,15-Heptacosadiene	
<i>Alkenes</i>	9,17-Heptacosadiene	<i>Further esters</i>
(Z)-9-Heneicosene	9,17-Octacosadiene	Hexadecenyl decanoate*
(Z)-7-Heneicosene	7,17-Nonacosadiene*	Hexadecanyl dodecanoate*
(Z)-9-Docosene	7,19-Nonacosadiene	Hexadecenyl hexadecenoate*
(Z)-11-Tricosene	9,17-Nonacosadiene	Hexadecenyl octadecenoate*
(Z)-9-Tricosene	9,19-Nonacosadiene	Eicosyl octadecenoate*
(Z)-7-Tricosene	9,21-Nonacosadiene	Docosyl octadecenoate*
(Z)-9-Tetracosene	Triacontadiene*	Tetracosyl octadecenoate*
(Z)-7-Tetracosene	9,19-Hentriacontadiene	Hexacosyl octadecenoate*
(Z)-12-Pentacosene	9,21-Hentriacontadiene	
(Z)-11-Pentacosene	<i>Terpenes</i>	<i>Ketones</i>
(Z)-10-Pentacosene	Geranyl citronellol	2-Nonanone
(Z)-9-Pentacosene	Geranyl citronellyl decanoate	2-Undecanone
(Z)-8-Pentacosene	Geranyl citronellyl dodecanoate	
(Z)-7-Pentacosene		

* Position of double bond has not been determined.

Fig 4. Total amounts of volatiles in different odor samples of breeding queens, 3 functional worker groups and newly emerged workers. The total amounts of volatiles were highest in queens, and higher in dominant than in subordinate workers. The smallest amounts were found in newly emerged bees. In the headspace samples the amount of volatiles released during 30 min is shown (* significant difference, analysis of variance, $p < 0.05$).

ent worker groups, but differed from those of the queens. In the cuticular washings approximately 270 µg could be identified and about half the amount (130 µg) in the head extracts of queens. The total amount of volatiles in the Dufour's gland was smaller (fig 4).

A canonical discriminant analysis significantly separated queens and worker groups for each sample type for absolute volatile amounts (DG: χ^2 : 331.38, DF: 52, $p < 0.0001$; HE: χ^2 : 271.58, DF: 44, $p < 0.0001$; CWS: χ^2 : 472.73; DF: 72; $p < 0.0001$). The first discriminant function explained 82% of the variation in the head extracts, 79% in the Dufour's gland and almost 100% in the cuticular washings. Geranyl citronellol (in HE and CWS), alkenes ((Z)-11-pentacosene, (Z)-7-pentacosene), branched alkanes (methyl tricosane, methyl pentacosene), alkadienes (heptacosadiene, octacosadiene, tricontadiene) and some

unidentified compounds contributed most to the overall discriminant functions; they showed large standardized canonical discriminant function coefficients in all sample types. Only the headspace samples of dominant and subordinate workers were compared with a discriminant analysis (χ^2 : 19.16, DF: 3, $p < 0.001$, fig 4) because of small sample sizes in queens and foragers.

Quantitative differences—relative amounts

Whereas dominant and subordinate workers showed very similar patterns of volatiles, they differed from queens, foragers and newly emerged workers. The proportions of branched alkanes in Dufour's gland and head extracts were higher in dominant bees than other workers (tables II and III). Foragers were different from all of the other

Fig 5. Occupants of bees of 4 worker sub-groups and queens plotted on the first, second and third canonical discriminant function (DSC-function). Relative proportions of single compounds of the cuticular washings of the surface were used to perform a canonical discriminant analysis, which showed significant differences (χ^2 : 272.4, DF: 48, $p < 0.0001$). The odor bouquets of the queens were clearly different from the ones identified in the worker bees. Among the workers dominant ones showed the most similar volatile patterns in comparison to the queens. Groups of females: ∇ young worker; $\triangle$ subordinate; $\diamond$ dominant; $\circ$ queen.

worker groups in having higher proportions of unsaturated acetates and alkadienes in Dufour's gland and cuticular washings (table IV). The odor bouquets of queens were significantly different from those identified in the worker bees and were characterized by the highest amount of branched alkanes and wax-type esters, while the amounts of saturated and unsaturated fatty acids were small. Dominant workers showed a pattern of volatiles similar to queens.

Canonical discriminant analyses successfully separated queens and worker groups for each sample type for relative

volatile amounts (DG: χ^2 : 363.03, DF: 24, $p < 0.0001$; HE: χ^2 : 273.42, DF: 52, $p < 0.0001$; CWS: χ^2 : 272.4, DF: 48, $p < 0.0001$; HS of dominant and subordinate workers: χ^2 : 43.58, DF: 6, $p < 0.0001$, tables II–V). More than 90% of the variance was explained by only 1 discriminant function for each sample type. Examination of standardized canonical discriminant function coefficients again showed branched alkanes, alkenes, alkadienes and geranyl citronellol of particular relevance in the separation among groups. The power of a discriminant function can also be shown by the

Table II. Volatile patterns of Dufour's gland secretions in queens and worker groups of the bumblebee *B hypnorum*.

Compound class	Queen		Dominant		Subordinate		Forager		Newly emerged		Analysis of variances LSD test ($p < 0.05$), significant difference ^a
	x n = 5	S _x	x n = 12	S _x	x n = 14	S _x	x n = 12	S _x	x n = 10	S _x	
<i>Relative proportions (%)</i>											
<i>n</i> -Alkanes	40.54	4.11	38.94	4.65	38.85	3.58	29.91	2.79	37.46	1.03	
Alkanes, branched	6.41	0.75	3.26	0.61	1.73	0.27	0.57	0.2	0.58	0.21	QD, QS, QF, QY, DS, SF, DY, SF
Alkenes	40.80	3.91	37.31	2.09	34.52	2.61	28.02	3.64	51.72	0.81	QF, QY, DF, DY, SY, FY
Alkadienes	2.12	0.43	0.95	0.15	1.11	0.31	1.32	0.33	0.36	0.14	QD, QS, QY, FY
Carboxylic acids, saturated	0.26	0.05	0.28	0.08	1.14	0.40	1.54	0.43	2.04	0.13	QF, QY, DS, DF, DY
Carboxylic acids, unsaturated	0.39	0.20	0.20	0.14	0.43	0.25	0.24	0.11	0.12	0.12	
Acetates, saturated	0.39	0.07	0.19	0.04	0.09	0.02	0.05	0.02	0.27	0.27	
Acetates, unsaturated	0.33	0.13	7.67	2.43	9.55	2.61	21.51	3.00	0.84	0.41	QS, QF, DF, SF, SY, FY
Terpenoid esters*	0.84	0.30	0.03	0.01	0.03	0.02	0.13	0.07	0	0	QD, QS, QF, QY
<i>Amount (μg)</i>											
<i>n</i> -Alkanes	17.27	6.99	5.22	1.05	2.45	0.81	1.07	0.41	0.27	0.04	QD, QS, QF, QY, DY
Alkanes, branched	3.29	1.53	0.50	0.14	0.12	0.05	0.03	0.02	0	0	QD, QS, QF, QY
Alkenes	22.53	10.51	5.97	1.50	2.98	1.41	1.08	0.57	0.37	0.05	QD, QS, QF, QY
Alkadienes	0.86	0.41	0.20	0.07	0.17	0.07	0.04	0.02	0	0	QD, QS, QF, QY
Carboxylic acids, saturated	0.10	0.04	0.02	0	0.02	0	0.03	0.01	0.01	0	QD, QS, QF, QY
Carboxylic acids, unsaturated	0.10	0.03	0.06	0.04	0.07	0.04	0.01	0.01	0	0	
Acetates, saturated	0.22	0.08	0.10	0.03	0.07	0.03	0.03	0.01	0	0	QD, QS, QF, QY, DY
Acetates, unsaturated	0.25	0.14	1.74	0.79	1.55	0.65	0.53	0.17	0.01	0	DY, SY
Terpenoid esters*	0.56	0.32	0.01	0	0.01	0.01	0.01	0.01	0	0	QD, QS, QF, QY
Total amount	48.46	20.35	16.49	4.25	9.35	3.77	3.35	1.11	0.72	0.10	QD, QS, QF, QY, DY

Altogether 67 single compounds were included to calculate relative and total amounts of chemical classes of compounds. Tests for significance of differences with an analysis of variances (Anova) followed by a multiple comparison test (LSD test). * Includes ethyl esters in trace amounts. Due to individual variation, the values for relative proportions and total amounts are not congruent in all cases. ^a Significant differences between: QD: queens and dominant workers; QS: queens and subordinate workers; QF: queens and foragers; QY: queens and newly emerged workers; DS: dominant and subordinate workers; DF: dominant workers and foragers; DY: dominant and newly emerged workers; SF: subordinate workers and foragers; SY: subordinate and newly emerged workers; FY: foragers and newly emerged workers.

Table III. Volatile patterns of head extract in queens and worker groups of the bumblebee *B hypnorum*.

Compound class	Queen		Dominant		Subordinate		Forager		Newly emerged		Analysis of variances LSD test ($p < 0.05$), significant difference ^a
	x	S _x	x	S _x	x	S _x	x	S _x	x	S _x	
	n = 7		n = 12		n = 14		n = 12		n = 10		
<i>Relative proportions (%)</i>											
n-Alkanes	16.41	1.86	16.10	0.77	19.48	2.11	18.29	1.57	29.27	1.81	QY, DY, SY, FY
Alkanes, branched	1.45	2.39	0.75	0.05	0.76	0.07	0.37	0.05	0.63	0.05	QD, QS, QF, QY, DF, SF, FY
Alkenes	18.53	2.17	14.71	1.03	16.15	1.05	13.24	1.13	35.54	2.06	QF, QY, DY, SY, FY
Alkadienes	0.42	0.13	0.20	0.04	0.23	0.03	0.75	0.11	0.48	0.06	QF, DF, DY, SF, SY, FY
Terpene	10.24	2.77	15.58	2.48	15.18	3.09	17.19	2.97	0.04	0.02	QY, DY, SY, FY
Carboxylic acids, saturated	0.10	0.03	0.35	0.07	0.22	0.05	0.51	0.31	1.50	0.31	QY, DY, SY, FY
Carboxylic acids, unsaturated	3.57	1.98	5.95	0.93	3.89	0.80	5.19	2.30	13.24	1.97	QY, DY, SY, FY
Acetates, saturated	0.25	0.11	0.12	0.02	0.13	0.02	0.19	0.03	0.22	0.05	QD, QF, QY
Acetates, unsaturated	0.30	0.11	0.07	0.04	0.13	0.08	0.07	0.02	0	0	QF, QY, DF, DY, SY, FY
Terpenoid esters*	32.44	4.91	26.32	2.23	25.09	2.55	19.39	2.04	1.03	0.28	
<i>Amount (μg)</i>											
n-Alkanes	20.91	3.41	8.34	0.65	7.65	0.89	8.26	1.65	4.29	0.38	QD, QS, QF, QY, DY, FY
Alkanes, branched	1.84	0.33	0.40	0.05	0.29	0.03	0.16	0.02	0.09	0.01	QD, QS, QF, QY, DY
Alkenes	23.70	4.07	7.65	0.72	6.31	0.59	5.72	0.88	5.22	0.46	QD, QS, QF, QY
Alkadienes	0.60	0.22	0.11	0.03	0.09	0.01	0.37	0.10	0.07	0.01	QD, QS, QF, QY, DF, SF, FY
Terpene	15.91	7.60	8.22	1.71	5.89	1.23	8.27	1.83	0.01	0	QS, QY, DY, FY
Carboxylic acids, saturated	0.12	0.04	0.18	0.05	0.09	0.02	0.12	0.03	0.21	0.03	DS, SY
Carboxylic acids, unsaturated	4.71	3.09	3.05	0.53	1.47	0.35	1.53	0.38	1.85	0.19	QS, QF
Acetates, saturated	0.30	0.10	0.06	0.01	0.05	0.01	0.08	0.01	0.03	0.01	QD, QS, QF, QY
Acetates, unsaturated	0.49	0.19	0.03	0.02	0.07	0.04	0.03	0.01	0	0	QD, QS, QF, QY
Terpenoid esters*	43.99	13.64	13.93	1.75	10.36	1.68	9.14	1.47	0.13	0.02	QD, QS, QF, QY, DY
Total amount	134.76	28.61	52.60	4.26	40.41	3.56	44.04	5.96	14.50	0.91	QD, QS, QF, QY, DY, SY, FY

* Includes ethyl esters in trace amounts. Due to individual variation, the values for relative proportions and total amounts are not congruent in all cases. ^a See table II for abbreviations.

Table IV. Comparison of volatiles in cuticular washings of the surface of various worker groups and breeding queens.

Compound class	Queen		Dominant		Subordinate		Forager		Newly emerged		Analysis of variances LSD test (p < 0.05), significant difference ^a
	x n = 7	S _x	x n = 12	S _x	x n = 14	S _x	x n = 12	S _x	x n = 10	S _x	
Relative proportions (%)											
n-Alkanes	56.49	5.33	57.85	1.21	54.95	1.18	55.28	3.17	54.86	2.44	
Alkanes, branched	0.98	0.26	0.62	0.10	0.46	0.05	0.37	0.04	0.77	0.14	QD, QS, QF, SF, SY
Alkenes	26.54	2.43	22.24	1.40	23.47	0.98	26.27	1.61	34.82	2.55	QY, DY, SY, FY
Alkadienes	1.47	1.13	0.08	0.02	0.09	0.05	0.47	0.18	0.20	0.05	QD, QS, QY
Terpene	0.20	0.12	0	0	0	0	0	0	0	0	QD, QS, QF, QY
Carboxylic acids, saturated	0.05	0.01	0.15	0.02	0.18	0.02	0.48	0.08	0.71	0.09	QF, QY, DF, DY, SF, SY, FY
Carboxylic acids, unsaturated	0.08	0.05	0	0	0.03	0.03	0	0	0	0	QD, QF
Acetates, saturated	0.68	0.21	4.17	0.34	4.41	0.66	1.95	0.83	0.77	0.20	QD, QS, QF, QY, SF, SY
Acetates, unsaturated	0.64	0.24	0.59	0.31	0.72	0.26	3.85	2.11	0.11	0.07	DF, SF, FY
Terpenoid esters*	0.47	0.21	0.06	0.01	0.03	0.02	0.22	0.12	0	0	QD, QS, QY
Amount (μg)											
n-Alkanes	159.65	46.54	42.27	7.38	26.72	3.04	25.36	14.90	7.10	0.97	QD, QS, QF, QY
Alkanes, branched	1.79	0.34	0.47	0.10	0.22	0.03	0.15	0.08	0.08	0.02	QD, QS, QF, QY, DF, DY
Alkenes	65.37	14.39	15.17	2.11	11.06	1.05	9.36	4.41	4.12	0.36	QD, QS, QF, QY
Alkadienes	4.82	4.16	0.05	0.02	0.03	0.01	0.08	0.03	0.02	0	QD, QS, QF, QY
Terpene	0.26	0.08	0	0	0	0	0	0	0	0	QD, QS, QF, QY
Carboxylic acids, saturated	0.11	0.03	0.08	0.01	0.07	0	0.09	0.01	0.08	0.01	QS
Carboxylic acids, unsaturated	0.19	0.16	0	0	0.02	0.02	0	0	0	0	QD, QS, QF, QY
Acetates, saturated	2.09	0.88	3.14	0.57	2.33	0.36	3.11	2.74	0.11	0.04	
Acetates, unsaturated	2.07	1.02	0.24	0.10	0.31	0.11	0.74	0.39	0.01	0.01	QD, QS, QF, QY
Terpenoid esters*	0.73	0.17	0.05	0.01	0.02	0.01	0.09	0.05	0	0	QD, QS, QF, QY
Total amount	274.23	66.56	71.44	11.40	48.31	4.85	46.16	27.25	12.55	1.37	QD, QS, QF, QY

* Includes ethyl esters in trace amounts. Due to individual variation, the values for relative proportions and total amounts are not congruent in all cases. ^a See table II for abbreviations.

Table V. Volatile patterns identified in headspace samples of different worker groups and queens.

Compound class	Queen		Dominant		Subordinate		Forager		Analysis of variances LSD test ($p < 0.05$), significant difference ^a
	x n = 4	S _x	x n = 11	S _x	x n = 16	S _x	x n = 22	S _x	
<i>Relative proportions (%)</i>									
n-Alkanes	48.90	4.90	47.11	2.51	41.01	3.01	40.35	4.60	
Alkanes, branched	1.81	0.50	0.96	0.14	1.00	0.21	0.27	0.27	QF
Alkenes	28.27	3.50	29.74	1.72	33.53	2.32	20.04	10.38	SF
Alkadienes	0.25	0.10	0.06	0.02	0.38	0.15	0	0	
Terpene	0.39	0.30	0.35	0.30	0.37	0.22	0	0	
Carboxylic acids, saturated	2.20	1.50	0.86	0.31	5.37	1.49	1.35	0.39	DS
Carboxylic acids, unsaturated	0.91	0.40	0.04	0.02	0.40	0.29	0	0	
Acetates, saturated	2.96	1.40	1.22	0.28	1.34	0.41	4.16	1.01	DF, SF
Acetates, unsaturated	0.06	0	1.02	0.3	0.87	0.29	3.23	1.91	QF, DF, SF
Terpenoid esters*	0.41	0.10	0.23	0.1	0.40	0.26	0	0	
<i>Amount (µg)</i>									
n-Alkanes	11.98	7.21	4.90	1.30	1.02	0.14	0.94	0.47	QD, QS, QF
Alkanes, branched	0.32	0.13	0.11	0.03	0.03	0.01	0.01	0.01	QD, QS, QF
Alkenes	5.84	2.84	2.83	0.67	0.88	0.17	0.45	0.29	QD, QS, QF, DS
Alkadienes	0.09	0.08	0.01	0	0.01	0	0	0	QD, QS, QF
Terpene	0.05	0.02	0.02	0.01	0.02	0.01	0	0	
Carboxylic acids, saturated	0.19	0.05	0.05	0.01	0.08	0.02	0.03	0.02	QD, QS, QF
Carboxylic acids, unsaturated	0.18	0.11	0.01	0	0.01	0.01	0	0	QD, QS, QF
Acetates, saturated	0.58	0.29	0.16	0.07	0.04	0.01	0.10	0.05	QD, QS, QF
Acetates, unsaturated	0.01	0	0.09	0.03	0.02	0	0.08	0.06	DS
Terpenoid esters*	0.06	0.03	0.03	0.01	0.02	0.01	0	0	
Total amount	21.90	11.80	10.17	2.56	2.47	0.35	2.34	1.18	QD, QS, QF

* Includes ethyl esters in trace amounts. Due to individual variation, the values for relative proportions and total amounts are not congruent in all cases. ^a See table II for abbreviations.

overall percentage of cases classified correctly. For the head extracts, almost 80% of the cases were classified correctly, whilst in the Dufour's gland extracts only 50% were classified correctly. While all of the queens in every sample type were classified correctly, dominant workers were more difficult to classify. Some of them were classified in the group of subordinate workers, suggesting overlap of the patterns of volatiles of dominant and subordinate workers, as can be seen in figure 5.

In all of the sample types, the total amount of volatiles was correlated with the head width (Spearman, DG: $r = 0.49$, $p < 0.0001$, $n = 55$; HE: $r = 0.64$, $p < 0.0001$, $n = 55$; CWS: $r = 0.49$, $p < 0.0001$, $n = 55$) and with the size of the ovaries (DG: $r = 0.67$, $p < 0.0001$, $n = 54$; HE: $r = 0.59$, $p < 0.0001$, $n = 54$; CWS: $r = 0.67$, $p < 0.0001$, $n = 54$) but not with the size of the fat body. In the headspace samples, a significant correlation was detected only between the size of the females and the size of the ovaries ($r = 0.45$, $p < 0.01$, $n = 33$). When the queens were excluded from the analysis, the total amount of volatiles in the CWS was still correlated with the size of the ovarioles ($r = 0.58$, $p < 0.0001$, $n = 55$), but not with the size of the females ($r = 0.26$, $p > 0.05$, $n = 48$).

DISCUSSION

Bumblebees are good candidates for investigations of the existence and evolution of queen pheromones and on the occurrence of chemical dominance signals among workers, because dominance hierarchies have been found to be based on aggressive behavior, chemical cues or both. In the bumblebee *B. terrestris*, a dominance hierarchy is established by aggressive interactions at emergence of the first workers (van Doorn, 1989). The queen dominance is maintained by the influence of pheromonal

cues (van Honk *et al*, 1980; Röseler *et al*, 1981). Our behavioral observations support the finding of Bollingmo (1989) that a dominance hierarchy is also established among workers in *B. hypnorum*. We could classify 3 worker groups: dominant workers, subordinate workers and foragers, that are characterized also by size and ovariole status. Dominant workers often showed aggressive behavior like bumping and biting threats towards other workers or even actual biting. Furthermore, they laid eggs. Accordingly, physical interactions seem to be of major importance in the establishment and maintenance of the dominance hierarchy among *B. hypnorum* workers. Observations of direct aggressive interactions between the queen and the workers were rare, however. Possibly, pheromonal queen recognition cues, acting as a queen control signal, may contribute to the maintenance of her dominant position.

A queen or worker pheromonal recognition, as hypothesized for primitively eusocial bees of more populous colonies, should be based on odor, and expressed as variation in patterns of volatile compound compositions (Hölldobler and Michener, 1980). Actually, in *B. hypnorum* we found group-specific odor bouquets in Dufour's gland and head extracts, cuticular washings, and headspace samples. Recently, caste- and subcaste-specific volatile patterns were also identified by chemical analyses in the primitively eusocial sweat bee *Lasiglossum malachurum*, (Ayasse *et al*, 1991, 1993). In both cases, bioassays prove the existence of pheromonal recognition and dominance signals remain to be performed.

Our knowledge on the glandular origin and the chemical composition of the queen pheromones in social insects is still in an early stage. Indeed, the honeybee is the only social bee species, for which queen pheromones have been characterized by chemical analyses. In the mandibular glands, a 5-component blend, consisting of

3 acids and 2 aromatic compounds has been identified (Winston and Slessor, 1992). In *B terrestris*, a queen pheromone is suggested to be produced in the mandibular glands and spread over the body surface (Röseler *et al*, 1981). Interestingly, in *B hypnorum* we found the most striking differences between the queen and worker groups in head extracts and cuticular washings, which might indicate the occurrence of a queen signal. However, abdominal glands may also contribute to characterize a queen odor. In honeybees, the mandibular glands are not the only site for production of queen pheromones; tergite glands are also involved in the secretion (Renner and Baumann, 1964). The results of our discriminant analyses showed the odor bouquet of *B hypnorum* queens to differ more from those of all groups of workers than these diverge from each other. Branched alkanes, alkadienes and geranyl citronellol were of particular relevance in characterizing queens. Geranyl citronellol was present in the cuticular washings of the queens but absent in those of workers. The total amount of volatiles in queens was 3–7-fold higher than in workers (fig 4). However, the importance of qualitative or quantitative differences in odor between queens and workers and their function in signaling pheromonal dominance is presently a matter of speculation.

According to observations of behavior within *B terrestris* colonies, "dominant workers did not have the same dominance signal as the queens, but they were nevertheless recognizable as highly dominant workers, probably by some pheromonal support" (van Doorn, 1987). In *B pratorum*, a dominant worker from a queenless group introduced into another queenless group, was recognized and attacked by the dominant worker of the latter group (Free, 1955). The pattern of volatiles of queens of *B hypnorum* was more similar to that of the dominant workers than to those of subordinate workers, for-

agers or young workers, possibly indicating that dominant workers, besides aggressive interactions, also use a chemical signal to establish and maintain a dominance position.

In queenless colonies of *B terrestris*, size was found to be the most important correlate of the rank of a worker within the dominance hierarchy (Röseler and van Honk, 1990). In queenright colonies, the size of a worker is not as important for becoming an egg-layer (van Honk *et al*, 1981; van Doorn and Hogeweg, 1985; van Doorn and Heringa, 1986). The activity of the corpora allata and ovariole status correlate with rank in *B pratorum*, as dominant bees show the most developed ovaries (Free, 1955), while there was no correlation between the size of a bee and its dominance status. In *B hypnorum* we found dominant workers to be significantly larger in comparison to subordinate ones or foragers. Furthermore, they had the largest ovaries. Interestingly, we found a stronger correlation between the size of the ovaries and the total amount of identified volatiles than between the size of a bee and the total amount of volatiles. When excluding queens from the statistical analysis, there was no correlation between the head width (representing body size) and the volatile amount on the cuticular surface, while the size of the ovaries was highly correlated with the amount of detected odor. Free (1955) previously suggested that the scent of an individual bumblebee is possibly associated with the degree of development of its ovaries.

Our data are in congruence with prevailing hypotheses on the evolution of pheromonal dominance signals in social insects. Hölldobler (1984) suggested that in bumblebees "aggressive superiority is linked to individual discriminators, thus enabling nest mates to identify the dominant individual and thereby avoiding costly physical conflicts". The queen would also profit from avoiding investment of time and

energy in repeated conflicts by the use of a chemical signal. The differences in volatile bouquets of queens and workers of *B hypnorum* might constitute the basis for a pheromonal recognition signal. Behavioral experiments to test the hypothesis of the existence of pheromonal recognition and dominance signals in *B hypnorum* are presently under investigation.

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Résumé – Existe-t-il chez le bourdon *Bombus hypnorum* L (Hymenoptera, Apidae) des signaux phéromonaux de dominance ? Nous avons identifié et comparé les bouquets odorants de reines, de divers groupes de dominance d'ouvrières et d'ouvrières fraîchement écloses de *Bombus hypnorum*. Dans un premier temps le comportement individuel des ouvrières de bourdons a été observé, ce qui a permis de les classer en dominantes, subordonnées et butineuses. Par rapport aux ouvrières subordonnées et aux butineuses, les ouvrières dominantes se sont montrées plus agressives dans leurs interactions avec les autres ouvrières (fig 1). Elles avaient des ovaires significativement plus gros (fig 2) et une tendance à avoir un corps gras développé ; en revanche les butineuses avaient les plus petits ovaires et un corps gras peu développé. On a réalisé des extraits de glandes de Dufour (DG), des extraits de têtes (HE), des lavages de cuticules (CWS) et des échantillons de phase vapeur (HS)

et identifié 132 composés (tableau I) par analyse en CG/MS (chromatographie phase gazeuse/spectrométrie de masse) et par la technique de co-injection. Les reines et les groupes d'ouvrières ont présenté des différences significatives, en fonction de leur comportement et de leur âge, dans la quantité totale de composés volatils et dans leurs proportions (fig 4, tableaux II-V). Les quantités absolues de produits volatils étaient maximales chez les reines et plus élevées chez les ouvrières dominantes que chez les subordonnées. De petites quantités ont pu être détectées chez les butineuses et les quantités les plus faibles ont été trouvées chez les ouvrières fraîchement écloses. La meilleure discrimination des sous-groupes d'ouvrières a été obtenue par les profils volatils des extraits de têtes et des lavages de cuticules. Les ouvrières dominantes et subordonnées présentent des profils très proches l'un de l'autre mais nettement différents de ceux des reines, des butineuses et des ouvrières fraîchement écloses. Les proportions relatives des alcanes ramifiés dans les extraits de glandes de Dufour et de têtes sont plus élevées chez les abeilles dominantes que chez les autres ouvrières. Les butineuses se différencient de tous les autres groupes d'ouvrières par des proportions plus fortes d'acétates insaturés et d'alkadiènes dans les extraits de glandes de Dufour et dans les lavages de cuticules. Les bouquets odorants des reines se caractérisent par la proportion la plus élevée d'alkanes ramifiés, d'alcools terpéniques et d'esters de cire et diffèrent significativement de ceux des ouvrières. Les ouvrières dominantes présentent un profil volatil semblable à celui des reines (fig 5). La discussion porte sur la signification biologique de ces résultats en relation avec l'hypothèse actuelle de l'évolution des signaux phéromonaux de dominance chez les insectes sociaux.

***Bombus hypnorum* / dominance / composé volatil / bouquet phéromonal / composition chimique**

Zusammenfassung — Gibt es pheromonale Dominanzsignale bei der Hummelart *Bombus hypnorum* L (Hymenoptera, Apidae)? Bei der Hummelart *Bombus hypnorum* wurden die Duftmuster von Königinnen, verschiedenen Arbeiterinnen-Dominanzgruppen und frisch geschlüpften Arbeiterinnen identifiziert und verglichen. In einem ersten Schritt wurden in Labornestern die Interaktionen von einzelnen Arbeiterinnen 3 mal täglich während Zeiträumen von je 20 min beobachtet. Anhand der dabei registrierten Verhaltensmuster konnten 3 unterschiedliche Arbeiterinnen-Dominanzgruppen identifiziert werden. Dominante Bienen zeigten bei Begegnungen mit subordinaten Arbeiterinnen oder Sammlerinnen aggressives Verhalten wie Beißen und Stoßen mit dem Kopf. Außerdem zeichneten sie sich durch größere Ovarien und einen großen Fettkörper aus. Sammlerinnen hingegen hatten die kleinsten Ovarien und einen kleinen Fettkörper. In Dufourdrüsen-, Kopf- und Kutikulaoberflächenextrakten sowie Headspaceproben konnten mittels GC/MS Koppelung und Koinjektionen 132 Substanzen identifiziert werden. Königinnen und durch unterschiedliche Verhaltensmuster und Alter charakterisierte Arbeiterinnengruppen zeigten signifikante Unterschiede in den relativen und absoluten Anteilen einzelner Volatile. Die Gesamtmenge an Duftstoffen war bei Königinnen am größten und bei dominanten Arbeiterinnen größer als bei subordinaten. Kleinere Substanzmengen fanden wir bei Sammlerinnen und die kleinsten bei frisch geschlüpften Arbeiterinnen. Innerhalb der Arbeiterinnengruppen ergaben sich die deutlichsten Unterschiede der Duftbouquets bei den Kopfextrakten und den der Kutikula aufgelagerten Volatilen. Dominante und subordinate Arbeiterinnen zeigten ähnliche Duftstoffmuster, während Königinnen, Sammlerinnen und frisch geschlüpfte Arbeiterinnen durch davon verschiedene Volatilbouquets charakterisiert werden konnten. In den Kopf- und Dufourdrüsenextrakten

von dominanten Arbeiterinnen waren die Anteile an verzweigten Alkanen größer als bei allen anderen Arbeiterinnen. Sammlerinnen zeichneten sich durch höhere Anteile an ungesättigten Acetaten und Alkadienen in den Dufourdrüsensekreten und den Volatilen auf der Kutikulaoberfläche aus. Die Volatilbouquets von Königinnen waren durch höhere Anteile an verzweigten Alkanen und Wachsestern signifikant verschieden von denen der Arbeiterinnen. Von allen Arbeiterinnengruppen waren die Duftstoffmuster von dominanten Bienen denen der Königinnen am ähnlichsten. Die biologische Bedeutung der Ergebnisse wurden anhand der momentan aktuellen Hypothesen zur Evolution von pheromonalen Dominanzsignalen bei sozialen Insekten diskutiert.

***Bombus hypnorum* / Dominanz / Volatilbouquet / chemische Zusammensetzung**

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