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TASK-RELATED CHEMICAL ANALYSIS OF LABIAL GLAND VOLATILE SECRETION IN WORKER HONEYBEES (*Apis mellifera ligustica*)

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Abstract—Chemical analyses revealed that the labial gland complex of worker honeybees possesses a series of hydrocarbons dominated by odd-numbered carbon chain alkanes along with minor amounts of alkenes and branched alkanes. Foragers contained significantly more secretion than nurse bees. Experiments with bees from colonies induced to have a division of labor independent of age revealed that the differences in the amount of secretion were task, but not age dependent.

Key Words—Honeybees, labial glands, exocrine glands, secretion, hydrocarbons, forager bees, task specificity.

INTRODUCTION

The labial (salivary) gland complex comprises two pairs of glands, one in the head and one in the thorax, both of which connect through a common duct to the mouth (Cruze Landim, 1967). They are intermittently developed in bees, and little is known about the chemistry or function of their secretion. In the mason bee, *Chalicodoma siculum*, the head gland secretion is composed of hydrocarbons that are used to waterproof brood cells (Kronenberg and Hefetz, 1984). Male carpenter

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bees, *Xylocopa varipuncta*, presumably use the secretion from the thoracic gland as a long-range sex attractant, but the chemistry remains elusive (Minckley et al., 1991). The chemistry and function of the labial gland secretion of male bumble bees was extensively studied, revealing a plethora of compounds (Bergström et al., 1981; Genin et al., 1984). The secretion is species-specific, and in the species studied it is used for marking the flight path to which queens and other males are attracted (Kullenberg et al. 1970, 1973; Bergman and Bergström 1997; Hovorka et al., 1998; Kindl et al., 1999). The labial glands are also well developed in *Bombus terrestris* queens and contain mostly a series of dodecyl esters (Hefetz et al., 1996).

The posterior part of the head gland and the thoracic gland were reported to be involved in partial food digestion in the honeybee. The secretion of the thoracic gland contains watery saliva that dissolves sugars, whereas the head gland produces an oily secretion of unknown function (Simpson, 1960; Arnold and Delge-Derachen, 1978). We present here a comparative analysis of the volatile constituents of the head and thoracic gland secretions of worker honey bees as a function of age and task.

METHODS AND MATERIALS

Bee and Hive Manipulations. Workers of European honeybees (*Apis mellifera ligustica*) were obtained mostly from the Tzrifin apiary (Ministry of Agriculture), Israel, and from one colony from an apiary at the University of Illinois Bee Research Facility. Nurse bees were collected on brood combs and foragers returning with pollen were collected at the hive entrance. To dissociate possible effect of age and behavioral status on glandular composition, a single-cohort colony was established with about 1000 1-day-old adult bees from a field colony. These bees were obtained by taking frames containing old pupae and placing them in an incubator (34°C and 80% relative humidity). Newly emerging adults were marked with paint dots on the thorax. The colony was given a single frame of food and a mated queen. Under these conditions some of the workers become precocious foragers, whereas three weeks later some workers still remained as old nurses (Robinson et al., 1989). Four groups of bees were collected for comparative analyses: young (precocious) foragers and young nurses were collected after one week, and old foragers and old (over-aged) nurses were collected after three weeks. Bees for the GC-MS analyses were obtained from several commercial colonies at the Tzrifin apiary. Quantitative analyses were conducted using bees from one "typical colony" and one "single cohort colony."

Chemical Analyses and Compound Identification. Head and thoracic labial glands were dissected under a stereo microscope ($\times 20$) and extracted in 100 μl dichloromethane (to enable proper detection of the peaks by gas chromatography

each sample comprised a pool of glands from five bees). The head glands were separated from the hypopharyngeal glands and the thoracic glands were separated from the thoracic muscles. Extracts were analyzed by combined capillary gas chromatography–mass spectrometry (EI 70 eV, and CI using methane as a reagent gas) using a 30-m DB-5 fused silica column that was temperature programmed from 120°C to 300°C at a rate of 3°/min with initial hold of 3 min. Quantitative analyses were done by gas chromatography using a 30-m SE-54 or DB-1 capillary column that was temperature programmed from 60°C to 100°C at 20°/min and to 270°C at 5°/min (final hold: 30 min). Identity of the components was verified by comparing their retention time with standard compounds. Quantification of the glandular secretion was performed by peak integration (FID detector) using eicosane (1 μg/sample) as an internal standard.

RESULTS AND DISCUSSION

Table 1 presents a list of the volatile components found both in the head and thoracic labial gland secretion of honeybee, nurses, and foragers. The main components in both worker groups were straight-chain alkanes ranging from C_{21} to C_{35}, accompanied by minor amounts of alkenes and methyl-branched alkanes. In most cases the major components in all groups of workers were the same (e.g., odd-numbered carbon chain alkanes), but there was a degree of specificity in the minor components.

Quantification of the glandular extracts revealed that the total amount of secretion was normally distributed among samples (Kolmogorov-Smirnov $P > 0.2$, for both head and thoracic glands). We assessed the differences between treatments (age and task) by ANOVA followed by Fisher post-hoc test. There was a significant effect of task, but not age on the total amount of secretion for both the head and thoracic glands (Table 2, ANOVA). Foragers had higher amounts of secretion than nurses, irrespective of their age.

For the head glands, specific comparison between the group of bees showed that among the hive bees (of undetermined age) foragers had larger amounts of secretion than nurses in the head but not in the thoracic glands (Table 3, typical colony, ANOVA followed by Fisher's PLSD; $P < 0.0001$). Analyses of bees from a single-cohort colony indicated that the quantitative differences in the head gland secretion are mostly related to differences in behavior, and not to age (Table 3, single cohort colony bees). Although old foragers that originated from the single cohort colony tended to have less secretion in the head labial glands as compared to foragers from a typical colony, it was not significant ($P = 0.057$). The amount of secretion in precocious foragers was lower than that of foragers from a typical colony, but not than of old foragers from the single cohort colony ($P = 0.03$ and $P = 0.68$, respectively). Foragers from the single-cohort colonies had greater

TABLE 1. CHEMICAL COMPOSITION OF LABIAL GLAND SECRETIONS OF NURSE AND FORAGER HONEYBEES[a]

	Head gland		Thoracic gland	
Compound	Nurses	Foragers	Nurses	Foragers
Alkanes				
C_{17}	—	*	—	—
C_{19}	*	t	*	—
C_{21}	—	*	*	*
C_{22}	*	t	—	***
C_{23}	***	***	**	**
C_{24}	*	*	*	*
C_{25}	**	****	**	**
C_{26}	*	*	**	**
C_{27}	**	***	**	**
C_{28}	—	*	—	—
C_{29}	**	*	*	*
C_{30}	—	*	—	—
C_{31}	***	*	*	*
C_{33}	**	t	**	*
C_{35}	**	t	**	—
Alkenes				
$C_{23:1}$	—	t	—	*
$C_{25:1}$	*	t	—	—
$C_{31:1}$	—	t	—	—
$C_{33:1}$	—	*	—	*
Methylalkanes				
4-MeC_{22}	*	—	—	—
3-MeC_{24}	—	—	—	*
9-MeC_{25}	*	—	—	—
3-MeC_{25}	—	—	—	*

[a] The results are presented in relative proportions: —, not detected; t, trace; *1–5%; **5–15%; ***16–25%; ****25–50%.

TABLE 2. STATISTICAL ANALYSES OF SECRETION AMOUNT IN HEAD AND THORACIC GLANDS

	ANOVA	
	Head glands	Thoracic glands
Total	$P = 0.0004$	$P = 0.006$
Effect of age	$P = 0.15$	$P = 0.12$
Effect of task	$P < 0.0001$	$P = 0.03$

TABLE 3. TOTAL AMOUNT OF VOLATILE COMPOUNDS FROM HEAD AND THORACIC LABIAL GLANDS OF WORKER HONEYBEES BELONGING TO DIFFERENT AGE AND TASK GROUPS.

Type of worker	Volatile (μg/glands of 5 bees; mean $\pm$ SD)	
	Head Glands	Thoracic glands
From typical colony		
Foragers	8.1 ± 2.8 (4)[a] a[b]	2.7 ± 0.9 (4) ac
Nurses	2.8 ± 0.6 (4) b	2.4 ± 0.5 (4) a
From single cohort colony		
Old foragers	6.1 ± 1.8 (9) ac	3.2 ± 0.3 (9) bc
Young (precocious) foragers	5.8 ± 1.5 (9) cd	2.1 ± 0.4 (8) a
Old (overaged) nurses	4.0 ± 1.8 (9) b	2.4 ± 0.9 (9) a
Young nurses	4.4 ± 1.4 (9) bd	2.0 ± 0.5 (9) a

[a] (N)-number of replicates.
[b] Values accompanied by the same letter are not statistically different (ANOVA followed by Fisher's PLSD).

amounts of secretion than nurse bees that originated from the same cohort colonies, irrespective of age. While the differences between the old foragers and the two types of nurses were significant, secretion of precocious foragers was higher than that of old nurses, but not that of young nurses.

Typical colony and single-cohort colony nurse bees did not differ in secretionary quantities in the head gland (ANOVA Fisher's PLSD $P = 0.97$ and $P > 0.99$ for young and old nurses, respectively).

For the thoracic glands, there were no differences between typical colony foragers and foragers from the single-cohort colony, whether old or precocious foragers, in the amount of glandular secretion (ANOVA Fisher's PLSD $P = 0.87$ and $P = 0.82$ for old and precocious foragers, respectively). In contrast, there were differences in the thoracic gland secretion between nurses from a typical colony and old foragers from a cohort colony, but not from young or overaged nurses, ($P = 0.04$ for old foragers and $P = 0.38$ and $P = 0.67$ for young and old nurses, respectively).

Differences in the amounts of material in the head gland suggest that as bees begin to forage, their glands start to fill up with hydrocarbons. Although this process appears to be largely age independent, there does seem to be an effect of a maturation or experience maturity component. The somewhat lower amounts found in precocious foragers may be explained by the short time (just a few days) that they had spent as foragers. The tendency of task-related greater amount of secretion was also noticeable in thoracic gland, although it was not statistically different.

Honeybee labial glands were previously thought to be solely involved in processing sugar. The secretion was thought to be mostly water soluble and to

contain digestive saliva (Simpson, 1960). The presence of copious amounts of hydrocarbons in the glands, suggests that the glandular secretion may have additional functions. There are only a few studies pertaining to the chemistry and function of the labial glands in other bee species. The gland is intermittently developed in bees and its use as well as its chemistry may have evolved several times independently. The head gland in the mason bee, *Chalicodoma siculum*, possesses an array of hydrocarbons similar to that of honeybees, used in this species in nest construction. The secretion is mixed with saliva and provides the brood cells with a waterproof layer (Kronenberg and Hefetz, 1984). It may have a similar function in the honeybee, e.g., assisting the preforager bees to manipulate the wax while constructing the brood comb. We think that this function is unlikely since foragers had larger amounts than nurses, opposite to what is expected from the above function.

The hydrocarbons detected in the labial glands of the honeybee are also present on the epicuticule (Arnold et al., 1996, and personal observation). This raises the possibility of a link between these two body parts. If this is so, the honeybee labial gland may function in a manner comparable to the postpharyngeal gland (PPG) of some ant species in which it serves as a reservoir of hydrocarbons that arise both from internal sources and through exchanges with nestmates (Soroker et al., 1994; Soroker and Hefetz, 2000). The postpharyngeal gland in the ant *Cataglyphis niger* is the source of hydrocarbon nestmate recognition cues (Lahav et al., 1999). This implies a hydrocarbon exchange between the labial gland and the cuticle of individual bees, as well as interindividual exchanges via trophallaxis and allogrooming. Worker honeybees can discriminate members of the same subfamily (super sister) from workers of other subfamilies (half sisters) (Moritz and Hillesheim, 1990). This is thought to be based partly upon cuticular hydrocarbons (Page et al., 1991). Different studies have revealed that none of the major cuticular hydrocarbons gave a positive result in recognition bioassays, whereas hydrocarbons present in smaller quantities gave positive results. The complete recognition mixture probably contains minute amounts of hydrocarbons, perhaps along with other as yet unidentified components that can be either produced by the workers or acquired from external sources, e.g., flowers, the queen, or the wax comb (Breed, 1998).

This study shows that the labial glands constitute yet another set of exocrine glands that are affected by honeybee age polyethism. The results suggest that the glandular secretion has a role that is specific to foragers. One possibility is that the secretion may be involved in regulation of the age at which bees begin to forage. One regulatory model postulates the presence of an inhibitor (as yet unidentified) that inhibits the rate of behavioral development and delays the age and onset of foraging (Huang and Robinson, 1992, 1996). The inhibitor is hypothesized to be produced and/or transferred in greater amount by old bees (Huang and Robinson, 1992), and recent results indicate that this transfer is done by trophallaxis (Huang et al; 1998; Schultz et al., 1998). This idea fits with the hypothesis,

based on behavioral observations, that trophallaxis functions in communication as well as in food transfer (Korst and Velthuis 1982). Because the labial glands open to the buccal cavity, it is tempting to speculate that the secretion in foragers constitutes part of the hypothesized inhibitory system, but this awaits further experimentation.

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