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Abstract

So far only a few studies have been conducted on the cuticular lipids of honey bees. Eggs and some body parts have been previously used in some chemical studies for certain purposes (e.g. the identification of egg marking pheromones and for investigating the role of hydrocarbons in nestmate recognition). In the present study, cuticular lipids of body parts (head, thorax and abdomen) were analyzed qualitatively by gas chromatography/mass spectrometry to identify the pattern of cuticular lipids on these parts for two main honey bee castes (workers and queens) and for two subspecies. The obtained results showed that the abdomen had the highest number of lipids in general and relatively minor differences between head and thorax lipids were found. Each subspecies had a specific lipid profile. Thus, the cuticular lipids could aid in the discrimination between honey bee subspecies. The conundrum behind the differences between body parts in lipid profiles may be related to the specific functions of each body part, and to role of lipids in protecting the body from desiccation.

Key words: *Apis mellifera*, cuticle honey bee, GC/MS, lipids.

Introduction

The body of honey bee castes (queen, workers and drones) is covered with cuticular lipids. Lipids contain various compounds, including wax esters, alcohols, fatty acids, and monoacylglycerols (Nation 2002). Roles for these lipids have been suggested, including nestmate recognition (Breed, Stiller 1992) and protecting the body from desiccation (Jones 1954; Gibbs 1995) especially regarding the long-chain hydrocarbons (Gibbs 1998). Similar characteristics have also been detected in the lipids of honey bee eggs (worker-laid eggs and queen-laid eggs). The role of the egg surface chemicals has been suggested to help workers in the discrimination between worker- and queen-laid eggs (Oldroyd, Ratnieks 2000) as well as in protecting the eggs from desiccation (Martin et al. 2004). Different components have been detected on the egg surface including: hydrocarbons, eicosanol, alcohols, aldehydes, terpenes and esters (Katzav-Gozansky et al. 2001; Katzav-Gozansky et al. 2003; Martin et al. 2004). Moreover, differences between the elemental composition of eggs from two honey bee subspecies have been found (Abou-Shaara et al. 2013).

Relatively few studies have been conducted on honey bees in regard to cuticular lipids (e.g. the studies of McDaniel et al. 1984; Carlson 1988; Schmitt et al. 2007; Kather et al. 2011) and comparison between honey bee subspecies in their cuticular lipid composition is needed. Honey bee subspecies are distributed within a wide range of environmental conditions and the discrimination between honey bee subspecies is basically based on morphological characters, including body and wing venation characters (e.g. Abou-Shaara, Al-Ghamdi 2012; Abou-Shaara 2013), as well as genetic methods (e.g. Kandemir et al. 2006). However, honey bee subspecies live under the same environmental conditions (e.g. harsh conditions) have been noticed to differ in thermal tolerance ability (Atmowidjojo et al. 1997; Abou-Shaara et al. 2012). Thus, it is expected that distinctive differences in cuticular lipids may exist between honey bee subspecies.

Therefore, the study was aimed to determine the cuticular lipid profile of body parts (head, thorax and abdomen) for two main castes in the colony (workers and queens), and for two honey bee subspecies. Regarding differences between body parts and castes the relations between lipids and function were determined. Also, differences between two honey bee subspecies in cuticular lipid composition was investigated.

Materials and methods

Sample collection

Two honey bee subspecies living under the same environmental conditions were used in the study: hybrids

of Carniolan (*Apis mellifera carnica* Pollmann) and hybrids of Italian (*Apis mellifera ligustica* Spinola) honey bees. Eighteen forager workers were collected in front of Carniolan and Italian colonies (nine per subspecies from three colonies, and three workers per colony). As differences between newly emerged, nurse and forager workers in their cuticular lipids have been found, with higher alkanes in forager bees (Kather et al., 2011), the forager bees were chosen in this study for comparison between body parts and between the two subspecies. Eight mated queens (four per each subspecies from four different colonies) with age more than one year were used in the analysis. The collected bees were killed in a freezer and the body parts (head, thorax and abdomen) were separated using scissors.

Chemical analysis

The separated body parts were used for cuticular lipid analysis using GC/MS. The qualitative analysis was done using a method similar to that of Kather et al. (2011). As the concentrations of cuticular lipid compounds differs with age and between bee within colonies, only qualitative analysis was performed to characterize the general profile of each subspecies. Heads, thoraxes and abdomens were placed separately in vials containing 3 mL HPLC-grade hexane for 10 min. The separated parts of each subspecies were mixed together to obtained detectable and representative compounds, and to minimize any differences between individuals or colonies within each subspecies. The body parts were subsequently removed and 30 µL of the extract from each sample was injected (the splitless mode was applied) in an Agilent 7890A GC system coupled to a 5975C MS (triple-axis detector). The used column was Agilent 19091S (250 µL ID × 30 m, film thickness: 0.25 µL; Agilent Technologies Inc., USA). A temperature programme from 70 to 200 °C at 40 °C min^{-1} and to 320 °C at 25 °C min^{-1} was used in the analysis. Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}.

Data analysis

The identification of the compounds was performed using an Mass Spectral Library database (08. L; National Institute of Standards and Technology) considering only those compounds with a probability above 80%. In the present method the samples may be contaminated by internal body contents because the body parts were separated. Therefore, only the compounds which had been previously identified as cuitular chemicals for honey bees by Kather et al. (2011) and Schmitt et al. (2007) were considered.

Results

The analysis of different body parts for queens and workers of Carniolan honey bees showed the presence of more compounds in the abdomen (Table 1). For queen body parts, the thorax contained more compounds than the head while the worker heads contained more compounds than the thorax. The worker body in general had higher alkene and fatty acid numbers than did the queen body. The worker abdomens also had the highest alkene and fatty acid numbers than the other parts. There were clear differences in the cuticular profile of queens and workers for the body parts. Also, there were more 22 compounds detected for the worker body than for the queens.

In the case of Italian honey bees, the abdomen contained the highest number of compounds than other body parts for queens and workers (Table 1). The highest alkane compound number was detected in the thorax for queens and workers. The queen body parts contained higher proportions of alkenes and fatty acids than the worker body parts. In contrast with Carniolan honey bees, the Italian queens had a higher number of compounds than the workers by nine compounds. Also, a clear difference in cuticular lipids was observed for queen and worker body parts.

The Italian queens had higher compound number than the Carniolan honey bees in the head and the abdomen. In the thorax, the Carniolan queens had higher compound number than the Italian bees. On the other hand, the detected compounds for the Carniolan workers were higher than the Italian workers for all body parts. Fig. 1 shows the differences between the numbers of different chemical groups per each body part for the two subspecies. It is clear that the Carniolan honey bees had a higher number of alkane, alkene and fatty acid compunds than the Italian honey bees for all body parts.

Discussion

Differences in chemical compunds between Carniolan honey bee body parts and castes (workers and queens) were found: a higher number of detected compounds was recorded in the abdomen for the two castes, indicating specific roles for these compounds in the abdomen. It is known that the abdomens of the workers contain wax glands for beeswax secretion, as well as scent glands, and the sting apparatus and associated glands (Snodgrass 1956). These specific glands may cause the abundance of cuticular lipids on the abdomen. On the other hand, the queens have less numbers of glands on the abdomen than the workers, but the presence of the cuticular lipids may have a role in the communication between workers and the queen. It was particularly suggested that alkanes have a role in protecting the body from desiccation while alkenes and fatty acids may have a role in the communication process (Chaline et al. 2005). Therefore, the presence of the cuticular lipids in the head and the thorax is likely related to the role of each part, mainly head in communication and thorax in thermoregulation. Nestmate recognition is based mainly on genetically-acquired cues (Breed 1983). Also, beside the role of cuticular lipids in nestmate recognition, other factors have

Table 1. Chemical analysis of cuticular lipids for different body parts of Carniolan and Italian honey bees. +, presence of the compound; –, absence of the compound. H, head; T, thorax; A, abdomen.

Substance	Carniolan honey bees						Italian honey bees					
	Queen			Worker			Queen			Worker		
	H	T	A	H	T	A	H	T	A	H	T	A
Alkanes:												
Octadecane	+	+	–	+	+	+	+	+	+	+	+	+
Hexadecane	–	–	+	–	+	+	–	+	–	–	–	–
Nonadecane	–	–	+	–	–	–	–	–	+	–	–	–
Eicosane	–	+	+	+	+	+	+	+	+	+	+	+
Heneicosane	+	+	+	+	–	+	+	–	–	–	–	+
Pentacosane	–	+	+	+	–	–	–	+	–	+	+	–
Docosane	–	–	–	–	–	+	–	–	+	–	–	–
Hexacosane	–	+	–	+	+	+	–	–	–	–	–	–
Heptacosane	–	+	–	+	+	–	–	+	+	–	+	–
Dodecane	–	+	–	–	–	–	–	–	+	–	–	–
Nonacosane	–	–	–	+	+	+	–	–	–	–	–	–
Tetracosane	–	–	+	+	+	+	+	–	–	–	–	+
Tetradecane	–	–	–	+	–	+	–	–	–	–	–	–
Pentadecane	–	–	+	–	–	–	–	–	+	–	–	–
Heptadecane	–	–	+	+	–	+	+	–	+	+	–	+
Alkenes:												
Docosene	–	–	–	–	–	+	–	–	–	–	–	–
Nonadecene	–	–	–	+	+	+	–	–	+	–	–	–
Tricosene	–	–	–	+	+	+	–	–	+	+	–	–
Fatty acids:												
Palmitic acid	–	–	+	+	+	+	–	–	+	–	–	–
Stearic acid	–	–	–	+	+	+	+	+	+	–	–	+

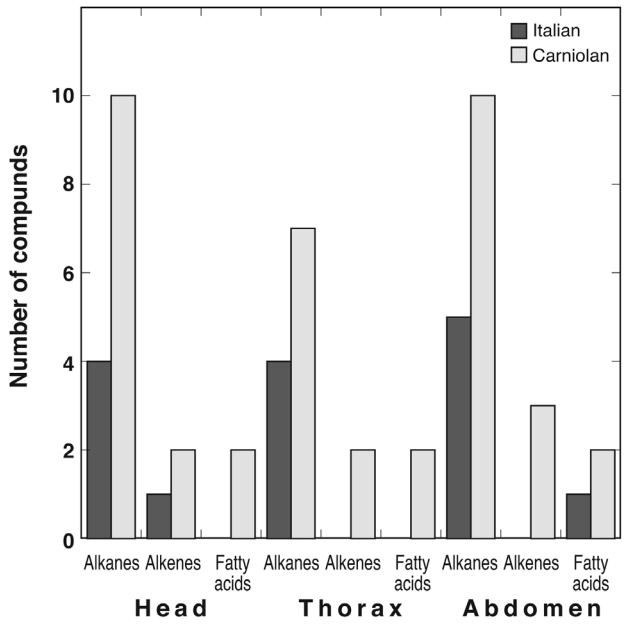

Fig. 1. Diversity of chemical compounds belonging to alkanes, alkenes and fatty acids on different body parts of workers for the two subspecies (Italian and Carniolan).

been found to mediate nestmate recognition,including wax combs by guard bees (D'ettorre et al. 2006). Unfortunately, few studies have been conducted on the role of cuticular lipids of honey bee workers and queens. The Italian honey bees showed similarity to Carniolan honey bees in having higher number of compounds in the abdomen region for the two castes. In contrast with the Carniolan honey bees, Italian honey bee queens had generally higher alkene and fatty acid compound numbers than the workers, and the higher alkane number was detected in the thorax region of the two Italian honey bee castes. This could be attributed to some genetic factors. The higher alkene and fatty acid compund number in the worker body thanin the queen can be explained by the nature of the worker body, as more glands, in particular in the abdomen (see, Snodgrass 1956).

Cuticular lipid compund number differed between body parts to subspecies, as well as within the castes of the same subspecies. Thus, the cuticular lipids for the two castes can be used for the discrimination between the two subspecies. This result is in line with Carlson (1988), who found differences between European and African bees in alkenes and alkadiene composition. Also, it was found that lipids are genetically controlled (Carlson 1988). The number of queens in the colony is very low, and only one

queen can be found in the colony under normal conditions. Therefore, the discrimination between the two subspecies can be done based on the cuticular lipids profile of the workers. Also, the whole body of forager bees had more alkanes than the nurse or newly emerged workers (Kather et al., 2011). Thus, forager bees are recommended for the discrimination between honey bee subspecies, as used in the current study.

In conclusion, cuitcular lipids of the workers, in particular, could be used for the discrimination between honey bee subspecies. To obtain a detailed profile for each subspecies, the analysis of each body part separately is advisable. However, the honey bee sampling technique and the chemical analysis method require more investigations to be standardized.

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