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Honeybee nestmate recognition: the thermal behaviour of guards and their examinees

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Summary

In honeybee colonies, guards protect their nest from various robbers including bees from other colonies. Infrared thermography showed that the guards and the bees examined by them (examinees) differ considerably in their thermal behaviour according to their particular role in the nestmate recognition process. The thorax surface temperature was on average higher and more variable in the examinees (36.1 °C, S.D.=4.14, *N*=1545, 303 bees) than in the guards (34.0 °C, S.D.=2.00, *N*=1681, 772 bees). During thorough examinations lasting longer than 30 s, more than 60 % of the examinees showed phases of intense thoracic heating of more than 2 °C (maximum temperature 48.5 °C), whereas most guards cooled down. Our data suggest that these examinees heat up their surface to enhance chemical signalling during examinations.

Key words: honeybee, *Apis mellifera carnica*, guard bee, examined bees, nestmate recognition, thermoregulation, thermal behaviour, thermography.

Introduction

The honeybee is a social insect that lays large quantities of protein (pollen) and energy resources (honey) in a stock. Beside the pollen and the brood, it is the honey that attracts not only robbers like man, other mammals (e.g. bears and badgers) and other insects (e.g. wasps) but also bees of their own species. That is why honeybees developed their typical guarding behaviour (Butler and Free, 1952; Moore et al., 1987). Their ability to produce endothermic heat by means of their flight muscles plays an important role in colony defence (Esch, 1960; Heinrich, 1979a; Ono et al., 1995). To defend their colony properly against other bees, guards are thought to need a higher or at least the same motility than the bees examined by them (examinees). The main parameter influencing the motility of honeybees is the thorax temperature. They use their highly developed endothermy (Esch, 1960; Heinrich, 1979a, 1993; Schmaranzer and Stabentheiner, 1988) to improve the function of their flight muscles (Esch, 1976; Coelho, 1991) and their general mobility (Stabentheiner et al., 1995; Stabentheiner and Crailsheim, 1999; Crailsheim et al., 1999). Therefore, we measured the body temperature of both the guards and the examinees simultaneously by real-time infrared thermography (Schmaranzer and Stabentheiner, 1988) without disturbing their interaction. The measurements show that guards and examinees differ considerably, and in an unexpected way, in their thermal behaviour.

The observation of unexpected thoracic heating phases in the examinees led to several hypotheses about their biological significance. One suggestion was that examinees heat up to get rid of guards' pheromones, which marks them as possible intruders. One substance that is known to have pheromonal function in honeybees and is suspected to be a marking pheromone is 2-heptanone from the mandibular glands (Shearer and Boch, 1965; Free, 1987). We tested 2-heptanone for its effect on the thorax temperature of bees.

Guards examine other bees not only at the nest entrance but also inside the nest (Butler and Free, 1952; Stabentheiner, 1994). The behaviour of a guard examining a bee resembles to some extent the behaviour of grooming bees during social grooming interactions inside the colony. In order to see whether or not the thoracic heating phases of the examined bees also exist in groomed bees, that is, whether they are required for grooming, we measured the thorax temperature of bees performing grooming dances and of groomed and grooming bees.

Materials and methods

Guarding behaviour can be observed at the nest entrance as well as inside a colony (Butler and Free, 1952; Stabentheiner, 1994). Therefore, measurements were made at the entrance of standard colonies of *Apis mellifera carnica* Pollmann (15,000–30,000 bees) located in an apiary and in observation hives (3000–6000 bees), which were covered by infrared transmissive plastic foils. Thermograpic measurements of the bees' body surface temperature were made using an AGA 782 SW (summers 1989–1992, 1998) and a ThermaCam SC2000 (summers 2000, 2001) thermographic system (FLIR, Inc.). The temperature was calibrated to the nearest 0.7 °C using an infrared emissivity of 0.97 of the honeybee cuticle and a reference source of known temperature and emissivity (Stabentheiner and Schmaranzer, 1987; Schmaranzer and Stabentheiner, 1988). Air temperature near the bees was measured using thermocouples inside the hive and thermistor thermoprobes at the hive entrance. Temperature data were read directly from thermocouple thermometers (Technoterm) or stored with Almemo data loggers (Ahlborn).

To test for the effect of 2-heptanone on thoracic heating, we applied 0.9 or 9 μg of 2-heptanone dissolved in small droplets (100 nl) of paraffin oil to bees in an observation hive and measured their body temperature thermographically. These amounts were chosen on the basis of total content of 2-heptanone in honeybee heads (Boch and Shearer, 1967; Sakamoto et al., 1990). For comparison, the same amount of pure paraffin oil was applied.

Results

At the nest entrance, the guards examined other bees for periods ranging from less than 1 s to several minutes. During examination, they made close contact with the examinees. All guards and examinees were in an endothermic state (Fig. 1), the thorax surface temperature (T_{ths}) ranging from 25.2 to 42.4 °C in the guards and from 27.0 to 48.5 °C in the examinees. When the guards made contact with the examinees, their T_{ths} (34.8 °C) was similar to that of the examinees (34.4 °C) but lower than in bees landing and entering the colony (potential examinees, 36.2 °C; Table 1A). If the guards decided to examine a bee thoroughly, the guards' T_{ths} changed considerably in most cases. Although they were moving agitatedly around their more calmly sitting examinees, many of them decreased their T_{ths} with increasing duration of interaction (Fig. 2) at an average rate of –0.28 °C 10 s^{-1} (r=–0.6573, N=129; P<0.0001, t-statistics). This explains in part why the total mean of T_{ths} was lower in the guards than in the examinees (Table 1A).

Especially during the longer lasting examinations, one examinee was usually inspected by several guards, who took turns to examine them (Fig. 2). A surprising finding was that the examinees showed a much higher variation in T_{ths} than the guards (Table 1A). Though some examinees decreased their T_{ths} during the initial phase of examination (Fig. 2), the high variation resulted for the greater part from phases of intense thoracic heating (Figs 1A$_1$, A$_2$, 2). This way, 21 out of 100 examinees that were examined for periods of more than 30 s reached a T_{ths} as high as 43–48.5 °C! One or several heating phases of more than 2 °C was observed in 64 % of these long lasting examinations (Fig. 3). The delay between the first contact of the first guard with an examinee and the beginning of the first heating phase ranged from approximately 1 s to several minutes. Because of the heating phases, the total mean of the examinees' T_{ths} (36.1 °C) was higher than their T_{ths} at the time when the first guard made contact with them (35.1 °C; Table 1A). The mean increase of T_{ths} during heating phases >2 °C amounted to 4.9±2.48 °C (mean ± S.D.) (maximum: 13.5 °C), and the mean heating rate was 7.0±2.72 °C min^{-1} (range: 2.8–15.5 °C min^{-1}; N=49). During shorter examinations of 10–30 s duration, only in 3 % of the examinees were heating phases of more than 2 °C observed, but 91 % kept their thorax temperature within ±2 °C (Fig. 3).

Since it is impossible for the guards to examine all bees at the nest entrance, many returning foragers enter the colony without having contact with them. Consequently, guards are

Table 1. *Thorax surface temperature* (T_{ths}) *of guard bees, examinees and landing bees at the hive entrance of standard colonies located in an apiary and inside observation hives*

	Temperature (°C)	N	Bees
(A) Colony entrance			
(a) Guards, first contact with examinees	34.8±1.88	282	179*
(b) Examinees, first contact of guards with examinees	34.4±2.92	282	282
(c) Guards total	34.0±2.00	1681	772
(d) Examinees total	36.1±4.14	1545	303
(e) Examinees, first contact with first guard	35.1±2.39	270	270
(f) Landing bees	36.2±2.00	50	50
Ambient air temperature	27.4±2.00	1580	
(B) Inside colony			
(g) Guards total	36.6±0.53	702	45
(h) Examinees total	42.2±0.95	587	34
Ambient air temperature	31.1±2.27	34	

Temperature values are means ± S.D.

The mean T_{ths} were significantly different at P<0.0001 at the colony entrance and inside the colony, except for b:c, e:f (P<0.01) and a:b, d:f (NS, t-test). S.D. values of T_{ths} (variances) were significantly different at P<0.001 except for a:c, a:f, c:f and e:f; all S.D. values of T_{ths} were smaller inside the colony than at the colony entrance (F-test). *The number of bees is smaller than N because during short inspections (<1 s) one guard often examined several examinees in rapid succession.

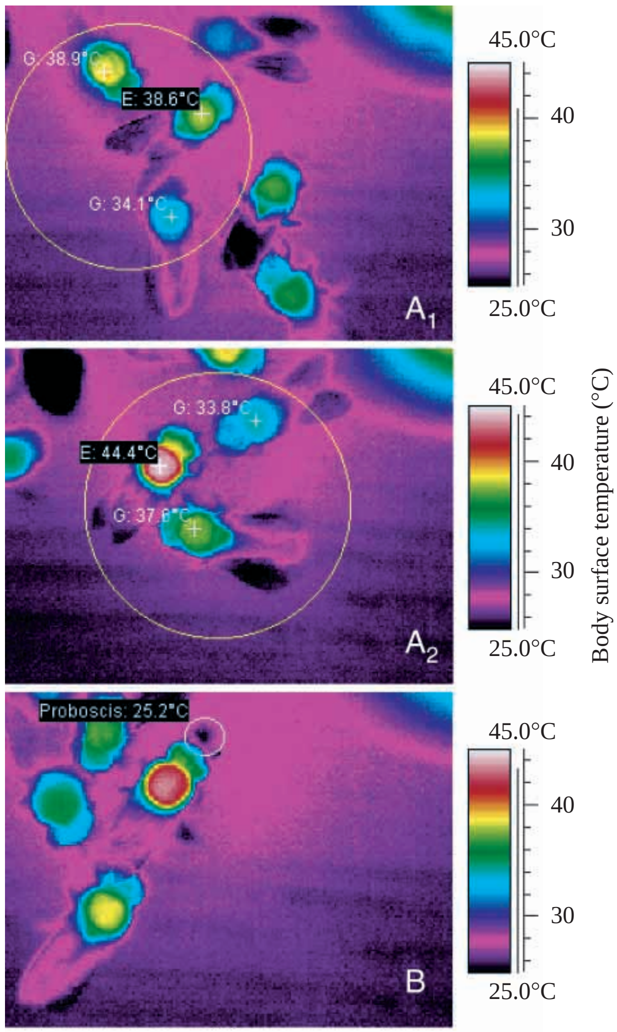

Fig. 1. Colour thermograms of guard bees and their examinees at the hive entrance. (A_1, A_2) Increase in thorax temperature during a heating phase of an examinee (E) as a reaction to a long-lasting examination (duration >30 s) by guards (G). In contrast to the examinee, the guards did not heat up; time difference between thermograms A_1 and A_2 is 1 min 24 s. (B) Cooling of the proboscis of an examinee by regurgitated fluid during tongue stropping in the course of a thoracic heating phase. Ambient air temperature: 18.5 °C.

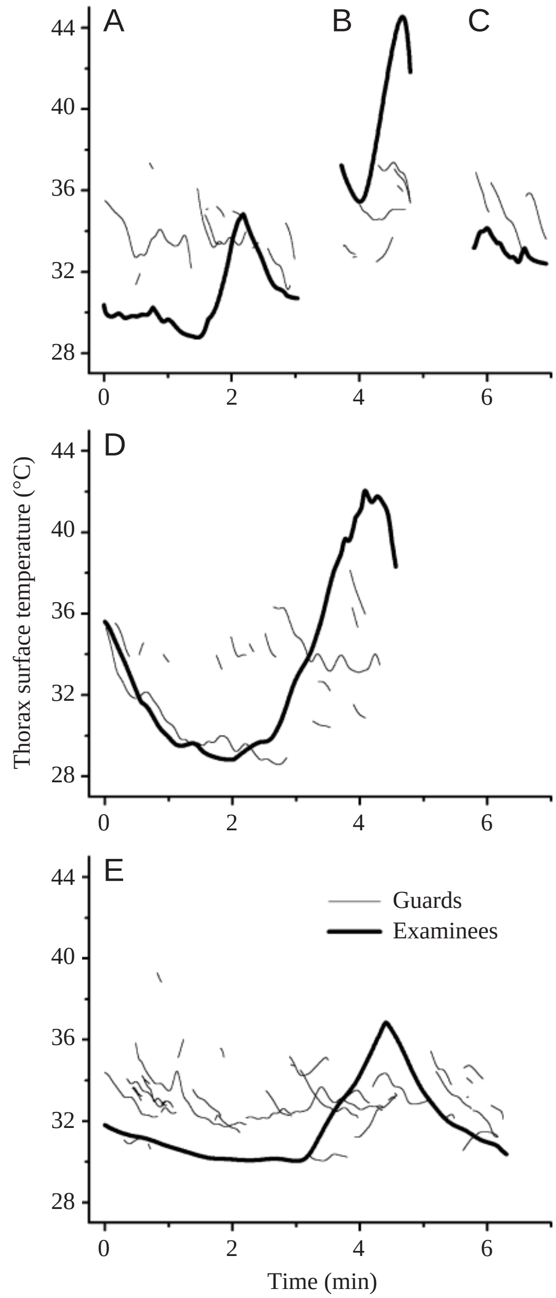

Fig. 2. (A–E) Sample curves of the thorax temperature of guard bees and their examinees at the hive entrance during interactions of more than 30 s duration. Note the phases of intense thoracic heating of the examinees, but not of the guards, in all curves except for C. Ambient air temperature: 25.5–30.7 °C.

also active inside the nest (Butler and Free, 1952; Stabentheiner, 1994). Because of the higher air temperature inside the colony, the lower limits of thorax temperature were at a higher level, with T_{ths} ranging from 33.5 to 39.4 °C in the guards and from 35.4 to 46.7 °C in the examinees. Like at the hive entrance, the guards' mean T_{ths} (36.6 °C) was below that of the examinees (42.2 °C; Table 1B). The examinees showed similar heating phases to those at the nest entrance (Fig. 4). In contrast to examinees and guards, the grooming dancers, groomed and grooming bees increased their thorax temperature only slightly above ambient temperature (Table 2). Heating phases of groomed bees comparable to those of examinees were never observed.

Examinees intercepted by a guard for thorough examination exhibited a typical submissive posture, with bended abdomen to expose their tergal glands, and after some time, they stood on their four hindlegs to display self-grooming of the head and tongue stropping with the forelegs (cf. Butler and Free, 1952). Thermography showed in many of these examinees that the proboscis was similarly warm as the head when they started tongue stropping, but after some time it became visible as a cold spot (Fig. 1B). We conclude that it cooled down *via* evaporation because it was wet. This interpretation is

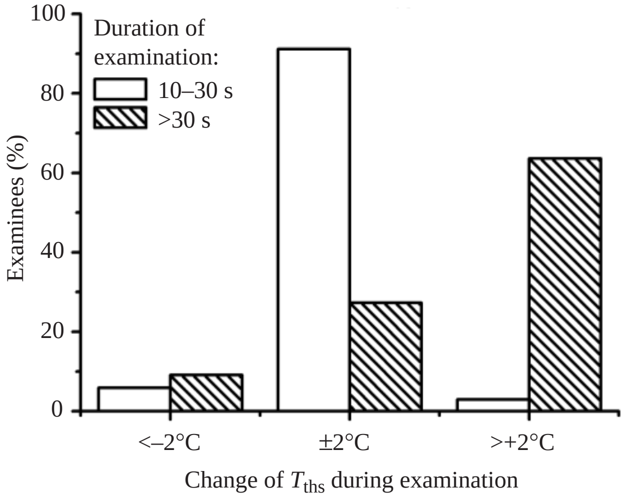

Fig. 3. Relative frequency of examinees where the largest change of thorax temperature during examinations of medium (10–30 s) or long (>30 s) duration was a phase of thoracic heating (T_{ths}>+2 °C), approximately constant temperature (T_{ths}±2 °C) or cooling (T_{ths}<–2 °C). 100 % = 34 examinees and 66 examinees for durations of 10–30 s and >30 s, respectively.

supported by the observation that when some bees touched the hive wall with their proboscis they left behind a cool spot. Head surface temperature during tongue stropping, as measured between the ocelli, remained below 43 °C even in the most intensely heating bee with a T_{ths} of 48.5 °C.

In the experiments where 2-heptanone was applied to hive bees, the largest increase of T_{ths} within a period of 2 min was evaluated. We were not able to elicit thoracic heating of more than 1 °C at higher frequency in bees treated with 0.9 or 9 μg

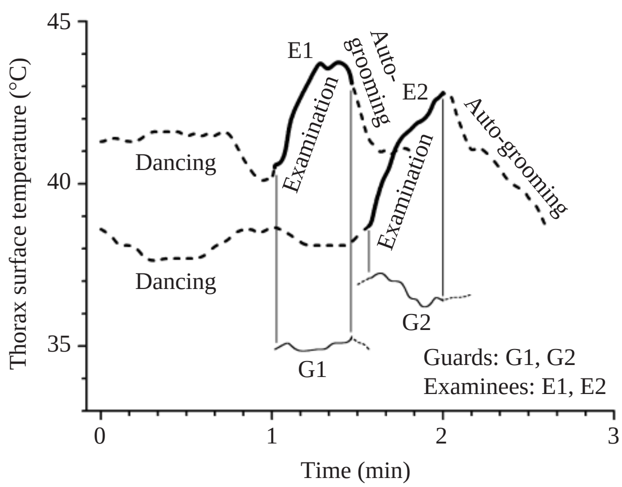

Fig. 4. Sample curves of the thorax temperature of guard bees and their examinees (dancing foragers) inside an observation hive. These examinees heated their thorax immediately after the guards had made contact with them (E1, 4 s; E2, 1 s). E1 was a forager dancing for a natural source of nectar (Stabentheiner, 2001), and E2 was a marked forager dancing for 1 mol l^{-1} sucrose on a feeding place 500 m south of the hive (Stabentheiner et al., 1995). Air temperature near the bees for E1, 34.0 °C; for E2, 29.7 °C.

2-heptanone/100 nl paraffin oil (26.7 %, N=15, with both concentrations) in comparison with bees treated with pure paraffin oil (30 %, N=10; χ^2=2.619, d.f.=1).

Discussion

The experiments showed that guard bees and examinees differ considerably in their thermal behaviour according to their role in the nestmate recognition process. Although some guards increased their T_{ths}, most of them cooled down with increasing duration of the contact with the examinees. This surely reduces their energy loss. The temperature decrease of the guards during longer lasting examinations, however, is remarkable in so far as the power output of the flight muscles – and thus the ability of the guards to take off immediately – decreases with decreasing flight muscle temperature (Esch, 1976; Coelho, 1991). Obviously, the guards expected their examinees not to try to escape by flight during longer lasting examinations, which indeed was observed only rarely (<1 % of examinees).

In many of the examined bees, their mouthparts were warm in the beginning and then cooled down, very probably because they became wet during examination. This resembles the situation in hive bees receiving food from foragers. The recipient's proboscis becomes warmer when the warm nectar of the donors reaches it, and it cools down afterwards because of evaporation (Stabentheiner et al., 1995; Farina and Wainselboim, 2001). Regurgitated droplets of fluid have a significant cooling effect not only on the mouthparts but also on the whole head and even the thorax (Heinrich, 1979b). Therefore, our data suggest that evaporative cooling *via* regurgitated fluid helped the examinees to prevent their brain from overheating during intense thoracic heating. However, because tongue stropping with a wet proboscis in many examinees preceded thoracic heating and was accompanied by self-grooming, which distributes the fluid from the mouthparts over the head, its exact role in the identification process remains to be investigated.

Several hypotheses for the biological significance of the examinees' phases of thoracic heat-up have to be considered: (1) Although honeybees increase the temperature of their thoracic flight muscles to facilitate take-off (Esch, 1960; Heinrich, 1979a, 1993; Schmaranzer and Stabentheiner, 1988), it is improbable that the heating phases serve as a preparation for easier escape because the heated examinees remained in the typical submissive posture and did not attempt to fly away. Inside the nest on the combs, escape by flight is prohibited by the narrow space anyway. Both outside and inside the nest, most examinees that had heated up did not even run away immediately after all guards had terminated examination. Many of them showed phases of auto-grooming after all guards had left (Fig. 4). (2) We exclude the hypothesis that the heating phases are a request for grooming, because the interaction between grooming and groomed bees was observed to be more leisurely than between guards and examinees, and because the interaction started before the thorax temperature increased. The

Table 2. *Increase of thorax surface temperature (*T_{ths}*) above air temperature (*T_a*) of bees inside an observation hive performing grooming dances and during grooming interactions*

	Temperature (°C)		
	$T_{ths}-T_a$	Maximum	*N*
Grooming dancers	1.2±0.15	1.4	11
Groomed bee	1.1±0.3	1.7	21
Grooming bee	0.9±0.34	1.5	25

Values for $T_{ths}-T_a$ are means ± S.D.
T_a=30.2–32.6 °C.
N=measurements and bees.

thorax temperature of grooming dancers, groomed and grooming bees increased only slightly above ambient temperature (Table 2). The examinees never showed an active behavioural pattern such as grooming dances, which stimulate the grooming behaviour of hivemates (Haydak, 1945). (3) The remaining hypothesis is that the heating phases play a role in the identification process. The examinees might heat up because they want to get rid of (3a) pheromones from the guards that mark them as possible intruders or (3b) volatiles that disturb identification or (3c) because they want to increase evaporation of substances that help guards to identify them as hivemates. 2-heptanone from the mandibular glands has a pheromonal function in honeybees (Shearer and Boch, 1965), and it is under suspicion to be used as a marking pheromone (Free, 1987). However, because we were not able to elicit an increased frequency of thoracic heating through treatment with 2-heptanone we do not attribute hypothesis (3a) a high priority, though we cannot completely disprove it at present.

Some support for the hypothesis (3b) that the examinees want to get rid of chemicals that disturb recognition comes from a recent observation that it takes longer for guards to reject examinees that are treated with floral oils (Downs et al., 2000). This, however, does not contradict the hypothesis (3c) that the examinees enhance their chemical signalling by increasing the evaporation of compounds from their surface by increasing its temperature. From the whole blend of odours of a honeybee that may play a role in nestmate recognition (e.g. Page et al., 1991; Breed and Julian, 1992; Arnold et al., 1996; Breed, 1998; Fröhlich et al., 2000), it is some of the fatty acids or their esters and some of the short chained alkanes and alkenes that gave positive results in recognition bioassays (Breed and Julian, 1992; Breed, 1998). Honeybees are also especially sensitive to fractions containing the more polar components of comb waxes (hydroxy alkyl esters, acids and primary alcohols) in tests using the proboscis extension reflex (Fröhlich et al., 2000). The vapour pressure of these substances at the thorax temperature of returning foragers and examinees (28–48 °C) is low but depends on temperature. In the liquid phase of *cis*-9-octadecenoic acid (oleic acid), for instance, it increased by a factor of about 5.9 between 30 and 40 °C (approx. 3.63×10^{-5} to 2.16×10^{-4} Pa) (Dykyj et al., 2000). In an alkane that yielded a positive bioassay result (hexadecane), the vapour pressure of the liquid phase increases by 2.7 times in the same temperature interval (0.374–1 Pa) (Dykyj et al., 2000). An increase of this magnitude may enhance detectability for the guards, all the more so as many of the substances that play a role in nestmate recognition are acquired in minute quantities from the environment inside the colony (Breed, 1998). Although it is clear that substances other than those tested by Breed and coworkers (1992, 1998) play a role in nestmate recognition, our results show that body temperature is a parameter that has to be considered in research on nestmate recognition in honeybees.

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