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Research article

Honey bee workers as mobile insulating units

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Summary. Heat-shielding is a method used by honey bee workers to insulate temperature sensitive brood from localized heat stress during development. Due largely to data collection techniques, heat-shielding has been defined as *stationary* bees congregating with their *ventral* side facing the *heat* stress. We conducted tests to determine if shielding behavior was limited to bees fitting this description. Specifically, we examined the behavior in response to heat and cold stress, and recorded both stationary and moving workers on the hive wall (ventral side visible) and on the brood comb (dorsal side visible). Our observations strongly suggest that stationary bees on the brood comb shield the developing brood from both localized heat and cold stress: after temperature-stress, the number of bees under the stressor significantly increased. A uniform response from stationary bees on the hive wall, however, was not observed: stationary bee number increased significantly after heat stress but tended to decrease after cold stress. Movement of bees on both the hive wall and brood comb decreased in response to cold stress. Movement of bees on brood comb decreased after heat stress, whereas the movement of bees on the hive wall increased in response to heat stress. This latter result raises the possibility that these bees are creating currents used to dissipate the heat and/or are absorbing heat near the source and moving it to non-sensitive areas. Our data indicate that 'heat-shielding', as previously defined, is a category within a broader response of honey bees to localized temperature stress: *Apis mellifera* appear to respond adaptively to all localized temperature stressors.

Key words: *Apis mellifera*, thermoregulation, heat-shielding.

Introduction

Honey bee workers (*Apis mellifera*) expend a great deal of time and energy maintaining hive conditions (Winston, 1987). The most important activity in maintaining the health of hive may be ensuring that the temperature is tightly regulated. A healthy honey bee hive will maintain the brood comb within 32–35 °C (Heinrich, 1980, 1985; Seeley, 1985; reviewed in Winston, 1987). If the brood comb drops below optimum temperature, it can increase both brood development time and disease susceptibility (Fukuda and Sakagami, 1968; Bailey and Ball, 1991). Conversely, if temperature is too high, brood are susceptible to developmental deformities (reviewed in Winston, 1987).

Several honey bee behaviors have been described that serve to maintain constant hive temperature. To decrease temperature, bees fan wings and spread water, and to increase temperature, bees isometrically contract muscles (Heinrich, 1980, 1985). Localized temperature increases have been shown to result from individual bees elevating body temperature and then pressing thoraces over capped brood (Bujok et al., 2002) or entering empty brood comb cells (Kleinhenz et al., 2003). Recently, an additional temperature regulation behavior called 'heat-shielding' was identified (Starks and Gilley, 1999). In response to localized temperature increase, honey bees shield the inner hive by clinging to the inner surface of the hive wall. This behavior effectively limits the temperature increase of brood comb thus protecting it from large temperature fluctuations (Starks and Gilley, 1999). Investigations of heat-shielding have shown that (1) only workers perform the behavior, (2) workers 12 to 14 days old are significantly more likely to perform the behavior than other age classes, and (3) the number of heat-shielders is positively correlated to colony size, temperature increase, and number of capped brood under the heat source (Starks and Gilley, 1999; Starks et al., 2005).

Due largely to data collection techniques, heat-shielding has been defined as *stationary* bees congregating with their *ventral* side facing the *heat* stress. However, it is possible that bees not fitting this definition are also behaving in ways that serve to limit temperature fluctuations around developing brood. For example, moving bees or bees stationary on the brood comb in the immediate vicinity may also be responding to the temperature stress. In the latter case, increasing bee density on the comb would serve to further insulate the temperature-sensitive brood. In the former case, movement of bees, particularly on the surface close to the heat stress, may serve to dissipate the heat.

An additional and as yet unexplored aspect of 'heat-shielding' behavior is the response of bees to localized cold stress. *A. mellifera* from temperate locations is known to clump over the brood during cold winter months (Simpson, 1961). A localized clumping in response to a focused cold stress would be consistent with both winter thermoregulation (chronic temperature stress) and the heat-shielding behavior (a response to an acute temperature stress). In this study, we further investigate the behavior described as 'heat-shielding' with a view to explore the degree to which it is expressed. To this end, we examined the behavior in response to heat and cold stress, and recorded both stationary and moving workers on the hive wall (ventral side visible) and on the brood comb (dorsal side visible).

Methods

Eight two-frame observation hives (inner dimensions: 53 × 48 × 5 cm) with Plexiglas® hive walls were constructed by PTS and installed at the Tufts University ISIRF in Medford, MA. In July 2003, the hives were queenright and contained 1432 ± 92.2 worker bees (mean ± standard error; Starks et al., 2005). The queen was restricted to the lower frame using queen excluder so that distinct honey and brood comb frames were produced. The colonies were kept inside a temperature-controlled room (~20 °C) during the experimental period. Observation hives contained tubes leading outdoors, thus workers were allowed to forage naturally in the surrounding area.

To simulate the dark conditions of a wild *A. mellifera* hive, black construction paper with a 10 × 13 cm rectangular cutout in the brood comb area was secured to the hive walls of each experimental colony. A type K (nickel-chromium and nickel-aluminum) Teflon®-insulated thermocouple sensitive to 0.1 °C was attached to the outer surface of the hive wall in the center of the window. Temperature data were recorded with an Omega HH22 hand-held digital thermometer. A black paper cover was placed over the central window (Fig. 1).

Experiment 1: Location and movement status of heat-insulators

All behavioral data were gathered in September and October 2003. All eight hives were used for this experiment. One hive was suffering from an *Ascosphera apis* (Chalkbrood) infestation during this test. The hive was included in analysis as this fungal pathogen causes a common disease (Bailey and Ball, 1991; Starks et al., 2000) and results from this hive were consistent with those of the other hives. A small (10 × 13 cm) electric heating pad (Repti Therm MiniR 4 watt heater) was used to increase the temperature on the hive walls of the observation hives. Temperature data were recorded from under the heating pad before and after each 4 minute heating period.

The orientation (ventral or dorsal side facing the hive wall) and status (stationary or moving) of all bees in the window were recorded before and after each heating session. Two people (AJS and JH) were needed to count four categories of bees. One counter was designated the "hive wall counter" and one the "brood comb counter". Each researcher counted moving bees with a mechanical counter in one hand, and stationary bees with a mechanical counter in the other hand. The counts were conducted immediately preceding and following heating periods, and each took approximately 30–45 seconds. Data on bees on the comb were more difficult to collect because bees on the glass obstructed the view. Because of this the number of bees present in this location were estimated based on those that were visible. It was assumed that the density of bees in visually obstructed areas was the same as the density in visible areas adjacent to the obstructed areas. We collected 29 before and after treatment counts on the hive walls and 27 before and after treatment counts on the brood combs. It was not possible to make an accurate estimate of bees for two runs on the comb in one hive, due to the large number of bees in this hive. Each hive was allowed at least 30 minutes to reacclimatize between trials.

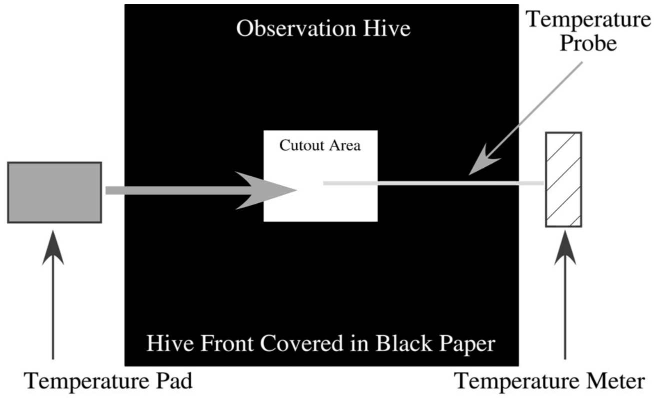

Figure 1. Experimental design for application of heat or cold stress. The cut out area was completely covered with a paper flap in the actual experiment to decrease light introduced to the hive. All experiments took place over the brood comb region

Experiment 2: Presence of cold-insulators

The experimental design was identical to Experiment 1 with the following exceptions: we used seven of the eight observation hives (one hive was excluded due to advanced colony illness) and a small gel based frozen cold pack (ICN Reusable non-toxic Cold Pack) to decrease hive wall temperature. We collected 30 before and after treatment counts on the hive wall and 26 before and after treatment counts on the brood comb. Each hive was allowed at least 30 minutes to reacclimatize between trials.

Statistical methods

Paired t-tests were used to compare the before and after number of bees for each category (e.g., stationary on the brood comb). Student's t-tests were used to compare across categories. All results are presented as means ± standard errors. Statistical analysis was performed using the software Microsoft Excel X and DataDesk 6.2.

Results

Heating experiments

The outside surface of the hive wall had a mean temperature of 26.3 °C before heating and 41.7 °C after heating. No hive had less than a 12.3 °C increase. *Stationary bees:* The number of stationary bees increased significantly on both the hive wall (mean increase, 6.62 ± 2.96; paired $t_{26} = -2.24$; $p < 0.05$) and brood comb (13.00 ± 3.52, paired $t_{26} = -3.69$; $p < 0.001$; Fig. 2) after heating. No statistical difference in the increase in stationary bee number was detected between the hive wall and brood comb regions (paired $t_{24} = -1.20$; $p = 0.24$; Table 1). *Moving bees:* The number of moving bees increased significantly on the hive wall after heating (mean increase, 4.72 ± 1.83, paired $t_{26} = 2.58$, $p < 0.02$; Fig. 3). The number of moving bees on the brood comb decreased after heating, but this decrease was not significant (mean decrease, –5.48 ± 3.01, paired $t_{24} = 1.82$, $p = 0.080$). The change in the number of moving bees was significantly different between the hive wall and brood comb regions (paired $t_{24} = -2.55$; $p < 0.02$; Table 1).

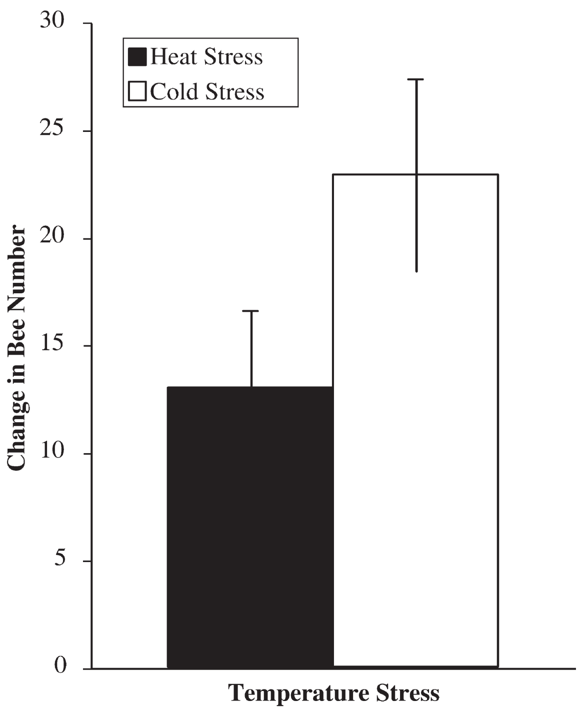

Figure 2. Mean increase (±standard error) in number of stationary bees on brood comb after application of localized heat and cold stress. There was a significant increase in stationary bees on the brood comb in response to both types of temperature stress

Chilling experiments

The outside surface of the hive wall had a mean temperature of 23.8 °C before cooling and 6.7 °C after cooling. No hive had less than an 8 °C decrease and most had a decrease that was greater than 15 °C. *Stationary bees:* The number of stationary bees on the hive wall decreased significantly after chilling (mean decrease, –17.46 ± 4.3 paired $t_{28} = 12.00$, $p < 0.0001$) while the number on the brood comb increased significantly after chilling (mean increase = 22.88 ± 4.49, paired $t_{24} = -5.09$, $p < 0.0001$; Fig. 2). The change in the number of stationary bees was significantly different between the hive wall and brood comb regions (paired $t_{24} = 2.05$; $p < 0.0001$;

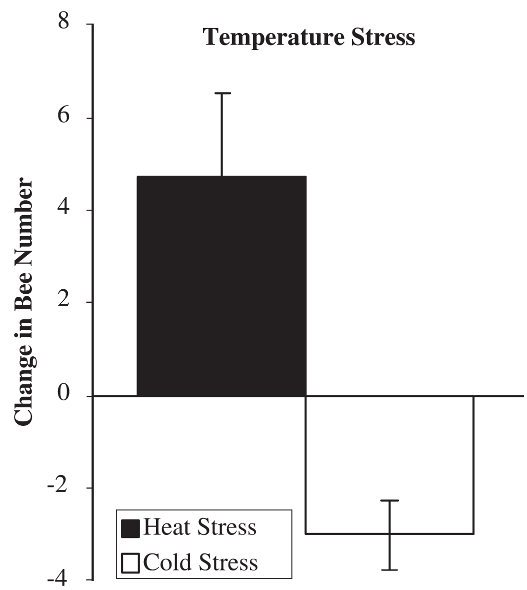

Figure 3. Mean change (±standard error) in number of moving bees on the hive wall after application of localized heat and cold stress. There was a significant increase in moving bees with the application of heat stress, and a significant decrease with the application of cold stress

Table 1. Response of bees in each location observed to localized hot and cold stress, and comparison of change on the glass and comb. Percent changes in bee number are given

Treatment	Behavior	Location	Direction (and %) of change in bee number after treatment	Comparison of change on Glass vs. Comb
Localized Heat Stress	Stationary Bees	Hive Wall Comb	↑ (117.7) ↑ (66.6)	Not Significant
	Moving Bees	Hive Wall Comb	↑ (87.2) ↓ (7.7)*	Significant
Localized Cold Stress	Stationary Bees	Hive Wall Comb	↓ (28.3) ↑ (38.1)	Significant
	Moving Bees	Hive Wall Comb	↓ (23.6) ↓ (30.5)	Not Significant

* $p = 0.08$, all other direction of change after treatment $p \leq 0.05$.

Table 1). *Moving bees:* The number of moving bees decreased significantly on both the hive wall (mean decrease, –3.03 ± 0.75, paired t_{28} = 3.80, p < 0.001; Fig. 3) and brood comb (mean decrease, –5.19 ± 2.46, paired t_{24} = 2.11, p < 0.05) after chilling. No statistical difference in the decrease in moving bee number was detected between the hive wall and brood comb regions (paired t_{24} = 0.96, p = 0.35; Table 1).

Discussion

Heat investigation

Data presented here indicate that in response to localized heat stress (1) the number of stationary bees on the hive wall and brood comb increases and (2) the number of moving bees on the hive wall, but not on the brood comb, increases. These results are consistent with both previous studies of heat-shielding (Starks and Gilley, 1999; Starks et al., 2005), and are highly suggestive that the behavior plays an important role in hive thermoregulation.

Starks and Gilley (1999) showed that heat-shielding bees decreased the amount of heat transferred from outside to inside the hive. An increase in bee number over the heat source on the hive wall and brood comb is likely to create a layered 'heat sink' with two layers of bees and an additional layer of insulating air. This arrangement will significantly decrease the amount of heat transferred to the developing brood and thus may explain the mechanism of the results described by Starks and Gilley (1999). As developing bee pupae are very heat-sensitive (Fukuda and Sakagami, 1968; Bailey and Ball, 1991), and heat sinks serve to protect sensitive components (Biber, 1995), this arrangement appears to have functional significance.

Our data also indicate that in response to localized heat stress the number of moving bees increases on the hive wall but decreases over the brood comb. This increased movement near the thermal radiation may increase airflow, which would facilitate cooling of the immediate area (Biber, 1995). As bees are known to flap wings to create cooling currents when the brood comb region is excessively hot (Winston, 1987), it is not unrealistic to hypothesize that this movement is designed to dissipate unwanted heat. In addition to creating currents, bees may be moving away from the heat source, taking absorbed heat with them, and releasing it elsewhere (perhaps outside the hive). Alternatively, instead of actively producing air currents or sequestering heat to be dumped in less sensitive areas, bees might simply be attracted to warm areas, which would naturally result in increased movement on heated hive wall sections. This hypothesis, however, would not explain why movement decreases on the brood comb. Increased movement on the brood comb would disrupt the shield and would not increase airflow near the heat source. While it is likely that movement on the hive wall increases airflow, thus dissipating excess thermal energy, it remains unclear if this behavior is designed specifically for this purpose.

Another possible explanation to the movement data is that bees are scattering on the hive wall in response to light exposure during counting. During counts the bees were exposed to white light, which included wavelengths within their visible spectrum. This explanation, however, is unlikely to be valid. If the movement were due to the introduction of white light during counting, increased movement would be expected in all light exposed areas. Increased movement was observed on the glass during the count after heating, but not on the comb, which was also exposed to light. Furthermore, increased movement was not observed anywhere after cooling even though the bees were exposed to the same levels of light as during the heating experiment. Further experimentation would conclusively rule out the possibility that the results are due to the experimental design. For example, the experiment could be repeated using red light, which is not detectible by the bees.

To date, heat-shielding has only been observed in experimental hives (Starks and Gilley, 1999; Starks et al., 2005), which serve to mimic cavity nest conditions. Our study subspecies, *Apis mellifera ligustica*, is a temperate subspecies and colonies within this subspecies generally build cavity nests (Winston, 1987); thus our design appears suitable for this investigation. There are isolated instances, however, when colonies within temperate subspecies build exposed nests. It is possible that heat-shielding also occurs in hives exposed to direct solar radiation, such as the open nests of tropical *A. mellifera* subspecies (Winston, 1987). Indeed, many *Apis* species such as *A. dorsata*, and *A. florea* build only open nests, which are frequently exposed to thermal-radiation (Michener, 1974; Seeley et al., 1982). The fact that heat-shielding, which would appear to be more beneficial for open nesting bees, is observed in temperate cavity nesters raises the possibility that the trait evolved in a common *Apis* ancestor (Starks et al., 2005).

Much insight could be gained into the evolutionary history of this behavior by the study of non-cavity dwelling species. The dwarf honey bee, *Apis florea*, may be a good candidate as they build relatively small open colonies (~5000 individuals) and have docile workers (Michener, 1974). An investigation into how or if movement impacts airflow would also be a good compliment to the work presented here. At present it is not possible to discern whether the movement over heated regions is a specific adaptation or an adaptive by-product of attraction to excess heat. The method of heat release by the living heat sink remains unknown, although, it has been hypothesized that the location of release is outside the brood comb region (Starks et al., 2005). There may be a heat release mechanism as exposure to extreme heat is detrimental to the adult bee (Coelho, 1991).

Cold investigation

Data presented here indicate that in response to localized cold stress (1) the number of stationary bees on the hive hall decreases while the number of stationary bees on the brood comb increases and (2) the number of moving bees on both the hive wall and brood comb decreases. As with the heat-shielding results, these results are highly suggestive that the behavior plays an important role in hive thermoregulation.

The results of our 'cold-shielding' experiments are consistent with previously described responses to cold ambient temperatures. Behavioral responses of the honey bee to cold winter conditions include clumping over the brood comb, and decreased activity (Simpson, 1961; reviewed in Winston, 1987). Our data suggests that clumping may be a specific adaptation to protect the temperature sensitive brood comb even outside of the winter months. In this view, the decrease in bee number on the hive wall is a direct result of the need to clump onto the temperature-sensitive developing brood. In addition to shielding the brood from the cold, these clumped bees may be elevating body temperature to protect the developing brood (see Bujok et al., 2002)

Alternatively, the decrease in bee number observed on the hive wall after cold stress may be an attempt to minimize heat loss by the adult workers. If this was the case however, we would expect to see the bees clumping in an area of the hive that was removed from the cold pack. The bees could have clumped in a corner, or on the other side of the comb. Instead they clumped directly underneath the cold source. The decreased movement in all locations may help conserve energy in the hive. As our experimental colonies had live brood throughout the lower frame, not just under the cold pack, we speculate that the reduction in movement may keep the cold air from spreading throughout the brood comb and damaging developing brood.

It is currently unclear whether 'cold-shielding' is a distinct behavior or if the bees are demonstrating typical winter behavior under novel conditions. One way to distinguish between these possibilities would be to periodically record the comb temperature at the center of the experimental area of brood comb during extended exposure to localized cold stress. During time periods when brood is not produced (e.g., in late fall and early winter in New England) temperature is not as well maintained and areas can fluctuate ± 20 °C. In contrast, during brood rearing season there is very little temperature variation within the brood comb (Heinrich, 1980, 1985; Seeley, 1985; reviewed in Winston, 1987). Large temperature fluctuations in the center of cold-exposed brood comb would suggest that the behavior is an artificially induced early winter response, and not a brood protection response. Recording age of cold-shielders could also support the hypothesis that the behavior is a brood protection response: while all ages of workers clump during the winter, bees aged 12–14 days are overrepresented performing the brood protecting heat-shielding behavior (Starks et al., 2005).

Conclusions

In conclusion, with respect to heat-shielding we have demonstrated that under these experimental conditions (1) worker number increases on both the hive wall and brood comb in response to localized heat stress thus creating an effective heat sink, (2) worker movement increases on the hive wall in response to localized heat stress, which may increase airflow near the source of radiation and facilitate cooling, and (3) worker movement decreases on the brood comb in response to localized heat stress, which would serve to create a more solid shield. With respect to cold-shielding we have demonstrated that under these experimental conditions (1) worker number decreases on the hive wall in response to localized cold stress, which may minimize heat loss by adult bees, (2) worker number on brood comb increases in response to localized cold stress which may serve to insulate brood, and (3) worker movement decreases on both the hive wall and brood comb under cold stress, possibly serving to decrease energy loss by adult workers (Table 1). Combined these results suggest that honey bee workers display a fine-tuned response to local temperature stress and might best be considered 'mobile insulating units'.

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