Vasculature of the Hive: heat dissipation in the honey bee (*Apis mellifera*) hive

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Abstract

Eusocial insects are distinguished by their elaborate cooperative behaviors and are sometimes defined as superorganisms. As a nest-bound superorganism, individuals work together to maintain favorable nest conditions. Residing in temperate environments, honey bees (*Apis mellifera*) work especially hard to maintain brood comb temperature between 32°C and 36°C. Heat shielding is a social homeostatic mechanism employed to combat local heat stress. Workers press the ventral side of their bodies against heated surfaces, absorb heat, and thus protect developing brood. While the absorption of heat has been characterized, the dissipation of absorbed heat has not. Our study characterized both how effectively worker bees absorb heat during heat shielding, and where worker bees dissipate absorbed heat. Hives were experimentally heated for 15-minutes during which internal temperatures and heat shielder counts were taken. Once the heat source was removed, hives were photographed with a thermal imaging camera for 15-minutes. Thermal images allowed for spatial tracking of heat flow as cooling occurred. Data indicate that honey bee workers collectively minimize heat gain during heating, and accelerate heat loss during cooling. Thermal images show that heated areas temporarily increase in size in all directions, and then rapidly decrease to safe levels (< 37°C). As such, heat shielding is reminiscent of bioheat removal via the cardiovascular system of mammals.

Keywords colonies, group behavior, heat transfer, temperature dynamics

Introduction

Social insects are the most abundant of land dwelling arthropods and are found, among other habitats, in almost all forests around the world (Hölldobler and Wilson 2009). Such success and diversity is attributed to the division of labor and the cooperative behavior exhibited within these social groups. Eusocial insects (i.e. ants, termites, and colonial wasps and bees) exhibit such extreme levels of cooperation that the colony is sometimes classified as a superorganism. A superorganism (as first defined in reference to ants) is a group of individuals that collectively share the characteristics of an organism (Wheeler 1910; Hölldobler and Wilson 2009). In this regard, each individual in the colony is analogous to a single cell and each caste is analogous to an organ (Wheeler 1910; Hölldobler and Wilson 2009).

The nest serves as the skin and the skeleton of this superorganism. The nest provides a microhabitat that allows for social life to happen—it is where food is stored, brood are raised, and colony members interact. As such, the nest must be appropriately protected and maintained – we call behaviors designed to accomplish this social homeostatic mechanisms (Wilson 1971). As the nest is both built and maintained via a collective effort, research on nest architecture and social homeostasis is integral to understanding the evolution of social behavior (Hansell 1996).

Temperature maintenance is a social homeostatic mechanism that lends itself well to experimentation. Particularly important to brood development, temperature is consistently maintained in a variety of social insect nests, despite their structural diversity. The first step in creating a buffer between ambient and nest temperatures is selecting a nest location (Jones and Oldroyd 2006). As such, nest location is very much a part of “the nest.” Honey bees nest in cavities that provide insulation (Heinrich 1979; Jones and Oldroyd 2006) while termites and many ant species build intricate underground nests equipped with ventilation (Wheeler 1910;

Korb 2003; Jones and Oldroyd 2006; Wilson 2009). Social wasps construct enveloped and unenveloped nests in diverse locations including cavities found both above and below ground (Jones and Oldroyd 2006; Jeanne and Morgan 1992).

When ambient temperatures are not favorable for brood development, both ants and termites actively move brood to more protected areas (Wheeler 1910; Wilson 1971; Korb 2003; Wilson 2009). In contrast, honey bees and social wasps cannot physically move their brood and must actively regulate temperature. For example, both honey bees and social wasps use evaporative cooling when ambient temperatures are too high for proper brood development (Wilson 1971; Prange 1996). Despite these similarities, honey bees stand alone when it comes to controlling even the slightest temperature fluctuations within their nest, the hive.

As part of the colony, adult honey bee workers can withstand hive temperatures up to 50°C (Coehlo 1991), but brood must remain between 32°C and 36°C with a specific preferred temperature is 34.5±1.5°C (Kronenberg and Heller 1982; Jones et al. 2005; Tautz 2008). This temperature range is necessary for proper larval as well as pupal development (Winston 1987; Kronenberg and Heller 1982). Temperatures higher than 36°C can increase brood mortality, delay development time, and cause malformations of the wings, stinger, and proboscis (Fukuda and Sakagami 1968; Winston 1987; Groh et al. 2004). Conversely, temperatures lower than 32°C can result in immune compromise and a decrease in foraging performance as an adult (Winston 1987; Tautz et al. 2003; Groh et al. 2004; Jones et al. 2005). Temperature is not as strictly maintained in all areas of the hive; stable temperatures are not as necessary for resources such as pollen and honey (Fahrenholz et al. 1989). Even within the broodcomb, temperature maintenance varies; pupae are more sensitive to variable temperatures (Starks et al. 2004; Jones et al. 2005; Tautz 2008).

To maintain brood comb temperature range during brood development, honey bees use a variety of thermoregulatory behaviors. For example, to increase temperature, workers create heat by isometrically contracting thoracic muscles, similar to shivering in mammals (Heinrich 1980, 1985; Bujok et al. 2002; Kleinhenz et al. 2003; Tautz 2008). To increase the overall temperature of the brood comb, multiple workers contract their muscles, simultaneously heating many larvae/pupae (Heinrich 1980, 1985). To increase the temperature locally, a single worker can enter an empty cell and warm the adjacent brood (Bujok et al. 2002; Kleinhenz et al. 2003; Tautz 2008). In order to survive long periods of cold temperature, workers exhibit clustering to maintain heat within the hive. During clustering, workers huddle together to retain heat actively produced by the workers at the center of the cluster (Simpson 1961; Kronenberg and Heller 1982; Stabentheiner et al. 2003).

During hot conditions, workers are able to decrease temperatures. To decrease the temperature on a large-scale, workers fan the hive with their wings, and may simultaneously spread water to induce evaporative cooling (Lindauer 1954; Heinrich 1979, 1980, 1985; Prange 1996; Tautz 2008). To decrease temperature on a fine-scale, workers use a behavior called heat shielding. To achieve heat shielding, young workers orient themselves between a heat source and brood comb, creating a physical barrier where they passively absorb heat (Starks and Gilley 1999; Siegel et al. 2005; Starks et al. 2005; Tautz 2008). Most workers heat shield by placing their ventral side directly against a heated surface (Starks and Gilley 1999). In conjunction, other workers have been observed orienting their ventral surface against potentially affected brood comb (Siegel et al. 2005). Research has shown that heat shielding is a context dependent response; changes in intensity of heat, placement of heat, and density of brood all influence the number of workers that engage in the behavior (Starks and Gilley 1999; Siegel et al. 2005; Starks et al. 2005).

Once workers have absorbed heat, it must be dissipated away from the brood. Studies have shown that foragers can cool their bodies by flying to simulate wind (Heinrich 1979, 1980; Fahrenholz et al. 1989) and by regurgitating nectar, allowing it to absorb excess heat (Prange 1996). While this provides a mechanism for heat dissipation, it does not provide information on where workers dissipate absorbed heat within a hive. To investigate this particular aspect of social homeostasis, we created localized heat stress in experimental honey bee hives and used thermal imaging to visualize movement following experimental heat stress. At least two types of heat movement are possible 1) pattern free dissipation and 2) pattern rich dissipation. Pattern free dissipation would be characterized by no trend in the direction in which heated workers move within the hive. Pattern rich dissipation would show some directed trend in movement of heated workers. This movement might be out of the hive or to a less regulated part of the hive (i.e. where there is stored honey or pollen).

Materials and Methods

Subjects

In June 2013, seven two-frame Plexiglas (6 mm thick) observation hives (53 cm x 48 cm x 5 cm) with active honey bee colonies were installed at the Tufts University International Social Insect Research Facility in Medford, MA. Each hive was re-queened before transportation, and queens were restricted to one frame using a queen excluder. This ensured that for the duration of the experiment, all brood would be laid and reared in only one of the two frames (see Siegel et al. 2005 and Starks et al. 2005; Fig. 1).

After installation, the facility was kept at a constant temperature of approximately 20°C. For one week, the newly installed hives were fed 100 ml of 1:1 sucrose water each day during orientation and acclimation. During the initial feeding period, each hive was censused for approximate number of bees. The approximate colony sizes were determined to be 1,000 – 2,500 adult bees (Sammataro and Avitabile 2011). Comb maps of capped and uncapped brood, capped and uncapped honey, pollen, and empty cells were generated to identify similar areas across hives for treatment (i.e., heating). Because workers preferentially shield brood comb (Starks and Gilley 1999; Starks et al 2005), we identified areas in each experimental hive that had similar amounts of brood. A 12.5 cm x 10 cm section at the left center of the lower frame was found to have similar quantities of brood comb in all hives and was subjected to heating during experimental trials (Fig. 1).

Collection of Temperature Data During Heating

To minimize heating of the surrounding hive areas, hives were fitted with insulation (Foamular 250 1-inch insulation, R = 5.0). A 12.5 cm x 10 cm rectangle was cut out of the lower left quadrant of each piece of insulation (see above). Since bees do not see red light, the experimental window was covered with red theater gel (Daumer 1956, as cited in von Frisch 1967; Gribakin 1969). This ensured that any observed change in behavior would be due to the presence of heat and not the presence of light. To heat the un-insulated experimental window, a theatre lamp with a heat bulb (GE 250W infrared heat reflector bulb) was pre-heated for 5-minutes (reaching 155.9°C – 173.0°C) and then placed 50 cm away from the window. From July 11, 2013 to July 19th, 2013, each of the seven experimental hives and a control hive were heated for 15-minutes using the methods described above. Two trials were run for each hive; all trials were done between the hours of 9:00 a.m. and 12:00 p.m. Additionally, all trials were run in the dark in order to mimic the natural nest environment and minimize possible light effects.

Internal temperature data were collected before heating, during heating, and immediately following heating. During heating, the temperature—both under the heated window and under the insulation—was taken every minute using an Omega handheld digital thermometer with type K Teflon insulated thermocouples sensitive to 0.1°C (see numbered thermocouples in Fig. 1).

A control hive—a hive with all comb characteristics but no honey bees—was heated to approximately normal brood comb temperatures (31.1 ± 4.7°C) using two small electric heating pads (ZooMed Repti-therm 4W heat pad). Coupled with the presence of the typical comb characteristics (brood, pollen and honey), the heating pads simulated a hive environment as if there were active bees in the hive. Once the control hive was stable at approximately normal brood comb temperatures, the hive was subjected to the heating protocol outlined above.

Observation of Heat Shielders

The number of heat shielders—as evidenced by individuals with the ventral side of their bodies placed against the heated Plexiglas—was counted before and immediately after each heating period (see methods in Starks and Gilley 1999; Siegel et al. 2005; Starks et al 2005).

Collection of Thermal Images During Cooling

Immediately after counting heat shielders, the insulation was removed and a thermal imaging camera (Fluke Thermal Imager model Ti32, emissivity=0.95) was used to take infrared images of the hives every thirty seconds for 15-minutes. In addition to general heat maps, the camera provided data on temperature extremes on the external Plexiglas surface of the observation hive. This procedure was repeated twice for each hive. Each hive was allowed a full day to recover between trials. Hives were never allowed to heat to an internal temperature above 41°C and were monitored for detrimental effects (i.e. increased mortality of workers and brood, abnormally slow workers) within the heated window – none were observed.

Statistical Analysis

Temperature gained during heating was analyzed using a 2x2 within subjects ANOVA (control vs. experimental, under window vs. under insulation); assumptions of equal variance and normal distribution were met. The change in heat-shielder number before (n=14) and after (n=14) heating was analyzed via a paired *t* test. One-sample Student's *t* tests were used to compare control (n=2) and experimental (n=14) hives for the average start and end temperatures post-heating. For each image generated during cooling, the area of heated regions at or above 37°C was determined (see the red and white areas in the images in Online Resource 1). The mean area measured in control (n=62) and experimental (n=434) hives at each time point was compared using a Welch Two-Sample *t* test. Regression analysis was run for both control (n=2) and experimental (n=14) hives to determine the rate in temperature decrease post-heating (time period= 0 – 9-minutes; on average, experimental hives had cooled to 37°C in 9-minutes). Statistics were performed using R Version 3.0.2 (R Core Team 2013).

Results

Heating period

During the 15-minute heating period, overall temperature gain was significantly greater in control relative to experimental hives (F_1=456.5, p<0.001; Fig. 2). The average internal temperature gain under the insulation was significantly lower than the average internal temperature gain under the heated window ($F_1 = 266.5$, $p < 0.001$; Fig. 2). This was seen in both control and experimental hives, indicating that the insulation was effective in creating localized heat stress. Consistent with the creation of localized heat stress, workers displayed heat shielding; there were significantly more heat shielders after heating than before ($t_{13}=2.82$, $p=0.01$; Fig. 3). Immediately after heating, the average external temperature of the Plexiglas window was significantly higher for the control hive (48.5 ± 0.8°C) than for experimental hives (46.5 ± 0.6°C) ($t_{15} = 5.73$, $p < 0.001$; Fig. 4).

Cooling period

During the 15-minute cooling period, the control hive did not reach temperatures safe for brood development (i.e., below 37°C; Online Resource 1; Fig. 4). By comparison, the mean temperature of the experimental hives reached safe levels within 10-minutes (Online Resource 1, Fig. 4). The mean temperature of the experimental hives at the end of the cooling period (35.2 ± 0.2°C) was significantly lower than that of the control hive (40.3 ± 0.7°C) (t_{13}=-25.85, p<0.001; Fig. 4). On average, the experimental hive cooled to a temperature less than 37°C almost twice as fast as the control hive ($\text{slope}[0{:}9\text{min}]_{exp}$=-1.87, $\text{slope}[0{:}9\text{min}]_{con}$=-1.07; Fig. 4).

When the thermal images of experimental and control hives were compared, differences in the area of regions above 37°C were observed (Online Resource 1; Fig. 5). Immediately after heating, control and experimental hives exhibited a similarly sized heated area (Online Resource 1; Fig. 5). However, as cooling continued, the area that showed temperatures above 37ºC increased in the experimental hives; no such effect was seen in the control hive (t_{372} = 4.32, p< 0.001; Fig. 5). By about 3-minutes post-heating, the average size of the high heat area in the experimental hive had increased dramatically in all directions (Δ area = 6660 ± 2630 pixels^2). Following this spike, the heated area of the experimental hives then decreased rapidly until the hive reached safe levels. By comparison, the high heat regions in the control hive decreased gradually without any increases and had not cooled even after 18-minutes (Online Resource 1, Fig. 5).

Discussion

Consistent with previous research, there was a significant increase in the number of heat shielders after heating, indicating that heat shielding occured (Fig. 3). As such, temperature dynamics within heated regions of the hive were likely influenced by this behavior. In addition, during the 15-minute heating period, temperatures remained lower in the heated window of the experimental hives than in the control hive (Fig. 2). Since the control hive did not have bees, differences in temperature were likely caused by the worker bees themselves. The significantly lower temperature gain in the experimental hives highlights the workers' ability to minimize temperature increases during localized heat stress (Fig. 2). Similarly, workers effectively lowered brood comb temperature back to safe levels within 10-minutes (Fig. 4).

While cooling, the change in area of the high heat regions between the experimental and the control hives differed markedly (Fig. 5). The experimental hives' sudden increase in high heat area—and the lack of an increase in the control hive—implies that the workers were actively moving heat out of the heated region (Fig. 5). Thermal images show that the area increases in *all* directions from the heated point in the hive, showing a radial movement of the workers to the periphery of the hive (Online Resource 1). In a natural hive, this movement would drive heat to less regulated areas of the hive, such as honey and pollen stores (Seeley and Morse 1976). Since feral honey bees build their comb in the same vertical fashion that is found in observation hives, our data are representative of what may occur in the field (Winston 1987).

This is the first study to characterize where heat is moved following heat shielding, and how effectively workers dissipate this absorbed heat. The results of this study demonstrate that workers are able to work in concert to inhibit localized temperature gain, and then work rapidly to dissipate what temperature gain is experienced over the developing brood. The initial expansion of the heated area – moving heat from hot to cool areas – is reminiscent of bioheat transfer via the cardiovascular system of mammals. Thus, these data provide additional support for the argument that a honey bee colony can be viewed as a superorganism as well as a concrete example of social homeostasis within a nest.

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Online Resource 1 Comparison of representative experimental and control infrared images taken pre- and post-heating. The color green indicates the presences of bees in the experimental hive and the heating pads in the control hive. Red and white areas indicate temperatures above 37°C. In the experimental hive, the red area grew significantly larger within 3-minutes of cooling and disappeared within 9-minutes. In contrast, the high heat area in the control hive gradually decreased in size and still persisted after 18-minutes of cooling. Such differences indicate that workers effectively cooled the hive by absorbing the heat moving it into the periphery.

Fig1 Diagram of the experimental set up and photo of the interior hive structure. Each hive contained two frames separated by a queen excluder. The characteristic pattern of hive structure is made clear by the dashed lines. After surveying all seven experimental hives, the area selected for heating was to the left center of the brood area. This particular area was selected as it had similar brood densities across all hives. For the purposes of temperature collection, one thermocouple (1) was situated on the Plexiglas that was to be covered with the insulation. The second thermocouple (2) was situated in the brood comb of the heated window so that internal temperatures before, during and immediately following heating could be recorded.

Fig2 Mean change in internal temperature for insulated and un-insulated regions of the observation hives. For both control (n=2) and experimental hives (n=14), the temperature increase was more gradual under the insulation than under the heated window ($p<0.001$). The heat gain in the experimental hive was significantly smaller than the gain in the control hive ($p<0.001$). Since the control hive lacked workers, these data demonstrate that the workers are responsible for regulating temperature changes within the hive. Data were taken from seven experimental hives and one empty control hive. Two trials were done for each hive. Error bars represent one standard error.

Fig3 Mean number of heat shielders before (n=14) and after heating (n=14). There were significantly more heat shielders observed after heating than before heating (*p*=0.01) indicating the heat shielding occurred. Data are from seven observation hives, two trials each.

Fig4 Mean change in surface temperature of the heated section of brood comb during the 15-minute cool down period. Immediately after heating (time=0), the control hive (n=2) had a significantly higher mean temperature than the experimental hive (n=14; *p*=0.005). After only 10-minutes of cooling, the experimental hives were able to reach a safe temperature (< 37°C) as highlighted by the shaded gray area. In contrast, the control hive did not reach safe temperatures until after the 15-minute cooling period (data not shown). Data were taken from seven experimental hives and one empty control hive with comb. Two trials were done for each hive. Error bars represent one standard error.

Fig5. The average percentage change in the area of the heated region for experimental (n=434) and control (n=62) hives over time. The heated region was defined as the red area above 37°C (see Online Resource 1) for each generated heat map. The percent increase in the experimental hives demonstrates a dramatic increase in the high heat area within the first 5-minutes; this pattern was not observed in the control hive. The high heat area within the experimental hives—but not the control hive—then rapidly decreased until the high heat region disappeared and the hives were cooled to safe levels (<37ºC). Data were from seven observation hives and one control hive with comb, two trials were done for each hive. Error bars represent one standard error.