Patterns of thermoregulatory mass evacuation in honey bees

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 9, 2025

Keywords: Thermoregulation, social physiology, collective behavior, nest evacuation, *Apis mellifera*

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Abstract

Honey bees are master thermoregulators, capable of maintaining nest homeostasis across fluctuating ambient temperatures. When workers must cool their nest, they use multiple thermoregulatory behaviors (e.g., fanning, collecting water), but bearding, where hundreds to thousands of workers evacuate their nest and form a bivouac outside, is relatively unexplored. Here, we (1) describe natural bearding patterns, (2) experimentally manipulate colonies to determine what impacts beard size and timing, and (3) explore how workers dissipate back into their nest. We show that bearding occurs daily in hot weather, but the largest beards consistently happen in the evening/night, between 1800 and 2400. Beards are located around the nest entrance, but workers bias their position towards the shaded-side of the nest box. As colony size increases, beard size and duration also increase, but the proportion of the colony bearding does not increase with colony size. Colonies with and without brood still cast beards; brood presence/absence did not impact beard size or duration. After noticing that beards tend to dissipate at sunrise, we experimentally showed that beards induced in the afternoon dissipate within 1-2 hrs, whereas beards induced in the evening remain overnight (10+ hrs). Bearding overnight, however, does carry risks for developing brood inside, as nest temperatures dropped below the optimal range, until the beard dissipated at sunrise. What cues workers use to depart the beard remain unknown, but experimentally illuminating colonies at night did not induce beards to dissipate. Our results suggest that bearding is an individual decision, not one that is coordinated across the colony. Still, these individual actions result in a dramatic collective response that colonies employ to reduce the temperature of their nest. Here, we show how and when colonies use bearding, despite its risks.

Acknowledgments

I would like to thank P. Prathibha and Peter R. Marting for their help on the paper. Morgan Pendergrass for loaning us a portable generator to illuminate apiaries at night, Clair Dickinson and Kaitlyn McInerney for their help with colony management, the Gardner family for providing apiary space, and RD Bassar for helpful comments on the manuscript.

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Chapter 1: Review of thermoregulatory behaviors: from individuals to integrated superorganisms

Temperature is a critical constraint for life. Organisms have a range of temperatures in which they can survive, and biological processes within organisms have a range of temperatures in which to function optimally (Heuy and Kingolver 1989). Maintaining body temperature outside of these limits can cause protein degradation and eventually death (Ferguson et al 1990, Orr 1955) To stay within these limits organisms can employ physiological or behavioral solutions (Heuy et al 2012). Thermoregulatory behaviors are present across many animal taxa and are expressed individually or in the context of a group.

Temperature impacts many biological processes broadly, including; development, sex determination, locomotion, metabolic rate, longevity, geographic range of a species, and daily activity patterns (Howe 1967, Matsumoto and Crews 2012, Lailvaux 2007, Schulte 2015, Keil et al 2015, Woodward 1990, Dillon et al 2010, Vieira 2010). Organisms have a particular thermal niche that they are able to successfully inhabit (Porte and Kearney 2009). Thermoregulation, the ability to balance temperature loss and gain, allows organisms to broaden their thermal niche. It has even been proposed that behavioral thermoregulation can buffer or amplify evolutionary forces in a process dubbed the Bogert effect (Muñoz 2021).

Animals can be divided by their ability to regulate body temperature via metabolic pathways. Endotherms use metabolic pathways to regulate body temperature while ectotherms do not (Hafez 1964, Kearney et al 2009). However, both endotherms and ectotherms use behavior to thermoregulate. While endotherms do so with a suite of other behaviors in tow, ectotherms rely primarily on microhabitat selection (Stevenson 1985). A single organism is able to behaviorally thermoregulate in a variety of different ways, and behavioral thermoregulation can be a solution for a variety of problems. Here, I explore various behaviors and their role in

thermoregulation. I will begin by discussing individual behaviors and then I will move into progressively more complex social groups, until reaching superorganisms.

Individual thermoregulation via movement

Past research has established critical thermal limits for many organisms (Lutterschmidt, et al 1997). These critical thermal limits mark the edges of survivable temperatures for an organism. To avoid these limits, organisms can engage in the simplest of behavior: move to a more suitable microclimate. Lizards, butterflies, and mammals have been shown to move away from areas that are close to their thermal limits, but probably most mobile animals will move away from extreme temperatures if able (Clench, 1966, Terrien et al 2011). Suitable microclimates to move to may include shade, sun, water, or higher or lower elevations and latitudes. Moving to shade or swimming has been shown to decrease body temperature in vervet monkeys (McFarland R et al 2020).

Animals may also move their bodies to produce heat or cool themselves without moving to a new micro-climate. Primates have been shown to increase movement when temperatures decrease (F. Aujard and F. Vasseur 2001). Many animals are capable of shivering to produce heat, including some insects, birds, and mammals (Heinrich, 1996, Hohtola, 2004). Elephants fan their ears to cool themselves (Wright, P. G. 1984). Some mammals have been shown to increase food consumption at low temperatures, providing more fuel to burn for thermogenesis (Terrien et al 2011). Any animal that is capable of movement can move itself to different thermal environments.

Thermoregulatory behavior towards offspring

Parents may use behavior to thermoregulate their young. Gravid female snakes have a preference towards temperatures optimal for their eggs (Shine 1993). Animals that carry their

eggs outside their body, like some frogs, likewise can have a preference for their young's optimal temperatures rather than their own (Lea Lange et al 2022). Famously, many species of birds incubate their eggs or young with their bodies. Nest building behavior can contribute to the thermoregulation of offspring (Grant 1982). Some birds build their nests differently under different climatic conditions, varying size or building materials to provide more stable and appropriate microclimates (Perez 2020). Larger nests tend to be warmer than smaller nests and there is evidence that climate drives variation in nest size in some species (Collias and Collias 1984, Perez 2020). The material and thickness of a nest can vary across the breeding season, presumably for thermoregulation in some species (Perez 2020), and some studies have experimentally shown this is due to changes in temperature (McGowan et al 2004).Some have even hypothesised that parental thermoregulation is a driving force in the evolution of endothermy in birds and mammals (Clavijo-Baque and Bozinovic 2012, Farmer 2000, Angilletta et al 2003).

Thermoregulatory behaviors for infection

Animals can use behavioral thermoregulation to fight parasitic infection. Just like their hosts, parasite fitness is related to its body temperature. By changing their body temperature, hosts can alter the temperature that the parasite is experiencing. Fever is an example of this, where body temperature is regulated above normal in order to fight infection (Rakus et al 2017). Behavioral fever can occur when organisms actively seek out micro-climates with higher temperatures, raising their body temperature in order to fight infection. This has been shown in insects, fish, amphibians and reptiles (See Rakus et al 2017 for large citation list for vertebrates, insect examples: Boorstein et al 1987, Adamo 1998, Lewis et al 1986). It has also been shown that newborn rabbits, who are unable to maintain their own body temperature, use behavioral

fever to fight infection (Satinoff et al 1976). Also of note is a reverse behavioral fever in bumble bees, where they seek out colder temperatures to kill an internal parasite (Müller, Christine B and Paul Schmid-Hempel).

Social Groups:

The previous examples describe solitary or parental pair behaviors. However, there are some behaviors that can only be exhibited in social groups. Hudding with conspecifics to thermoregulate is seen in many species of mammals and birds (Gilbert C et al 2010). Some animals also share nests which can contribute to more efficient thermoregulation (Ebensperger, Luis 2001, Williams et al 2013, Edelman 2007). Thermoregulation in the context of social groups probably allows organisms to access thermal niches that they would otherwise be unable to access.

As social groups become more integrated so do their suite of thermoregulatory behaviors. Simply aggregating in mass increases local temperature and therefore lowers development time in the larval stage of bot flies and blowflies (Charabidze et al 2011, Aubernon et al 2019). In comparison, the obligately social honey bee maintains its brood nest constantly between 33℃ and 35℃ by using a wide variety of behaviors including heat shielding, fanning, evaporative cooling, and bearding (see Chapter 2 for more details). Many species of ants, also obligately social, will move their brood around their nest to maintain optimal rearing temperature (Holldobler and Wilson 1990).

Superorganisms:

Superorganisms are groups of multicellular organisms that function together like a single organism (Holldobler and Wilson 2009). Honey bees, army ants, naked mole rats, and termites are all examples of superorganisms. In these systems, workers will cooperate together in order to help the colony with some task, for example workers maintain homeostasis, collect food, rear

young and defend the nest from parasites or predators. One of the most critical tasks of a superorganism is to produce workers. Developing workers in superorganism colonies often require a particular temperature in order to develop quickly and without malformaties. The colony maintains nest temperature for the developing workers through collective behavior, and nest architecture.

Just like for individuals, movement plays a critical role in behavioral thermoregulation for superorganisms despite the generally sessile nature of their nests. Because of this, colonies are unable to move and the workers of the colony must build the appropriate thermal environment inside their nest. For example, red wood ants build their nest in such a way that it provides a thermal gradient between the top of their nest and the bottom (Jones and Oldroyd 2009, Kadochová S and Frouz J 2013).. They are then able to move their brood up and down this gradient, allowing for appropriate thermoregulation even in a variable environment and with a immobile nest (Jones and Oldroyd 2009, Kadochová S and Frouz J 2013). Army ants on the other hand live in bivouacs, mobile nests made of the living bodies of their workers. They form a bivouac with ventilation tunnels, capable of maintaining 25∓1℃ temperature for their brood (Franks, Nigel R 1989). The Western Honey Bee will evacuate their nest en-mass under high ambient temperatures, likely to help cool the nest (Ostwald et al 2016).

Social insect colonies may also build their nest to aid in thermoregulation.Termites make giant nest mounds with well placed corridors that ventilate and cool their nest (Judith Korb 1993). One species builds its mounds to “point” north, maximizing shade on the mound (Jacklyn 1992). Another species builds its mound in such a way that the change in temperature throughout the day creates a flow of air that flushes out the CO2 inside the nest (King et al 2015). The

Western honey bee lives in tree cavities, but workers build two-sided wax cells, which allow for better thermoregulation than single-sided cells (Smith et al 2024).

Honey bee colonies provide particularly good examples of collective thermoregulation. Honey bee nests are made of wax and require a large amount of energy to produce and honey bees are unable to move their brood like the red wood ants. This means that after a nest has been constructed a honey bee colony must rely solely on the behavior of their workers to thermoregulate. Eggs, larvae, and pupae of the honey bee *Apis mellifera* must be maintained between 33℃ and 35℃ or the developing pupae will emerge with malformaties (Jones et al., 2005). In order to maintain this tight thermal range, worker honey bees exhibit a complex series of behaviors including; water foraging, fanning, evacuation, heat shielding, and huddling (Johnson 2023). Colonies dynamically respond to changes in environmental temperature through the behaviors of its workers. Workers will hold a droplet of water in their mandibles, when it evaporates it cools the area in the same way that sweating works. This requires water, so honey bees workers begin foraging for water, and others create air currents inside the nest by fanning their wings at a particular location. Using this strategy *Apis mellifera* colonies maintain a 2℃ range (33-35℃) from temperatures as cold as -40°C and as hot as 50°C (Johnson 2023). This has likely helped them to have a near global distribution, despite their origin as a tropical species (Hung et al 2018).

Conclusion:

Environmental temperature varies spatially and temporally. Animals must maintain their body within some range of temperatures or face fitness consequences. Animals may use physiology or behavior to regulate their body temperature (i.e., thermoregulate). Animals that can sense temperature and are mobile may simply move away from areas of temperature that are

unstable for them. Some animals thermoregulate their young, or may thermoregulate to fight parasites. As animals become more social, more complex behaviors may emerge. These behaviors include moving away from cold or hot areas, aggregating in mass, and moving young up and down a thermal gradient. An animal's ability to behaviorally thermoregulate plays a role in its geographic range, ecological niche, and can affect its evolution.

Chapter 2: Honey bee beards: internal and external factors driving mass thermoregulatory evacuation

Citation: Rowe, E.B., Prathibha, P., Marting, P.R. *et al.* Honey bee beards: internal and external factors driving mass thermoregulatory evacuation. *Insect. Soc.* (2025). https://doi.org/10.1007/s00040-025-01046-w

Introduction:

Homeostasis is critically important for biological function in a variable environment, and organisms use a combination of physiological and behavioral responses to maintain a consistent body temperature (Smith 1979, Clench 1996). For example, an organism can respond physiologically by sweating or panting, but also behaviorally, by seeking shade to avoid sun exposure. This combination of physiology and behavior helps define the range of ambient temperatures in which individual organisms can successfully thermoregulate, and survive (Muñoz and Losos 2018). When organisms gather into groups, they can also incorporate social behaviors to thermoregulate (Sukhchuluun et al., 2018). This can be as simple as huddling together in cold weather (Le Maho et al., 1976), and as groups become more integrated, so too can their suite of thermoregulatory behaviors.

Superorganisms are a unique level of biological organization, where a group of organisms form a cooperative unit to propagate their genes (e.g., colonies of ants, bees, wasps, and termites; Wheeler 1911, Hölldobler and Wilson 2009, Szathmáry and Smith 1995). In these highly integrated societies, the collective actions of the individual workers can give rise to thermoregulation at the colony-level (Seeley 1995). Colonies of the Western honey bee (*Apis mellifera*) are a classic example of a thermoregulating superorganism because their developing brood must be maintained between 33-36°C (Jones et al., 2005). Despite their brood's narrow temperature range, colonies of *A. mellifera* have an incredibly wide thermal niche, and are

capable of thermoregulation in ambient temperatures as cold as -40°C and as hot as 50°C (Johnson 2023). This wide thermal range is made possible by their collective behavior.

The behaviors that *A. mellifera* workers use to warm the nest are relatively simple – adult bees cluster around the brood, and generate heat with their thoracic muscles (Southwick 1983). To cool the nest, workers spring into action with multiple complementary behaviors. Workers fan their wings inside the nest and at the entrance, creating air currents to cycle out hot air, and replace it with cooler air from the environment (Gravish et al., 2015, Cook et al., 2016, Kaspar et al., 2018, Peters et al., 2017). Foragers collect water, which is spread throughout the nest for evaporative cooling, and even stockpiled in cells or the bees themselves (Kühnholz and Seeley 1997, Ostwald et al., 2016). The brood area, typically dense with workers, empties as workers move frantically throughout the nest (Jhawar et al., 2023). Some workers perform a behavior called heat shielding, where they use their bodies to absorb heat around the brood and will then disperse away from the brood (Starks et al., 2005). Finally, in a robust collective spectacle, workers evacuate the nest en-masse, aggregating outside the colony in a "beard", so-named because the bees clustered around the nest entrance resemble a beard (Fig. 1a).

Although many honey bee thermoregulatory behaviors have been studied extensively (water collection; Kühnholz and Seeley 1997, Kleinhenz et al., 2003, Ostwald et al., 2016, heat shielding; Starks et al., 2005, Bonoan et al., 2014, fanning; Cook et al., 2016, Kaspar et al., 2018) bearding is relatively unexplored (Johnson 2023). Colonies form beards when exposed to a heat stress, particularly when water is unavailable (Ostwald et al., 2016), but general bearding patterns in unmanipulated colonies are largely unknown. We also know that a colony's internal state impacts thermoregulation broadly (e.g., colonies without brood have a more variable metabolic rate than colonies with brood; Southwick 1982) but it is unclear how colony state may

influence bearding (e.g., colony size, brood status). Finally, given that bearding bees settle outside the nest, separated from workers and nest conditions within, it is unknown how bees in the beard know when to return to the nest (e.g., when brood temperature returns to normal).

The goal of this paper is to describe natural bearding patterns, and to experimentally manipulate colonies to understand what impacts the size and timing of beards. Our research investigations are: (1) How does beard size change throughout the day? (2) How does colony state (number of adult workers; presence of brood) impact bearding? (3) When do workers in the beard return to the nest?

Methods:

Apiaries:

Experiments were performed between May and August 2023 at the Smith Bee Lab at Auburn University (32.674, -85.511). Honey bee colonies (*A. mellifera*) were sourced from an apiary containing 20-25 colonies, all initiated as 3lb packages with a mated queen (Gardner Apiaries, Baxley, GA). For each experiment, colonies were matched according to queen status (all queenright), distribution of worker ages (workers obtained from multiple frames), similar nest contents (brood, pollen, nectar/honey stores), and were free from any visible signs of disease (e.g., chalk brood, American foulbrood, deformed wing virus; Hansen, 1987). Colonies housed in nest boxes (as opposed to observation hives, see below), were kept in one of two apiaries: the "concrete" apiary, or the "grass" apiary. In both apiaries, all colonies were fully exposed to the sun, with nest entrances facing south. We used the concrete apiary to raise ambient temperatures, so that colonies would be more likely to beard. When peak ambient temperatures were already high (>40°C), we kept colonies in the grass apiary, located 200m from the concrete apiary. For

any given experiment, all colonies were kept in the same apiary, in the same type of nest boxes (5- or 8-frame Langstroth “deep”).

Measuring beard size:

To study the dynamics of bearding behavior, we established a standard method to measure beard size. Bearding bees form a bivouac around the nest entrance, but this cluster can aggregate beneath the nest box, making it difficult to estimate the number of bees that have evacuated the nest and joined the beard (Fig. 1a). To accurately measure the number of bees in the beard, we modified our nest entrances with plywood, creating a flat vertical surface upon which the bees could aggregate (see Fig. 7). This also helped standardize beards across colonies housed in 3-dimensional nest boxes and colonies housed in observation hives.

To estimate the number of bees in a beard, we photographed the beard from four directions (facing the nest entrance, left side, right side, from above; with ruler for scale, see Fig. 7). We identified bearding bees by their behavior, which includes a characteristic stance (immobile, flattened upon the surface, often hanging in aggregations with other bearding bees). This makes them easily distinguishable from other bees located around the entrance (e.g., foragers quickly walk inside, guards stand alert with front legs lifted, fanning bees have beating wings). For each group of bearding bees (e.g., a single colony may have bees clustered above and below the nest entrance, but also on the left and right side of the nest box), we used ImageJ (Schneider et al., 2012) to measure beard volume (beard area x depth). We then divided beard volume by the average volume of a honey bee to estimate the total number of bees in the beard (average volume of a bee in a beard: 0.35 cm^3; length: tip of head to tip of abdomen, height: base to top of thorax, width: side to side of thorax). Bees on the nest entrance, but not clustered together, were counted from the photographs and added to the estimate. If the beard size was small and direct counting

was feasible, we counted the number of bees in the beard in the field during our observation. Our cutoff for a colony having a beard was 25 bees, which does not include forager traffic, or guards at the entrance. If the number of bees on the entrance was below the 25-bee cutoff, we considered the number of bees in the beard to be zero. Note that, in some experiments, we are working with small colony sizes (~2000 bees), in which case a beard size of 25 bees represents over 1% of the colony's workforce.

Investigation 1: How does beard size change throughout the day?

We first investigated the daily pattern of bearding in unmanipulated colonies (7–10 June; 31 July–3 August 2024). For 12 total colonies (concrete apiary n = 6; grass apiary n = 6), we measured beard size every 3 hrs for 66 hrs. All colonies were kept in 5-frame boxes, with a standardized entrance (2 cm x 2 cm). Apiary temperature was measured using J-type thermocouples and a HOBO 4-channel logger (Onset Computer Corporation, Bourne, MA) taped to the west side of a colony in the center of the apiary. We estimated colony size for each colony at the start of the observation period, and immediately after the final observation, using the Liebefeld method (Imdorf et al., 1987). At the start of this experiment (7 June 2024; timepoints 1200 and 1500), bees in the beard continued to cluster behind the modified entrances, making it difficult to estimate the total number of bees in the cluster (we could still record the presence/absence of a beard). We removed these two timepoints from the analysis involving beard size (but not beard presence/absence), and adjusted the entrances to prevent the clustering problem.

Investigation 2: How does colony state impact bearding?

Impact of colony size on bearding:

To experimentally test the impact of colony size on bearding, we established colonies with 2000, 6000, or 10000 workers (12 colonies total; four of each colony size. These are the same colonies as used for Investigation 1). All colonies were kept in 5-frame boxes, with two frames of brood, two frames of resources (honey, nectar, and pollen), and one empty comb frame. All colonies were given 48 hrs to acclimate before we began measuring beard size every 3 hrs for 66 hrs ("measuring beard size" method above). Brood temperature was monitored using broodminder temperature loggers placed in the center of the colony (Broodminder, Stoughton, WI). We measured colony size again at the end of the experiment to account for any changes during the experiment (Imdorf et al., 1987), and used this final measurement as our estimate of each colony's size. One 2000-worker colony failed to thermoregulate its brood (hive temperature outside the viable 33-36°C), so we removed this colony from our subsequent analyses as it was not indicative of a viable colony.

Impact of brood presence on bearding:

To experimentally test the impact of brood presence on bearding, we established colonies with and without brood. All colonies (n = 8; four per treatment group) were established in 5-frame boxes with 5000 workers. Colonies in the "brood" treatment group were given four bee-frames with a mixture of eggs, larvae, and pupae, and one frame of resources (honey, nectar, and pollen); colonies in the "no-brood" treatment group were given two frames of resources and three empty comb frames. All colonies were given 48 hrs to acclimate, after which we measured beard size every 3 hrs for 66 hrs. We then estimated colony size (Imdorf et al., 1987), to account for changes during the experiment.

To directly compare the effect of brood presence on beard size for each colony, we then swapped the brood status of each colony, and repeated the experiment ("brood" colonies became

"no-brood" colonies, and vice versa). Colonies were given 24 hrs to acclimate before we resumed beard measurements (every 3 hrs for 66 hrs). After the final beard measurement, we re-estimated colony size, and used this estimate of colony size for the second round of beard observations (i.e., each of the 8 colonies is represented in both the "brood" and "no-brood" treatment groups, but we account for any changes in colony size between the two trials).

Investigation 3: When do workers in the beard return to the nest?

How time-of-day influences beard formation and dissipation during a heat stress:

We next explored how heat stress occurring at different times of day (afternoon versus evening) impacted both the formation and dissipation of beards. For this experiment, we housed colonies in two-frame observation hives, so that we could reliably induce a controlled heat stress (each colony contained 2000 workers and a mated queen; observation hives as in Seeley 1995). To heat stress the colony, we positioned two 250 V heat lamps, one on either side of the observation hive, 38 cm from the glass walls. To maintain a consistent heat stress, and to avoid melting the wax combs, we turned the heat lamps off when the internal temperature rose above 42°C (the heat lamps were turned back on once the temperature dropped to 38°C). The total duration of the heat stress was 1.5 hrs. Temperature was measured using J-type thermocouples and a HOBO 4-channel logger (Onset Computer Corporation, Bourne, MA). One thermocouple was embedded in the center of the brood, one thermocouple was placed next to the nest entrance, and the final thermocouple was placed inside the observation hive room.

We experimentally heat stressed colonies in the afternoon (start time: 1500) or the evening (start time: between 1830 and 1900, one hour before sunset, so that the heat stress concludes after sunset). Each colony (n = 5), was subjected to one heat stress in the afternoon and one in the

evening, with the order randomized, and 48 hrs of rest between heat stress trials. Beards were measured every 15 min throughout the 1.5 hr heat stress and for 3 hrs post heat stress. If the colony still had a beard 3 hrs after the end of the heat stress, we measured beard size hourly until 2400, again at 0300, and every 15 min from 0500 until the beard dissipated completely (0 bees left on the colony entrance). We increased our measurement interval from 0500 onward because we noticed that beards often dissipated with the onset of sunrise (0530 – 0600). For these data, we report beard area instead of estimated bee count, because perfectly perpendicular photographs of the beard were not possible, making estimates of beard depth unreliable.

The impact of light on dissipation:

Our work showed that beards tend to dissipate rapidly with the onset of dawn, so we experimentally tested the effect of light on beard dissipation. We artificially illuminated the apiary at night, between 2300 and 0100, using four 1000-watt lights (Halogen Free Standing Construction Lights) placed in the middle of the apiary, shining on the beards at the nest entrances (distance from light: 3.4 ± 0.6m). Lights were positioned in the apiary across all three overnight trials (Aug 18-19th, 19-20th, 20-21st), but were only illuminated from the 19-20th (experimental test), whereas the other trial dates served as a control (18-19th, 20-21st). Across all three trials, we measured beard size before sunset, throughout the night, and after sunrise (beards were measured at: 1800, 2100, 2300, 0000, 0100, 0500, 0530, 0600, 0630, and 0700; time of sunset in Auburn AL across 18-21 Aug 2023: 1921-1924; sunrise: 0608-0609). The colonies used for this experiment (n = 8), were housed in 5-frame boxes, and were previously used for the brood experiment (see above). One colony did not beard at all during our experiment, so we removed this colony from subsequent analysis (bearding was a necessary precursor to investigate beard dissipation).

Statistics

We used linear mixed effect models to investigate the effect of colony size on the proportion of observations where bearding occurred, the daily peak beard size, and the colony proportion in daily peak beard (daily peak beard size divided by colony size). To control for differences between the two apiaries we included it as a fixed effect, and colony ID as a random effect. To understand the impact of brood status on bearding, we ran linear mixed effects models on the daily peak beard size, and the proportion of observation where bearding occurred, with colony ID as random effect. We also tested the effects of brood status on the internal nest temperature, and its variability, using a linear mixed effects model with colony ID as random effect. For the experiment inducing beards in the evening versus afternoon, we ran three paired t-tests, predicting the time spent bearding till the beard dissipated completely (with 0 bees on the modified entrance), the peak beard size, and the time to cast a beard from the onset of the heat stress based on treatment group (beard induced in the evening versus afternoon). To test the effect of light on beard dissipation, we ran a linear mixed effect model (lmer) predicting the number of bees that dissipated and the proportion of beard dissipated (change in beard size from 2300 - 0100, divided by the initial size of the beard at 2300), with treatment group (light versus no light) as the independent variable and colony ID as random effect. All statistical tests were done in R (Version 4.4.2), and the lme4 package (Bates et al., 2015; R Core Team, 2020).

Results:

Investigation 1: How does beard size change throughout the day?

To track daily beard patterns, we monitored naturally-occurring beards in 11 colonies. During our observation period (7–10 June; 31 July–3 August 2024), all of our colonies cast a beard daily except one (this colony bearded on the first and third day of observation, but not on the second day). In one colony (1 of 11) a beard was present every time we observed them. For the majority of colonies (9 of 11), beards began in the afternoon (1200–1500), persisted past 2400, and dissipated by 0600 the following day (Fig. 1b). Beard patterns did not align with ambient temperatures, which peaked during the day between 1200 and 1400. Instead, we found that beard size peaked at night, between 1800 and 2400 (Fig. 1b). The number of bees in the peak beard size ranged from 78 to 5174 bees (peak beard size: 1483.64±1526.84; mean ± SD), or 3.7 to 53.3% of the colony (percent of colony bearding at peak: 24±17.3%).

We also tracked the position of the beard upon the colony's nest box (Fig. 2). Across all timepoints and dates, the majority of the beard was located upon the south-facing wall of the nest box, where the entrance was located (94.5±16.1%). However, at 1200, when the sun was positioned in the east and cast a shadow on the west-facing wall, 22.2±38.1% of the bees in the beard were located on the west side of the nest box (beard position at 1200; east: <1%; south: 77.8±38.1%; west: 22.2±38.1%). Conversely, at 1800, when the sun was positioned in the west and cast a shadow on the east-facing wall, 8.2± 12.2% of the bees in the beard were located on the east side of the nest box (beard position at 1800; east: 8.2±12.2%; south: 91.8±12.2%; west: <1%). At night, when the sun was not shining upon the colonies, the beard position returned to the south-facing wall (beard position between 2100–0600; south: 99.5±1.6%; east: <1%; west: <1%). Therefore, while workers predominantly beard where the entrance is located (in our study, the south-facing wall), their position is also skewed towards the shaded side of the nest box.

Investigation 2: How does colony state impact bearding propensity and size?

Impact of colony size on bearding:

We experimentally manipulated colony size and found that larger colonies have more bees in their daily peak beard size (n = 11, slope =0.331, *P*=0.027); for every 1000 additional bees in a colony, daily peak beard size increased by 331 bees (Fig. 3a). Larger colonies also cast a beard more often than smaller colonies (n = 11, slope=0.00005, *P*=0.009); for every 1000 additional bees, beards are observed 5% more often (Fig. 3b). However, we found no impact of colony size on the proportion of the colony bearding in the largest beard for a colony in a day (n = 11, *P*=0.283). For each colony, its largest beard size represented 25±40% of the colony, but this did not increase significantly with colony size (Fig. 3c). Therefore, while larger colonies do beard more often and with more workers than do smaller colonies, the proportion of the colony that exits the nest to form a beard is similar across large and small colonies.

Impact of brood presence on bearding:

To determine the influence of brood on colony bearding, we experimentally manipulated colonies such that some had brood (brood colonies) and some did not (no-brood colonies; note that each colony was represented in both treatment groups, see Methods). Comparing brood and no-brood colonies, we found no difference in daily peak beard size (brood: 387.79±415.05 bees; no-brood: 252.04±352.62 bees; n = 8, *P*=0.213, lmer), or proportion of observations bearding (brood: 0.34±0.18; no-brood: 0.25±0.13; n = 8, *P*=0.111, lmer). No-brood colonies still thermoregulated their nests (Fig. 4), but we did find that no-brood colonies had lower and more variable internal nest temperatures than when the same colonies did contain brood (nest temperature in brood colonies: 35.61±0.94°C; no-brood colonies: 33.93±1.74°C; *P*<0.01, lmer). A carefully thermoregulated nest is critically important for brood rearing, but colonies still cast beards regardless of whether or not their colony contained brood.

Investigation 3: When do workers in the beard return to the nest?

How time of day influences beard formation and dissipation:

We next explored how heat stress occurring at different times of day (evening versus afternoon) impacted both the formation and dissipation of beards. Regardless of when beards were induced, we found no difference in peak beard size (evening beards: 301.15±200.61 cm^2; afternoon beards: 162.74±105.12 cm^2; P=0.152, n = 5, paired t-test), or the time to cast a beard from the onset of the heat stress (evening beards: 27±16.43 min; afternoon beards: 36±8.22 min; P=0.208, n = 5, paired t-test). We did, however, find that if colonies were heat stressed in the evening, the beards lasted significantly longer than if colonies were heat stressed during the day (duration of evening beards: 8.8±4.2 hrs; afternoon beards: 1.2±0.5 hrs, P=0.013, n = 5, paired t-test). Four of the 5 evening stressed colonies bearded over the entire night; the bees in these beards only fully dissipated with the onset of dawn, 10+ hrs after the end of the heat stress. In contrast, all of the afternoon stressed colonies (5 of 5) had beards that fully dissipated within 2 hrs of the end of heat stress (Fig. 5a). Note that each colony was represented in both treatment groups (see methods), and so the difference in beard duration is not due to the colony itself, but rather the timing of beard formation.

During the heat stress, as expected, brood temperature within the colonies increased. There was no difference between the two treatment groups in their temperature profiles pre-heat stress or during the heat stress (evening heat stress colonies, pre-heat stress: 33.35±1.26°C, during heat stress: 39.94± 2.39°C; afternoon heat stress colonies, pre-heat stress: 33.38 ±1.13°C, during heat stress: 39.94±1.96°C; Fig. 5b). Immediately after the heat stress concluded, the brood nest

temperature in both treatment groups dropped (Fig. 5b). However, whereas the temperature profile of the brood nest in the afternoon heat stress colonies returned to the optimal range (33–36°C), the temperature profile of the evening heat stress colonies dropped below the optimal range (brood nest temperature during hrs 2–12 post heat stress: evening heat stress colonies: 30.88 ±1.85°C; afternoon heat stress colonies: 33.55 ±0.91°C; $P<0.001$, n = 5, lmer; Fig. 5b). The brood nest temperature profile in the evening heat stress colonies only returned to its optimal range once the bearding bees had fully dissipated back into their nest the following morning (Fig. 5b). This shows that brood temperature falling back to its normal range, or even below, is not a cue for bearding bees to dissipate and return to their nest.

The impact of light on beard dissipation:

Given that beards dissipated in the morning (Fig. 1, 5), we next explored the potential role of illumination as a cue for workers to return to their nest, by shining bright lights on bearding colonies from 2300 to 0100 ("illuminated night"), and comparing that to the two adjacent nights without artificial lighting ("dark nights"). The number of bees that dissipated was not significantly different between nights with and without experimental lighting (illuminated night: 179.43±173.65 bees returned between 2300 and 0100; dark nights: 128.15±124.18 bees returned; n = 7, $P=0.242$, lmer). The percentage of the beard that dissipated between 2300-0100 was also not significantly different (illuminated night: 80.02±36.85% of the beard returned; dark nights: 49.11±38.16% of the beard returned; n = 7, $P=0.07$, lmer). Regardless of whether the apiary received artificial lighting or not, by 0530 (38-39 minutes before sunrise) only a few bees remained in the beard (illuminated night: 0 bees; dark nights: 4±10 bees). To confirm that the initial beard sizes were no different between the illuminated and dark nights, we compared beard

size at 1800 (i.e., before any experimental manipulation), and found no significant difference (beard size prior to illuminated night: 88.29±133.06; dark nights: 129.36±194.6; n = 7,

Discussion:

Bearding is a thermoregulatory behavior to cool the nest, where hundreds to thousands of workers leave their nest and form a bivouac near the entrance. Here, we investigated natural bearding patterns, how colony condition impacts bearding, and when beards dissipate.

We observed colonies bearding throughout the day, but beard size consistently peaked in the evening/night, between 1800 and 2400; a 4-6 hr lag behind the hottest daily temperature (Fig. 1b), and a 1-2 hr lag behind peak nest temperature (Fig. 4). This lag provides support for bearding as a "last resort" thermoregulatory behavior, occurring in earnest once nest temperature rises, and other thermoregulatory behaviors have reached their maximum. The nocturnal shift in bearding behavior, which was unexpected and only observed due to round-the-clock data collection, has two likely causes. First, colonies have more workers in the nest overnight, when foragers are no longer in the field (Crailsheim et al., 1996), and we have shown that larger colonies have larger beards (Fig. 3a). Second, bearding has been shown to increase when water is unavailable (Ostwald et al., 2016); at night, workers cannot forage for water and so must instead rely on bearding to cool the nest (though workers can also store water in cells; Ostwald et al., 2016). This combination of fewer thermoregulatory tools (e.g., no access to water), and more dense colonies (i.e., all foragers at home), may explain the nocturnal pattern of bearding behavior. It also shows how superorganisms can combine different thermoregulatory tools across different ambient conditions (e.g., day versus night). Indeed, workers spending the night outside the nest may be more cost effective for the colony than collecting and storing water for nighttime evaporative cooling, which would have reduced efficiency in the higher humidity.

If bearding does not coincide with peak ambient temperatures, can it still be considered a thermoregulatory behavior? We define a thermoregulatory behavior as a basic motivated behavior for nest temperature homeostasis. Given that we, and others, have consistently shown that experimentally heat stressing a colony will induce a beard (Ostwald et. al, 2016; this work), we do consider bearding to be a thermoregulatory behavior. However, it is part of an interconnected suite of behaviors, and how it contributes to thermoregulation is debatable. Whether bearding bees are responding to temperature per se, or some other correlate (e.g., increased movement; Jhawar et. al, 2023; CO_2 concentration; Seeley, 1974), is unknown. Bearding is likely linked to ventilation, as it reduces the number of bees inside the cavity, and presumably improves airflow. Bearding has also been observed during mite treatments, such as thymol, a vapor-releasing essential oil (Richards et. al, 2021; ApiGuard FAQ). If the primary function of bearding is to reduce the number of individuals inside the nest to improve ventilation, then this behavior may be employed in multiple contexts (e.g., during a heat stress, when exposed to volatiles). Similarly, other thermoregulatory behaviors can also be employed in multiple contexts (e.g., water collection for evaporative cooling, or brood rearing; CITE: Kühnholz and Seeley, 1997; Ostwald et. al, 2016).

Colonies formed beards regardless of whether or not their nest contained brood. We found no impact of a colony's brood status (brood versus no brood) on the size of their beard, or duration of bearding. Colonies without brood, however, did have a lower and more variable temperature profile than colonies with brood (Fig. 4). This shows that while brood is an important factor in nest temperature homeostasis, workers do not modify their bearding behavior based on the presence or absence of brood. Colonies without brood must still thermoregulate; the queen is sensitive to high temperatures (above 38°C, she begins to lose sperm viability in her

spermatheca; McAfee et al., 2020), and their precious wax nest begins to melt above 40°C (Buchwald et al., 2008). Bees that depart in a beard may simply be detecting their immediate thermal environment as being above a threshold, rather than modifying their behavior based on the contents of their nest. This implies a simpler decision making process for bearding bees (e.g., "if hot, then beard" instead of "if hot, and brood present, then beard"), and social insects are exemplars for how simple individual responses can give rise to robust group-level phenomena (Lutz et al., 2021).

Beard formation was no different in colonies that were induced to beard in the afternoon or the evening, but beard dissipation was extremely context dependent (Fig. 5a); afternoon beards dissipated within 1-2 hrs, but evening beards persisted overnight (10+ hrs). Bees bearding during the day experience a different environment than bees bearding at night (e.g., illumination levels, ambient temperatures, forager traffic), and these conditions may impact an individual's decision to return to the nest. We experimentally tested if illumination was the cue that bearding workers use to dissipate, but our artificial illumination did not significantly impact beard dissipation (Fig. 6). How workers in the beard determine to return to their nest remains an open question.

Inducing beards at different times of day also showed that bearding carries risks for colonies. When beards were induced in the evening, brood temperature dropped *below* the optimal range after the heat stress finished (Fig. 5b). This low-brood temperature persisted until the sun had risen and the bearding bees returned to their nest. Therefore, during a heat stress, bearding can cause colonies to "over-correct" the temperature profile of their nest. Brood temperature is also an unlikely cue for beard dissipation (e.g., if workers inside the nest would relay information to bees in the beard, but we did not observe this). These results suggest that there is limited information flow between internal nest conditions, and workers that have left the nest to join the

beard outside. One caveat, however, is that using heat lamps to raise the temperature in an observation hive may not fully replicate how colonies naturally experience high ambient temperatures.

Here, we present three lines of evidence that suggest bearding is an individual decision, not one that is coordinated across the colony: (1) bearding patterns do not respond to a colony's brood status, (2) beards follow shade (but only slightly), and (3) individuals in the beard do not return to the nest even when the brood is at lower-than-optimal temperatures. Nevertheless, individual behaviors are the basis upon which collective patterns in the superorganism take form, and indirect coordination can produce impressive higher-order processes.

Thermoregulation is an important feature of organisms living in a variable environment, and cooperative group living allows for thermoregulatory feats that would be impossible for individuals to achieve. In superorganisms, thermoregulation can be reflected in how workers build their nests (King et al., 2015; Kleineidam et al., 2001), adaptive behavioral responses to social conditions (Cook and Breed, 2013; Ostwald et al., 2016), or simply where individuals position themselves (Jhawar et al., 2023). Here, we show how workers use nest evacuation as part of their thermoregulatory toolkit, and how these patterns change with internal and external conditions.

Figure 1

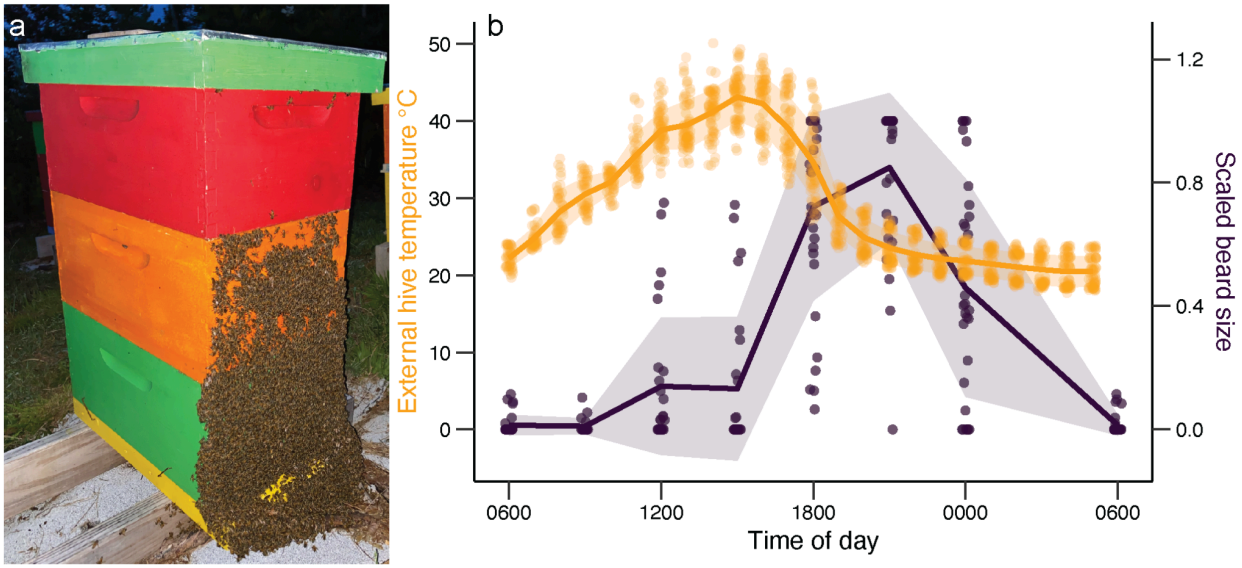

Fig. 1. Daily pattern of temperature and beard size. *(a) Photograph of a colony with a beard. (b) External hive temperature (left axis, in orange) shows the daily temperature measurements using a thermocouple attached to the outside of a nest box. Scaled beard size (right axis, in purple) is calculated for each colony on each day (number of bees in the beard at each timepoint is divided by the daily maximum beard size; 1.0 denotes the largest beard for a given colony on a given day, whereas 0.5 is a beard half that size; see also Fig. S2 for raw data counts per colony). Lines show mean value, shading shows SD, points show raw data.*

Figure 2

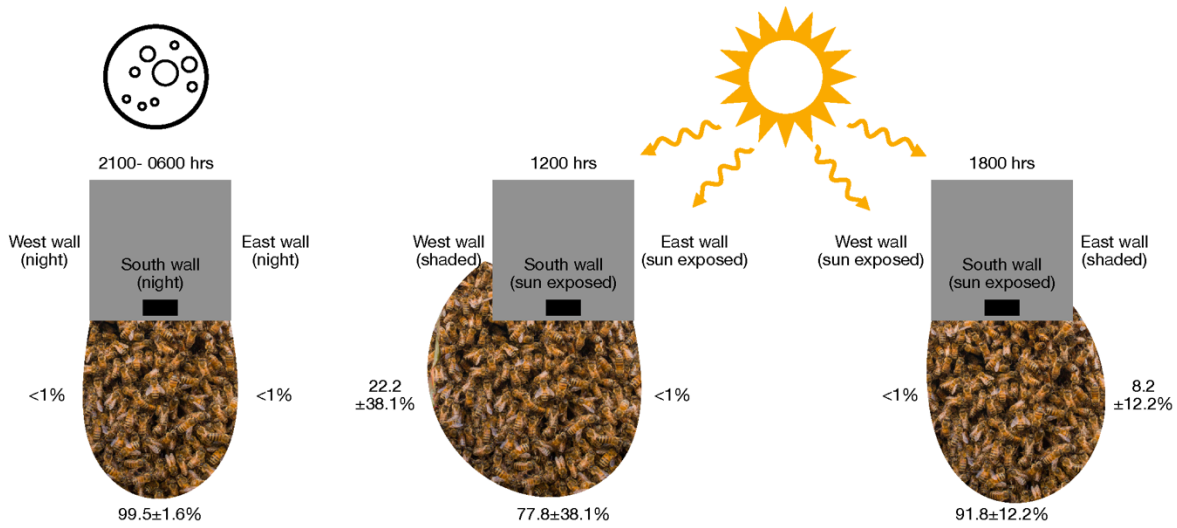

Fig. 2. Beard location throughout the day. *Illustration showing the position of the beard depending on time of day (moon symbol for nighttime; sun symbol for daytime, with rays indicating sun-exposed side of the nest box, in grey; nest entrance in black; all nest entrances faced south). Bees predominantly formed a beard on the south-facing wall (beneath/around the colony entrance), but also skewed towards the shaded side of the next box during the day (see also Fig. S1).*

Figure 3

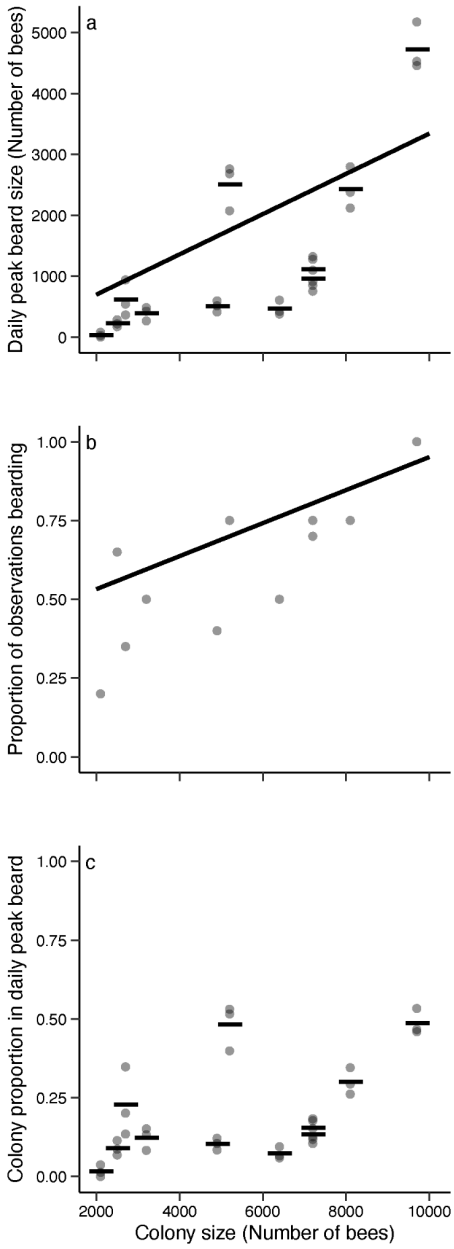

Fig 3. Effect of colony size on beard size, beard frequency, and proportion of the colony in the beard. *Estimated bee count for the daily peak beard size (a), how frequently colonies were observed bearding (b), and proportion of colony in daily peak beard (c). Trend lines in A and B show statistically significant relationships (P<0.05). The dash in a and c denoted the average value for each colony, individual points show raw data in all plots.*

Figure 4

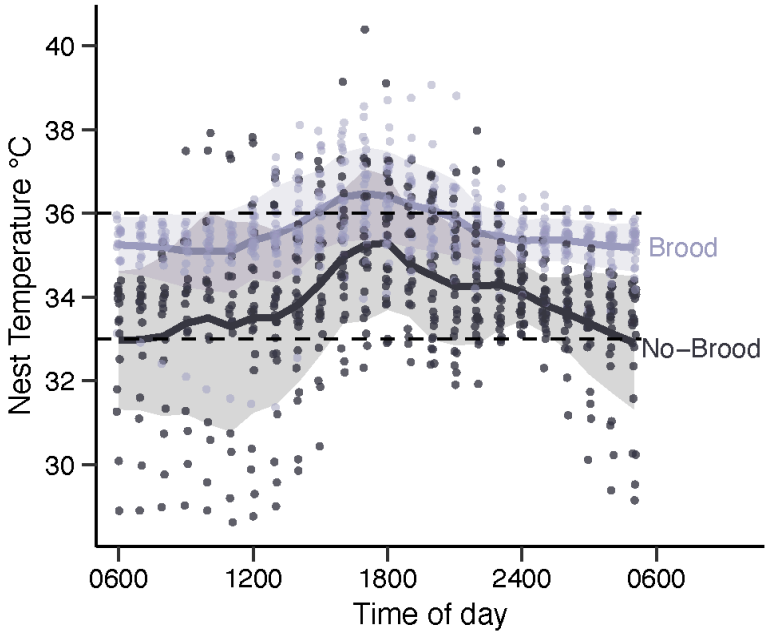

Fig. 4. Nest temperature of colonies in the "brood" and "no brood" treatment groups*. When colonies did not have brood, their nest temperature was consistently lower than when they had brood. Note that each colony is represented in both treatment groups (i.e. brood colonies become no-brood colonies, and vice versa). Dashed horizontal lines represent the range of optimal brood temperature. Solid lines show mean values per treatment group, shading shows SD, points show raw data.*

Figure 5

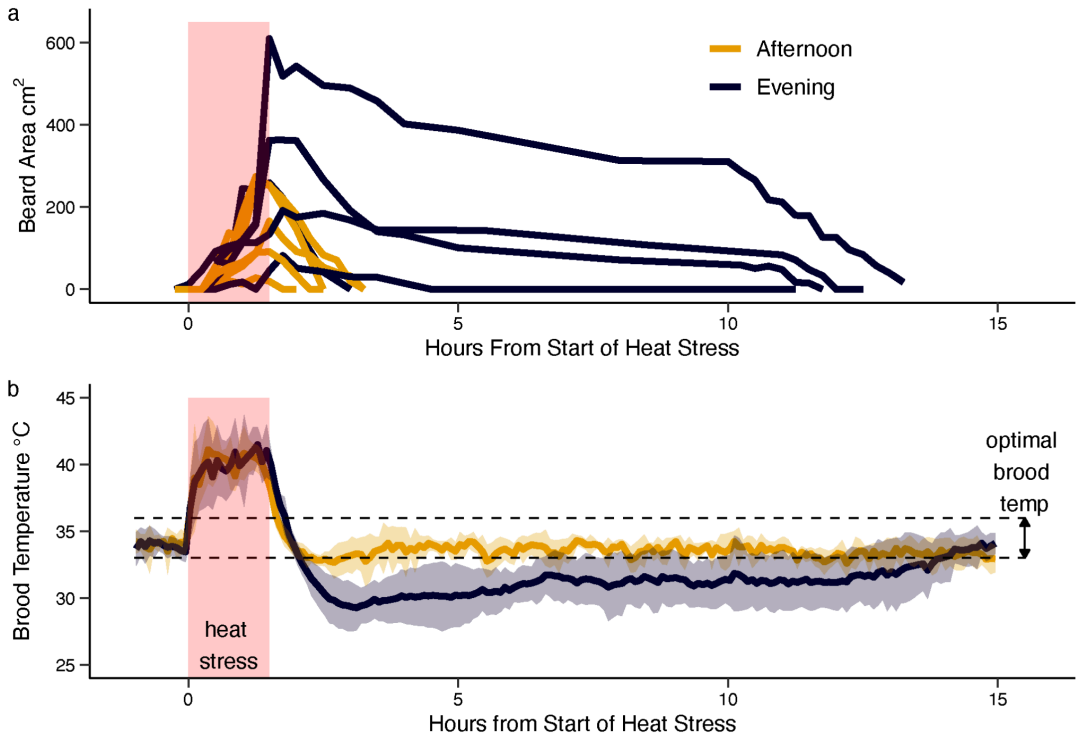

Fig. 5. Timing of heat stress and its impact on beard formation, dissipation, and brood temperature profile. *Beard size (a) and brood temperature (b) from colonies that were experimentally heat stressed in the evening (purple) or afternoon (yellow). Red rectangle shows heat stress (1.5 hrs). In a, each individual colony is shown. In b, solid lines show mean values per treatment group, shading shows SD. Dashed horizontal lines represent the range of optimal brood temperature.*

Figure 6

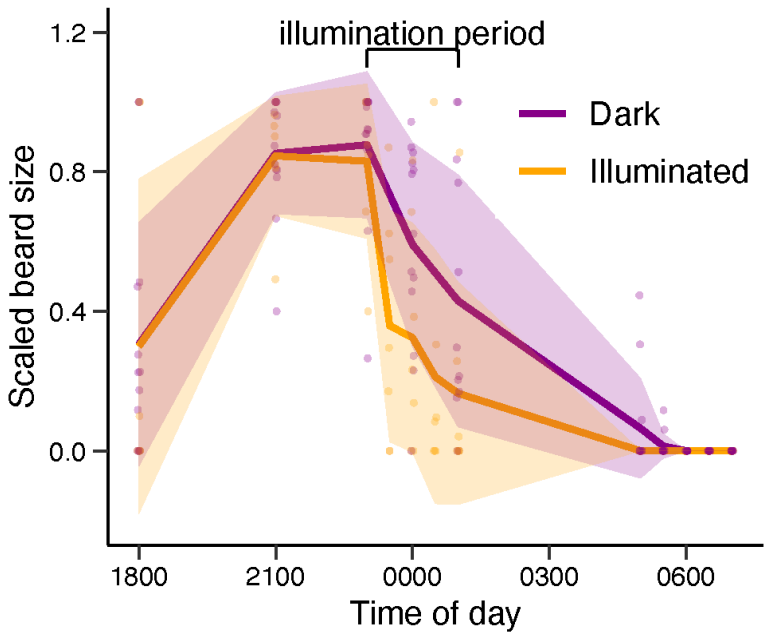

Fig. 6. Testing illumination as a cue for beard dissipation. *Pattern of beard dissipation when colonies are exposed to artificial lighting at night ("illuminated", in orange), versus no artificial lighting ("dark", in magenta). Colonies were illuminated from 2300 to 0100. Scaled beard size was calculated by dividing the beard size at a given time by the largest beard size for that particular day/colony. Lines show mean values per treatment group, shading shows SD, and points show raw data.*

Figure 7

Fig. 7. Photographs of modified nest entrance (top row, without bees; bottom row, with bees). *To prevent bearding bees from aggregating underneath the nest box, we modified our nest entrances using plywood. This created a flat vertical surface upon which the bees could aggregate, as shown in the bottom row. To measure beard size, we photographed the nest entrance from multiple angles (see methods). In the lower left image, beard size = 26 bees. In the bottom middle image, note how beard skews left at 1200, but by 1800 (bottom right image), the beard now skews right (same colony, different timepoints).*

References:

1. Adamo, S. A. 1998. “The Specificity of Behavioral Fever in the Cricket Acheta Domesticus.” *The Journal of Parasitology* 84 (3): 529–33. https://doi.org/10.2307/3284717.
2. Angilletta, Michael J., and Michael W. Sears. 2003. “Is Parental Care the Key to Understanding Endothermy in Birds and Mammals?” *The American Naturalist* 162 (6): 821–25. https://doi.org/10.1086/380921.
3. Aubernon, Cindy, Valery Hedouin, and Damien Charabidze. 2019. “The Maggot, the Ethologist and the Forensic Entomologist: Sociality and Thermoregulation in Necrophagous Larvae.” *Journal of Advanced Research* 16 (March):67–73. https://doi.org/10.1016/j.jare.2018.12.001.
4. Aujard, F., and F. Vasseur. 2001. “Effect of Ambient Temperature on the Body Temperature Rhythm of Male Gray Mouse Lemurs (Microcebus Murinus).” *International Journal of Primatology* 22 (1): 43–56. https://doi.org/10.1023/A:1026461914534.
5. Boorstein, Stephen Michael, and Paul W. Ewald. 1987. “Costs and Benefits of Behavioral Fever in Melanoplus Sanguinipes Infected by Nosema Acridophagus.” *Physiological Zoology* 60 (5): 586–95. https://doi.org/10.1086/physzool.60.5.30156132.
6. Charabidze, Damien, Benoit Bourel, and Didier Gosset. 2011. “Larval-Mass Effect: Characterisation of Heat Emission by Necrophageous Blowflies (Diptera: Calliphoridae) Larval Aggregates.” *Forensic Science International* 211 (1–3): 61–66. https://doi.org/10.1016/j.forsciint.2011.04.016.
7. Clavijo-Baque, Sabrina, and Francisco Bozinovic. 2012. “Testing the Fitness Consequences of the Thermoregulatory and Parental Care Models for the Origin of Endothermy.” *PLOS ONE* 7 (5): e37069. https://doi.org/10.1371/journal.pone.0037069.
8. Clench, Harry K. 1966. “Behavioral Thermoregulation in Butterflies.” *Ecology* 47 (6): 1021–34. https://doi.org/10.2307/1935649.
9. Dillon, Michael E., George Wang, and Raymond B. Huey. 2010. “Global Metabolic Impacts of Recent Climate Warming.” *Nature* 467 (7316): 704–6. https://doi.org/10.1038/nature09407.
10. Ebensperger, Luis. 2001. “A Review of the Evolutionary Causes of Rodent Group-Living.” *Acta Theriologica* 46 (June):115–44. https://doi.org/10.1007/BF03192423.
11. Edelman, Andrew, and John Koprowski. 2007. “Communal Nesting in Asocial Abert’s Squirrels: The Role of Social Thermoregulation and Breeding Strategy.” *Ethology* 113 (February):147–54. https://doi.org/10.1111/j.1439-0310.2006.01310.x.
12. Farmer, C. G. 2000. “Parental Care: The Key to Understanding Endothermy and Other Convergent Features in Birds and Mammals.” *The American Naturalist* 155 (3): 326–34. https://doi.org/10.1086/303323.
13. Ferguson, David L., James A. Guikema, and Gary M. Paulsen. 1990. “Ubiquitin Pool Modulation and Protein Degradation in Wheat Roots during High Temperature Stress 1.” *Plant Physiology* 92 (3): 740–46. https://doi.org/10.1104/pp.92.3.740.
14. Franks, Nigel R. 1989. “Thermoregulation in Army Ant Bivouacs.” *Physiological Entomology* 14 (4): 397–404. https://doi.org/10.1111/j.1365-3032.1989.tb01109.x.
15. Gilbert, Caroline, Dominic McCafferty, Yvon Le Maho, Jean-Marc Martrette, Sylvain Giroud, Stéphane Blanc, and André Ancel. 2010. “One for All and All for One: The Energetic Benefits of Huddling in Endotherms.” *Biological Reviews of the Cambridge Philosophical Society* 85 (3): 545–69. https://doi.org/10.1111/j.1469-185X.2009.00115.x.

16. Grant, Gilbert S. 1982. “Avian Incubation: Egg Temperature, Nest Humidity, and Behavioral Thermoregulation in a Hot Environment.” *Ornithological Monographs*, no. 30, iii–75. https://doi.org/10.2307/40166669.
17. Hafez, E. S. E. 1964. “Behavioral Thermoregulation in Mammals and Birds.” *International Journal of Biometeorology* 7 (3): 231–40. https://doi.org/10.1007/BF02187455.
18. Heinrich, Bernd. 1996. “How the Honey Bee Regulates Its Body Temperature.” *Bee World* 77 (3): 130–37. https://doi.org/10.1080/0005772X.1996.11099304.
19. Hohtola, Esa. n.d. “Shivering Thermogenesis in Birds and Mammals.”
20. “Honey Bee Biology | Princeton University Press.” 2023. June 6, 2023. https://press.princeton.edu/books/hardcover/9780691204888/honey-bee-biology.
21. Howe, R. W. 1967. “Temperature Effects on Embryonic Development in Insects.” *Annual Review of Entomology* 12 (Volume 12, 1967): 15–42. https://doi.org/10.1146/annurev.en.12.010167.000311.
22. Huey, Raymond B., Michael R. Kearney, Andrew Krockenberger, Joseph A. M. Holtum, Mellissa Jess, and Stephen E. Williams. 2012. “Predicting Organismal Vulnerability to Climate Warming: Roles of Behaviour, Physiology and Adaptation.” *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 367 (1596): 1665–79. https://doi.org/10.1098/rstb.2012.0005.
23. Huey, Raymond B., and Joel G. Kingsolver. 1989. “Evolution of Thermal Sensitivity of Ectotherm Performance.” *Trends in Ecology & Evolution* 4 (5): 131–35. https://doi.org/10.1016/0169-5347(89)90211-5.
24. Hung, Keng-Lou James, Jennifer M. Kingston, Matthias Albrecht, David A. Holway, and Joshua R. Kohn. 2018. “The Worldwide Importance of Honey Bees as Pollinators in Natural Habitats.” *Proceedings of the Royal Society B: Biological Sciences* 285 (1870): 20172140. https://doi.org/10.1098/rspb.2017.2140.
25. Hölldobler, B. and Wilson, E.O. 1990. “The Ants.” *Harvard University Press, Cambridge.*
26. Jacklyn, Peter M. 1992. “‘Magnetic’ Termite Mound Surfaces Are Oriented to Suit Wind and Shade Conditions.” *Oecologia* 91 (3): 385–95. https://doi.org/10.1007/BF00317628.
27. Jones, Julia C., Paul Helliwell, Madeleine Beekman, Ryszard Maleszka, and Benjamin P. Oldroyd. 2005. “The Effects of Rearing Temperature on Developmental Stability and Learning and Memory in the Honey Bee, Apis Mellifera.” *Journal of Comparative Physiology A* 191 (12): 1121–29. https://doi.org/10.1007/s00359-005-0035-z.
28. Jones, Julia C., and Benjamin P. Oldroyd. 2006. “Nest Thermoregulation in Social Insects.” In *Advances in Insect Physiology*, edited by S. J. Simpson, 33:153–91. Academic Press. https://doi.org/10.1016/S0065-2806(06)33003-2.
29. Kadochová, Štěpánka, and Jan Frouz. 2014. “Thermoregulation Strategies in Ants in Comparison to Other Social Insects, with a Focus on Red Wood Ants (Formica Rufa Group).” *F1000Research* 2 (March):280. https://doi.org/10.12688/f1000research.2-280.v2.
30. Kearney, Michael, Richard Shine, and Warren P. Porter. 2009. “The Potential for Behavioral Thermoregulation to Buffer ‘Cold-Blooded’ Animals against Climate Warming.” *Proceedings of the National Academy of Sciences of the United States of America* 106 (10): 3835–40. https://doi.org/10.1073/pnas.0808913106.
31. Keil, Gerald, Elizabeth Cummings, and João Pedro de Magalhães. 2015. “Being Cool:

How Body Temperature Influences Ageing and Longevity." *Biogerontology* 16 (4): 383–97. https://doi.org/10.1007/s10522-015-9571-2.
32. King, Hunter, Samuel Ocko, and L. Mahadevan. 2015. "Termite Mounds Harness Diurnal Temperature Oscillations for Ventilation." *Proceedings of the National Academy of Sciences* 112 (37): 11589–93. https://doi.org/10.1073/pnas.1423242112.
33. Koops, Kathelijne, William Mcgrew, Han De Vries, and Tetsuro Matsuzawa. 2012. "Nest-Building by Chimpanzees (Pan Troglodytes Verus) at Seringbara, Nimba Mountains: Antipredation, Thermoregulation, and Antivector Hypotheses." *International Journal of Primatology* 33 (April). https://doi.org/10.1007/s10764-012-9585-4.
34. Korb, Judith, and Karl Eduard Linsenmair. 1999. "The Architecture of Termite Mounds: A Result of a Trade-off between Thermoregulation and Gas Exchange?" *Behavioral Ecology* 10 (3): 312–16. https://doi.org/10.1093/beheco/10.3.312.
35. Lailvaux, Simon P. 2007. "Interactive Effects of Sex and Temperature on Locomotion in Reptiles." *Integrative and Comparative Biology* 47 (2): 189–99. https://doi.org/10.1093/icb/icm011.
36. Lange, Léa, François Brischoux, and Olivier Lourdais. 2022. "Benefits of Paternal Thermoregulation: Male Midwife Toads Select Warmer Temperature to Shorten Embryonic Development." *Behavioral Ecology and Sociobiology* 76 (April). https://doi.org/10.1007/s00265-022-03155-z.
37. Louis, C., M. Jourdan, and M. Cabanac. 1986. "Behavioral Fever and Therapy in a Rickettsia-Infected Orthoptera." *The American Journal of Physiology* 250 (6 Pt 2): R991-995. https://doi.org/10.1152/ajpregu.1986.250.6.R991.
38. Lutterschmidt, William, and Victor Hutchison. 1997. "The Critical Thermal Maximum: History and Critique." *Canadian Journal of Zoology-Revue Canadienne De Zoologie - CAN J ZOOL* 75 (October):1561–74. https://doi.org/10.1139/z97-783.
39. Matsumoto, Yuiko, and David Crews. 2012. "Molecular Mechanisms of Temperature-Dependent Sex Determination in the Context of Ecological Developmental Biology." *Molecular and Cellular Endocrinology* 354 (1–2): 103–10. https://doi.org/10.1016/j.mce.2011.10.012.
40. McFarland, Richard, Louise Barrett, Mary-Ann Costello, Andrea Fuller, Robyn S. Hetem, Shane K. Maloney, Duncan Mitchell, and Peter S. Henzi. 2020. "Keeping Cool in the Heat: Behavioral Thermoregulation and Body Temperature Patterns in Wild Vervet Monkeys." *American Journal of Physical Anthropology* 171 (3): 407–18. https://doi.org/10.1002/ajpa.23962.
41. McGOWAN, A., S. P. Sharp, and B. J. Hatchwell. 2004. "The Structure and Function of Nests of Long-Tailed Tits Aegithalos Caudatus." *Functional Ecology* 18 (4): 578–83. https://doi.org/10.1111/j.0269-8463.2004.00883.x.
42. Müller, Christine B., and Paul Schmid-Hempel. 1993. "Exploitation of Cold Temperature as Defence against Parasitoids in Bumblebees." *Nature* 363 (May):65–67. https://doi.org/10.1038/363065a0.
43. Muñoz, Martha M. 2022. "The Bogert Effect, a Factor in Evolution." *Evolution* 76 (S1): 49–66. https://doi.org/10.1111/evo.14388.
44. "Nest Building and Bird Behavior | Princeton University Press." 2016. April 19, 2016. https://press.princeton.edu/books/hardcover/9780691640228/nest-building-and-bird-behavior.
45. Orr, Paul R. 1955. "Heat Death. I. Time-Temperature Relationships in Marine Animals."

Physiological Zoology 28 (4): 290–94. https://doi.org/10.1086/physzool.28.4.30152190.
46. Ostwald, Madeleine M., Michael L. Smith, and Thomas D. Seeley. 2016. "The Behavioral Regulation of Thirst, Water Collection and Water Storage in Honey Bee Colonies." *Journal of Experimental Biology* 219 (14): 2156–65. https://doi.org/10.1242/jeb.139824.
47. Perez, Daniela M., Janet L. Gardner, and Iliana Medina. 2020. "Climate as an Evolutionary Driver of Nest Morphology in Birds: A Review." *Frontiers in Ecology and Evolution* 8 (December). https://doi.org/10.3389/fevo.2020.566018.
48. Porter, Warren P., and Michael Kearney. 2009. "Size, Shape, and the Thermal Niche of Endotherms." *Proceedings of the National Academy of Sciences* 106 (supplement_2): 19666–72. https://doi.org/10.1073/pnas.0907321106.
49. Rakus, Krzysztof, Maygane Ronsmans, and Alain Vanderplasschen. 2017a. "Behavioral Fever in Ectothermic Vertebrates." *Developmental & Comparative Immunology*, Neuroendocrine-immune system interactions: An evolutionary perspective, 66 (January):84–91. https://doi.org/10.1016/j.dci.2016.06.027.
50. ———. 2017b. "Behavioral Fever in Ectothermic Vertebrates." *Developmental & Comparative Immunology*, Neuroendocrine-immune system interactions: An evolutionary perspective, 66 (January):84–91. https://doi.org/10.1016/j.dci.2016.06.027.
51. Satinoff, E., G. N. McEwen, and B. A. Williams. 1976. "Behavioral Fever in Newborn Rabbits." *Science (New York, N.Y.)* 193 (4258): 1139–40. https://doi.org/10.1126/science.959829.
52. Schulte, Patricia M. 2015. "The Effects of Temperature on Aerobic Metabolism: Towards a Mechanistic Understanding of the Responses of Ectotherms to a Changing Environment." Edited by Jason E. Podrabsky, Jonathon H. Stillman, and Lars Tomanek. *Journal of Experimental Biology* 218 (12): 1856–66. https://doi.org/10.1242/jeb.118851.
53. Shine, R., and P. Harlow. 1993. "Maternal Thermoregulation Influences Offspring Viability in a Viviparous Lizard." *Oecologia* 96 (1): 122–27. https://doi.org/10.1007/BF00318039.
54. Stevenson, R. D. 1985. "The Relative Importance of Behavioral and Physiological Adjustments Controlling Body Temperature in Terrestrial Ectotherms." *The American Naturalist* 126 (3): 362–86.
55. Terrien, Jeremy, Martine Perret, and Fabienne Aujard. 2011. "Behavioral Thermoregulation in Mammals: A Review." *Frontiers in Bioscience (Landmark Edition)* 16 (4): 1428–44. https://doi.org/10.2741/3797.
56. Vieira, Emerson, Leandro Baumgarten, Gabriela Paise, and Rafael Becker. 2010. "Seasonal Patterns and Influence of Temperature on the Daily Activity of the Diurnal Neotropical Rodent Necromyslasiurus." *Canadian Journal of Zoology* 88 (February):259–65. https://doi.org/10.1139/Z09-142.
57. Wenseleers, Tom. 2010. "The Superorganism: The Beauty, Elegance, and Strangeness of Insect Societies . By Bert Hölldobler and E. O. Wilson ; Line Drawings by Margaret C. Nelson . New York : W. W. Norton . $55.00. Xxi + 522 p.; Ill.; Index. ISBN: 978‐0‐393‐06704‐0 . 2009." *Quarterly Review of Biology - QUART REV BIOL* 85 (March):114–15. https://doi.org/10.1086/650272.
58. Williams, Cory, Jamieson Gorrell, Jeffrey Lane, Andrew Mcadam, Murray Humphries, and Stan Boutin. 2013. "Communal Nesting in an 'asocial' Mammal: Social Thermoregulation among Spatially Dispersed Kin." *Behavioral Ecology and*

Sociobiology 67 (February). https://doi.org/10.1007/s00265-013-1499-4.
59. Woodward, F. Ian, Gordon Elliott Fogg, and U. Heber. 1997. "The Impact of Low Temperatures in Controlling the Geographical Distribution of Plants." *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* 326 (1237): 585–93. https://doi.org/10.1098/rstb.1990.0033.
60. Wright, P.G. 1984. "Why Do Elephants Flap Their Ears?" *South African Journal of Zoology* 19 (4): 266–69. https://doi.org/10.1080/02541858.1984.11447891.

Chapter 2:

1. Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw*. 67, 1–48. https://doi.org/10.18637/jss.v067.i01
2. Bonoan, R.E., Goldman, R.R., Wong, P.Y., Starks, P.T., 2014. Vasculature of the hive: heat dissipation in the honey bee (*Apis mellifera*) hive. *Naturwissenschaften* 101, 459–465. https://doi.org/10.1007/s00114-014-1174-2
3. Buchwald, R., Breed, M.D., Greenberg, A.R., 2008. The thermal properties of beeswaxes: unexpected findings. *J. Exp. Biol.* 211, 121–127. https://doi.org/10.1242/jeb.007583
4. Clench, H.K., 1966. Behavioral Thermoregulation in Butterflies. *Ecology* 47, 1021–1034. https://doi.org/10.2307/1935649
5. Cook, C.N., Breed, M.D., 2013. Social context influences the initiation and threshold of thermoregulatory behaviour in honey bees. *Anim. Behav.* 86, 323–329. https://doi.org/10.1016/j.anbehav.2013.05.021
6. Cook, C.N., Durzi, S., Scheckel, K.J., Breed, M.D., 2016. Larvae influence thermoregulatory fanning behavior in honey bees (*Apis mellifera L.*). *Insectes Sociaux* 63, 271–278. https://doi.org/10.1007/s00040-016-0463-5
7. Crailsheim, K., Hrassnigg, N., Stabentheiner, A., 1996. Diurnal behavioural differences in forager and nurse honey bees (*Apis mellifera carnica* Pollm). *Apidologie* 27, 235–244. https://doi.org/10.1051/apido:19960406
8. Gravish, N., Peters, J.M., Combes, S.A., Wood, R.J., 2015. Collective Flow Enhancement by Tandem Flapping Wings. *Phys. Rev. Lett.* 115, 188101. https://doi.org/10.1103/PhysRevLett.115.188101
9. Hansen, H., 1987. *Honey Bee Brood Diseases*. Wicwas Press, Ithaca (NY).
10. Hölldobler, B., Wilson, E.O., 2009. *The superorganism: the beauty, elegance, and strangeness of insect societies*, 1st ed. ed. W.W. Norton, New York.
11. Imdorf, A., Buehlmann, G., Gerig, L., Kilchenmann, V., Wille, H., 1987. Überprüfung der schätzmethode zur ermittlung der brutfläche und der anzahl arbeiterinnen in freifliegenden bienenvölkern. *Apidologie* 18, 137–146. https://doi.org/10.1051/apido:19870204
12. Jhawar, J., Davidson, J.D., Weidenmüller, A., Wild, B., Dormagen, D.M., Landgraf, T., Couzin, I.D., Smith, M.L., 2023. How honey bees respond to heat stress from the individual to colony level. *J. R. Soc. Interface* 20, 20230290. https://doi.org/10.1098/rsif.2023.0290
13. Johnson, B.R., 2023. *Honey bee biology*, First edition. ed. Princeton University Press, Princeton, New Jersey.
14. Jones, J.C., Helliwell, P., Beekman, M., Maleszka, R., Oldroyd, B.P., 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey

bee, *Apis mellifera*. *J. Comp. Physiol. A* 191, 1121–1129. https://doi.org/10.1007/s00359-005-0035-z
15. Kaspar, R.E., Cook, C.N., Breed, M.D., 2018. Experienced individuals influence the thermoregulatory fanning behaviour in honey bee colonies. *Anim. Behav.* 142, 69–76. https://doi.org/10.1016/j.anbehav.2018.06.004
16. King, H., Ocko, S., Mahadevan, L., 2015. Termite mounds harness diurnal temperature oscillations for ventilation. *Proc. Natl. Acad. Sci.* 112, 11589–11593. https://doi.org/10.1073/pnas.1423242112
17. Kleineidam, C., Ernst, R., Roces, F., 2001. Wind-induced ventilation of the giant nests of the leaf-cutting ant *Atta vollenweideri*. *Naturwissenschaften* 88, 301–305. https://doi.org/10.1007/s001140100235
18. Kleinhenz, M., Bujok, B., Fuchs, S., Tautz, J., 2003. Hot bees in empty broodnest cells: heating from within. *J. Exp. Biol.*206, 4217–4231. https://doi.org/10.1242/jeb.00680
19. Kühnholz, S., Seeley, T.D., 1997. The control of water collection in honey bee colonies. *Behav. Ecol. Sociobiol.* 41, 407–422. https://doi.org/10.1007/s002650050402
20. Le Maho, Y., Delclitte, P., Chatonnet, J., 1976. Thermoregulation in fasting emperor penguins under natural conditions. *Am. J. Physiol*.-Leg. Content 231, 913–922. https://doi.org/10.1152/ajplegacy.1976.231.3.913
21. Lutz, M.J., Reid, C.R., Lustri, C.J., Kao, A.B., Garnier, S., Couzin, I.D., 2021. Individual error correction drives responsive self-assembly of army ant scaffolds. *Proc. Natl. Acad. Sci*. 118, e2013741118. https://doi.org/10.1073/pnas.2013741118
22. McAfee, A., Chapman, A., Higo, H., Underwood, R., Milone, J., Foster, L.J., Guarna, M.M., Tarpy, D.R., Pettis, J.S., 2020. Vulnerability of honey bee queens to heat-induced loss of fertility. *Nat. Sustain.* 3, 367–376. https://doi.org/10.1038/s41893-020-0493-x
23. Muñoz, M.M., Losos, J.B., 2018. Thermoregulatory Behavior Simultaneously Promotes and Forestalls Evolution in a Tropical Lizard. *Am. Nat.* 191, E15–E26. https://doi.org/10.1086/694779
24. Ostwald, M.M., Smith, M.L., Seeley, T.D., 2016. The behavioral regulation of thirst, water collection and water storage in honey bee colonies. *J. Exp. Biol.* 219, 2156–2165. https://doi.org/10.1242/jeb.139824
25. Peters, J.M., Gravish, N., Combes, S.A., 2017. Wings as impellers: honey bees co-opt flight system to induce nest ventilation and disperse pheromones. *J. Exp. Biol.* 220, 2203–2209. https://doi.org/10.1242/jeb.149476
26. R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
27. Richards, E.D., Tell, L.A., Davis, J.L., Baynes, R.E., Lin, Z., Maunsell, F.P., Riviere, J.E., Jaberi-Douraki, M., Martin, K.L., Davidson, G., 2021. Honey bee medicine for veterinarians and guidance for avoiding violative chemical residues in honey. *J. Am. Vet. Med. Assoc*. 259, 860–873. https://doi.org/10.2460/javma.259.8.860
28. Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. https://doi.org/10.1038/nmeth.2089
29. Seeley, T.D., 1974. Atmospheric carbon dioxide regulation in honey-bee (*Apis mellifera*) colonies. *J. Insect Physiol*. 20, 2301–2305. https://doi.org/10.1016/0022-1910(74)90052-3
30. Seeley, T.D., 1995. *The wisdom of the hive: the social physiology of honey bee colonies.* Harvard University Press, Cambridge, Mass.

31. Smith, E.N., 1979. Behavioral and Physiological Thermoregulation of Crocodilians. *Am. Zool.* 19, 239–247. https://doi.org/10.1093/icb/19.1.239
32. Southwick, E.E., 1983. The honey bee cluster as a homeothermic superorganism. *Comp. Biochem. Physiol. A Physiol.* 75, 641–645. https://doi.org/10.1016/0300-9629(83)90434-6
33. Southwick, E.E., 1982. Metabolic energy of intact honey bee colonies. *Comp. Biochem. Physiol. A Physiol.* 71, 277–281. https://doi.org/10.1016/0300-9629(82)90400-5
34. Starks, P.T., Johnson, R.N., Siegel, A.J., Decelle, M.M., 2005. Heat shielding: a task for youngsters. *Behav. Ecol.* 16, 128–132. https://doi.org/10.1093/beheco/arh124
35. Sukhchuluun, G., Zhang, X.-Y., Chi, Q.-S., Wang, D.-H., 2018. Huddling Conserves Energy, Decreases Core Body Temperature, but Increases Activity in Brandt's Voles (*Lasiopodomys brandtii*). *Front. Physiol.* 9. https://doi.org/10.3389/fphys.2018.00563
36. Szathmáry, E., Smith, J.M., 1995. The major evolutionary transitions. *Nature* 374, 227–232. https://doi.org/10.1038/374227a0
37. Wheeler, W.M., 1911. The ant-colony as an organism. *J. Morphol.* 22, 307–325. https://doi.org/10.1002/jmor.1050220206