

## RESEARCH ARTICLE

# Thermoregulatory morphodynamics of honeybee swarm clusters

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## ABSTRACT

During reproductive swarming, honeybee clusters of more than 10,000 individuals that hang from structures in the environment (e.g. tree branches) are exposed to diurnal variations in ambient temperature for up to a week. Swarm clusters collectively modulate their morphology in response to these variations (i.e. expanding/contracting in response to heating/cooling) to maintain their internal temperature within a tolerable range and to avoid exhausting their honey stores prematurely. To understand the spatiotemporal aspects of thermoregulatory morphing, we measured the change in size, shape and internal temperature profiles of swarm clusters in response to dynamic temperature ramp perturbations. Swarm clusters showed a two-fold variation in their volume/density when heated from 15°C to 30°C. However, they did not reach an equilibrium size or shape when held at 30°C for 5 h, long after the core temperature of the cluster had stabilized. Furthermore, the changes in cluster shape and size were hysteretic, contracting in response to cooling faster than expanding in response to heating. Although the base contact diameter of the cluster increased continuously when the swarm was heated, the change in length of the swarm (base to tip) over time was non-monotonic. Consequently, the aspect ratio of the swarm fluctuated continuously even when held at a constant temperature. Taken together, our results quantify the hysteretic and anisotropic morphological responses of swarm clusters to ambient temperature variations while suggesting that both mechanical constraints and heat transfer govern their thermoregulatory morphodynamics.

**KEY WORDS:** *Apis mellifera*, Collective behavior, Thermoregulation

## INTRODUCTION

Many social hymenopterans (i.e. ants, wasps and bees) engage in a class of collective behaviors in which individuals link their bodies together to form self-assembled structures such as rafts (Mlot et al., 2011), bridges (Reid et al., 2015), droplets (Bonabeau et al., 1998) and clusters (Heinrich, 1981a). The ability to control the microenvironment within these aggregates leads to a range of extended phenotypes of the colony that allows for emergent and adaptive physiology at the super-organismal level, aiding in flood survival, predator evasion, food transport, navigation of complex environments, thermoregulation, etc. (see Anderson et al., 2002 for a review). Studying these individual and collective behaviors while monitoring the microenvironment can teach us how colony-level

physiology arises, what its limits are, and how it might have developed, evolved or be learnt. More specifically, an important goal is to understand how individual responses to local stimuli can lead to the emergence of functional collective behavior on scales much larger than individuals. The present study aims to understand how an aggregation of social insects can collectively modulate its internal state by dynamically changing its morphology in response to fluctuating ambient conditions.

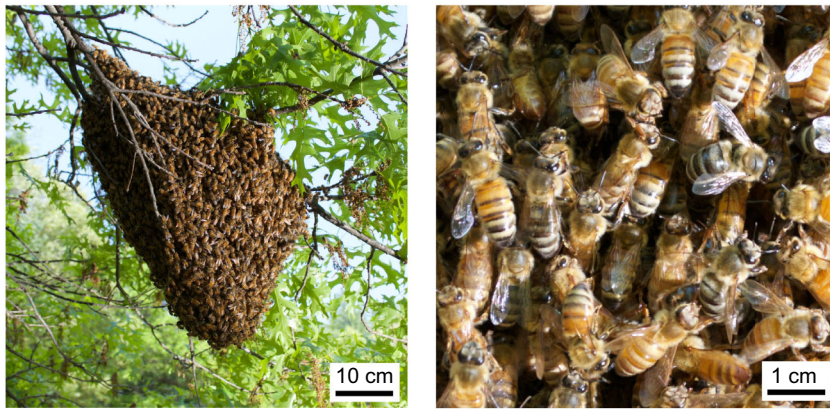
Honeybee swarm clusters can have of the order of  $\approx 10,000$  bees that settle on a tree branch (Fig. 1). Exposure to a variable environment for days while scouts seek new nest sites (Seeley, 2010) causes the cluster to modulate its morphology in an attempt to maintain a relatively stable microenvironment. For example, when exposed to rain, the bees on the surface of the cluster adjust their posture to collectively shed water much like a tiled roof does (Cully and Seeley, 2004), while in response to mechanical perturbations (such as being shaken by the wind), the cluster collectively flattens out and increases its attachment area to reduce mechanical strain on the cluster and increase its overall stability (Peleg et al., 2018). Swarm clusters also modulate their morphology in response to changes in ambient temperatures in order to maintain relatively stable internal temperatures, expanding to dissipate heat when ambient temperatures rise and contracting to conserve heat when ambient temperatures drop (Heinrich, 1981a,b). This ability to change the micro-structure of an aggregate and thence its active macroscopic properties in thermoregulatory behavior, a pervasive example of collective physiology, is also seen in bivouacs and swarms formed by army ants (Franks, 1989), and other social organisms such as penguins (Ancel et al., 1997) and rats (Alberts, 1978). Here, we investigate this behavior in bees by quantifying the extent of thermoregulatory adaptation via morphological variation.

Early experiments pioneered by Heinrich (1981a) measured the core and surface temperature of *Apis mellifera* swarm clusters across a broad range of fixed ambient temperatures (1–35°C). In large clusters (>15,000 bees), the core temperature of large swarms was relatively stable across this range at 35–36°C, while in small swarms (<10,000 bees), the temperature was more variable, ranging from 20 to 35°C. When the ambient temperature was below  $\sim 17^\circ\text{C}$ , the surface temperatures were actively maintained at  $\sim 17^\circ\text{C}$  through shivering (i.e. vibration of the thorax) in order to prevent chill coma (i.e. cold-induced immobilization). At ambient temperatures greater than  $\sim 17^\circ\text{C}$ , the surface temperatures were maintained a few degrees above ambient temperature. Heinrich's observations showed that the cluster increased its volume and surface area dramatically as ambient temperatures rose from 17 to 26°C, allowing more metabolic heat to dissipate and avoiding overheating. As ambient temperature dropped below 17°C, the cluster reduced its surface area and increased its density to conserve metabolic heat. The change in size/shape below 17°C was subtle, suggesting that bees may approach their maximum density. Heinrich (1981a) also measured the collective metabolic rate of the cluster and showed that metabolic rate was minimal at 17°C and increased linearly as ambient temperature rose above 17°C.

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**Fig. 1. A feral honeybee swarm forming a cluster on a tree branch at the Concord Field Station at Harvard University.** A view of the entire cluster shows its conical shape (left). A close-up view of the surface of the cluster highlights the grasping connections between neighboring bees (right).

Metabolic rate increased even more steeply as ambient temperatures dropped below 17°C, indicating that the bees increased their metabolic heat production. Taken together, these results suggested that the swarm modulates its metabolic rate below 17°C to prevent chill coma and modulates its morphology above 17°C to minimize energetic costs and prevent overheating (Heinrich, 1981a). However, little is known about how this collective morphological response is coordinated.

Thermoregulation in swarm clusters is an intriguing multi-scale control problem wherein individual bees (2 cm) presumably sense or respond only to local information, and yet collectively they achieve relatively stable cluster (10–30 cm in diameter) temperatures. A simple way to explain this global regulation is to recognize that it can emerge from coupling between local behaviors, the (geometrical and topological) structure of the cluster and the environmental physics of heat transfer that both enables and constrains function, as studied quantitatively in bees, termites, etc. (Ocko and Mahadevan, 2014; King et al., 2015; Camazine, 2003). The specific question of how honeybee clusters coordinate thermoregulation has been the subject of many experimentally motivated theoretical studies (Omholt and Lonvik, 1986; Omholt, 1987; Lemke and Lamprecht, 1990; Eskov and Toboev, 2009; Myerscough, 1993; Watmough and Camazine, 1995; Sumpter and Broomhead, 2000; Ocko and Mahadevan, 2014). Each model involves the conduction and convection of heat in a metabolically active material (the cluster) and some include ‘thermotactic’ behaviors in which bees modulate their activity according to local temperature (Myerscough, 1993; Watmough and Camazine, 1995; Sumpter and Broomhead, 2000; Ocko and Mahadevan, 2014) and/or a local mechanical pressure (Ocko and Mahadevan, 2014). However, these models focus primarily on the steady-state behavior of the loaded network of bees that are assumed to be able to equilibrate with the environment by breaking and reforming attachments between neighboring bees on relatively short time scales. This is because experimental studies reported the size and shape of swarm clusters at fixed ambient temperatures (Heinrich, 1981a,b), and steady-state behaviors are easier to analyze, both experimentally and theoretically. However, natural swarm clusters experience unpredictable fluctuations in ambient temperature, and this leads to the question of how swarm clusters dynamically vary their shape to maintain a stable internal temperature in fluctuating environments. Are there limits to how quickly swarm clusters can expand and contract? If so, how do they cope with these limitations?

To understand the dynamics of swarm thermoregulation, here we focus on measuring the change in size, shape, density and temperature profiles of swarm clusters over time in response to

dynamic temperature ramp perturbations with varying heating and cooling rates. This allows us to measure the dynamical morphological response of swarm clusters and thus shed light on the temporally transient and spatially anisotropic collective behaviors that arise from local responses of individual bees.

## MATERIALS AND METHODS

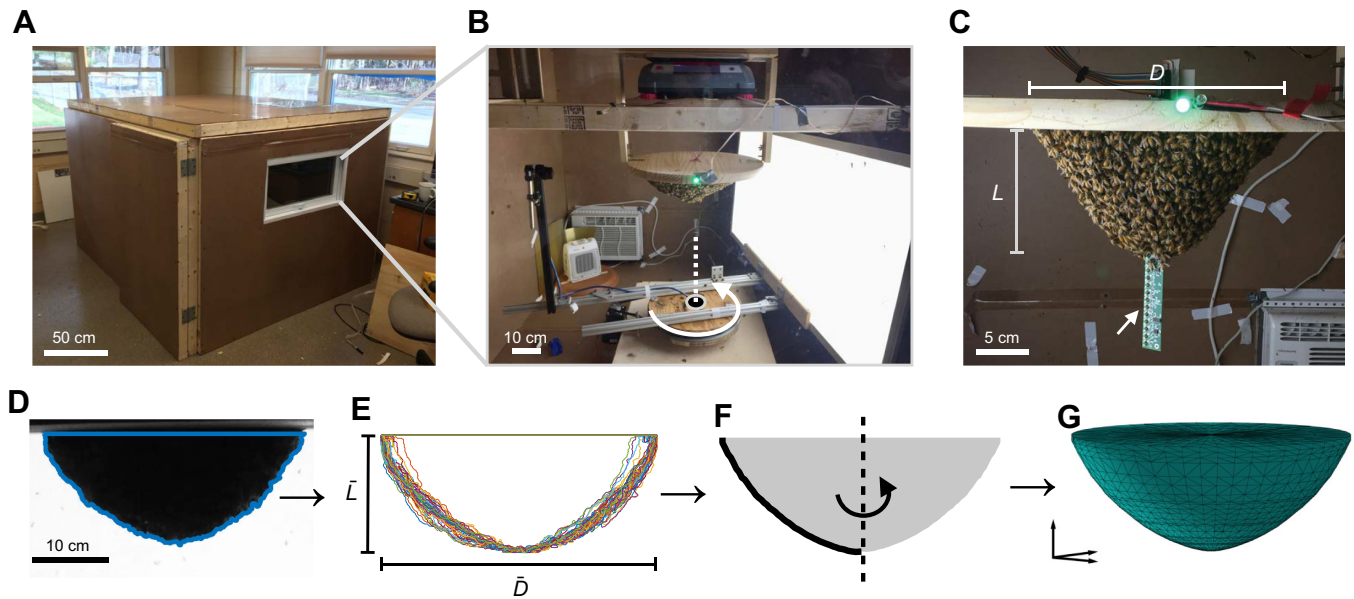
### Artificial swarms

The artificial swarm clusters used in experiments consisted of packages of honeybees purchased from New England Beekeeping in Tyngsboro, MA, USA, in the spring of 2017 and 2018. These packages consisted of approximately 1 kg of European honeybees *Apis mellifera* Linnaeus 1758 and a caged queen. For at least 2 days prior to the experiments, the bees were fed *ad libitum* a 2:1 sugar: water solution, which is known to induce natural swarming behavior (Seeley and Buhrman, 1999). At the beginning of the experiments, the swarm clusters had a mean mass of 0.66 kg (s.d.=0.30 kg). The average mass of an individual bee is 110 mg, leading to an estimate of the average number of bees in a swarm to be ~6000 bees. In Table S1, we list the initial conditions (e.g. size, shape, mass) of the swarms we studied.

### Experimental apparatus

The experimental setup consisted of a 1.2×1.5×1.2 m box constructed from 3.8×8.9 cm dimensional lumber and insulated with a double layer of press-fit 2.5 cm Styrofoam sheets (Fig. 2A). Two window air conditioners were inserted into holes at the bottom left and right corners of one of the walls of the box, and three small space heaters were placed in the corners of the box. Four temperature sensors were suspended in the airspace of the box and connected to an Arduino microcontroller. The heaters and air conditioners were toggled on and off in response to this temperature feedback in order to maintain a desired set-point temperature. This set-point temperature was modulated by a MATLAB program to achieve controlled heating and cooling cycles.

Two wooden struts were used to support a scale (Ohaus Ranger 3000) approximately 10 cm from the roof of the box, and a 60-cm diameter wooden board was hung from the scale (Fig. 2B). The queen cage was fixed to the underside of this board with wires. The rest of the bees were allowed to form a cluster around the queen until the mass reported by the scale was stable, indicating that all of the bees had joined the swarm cluster. The bees were allowed to fly freely, both within the box and in/out of a 0.25×1 m window in the side of the box which connected to the outdoors. This allowed the swarm to send scouts in search of new nest sites. In preliminary attempts using a completely closed box, we found that the bees



**Fig. 2. Experimental setup for quantifying the thermoregulatory response of swarm clusters.** (A) Swarms were housed in a large insulated box, outfitted with heaters and coolers that were toggled on and off to produce controlled temperature perturbations. (B) Within the box, a board (to which the swarm was attached) was suspended from a scale. A motor was used to revolve a camera and an opposing back light 360 deg around the swarm. (C) In some trials, a printed circuit board with a 32-sensor array (white arrow) was inserted in the swarm cluster to record the temperature gradient within the swarm. Bars corresponding to  $D$  and  $L$  indicate the diameter and length of the cluster, respectively. (D) During each rotation, 40 images of the swarm were captured from different angles. (E) The swarm was segmented from the background of each image using thresholding. (F) The silhouettes of the 40 images were transformed so that diameter  $D$  and length  $L$  matched the average values for the whole set,  $\bar{D}$  and  $\bar{L}$ . (G) An average shape was calculated from the 40 silhouettes, and this shape was revolved around the z-axis to obtain an axisymmetric, 3D approximation of the swarm's shape.

would continuously search for an opening in the box to escape from if they were not given freedom to fly in/out of the window and the mass of the cluster would not stabilize.

Each experiment was 10–16 h long; initially, the air temperature in the box was held at 15°C for 4 h to equilibrate the system, and then ramped up to 30°C at a fixed rate, held at 30°C for 5 h, and then ramped down at a fixed rate to 15°C, and held at 15°C for an additional 4 h. Both the ramp-up and ramp-down were included to capture any hysteresis in the response of the bees. Three heating/cooling rates were tested: 33.4°C h<sup>-1</sup> ( $n=6$ ), 8.6°C h<sup>-1</sup> ( $n=9$ ) and 4.3°C h<sup>-1</sup> ( $n=5$ ). On occasion, e.g. on warm, sunny days, the bees would fly off as if to a new nest site, only to return to cluster around the caged queen and thus change the morphology of the clusters. To minimize this effect, the experiments were conducted overnight. In Table S1, we provide a list of dates/times of individual trials and of the swarms used.

### Image acquisition and analysis

To quantify how the morphology of the swarm cluster changed over time, a machine vision camera (1.3 MP, Point Grey Chameleon3) was placed 50 cm from the board supporting the cluster and an LED back light (50×100 cm) was placed orthogonal to the camera on the other side of the swarm. During each data collection bout, the back light was switched on and the camera was rotated 360 deg around the swarm (Fig. 2B, Movie 1). Images were captured every 9 deg of rotation, resulting in 40 unique views of the swarm. When the revolution was complete, the back light was turned off to avoid luring bees away from the cluster. This filming routine was executed approximately every 4 min during the experiment. The mass of the swarm was also continuously recorded throughout the experiment. An example time lapse video from a single camera angle is shown in Movie 2. This imaging system was used to quantify the shape of the swarm clusters. The movements of individual bees within the

clusters could not be observed with our system owing to visual occlusions and are therefore beyond the scope of this study.

Prior to analysis, each image of the swarm was undistorted using MATLAB's checkerboard calibration tool (to correct for wide-angle lens geometry). The checkerboard was placed orthogonal to the camera at the axis of rotation so that images in that plane could be converted from pixels to centimeters. A custom MATLAB image segmentation program was then used to define a wire frame silhouette of the cluster in each of the 40 views (Fig. 2D). Because the cluster was rarely centered at the camera's axis of rotation, the silhouette of the cluster appeared to loom and recede during the camera's rotation (Movie 1). To correct for this, each silhouette was transformed such that its dimensions (i.e. diameter and length) equaled the average dimensions of the set of 40 silhouettes (Fig. 2E). The silhouettes were then averaged to achieve a single 2D approximation of the profile shape of the cluster centered at the axis of rotation (Fig. 2F). The diameter  $D$  of the swarm and the length  $L$  cluster were determined using this averaged silhouette. The perimeter of the averaged silhouette was revolved around the vertical axis to create an axisymmetric, 3D approximation of the shape of the cluster, and to determine its surface area  $A$  and volume  $V$  (Fig. 2G, Movie 3). We chose to use this method rather than hull reconstruction because it was more robust to bees flying in front of the camera (which was a persistent problem).

### Recording internal temperature profiles

We used a custom sensor array (Fig. 2C) to record the internal temperature profile of a single swarm cluster over the course of two experiments with two different heating/cooling rates (i.e. 33.4°C h<sup>-1</sup> and 8.6°C h<sup>-1</sup>). We also attempted to record the internal temperature of a cluster at a heating/cooling rate of 4.3°C h<sup>-1</sup>, but we later noticed that the bees had begun building comb on the sensor array. Bees elevate their body temperatures in



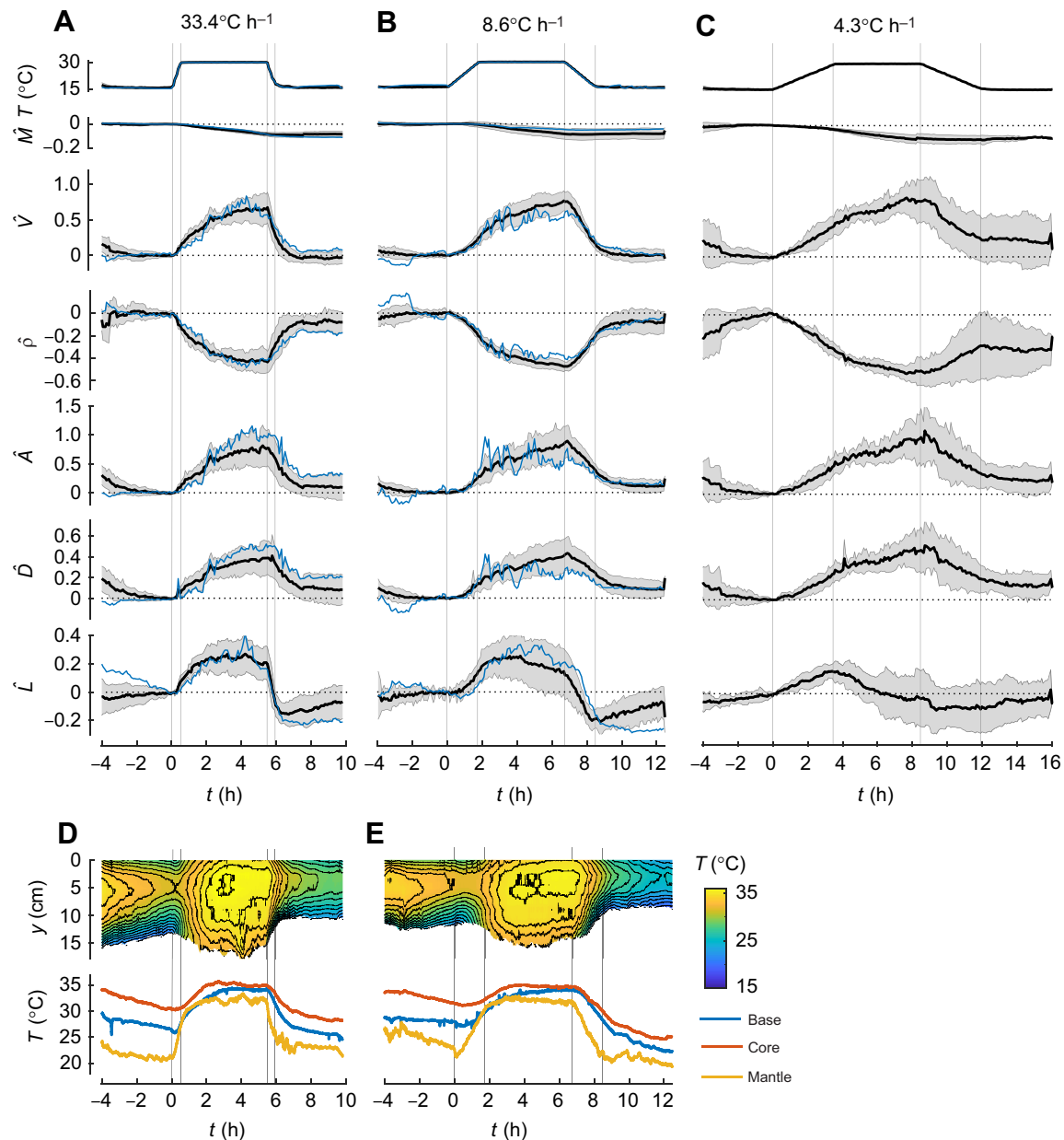
order to build comb, which would confound our experiments. Therefore, we excluded this trial.

The sensor array consists of a printed circuit board (PCB) with 32 temperature sensors (i.e. 10k-ohm glass bead NTC linear response thermistors) spaced every 0.89 cm along the length of the PCB. To ensure that the sensors measured the temperature of the air and/or bees within the cluster rather than the surface temperature of the PCB itself, the sensors were suspended by their lead wires inside of 0.32 cm holes in the PCB, such that the glass bead was not in direct contact with the PCB. In order to avoid self-heating, a digital switch

was used to ensure that the sensors received power only while the measurement was being made (i.e. about 500 ms). Measurements from all 32 sensors were recorded every 10 s throughout the experiment. The sensors were calibrated by running a temperature ramp experiment without the swarm present and fitting a linear model relating the output voltages to air temperature.

## RESULTS

The scaled change in each response variable (i.e. mass  $\hat{M}$ , volume  $\hat{V}$ , density  $\hat{\rho}$ , area  $\hat{A}$ , diameter  $\hat{D}$ , length  $\hat{L}$ ) is plotted in Fig. 3A–C.



**Fig. 3. The dynamic response of honeybee swarm clusters to controlled temperature ramp perturbations.** Each response variable  $r$  is scaled such that  $\hat{r} = [r(t) - r(0)]/r(0)$ . The scaled change in volume  $\hat{V}$ , surface area  $\hat{A}$ , diameter  $\hat{D}$ , length  $\hat{L}$ , density  $\hat{\rho}$  and mass  $\hat{M}$  are plotted for three treatments with varying heating/cooling rates: (A) 33.4°C h<sup>-1</sup> ( $n=6$ ), (B) 8.6°C h<sup>-1</sup> ( $n=9$ ) and (C) 4.3°C h<sup>-1</sup> ( $n=5$ ). The treatment mean is plotted in black and the gray error band indicates standard deviation. In A and B, the blue line indicates the response for trials in which a sensor array was used to record internal temperature data (as shown in D, E). Continuous measurements of the temperature profile within swarm clusters. Internal temperature data are provided for one trial for two heating/cooling rates: (D) 33.4°C h<sup>-1</sup> and (E) 8.6°C h<sup>-1</sup>. The heatmap (top panel) represents the temperature at various positions  $y$  within the cluster over time  $t$ , where  $y=0$  at the base of the swarm and increases towards the tip of the swarm. Data recorded from sensors external to the swarm are omitted. In the lower panel, the temperature at the base, core and mantle of the swarm are plotted over time.  $T$ , temperature.

**Table 1. Table of symbols and abbreviations**

Symbol	Description	Equation	Units
$\hat{M}$	Scaled change in mass	$\hat{M} = [M(t) - M(0)]/M(0)$	Dimensionless
$\hat{V}$	Scaled change in volume	$\hat{V} = [V(t) - V(0)]/V(0)$	Dimensionless
$\rho$	Density	$\rho = V/M$	$\text{g cm}^{-3}$
$\hat{\rho}$	Scaled change in density	$\hat{\rho} = [\rho(t) - \rho(0)]/\rho(0)$	Dimensionless
$\hat{A}$	Scaled change in area	$\hat{A} = [A(t) - A(0)]/A(0)$	Dimensionless
$\hat{D}$	Scaled change in diameter	$\hat{D} = [D(t) - D(0)]/D(0)$	Dimensionless
$D$	Mean diameter (from 40 views)	$D = \frac{1}{40} \sum_{i=1}^{40} D_i$	cm
$\hat{L}$	Scaled change in length	$\hat{L} = [L(t) - L(0)]/L(0)$	Dimensionless
$\bar{L}$	Mean length (from 40 views)	$\bar{L} = \frac{1}{40} \sum_{i=1}^{40} L_i$	cm
$R$	Aspect ratio	$R = D/L$	Dimensionless
$\hat{R}$	Scaled change in aspect ratio	$\hat{R} = [R(t) - R(0)]/R(0)$	Dimensionless

For a list of symbols and their descriptions, see Table 1. Each response variable  $r$  is scaled by dividing the change in that variable by the initial value,  $\hat{r} = (r(t) - r(0))/r(0)$ . This removes the dependence on the sizes of the clusters, which were variable. See Table S1 for initial conditions for each response variable. Statistical comparisons of each morphological response variable were made at key time points in the temperature ramp experiments (i.e.  $t_0$ , the end of the 30°C plateau, and the end of the experiment) using paired  $t$ -tests (Fig. S2, Tables S2, S3).

### Changes in the mass of the clusters

The clusters lost 8–10% of their initial mass over the course of a heating/cooling experiment (Fig. 3A–C). Although we did not quantify the source of this mass loss, we noticed several potential sources of mass loss. First, we noticed that wax scales accumulated on the floor of the box during the experiment. Honeybees regularly produce wax scales in preparation for colonizing a new nest site (Grozinger et al., 2013). In addition, some of the loss may be attributed to attrition of bees, as some dead bees accumulated on the floor of the box. However, this did not appear to be enough to account for the 8–10% mass loss. It is also possible that some of the mass loss resulted from bees leaving the box and failing to return to the swarm, though scouts appeared to move in and out of the box with ease. In fact, on a few occasions between experiments the entire swarm flew out the window (likely toward a newly selected nest sight), only to return to the box and aggregate around the queen. In these instances, the mass of the swarm was similar before and after its departure, suggesting that the bees did not struggle to find their way back to the box.

Although the rate of mass loss was affected by the ambient air temperature (Fig. 3A–C, change in  $M$ ; Fig. S1), the mean mass loss rate across all trials during the 30°C plateau was 2.8%  $\text{h}^{-1}$  (s.d.=1.7)

(paired  $t$ -test,  $P=1.94\text{e-}05$ ) and was not significantly different from zero ( $-0.1 \pm 0.48$  s.d.) during the 15°C plateau ( $t$ -test,  $P=0.52$ ). This temperature dependent mass loss is not unexpected, as earlier observations (Heinrich, 1981b) demonstrated that the metabolic rate of swarms increases two-fold from 15°C to 30°C. Both behavioral (e.g. bees getting lost) and physiological (e.g. wax excretion, metabolic water loss, respiration, death of individuals) mechanisms of mass loss are likely to be affected by metabolic rate.

### Morphological response

To characterize the morphology of the swarm clusters, we measured the volume and surface area of the clusters as well as their diameter and length as a function of the imposed temperature perturbations, as shown in Fig. 3A–C (see Table 2 for summary statistics). When the temperature was raised from 15°C to 30°C, the clusters nearly doubled in volume, but never actually reached a steady state, even after a 5 h heating phase. However, when the temperature was decreased to 15°C during the cooling phase, the volume returned to the initial volume within 2 h for the fast (33.4°C  $\text{h}^{-1}$ ) and medium (8.6°C  $\text{h}^{-1}$ ) ramp treatments, but did not return to the initial volume for the slowest treatment (4.3°C  $\text{h}^{-1}$ ). Similar dynamics were observed for surface area, and the surface area did not return to its initial value by the end of the experiments. As expected given the doubling in cluster volume, the density of the clusters was approximately halved by the end of the heating phase.

When we measured the diameter and the length of the clusters during heating and cooling, while the diameter increased monotonically during heating and increased nearly 50% relative to its initial value, the length showed a non-monotonic response. In fact, the length initially increased upon heating but reached a peak and began to fall before the end of the 30°C plateau. During cooling,

**Table 2. Means and standard deviations of each scaled response variable ( $\hat{r}$ ) at the end of the heating phase  $\hat{r}_h$  (i.e. at the end of the 30°C plateau) and cooling phase  $\hat{r}_c$  (i.e. at the end of the final 15°C plateau)**

Rate (°C $\text{h}^{-1}$ )	$\hat{V}_h$	$\hat{V}_c$	$\hat{A}_h$	$\hat{A}_c$	$\hat{\rho}_h$	$\hat{\rho}_c$
33.4	0.66±0.25	-0.03±0.10	0.78±0.34	0.10±0.24	-0.47±0.07	-0.12±0.08
8.6	0.76±0.12	0.01±0.01	0.88±0.28	0.13±0.11	-0.47±0.04	-0.08±0.11
4.3	0.80±0.27	0.20±0.31	0.93±0.44	0.21±0.22	-0.50±0.13	-0.31±0.14
	$\hat{D}_h$	$\hat{D}_c$	$\hat{L}_h$	$\hat{L}_c$	$\hat{M}_h$	$\hat{M}_c$
33.4	0.40±0.18	0.09±0.16	0.22±0.13	-0.05±0.12	-0.08±0.02	-0.08±0.02
8.6	0.42±0.15	0.09±0.07	0.12±0.17	-0.08±0.12	-0.08±0.04	-0.08±0.04
4.3	0.47±0.21	0.14±0.11	-0.02±0.20	-0.03±0.15	-0.11±0.04	-0.10±0.01

the diameter decreased monotonically and nearly reached its initial value by the end of the experiment. The length, however, rapidly decreased upon cooling, overshooting its initial value and finally began to rise again during the 15°C plateau, almost reaching its initial value.

### Internal temperature variation

To link the response of individual bees in the cluster that can most likely sense and respond to their local temperatures at various depths within the swarm, we measured the internal temperature profile along the central axis of a swarm cluster. In experiments at two different heating/cooling rates (i.e. 33.4°C h<sup>-1</sup> and 8.6°C h<sup>-1</sup>) using the setup depicted in Fig. 2C, we found that the internal temperature data are as shown in Fig. 3D,E. In both experiments, the core of the swarm was generally less variable and warmer (30–36°C) than the mantle (19–33°C), which typically remained several degrees above the ambient temperature when above 15°C. These results are in agreement with previous reports (e.g. Heinrich, 1981b). The core of the cluster is insulated from the fluctuations in the environment by the surrounding bees and is influenced by the metabolic heat production of the whole swarm. However, the density of the swarm cluster decreases in response to rising ambient temperatures and increases with lowering ambient temperatures (Fig. 3A–C), thereby modulating the rate of metabolic heat loss and stabilizing the core temperature of the swarm.

From a dynamical perspective, at the beginning of a heating protocol, even as the mantle began to warm, there was a lag in the onset of heating at the core of the swarm of approximately 0.5 h for the 33.4°C h<sup>-1</sup> experiment and 1 h for the 8.6°C h<sup>-1</sup>. This lag was reflected by a corresponding lag in the morphological response of the cluster as evidenced by the delayed increase in volume and surface area. Although the movements of individual bees were not monitored, these observations are consistent with the morphological response to heating being triggered/driven by bees in the interior of the swarm responding to a gradually rising local temperature owing to the conduction and convection of heat from the boundary.

At the end of the experiments, the core temperature of the swarm was significantly lower than the initial core temperature. This suggests that the swarm cluster is particularly susceptible to cooling after extended periods at high temperatures, consistent with the fact that clusters reduced their density by approximately 50% and increased their aspect ratio during the 30°C plateau. The cluster is able to rapidly decrease its length in response to cooling in order to increase its density and conserve heat. However, this results in a high aspect ratio shape (short and wide) with a high surface-area-to-volume ratio, and the cluster is thus susceptible to cooling. When the cluster is at high density, recovering towards a lower aspect ratio shape with a low surface-area-to-volume ratio is

slow relative to the falling edge of the heating ramp as can be seen in Fig. 5A,B.

### Hysteresis

To better understand the response of the cluster to dynamic temperature perturbations, we fitted the mean  $\hat{V}$  and  $\hat{A}$  to a simple first-order kinetic response functions associated with the slowest time scale  $\tau$  in the relaxation processes at play (Edelstein-Keshet, 1988). Then, the response  $r(t)$  that follows a stimulus  $s(t)$  would be governed by a differential equation of the form:

$$dr/dt = \int_{-\infty}^t K(t-t')[s(t') - r(t')]dt', \quad (1)$$

with a kernel  $K(t-t')$  that is assumed to be time-translation invariant. For simplicity, we chose a kernel of the form  $K(x)=\delta(x)/\tau$  with a relaxation time  $\tau$  (see Fig. 4A,B), so that the dynamical response is then given by the equation  $dr/dt=[r(t)-s(t)]/\tau$ . As we will see, our experiments are reasonably well fitted by this minimal model.

For the response to a step change in the temperature (i.e. expansion of the cluster), we fitted the response to:

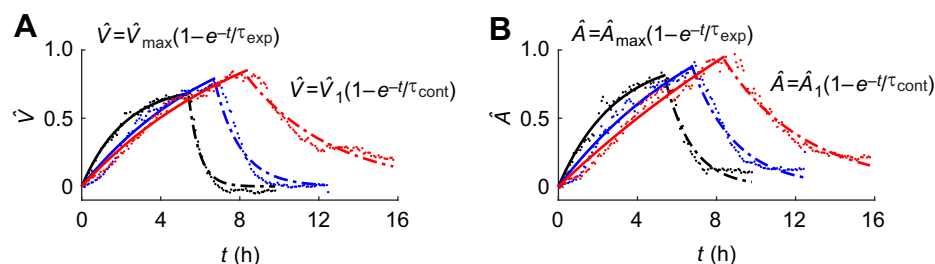
$$r_{\text{exp}} = r_{\text{max}}(1 - e^{-t/\tau_{\text{exp}}}), \quad (2)$$

where  $r_{\text{exp}}$  is the response variable,  $\tau_{\text{exp}}$  is the time constant of the response and  $r_{\text{max}}$  is the value at which  $r$  would eventually saturate if allowed enough time. For the response to a step decrease in the temperature (i.e. contraction of the cluster), we fitted the response variable to:

$$r_{\text{cont}} = r_1(1 - e^{-t/\tau_{\text{cont}}}), \quad (3)$$

where  $r_1$  is the value of  $r$  at the start of the cooling ramp. For all trials, the time scale of volumetric expansion was higher than that of contraction, indicating that expansion occurs more slowly than does contraction (Table 2). This difference in expansion/contraction time scales was more pronounced for slow heating/cooling treatments than for fast heating/cooling treatments. We observed an even more dramatic difference in surface area expansion and contraction timescales. Taken together, this suggests that the expansion/contraction response shows strong hysteresis.

Neither  $\hat{V}$  nor  $\hat{A}$  reached saturation during the 5 h 30°C plateau. The parameters  $\hat{V}_{\text{max}}$  and  $\hat{A}_{\text{max}}$  from the response model are estimates of the maximum values that  $\hat{V}$  and  $\hat{A}$  would have reached if the 30°C plateau was sufficiently long (Table 2). We expected that  $\hat{V}_{\text{max}}$  and  $\hat{A}_{\text{max}}$  would be constants; however, they appear to decrease with the heating rate of the treatment. This suggests that the final volume and surface area of the cluster are greater if heated more slowly.



**Fig. 4. Hysteresis in the thermoregulatory expansion (solid line) and contraction (dotted line) responses of swarm clusters.** The mean (relative) change in cluster (A) volume  $\hat{V}$  and (B) area  $\hat{A}$  over time for each treatment group: 33.4°C h<sup>-1</sup> (black), 8.6°C h<sup>-1</sup> (blue) and 4.3°C h<sup>-1</sup> (red). The rising edge of the response (expansion) is fitted to  $r=r_{\text{max}}(1-e^{-t/\tau_{\text{exp}}})$  and the falling edge of the response (contraction)  $r=r_1(1-e^{-t/\tau_{\text{cont}}})$ . Filled circles represent means at each time step and lines indicate the fitted model. The timescales for the rising and falling edge are shown in Table 3.

**Table 3.** Time scales (in hours) associated with the relatively slow expansion  $\tau_{\text{exp}}$  and fast contraction  $\tau_{\text{cont}}$  of swarm clusters are provided for both relative volume and relative area

H/C rate ( $^{\circ}\text{C h}^{-1}$ )	Scaled rate $\left(\frac{dT}{dt} \cdot \frac{1}{T_0}\right)$	$\hat{V}$			$\hat{A}$		
		$\tau_{\text{exp}}$	$\tau_{\text{cont}}$	$\hat{V}_{\text{max}}$	$\tau_{\text{exp}}$	$\tau_{\text{cont}}$	$\hat{A}_{\text{max}}$
4.3	0.3	2.2	0.6	0.7	2.9	1.4	1.0
8.6	0.6	7.1	1.3	1.3	8.2	2.2	1.6
33.4	2.2	8.9	4.3	1.4	21.2	4.1	2.9

The projected maximum relative volume  $\hat{V}_{\text{max}}$  and maximum relative area  $\hat{A}_{\text{max}}$  are also provided.

### Anisotropy and shape changes

The changes in diameter  $\hat{D}$  and length  $\hat{L}$  are plotted together in Fig. 5A–C to highlight their different but coupled dynamics. The scaled change in aspect ratio  $\hat{R} = [R(t) - R(0)]/R(0)$  is plotted, where aspect ratio is the ratio of width to length  $R=D/L$ . Key transitions in the shape/size of the clusters are denoted in Fig. 5 by Roman numerals (I–V). We note that these transitions are related to key events in the heating/cooling ramps, but they are not necessarily coincident. The aspect ratio stays approximately constant at the start of each experiment as diameter and length increase together (I–II). However, length begins to decrease before the end of the  $30^{\circ}\text{C}$  plateau, causing the aspect ratio to increase (II–III). At the onset of the cooling ramp, aspect ratio undergoes a sharp increase as cluster length decreases dramatically faster than diameter, leading to an even flatter shape (III–IV). This sharp increase in aspect ratio (i.e. flattening) is most pronounced for the fastest treatment and is nearly absent for the slowest treatment. Finally, the cluster begins to slowly return to its original shape during the  $15^{\circ}\text{C}$  plateau (IV–V). Aspect ratio does not quite return to its initial value by the end of the experiment (V).

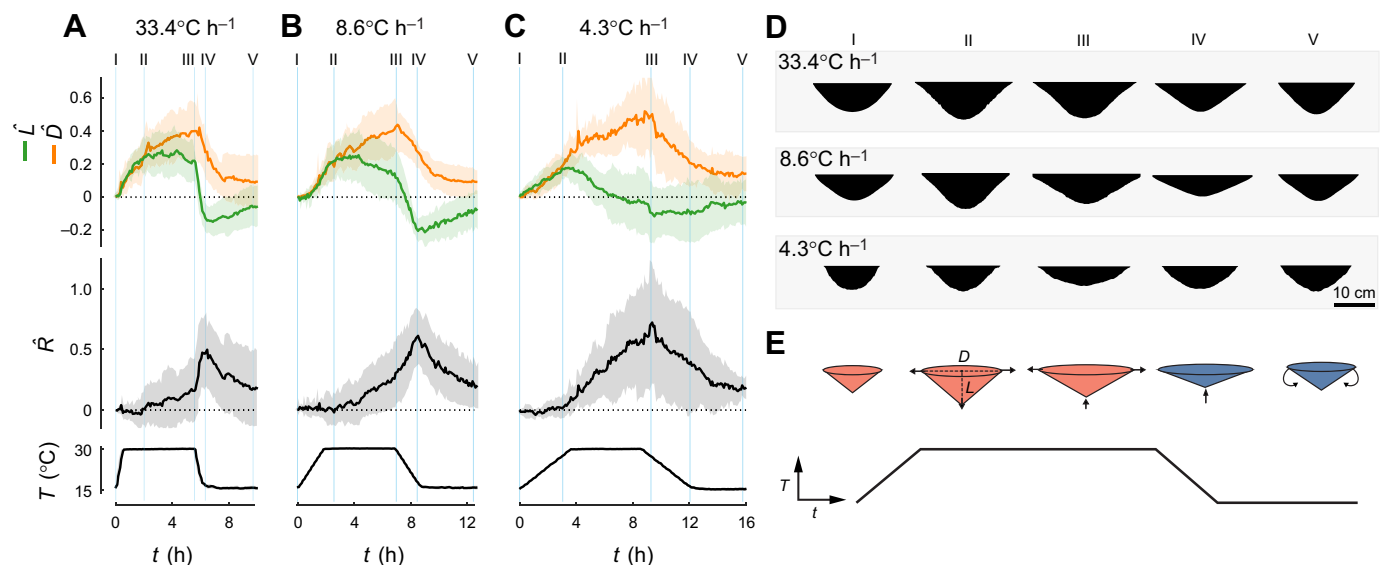
These dynamics suggest that there are two distinct phases of the response to heating (Fig. 5E). In the first phase, as heating begins

the bees begin to increase their volume and thus decrease their density relatively quickly, allowing for convective cooling, keeping the shape of the cluster invariant. This of course cannot continue forever owing to the mechanical constraints of keeping the cluster contiguous and strong enough to support itself. The second phase involves changing the shape of the cluster subject to keeping the volume (and the mass) constant, but occurs much more slowly.

To quantify the morphological changes, we recall that for a generalized conically shaped cluster with a base of radius  $r$  and length  $L$ , we can write:

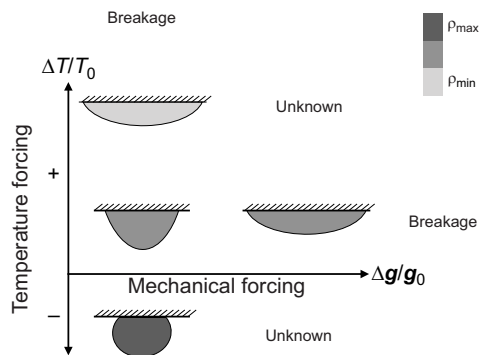
$$V = Lr^2\alpha, \quad (4)$$

where  $\alpha$  is a constant associated with the shape of the cluster (for a right cone  $\alpha=\pi/3$ , while for a cylinder  $\alpha=\pi$ ). The diameter and length of the cluster initially increase together to achieve this increase (decrease) in volume (density), preserving the shape but increasing the size. However, as the density of the cluster approaches a minimum (below which the structural integrity of the swarm would likely be compromised), diameter and length can no longer increase together. Instead, the cluster increases in diameter and decreases in length, i.e. the shape now changes. This allows the surface area to



**Fig. 5.** Dynamic changes to the shape of swarm clusters in response to temperature perturbations. In the top panel, the change in length  $\hat{L}$  (green) and  $\hat{D}$  (orange) are plotted over time for each treatment group: 33.4  $^{\circ}\text{C h}^{-1}$  ( $n=6$ ), (B) 8.6  $^{\circ}\text{C h}^{-1}$  ( $n=9$ ) and (C) 4.3  $^{\circ}\text{C h}^{-1}$  ( $n=5$ ). Each line represents the mean and error bands span the mean  $\pm$  s.d. In the middle panel, the change in aspect ratio [ $\hat{R} = R/R(0) - 1$ ] relative to the initial aspect ratio ( $R=D/L$ ) is shown. The temperature perturbations are plotted in the bottom panel for reference. Key transitions are denoted by Roman numerals (I–V). They are not necessarily coincident with the transitions in the heating/cooling ramps. (D) Averaged silhouettes of clusters at these transitions are shown for a representative trial from each treatment group. (E) The shape of the swarm cluster is represented with a series of cones which change dimensions over time in response to a temperature ramp perturbation (depicted below). In response to heating (red cones), the cluster initially expands in diameter and in length (I–II). Later, as the cluster's diameter continues to increase, its length begins to decrease (II–III). During cooling (blue cones), the length of the cluster initially decreases rapidly (III–IV), but the diameter decreases very slowly (V).





**Fig. 6. A schematic of the known phase space of honeybee swarm cluster density/shape according to this and previous studies (Peleg et al., 2018; Heinrich, 1981b).** Mechanical forcing on the x-axis is reported as  $\Delta g/g_0$ , where  $g_0=9.8 \text{ m s}^{-2}$ , the acceleration due to gravity. Temperature forcing on the y-axis is reported as  $\Delta T/T_0$ , where  $T_0=17^\circ\text{C}$ . Below  $T_0$ , the cluster reaches a maximum density and thermoregulates primarily by modulating metabolic heat production through shivering. The shape of the cluster is represented by cartoons (not to scale) and the density of the cluster is represented by the color axis. How the swarm integrates both temperature information and mechanical information to maintain stability when temperature and mechanical forcing are decoupled is unknown. This provides a unique opportunity for future study of multi-sensory integration at the colony scale.

continue to increase while slowing the increase (decrease) in volume (density), thereby preventing mechanical failure. An additional benefit of this change in the shape (i.e. flattening of the cluster) is the advantage that it proffers from a mechanical perspective, consistent with a recent experimental and theoretical study (Peleg et al., 2018). This study demonstrated that flatter swarm clusters with broad attachment areas are more stable than elongated clusters with small attachment areas (Fig. 6A) because they more efficiently distribute the load, and further, that clusters are able to increase their aspect ratio (and attachment area) in response to imposed mechanical perturbation.

## DISCUSSION

Our study complements and builds on the pioneering experimental work of Heinrich (1981b) on honeybee swarm clusters that demonstrated their ability to maintain a relatively stable internal temperature despite changes in ambient temperature. The proposed mechanism for this was postulated to be the modulation of metabolic heating (through shivering) at low temperatures and by controlled heat dissipation at high temperatures (through collective expansion and contraction of the cluster). This inspired many mathematical models that treat honeybee swarm clusters as a metabolically active material which generates heat and also responds by changing its size, shape and density (Omholt and Lonvik, 1986; Omholt, 1987; Lemke and Lamprecht, 1990; Eskov and Toboev, 2009; Myerscough, 1993; Watmough and Camazine, 1995; Sumpter and Broomhead, 2000; Ocko and Mahadevan, 2014) in response to local temperature information. These models demonstrate that thermoregulation is possible even without direct communication among bees owing to physical coupling between individuals. However, these studies typically consider the steady state of the swarm because previous experiments measured the temperature, metabolic activity and size of the clusters only at fixed temperatures (Heinrich, 1981a,b).

Our work generalizes this and contributes to the understanding of collective thermoregulation in swarm clusters by measuring their morphological responses to time-varying temperature perturbations.

Our results show that expansion and contraction of swarm clusters has two phases, a fast and a slow phase (Fig. 5).

At the start of the heating ramp the clusters initially increase rapidly in both diameter and length (phase 1), but then the clusters begin to shorten in length while continuing to increase in diameter (phase 2). The simultaneous expansion in length and diameter in phase 1 is possible only until the density of bees approaches a minimum below which the bonds between bees begin to strain and would eventually break. Perhaps to avoid this limit, the clusters begin to shorten while increasing their diameter in phase 2. This allows them to continue to increase their surface area without a dramatic decrease in density. Alternatively, it is possible that phase 1 of expansion occurs in response to the derivative of temperature with respect to time, suggesting that monotonic increases in diameter and length are a strategy used to respond to increasing temperature rather than to the absolute temperature. For the slower heating rates,  $8.6^\circ\text{C h}^{-1}$  and  $4.3^\circ\text{C h}^{-1}$ , this transition from phase 1 to phase 2 occurs when the heating ramp ends and the plateau begins, suggesting that phase 2 may be a strategy used in response to high absolute temperatures. If this is true, the transition may be delayed in the fastest treatment ( $33.4^\circ\text{C h}^{-1}$ ) because the internal temperature of the swarm changes more slowly than does the ambient air (Fig. 3D).

The contraction of the swarm cluster in response to cooling also follows a two-phase response. During the cooling ramp, the cluster decreases rapidly in length while decreasing more slowly in diameter (phase I) leading to a dense but relatively high surface area-to-volume ratio by the end of the cooling ramp (Fig. 5A,B). When the temperature reaches the  $15^\circ\text{C}$  plateau, the cluster begins to increase in length as its diameter continues to slowly decrease leading to a decrease in surface area as it recovers its original shape (phase 2). Why does this two-phase contraction response occur? One hypothesis is that it is easier for the swarm cluster to reduce its length than it is to reduce its diameter owing to mechanical constraints, so it shortens more quickly to compensate in response to rapid decreases in temperature. Although we did not observe the movement of individual bees in this study, it is relatively easy for unloaded bees at the surface to climb up chains of bees toward the center of the cluster to avoid cooling, which could explain the rapid shortening. However, it is likely more difficult for bees at the perimeter of the cluster's base to move inward because they are bearing the load of other bees, which may explain why reducing the cluster's diameter is a slower process. It is also possible that the initial rapid shortening of the cluster is accomplished by shortening the connections between bees (i.e. retracting the legs) rather than by topological rearrangements of bees (i.e. bees moving relative to others by breaking and reforming connections). These leg contractions would not reduce the diameter of the cluster, which could explain why the reduction in length is much faster than the reduction in diameter in phase 1. The transition from phase 1 to phase 2 may occur when the cluster approaches a maximum density beyond which shortening of the cluster is no longer possible. Alternatively, this transition may coincide with the end of the cooling ramp because phase 1 may be a response to the rate of change in temperature (during the cooling ramp) and phase 2 may be a response to the absolute temperatures.

The slow, hysteretic and anisotropic nature of the morphological responses of the swarm clusters to heating and cooling events point to the presence of mechanical constraints on the topological rearrangements of these loaded networks of interconnected bees. The movement of a bee in response to local temperature gradients may be limited not only by the local density of bees, but also by the



network structure of the cluster and the loading of individual bees. Although the internal structure of swarm clusters has not been rigorously quantified owing to obvious methodological challenges, previous observational work has suggested the presence of a dense 'mantle' of bees on the surface which pushes toward the center of the cluster at cool temperatures (Heinrich, 1981b). The center of the cluster is thought to be composed by vertically oriented chains of loaded bees (which support the swarm) as well as caverns in which other unloaded bees are free to move around (Meyer, 1956; Heinrich, 1981b).

Peleg et al. (2018) recently discovered that swarm clusters are able to change their morphology in response to mechanical perturbations. When a swarm is subjected to horizontal oscillations (similar to those imposed by a tree branch swaying in the wind), the cluster spreads out to increase its attachment area, thereby reducing the overall mechanical strain experienced by the cluster. This collective response requires individual bees to sense local strains and move from regions of low strain to regions of higher strain in order to stabilize the cluster as a whole. Oscillations with low accelerations induce little spreading, while oscillations with high accelerations lead to lots of spreading, suggesting a graded response. Taken together, these experiments suggest that morphological responses of swarm clusters to their environmental conditions are multi-functional and require collective multi-sensory integration. Fig. 6 illustrates this phase space when the cluster reaches a steady state ( $t \rightarrow \infty$ ). At low temperature and low mechanical forcing, the swarm is relatively dense and conical (except when  $\Delta T/T_0 < 0$ ; Heinrich, 1981b). At high temperatures and mechanical forcing, the swarm tends to increase its attachment area and flatten out. When temperatures are too high, the cluster may break up, and when mechanical forcing is too high, the cluster may also break up (Heinrich, 1981b).

Our work shows that the dynamic thermoregulatory morphing behavior by honeybee swarm clusters in response to ambient temperature fluctuations is relatively slow, hysteretic and anisotropic. These results may help improve models of this phenomenon that previously considered only steady-state behavior of swarm clusters at fixed ambient temperatures. However, developing a complete understanding of how thermoregulation is achieved through the integration of individual stimulus-response behaviors that are coupled through a shared environment will require further empirical work to understand the movements of individual bees, the internal structure of the cluster and the integration of mechanical and temperature cues. There may also be additional mechanisms that do not involve morphodynamics that were not observed in this study, such as water foraging or fanning behaviors.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: J.M.P., O.P., L.M.; Methodology: J.M.P.; Validation: J.M.P.; Formal analysis: J.M.P., L.M.; Investigation: J.M.P., O.P.; Resources: J.M.P.; Data curation: J.M.P.; Writing - original draft: J.M.P.; Writing - review & editing:

J.M.P., O.P., L.M.; Visualization: J.M.P.; Project administration: L.M.; Funding acquisition: J.M.P., L.M.

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#### Data availability

Data included in this manuscript are available on github: [https://github.com/jacobmpeters/thermoregulatory\\_morphodynamics](https://github.com/jacobmpeters/thermoregulatory_morphodynamics)

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