

RESEARCH ARTICLE

Honey bee forager thoracic temperature inside the nest is tuned to broad-scale differences in recruitment motivation

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SUMMARY

Insects that regulate flight muscle temperatures serve as crucial pollinators in a broad range of ecosystems, in part because they forage over a wide span of temperatures. Honey bees are a classic example and maintain their thoracic muscles at temperatures (T_{th}) tuned to the caloric benefits of floral resources. Using infrared thermography, we tested the hypothesis that forager motivation to recruit nestmates for a food source is positively correlated with T_{th} . We trained bees to a sucrose feeder located 5–100 m from the nest. Recruiting foragers had a significantly higher average T_{th} (2.7°C higher) when returning from 2.5 mol l⁻¹ sucrose (65% w/w) than when returning from 1.0 mol l⁻¹ sucrose (31% w/w). Foragers exhibited significantly larger thermal fluctuations the longer they spent inside the nest between foraging trips. The difference between maximum and minimum temperatures during a nest visit (T_{range}) increased with total duration of the nest visit (0.7°C increase per additional min spent inside the nest). Bees that recruited nestmates (waggle or round danced) were significantly warmer, with a 1.4–1.5 times higher ΔT_{th} (difference between T_{th} and nest ambient air temperature) than bees who tremble danced or simply walked on the nest floor without recruiting between foraging bouts. However, recruiter T_{th} was not correlated with finer-scale measures of motivation: the number of waggle dance circuits or waggle dance return phase duration. These results support the hypothesis that forager T_{th} within the nest is correlated to broad-scale differences in foraging motivation.

Key words: thermoregulation, heterothermy, foraging, waggle dance, recruitment, honey bee.

INTRODUCTION

Insects that regulate their flight muscle temperatures can fly and forage at lower ambient temperatures than insects without the ability to generate metabolic heat (Kammer and Heinrich, 1978). Such thermoregulation is widespread in Hymenoptera such as wasps (Coelho and Ross, 1996; Eckles et al., 2008), solitary bees (Baird, 1986; May and Casey, 1983; Stone, 1993) and social bees (Nieh et al., 2006; Nieh and Sánchez, 2005; Stabentheiner, 2001). This ability to thermoregulate is a valuable adaptation that has allowed honey bees to occupy wide altitudinal gradients (Heinrich, 1993) in which they are also important facilitators of plant gene flow (Kearns et al., 1998). Thus, their ability to regulate flight muscle temperatures is an important factor in their ability to pollinate.

Insect muscles must achieve a minimum temperature to generate sufficient force for flight (Heinrich, 1993), a force that is correlated, within a range, to muscle temperature and metabolic rate (Coelho, 1991; Harrison and Fewell, 2002; Josephson, 2006; Woods et al., 2005). Thoracic temperature (T_{th}) is also tuned to food source profitability and the colony's need for carbohydrate and protein (Stabentheiner, 2001). The T_{th} of foragers remains elevated once foragers have returned inside the nest under several conditions: when food has a high sucrose concentration (Stabentheiner and Haggmüller, 1991), is close to the nest (Stabentheiner, 1996), or flows at a high rate during food exchange (trophallaxis) (Farina and Wainseboim, 2001; Farina and Wainseboim, 2005). In the field and within the nest (intranidal), increased thoracic temperatures may assist flight readiness by decreasing warm-up times.

Honey bees recruit nestmates to a food source through the waggle dance and round dance (von Frisch, 1967). In both dance types, forager motivation to recruit and the number of nestmates recruited is positively correlated with the number of dance repetitions (number of waggle phases or number of round dance cycles) (von Frisch, 1967; Seeley et al., 2000). Recruitment motivation is also influenced by colony need, forager genotype, relative food inflow rate, food availability, food quality, and the distribution of environmental resources (Barron et al., 2002; De Marco, 2006; De Marco et al., 2005; Dornhaus and Chittka, 2004; Dyer, 2002; Mattila and Seeley, 2007).

Studies that measure honey bee forager temperatures over relatively brief periods of time inside the nest reveal an interesting, but largely unexplored, phenomenon that may result from the physiology of heat production and forager motivation. Forager T_{th} fluctuates inside the nest (Stabentheiner et al., 1995). Between foraging trips, forager T_{th} changed in cooling and heating cycles with peak-to-peak amplitudes of 1–2°C while the forager was inside the nest (Stabentheiner and Haggmüller, 1991).

Our goal was to determine how forager recruitment motivation affects average T_{th} and fluctuations in T_{th} . We tested the hypothesis that a forager's T_{th} inside the nest is positively correlated with its motivation to recruit (Stabentheiner, 2001). Forager motivation was measured on two scales. On a fine scale, we measured T_{th} , the number of waggle dance circuits (positively correlated with motivation) and waggle dance return phase duration (inversely correlated with motivation) (Seeley et al., 2000). On a broad scale,

we compared the T_{th} of actively foraging bees that were waggle or round dancing (recruiting nestmates to a food source), tremble dancing (recruiting nestmates to assist in nectar handling inside the nest or communicating other food source conditions) (Seeley, 1992; Thom, 2003), simply walking around the nest, or remaining stationary after unloading their food and before leaving the nest. Bees that tremble dance, only walk around the nest or remain stationary after unloading their food do not recruit new nestmates (Seeley, 1992; von Frisch, 1967), although they can reactivate experienced foragers that experience a familiar food odor (Reinhard et al., 2004). To elicit a broader range of recruitment motivation, we used a range of food distances and food qualities that reliably elicited forager visitation at our site during the field season.

MATERIALS AND METHODS

We used three colonies of *Apis mellifera* (Linnaeus 1758) sequentially placed in a temperature-controlled room (25°C) at the University of California, San Diego, La Jolla, CA, USA (09° 09.890' N, 79° 50.201' W) from September to November of 2003 and 2004.

We conducted one trial per day, from 09:00h to 13:00h. In May 2010, we used a fourth colony for thermal calibration measurements (see below). We housed each colony in a three-comb (Langstroth, American Standard) observation hive (56.5×78.7 cm) with doors to keep the colony dark during non-observation. All colonies had approximately equal populations and stores of pollen and honey. For measurements, we covered two sides with clear, infrared-transmitting, plastic film (Polyolefin FDA grade 75 gauge film, catalog no. LS-2475, BCU Plastics, Temecula, CA, USA). This film reduced air-current disturbances and facilitated normal colony thermoregulation. Colonies had access to the outside through a 3.75 diameter, 0.5 m long vinyl tube exiting the lab wall.

To measure thorax temperatures (T_{th}), we used a Raytek PhotoTemp MX6 (close-focus model, accuracy of 1% of measured temperature, Raytek Corp., Santa Cruz, CA, USA) infrared (IR) thermometer equipped with True Spot laser sighting to delineate precisely the measured area. We adjusted the spot measurement size to the diameter of a honey bee thorax (Fig. 1A). The emissivity of the polyolefin film was measured and the IR sensor was calibrated

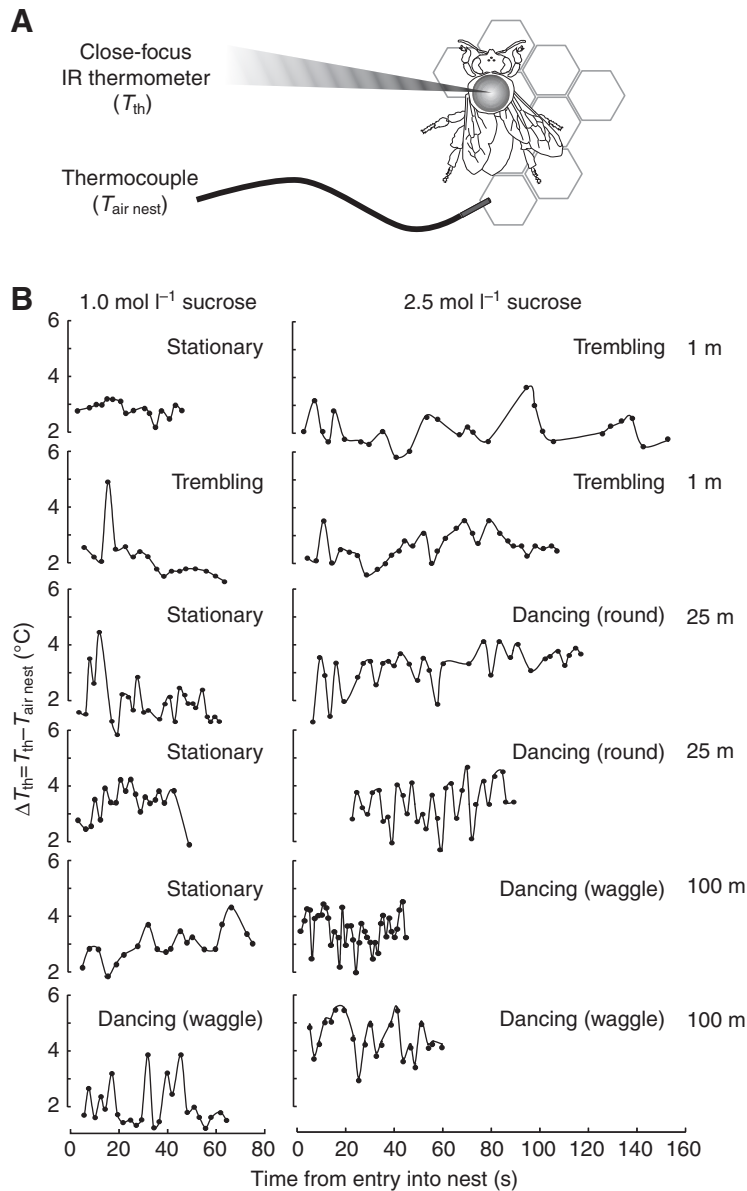


Fig. 1. Setup for recording honey bee thoracic temperatures and representative examples of thoracic temperature changes over time. (A) Diagram showing a forager on the nest dance floor with a thermocouple measuring the nest air temperature and the IR thermometer measuring the forager's thoracic temperature. (B) Changes in thoracic temperature over the nest visits of 12 different foragers returning from feeders at three different distances providing low (1.0 mol l⁻¹) or high (2.5 mol l⁻¹) sucrose solution. The ΔT_{th} of each nest visit is shown (filled circles) over time. Distance to the feeder is shown on the right side of the figure. Representative examples of the four different behaviors are provided.

as described by Mapalad et al. (Mapalad et al., 2008). Internal nest air temperatures ($T_{\text{air nest}}$, within 5 cm of the focal bee) were simultaneously measured with the PhotoTemp MX6 (100 cm long type-K thermocouple, 0.3 mm diameter tip). Ambient air temperatures at the feeder were recorded each 5 min with a Vantage Pro weather station (Davis Instruments, Hayward, CA, USA). To calibrate our IR measurements, we placed the thermocouple in direct contact with the thorax of a bee harnessed in a tube while we measured the uncorrected thoracic temperature through a sheet of our IR-transmitting plastic film placed 1 cm above the thorax, as it was in the bee colonies. Bees ($N=15$) were harnessed to allow direct, stable contact with the thermocouple. We then calculated a correction ($T_{\text{th,corrected}}=0.8165 \times T_{\text{th,IR raw measurement}}+9.0404$) that we applied to all IR measurements. Throughout this paper, we only report $T_{\text{th,corrected}}$ values, referring to them simply as T_{th} .

We trained bees to an inverted-jar feeder (von Frisch, 1967) and used unscented sucrose solution (catalog no. 821721, Ultra Pure, ICN Biomedicals, Irvine, CA, USA) and added 20 μl of lemon scent (lemon extract 98-0554, Kroger, Cincinnati, OH, USA) each hour to filter paper on top of the feeder. We trained approximately 10 bees (at a time) to distances of 5, 25, 50 and 100 m north of the colony, randomly alternating at each location between three concentrations [1.0, 1.5 and 2.5 mol l^{-1} , equivalent to 31, 43 and 65% sucrose (w/w)] (Kearns and Inouye, 1993). The lowest concentration to which bees would dance at all feeder distances and seasons at our site was 1.0 mol l^{-1} . Floral nectars occur at a variety of concentrations, and generalist bee foragers collect nectars ranging from 10–70% sugar (w/w) (Roubik et al., 1995). We individually marked all feeder foragers with plastic tags (0.2 mm thick, 2.5 mm diameter, 2 mg; Bee Works, Orillia, ON, Canada) glued with cyanoacrylate. These thin plastic tags do not interfere with IR thoracic temperature measurements (Mapalad et al., 2008). All foragers were verified on their return to our focal colony as colony members.

We videotaped behavior inside the nest with a Canon XL-1 digital camcorder (Canon USA, Lake Success, NY, USA). An assistant measured focal forager (T_{th}) and ambient nest air temperatures ($T_{\text{air nest}}$) approximately each 3 s and recorded these as voice notes while filming forager behavior. Thus, forager thoracic temperatures could be correlated with behavior and nest visit time. When the focal forager was obscured by other bees or positioned its thorax away from the assistant, we recorded T_{th} when it returned to a measurable position. We measured T_{th} each 3.2 ± 2.9 s (mean \pm s.d.).

We used iMovie v4.0.1 on an iMac G3 (Apple Computer, Cupertino, CA, USA) to analyze videos. The time of each temperature measurement, nest visit duration (time spent performing a behavior inside the nest between foraging trips), and focal forager behavior were recorded. We only followed foragers who consistently made multiple feeder trips during the day and randomly chose among these for our focal foragers. We did not select focal foragers based upon which intranidal behavior they performed. Thus, our data are based upon a random selection of these behaviors.

We examined four behaviors: dancing (round or waggle dancing, as determined by distance to the food source) (von Frisch, 1967), trembling (forager performs the tremble dance), walking (forager walks on comb without trembling or dancing), stationary (forager does not move). Round dances are approximately circular motions that communicate the presence of resources close to the nest (generally <100 m away). Waggle dances are looping figure-eight motions in which the forager waggles its body to communicate the distance and direction of the food source (generally ≥ 100 m away) (von Frisch, 1967). Tremble dancing can occur when nectar inflow

is high and foragers need additional nestmates to help process the nectar (Seeley, 1992). Tremble dancing can also be elicited by feeder conditions. Bees that return from crowded artificial feeders produce tremble dances and vibrational ‘stop signals’, behaviors that are highly correlated (Nieh, 1993; Thom, 2003). Stop signal recipients reduce their waggle dancing and recruitment is therefore inhibited (Kirchner, 1993; Nieh, 1993; Nieh, 2010; Pastor and Seeley, 2005; Thom, 2003). All of our foragers returned to the nest and unloaded their food, but stationary bees remained stationary until their departure. We will refer to round or waggle dancing as ‘dancing’ and tremble dancing as ‘trembling’. We also tested for the fine-scale effect of waggle dancer motivation on T_{th} by measuring the return phase (time between waggle phases), and the total number of waggle phases per nest visit. Return phase duration is inversely correlated and the number of waggle phases is positively correlated with dancer motivation (Seeley et al., 2000).

Previous studies have recorded T_{th} of a bee performing different behaviors during the same nest visit (Stabentheiner, 1996; Stabentheiner et al., 1995). We focused on foragers that only performed one of these four behaviors during their nest visit. To avoid pseudoreplication and provide independent data points, we recorded each forager’s nest visit only once. For example, a forager that entered the nest, unloaded food and then walked around without dancing (round or waggle) or trembling was classified as a ‘walking’ bee. We recorded its T_{th} only when it was walking. Like other investigators (Stabentheiner and Hagmüller, 1991), we observed fluctuations in forager thoracic temperatures during a nest visit. To quantify this, we calculated the temperature range ($T_{\text{range}} = \text{maximum } T_{\text{th}} - \text{minimum } T_{\text{th}}$ per nest visit) and variance (Zar, 1984) in T_{th} per nest visit (T_{variance}). Honey bees are poikilothermic and their body temperature is influenced by the surrounding air temperature (Heinrich, 1993). Thus, we calculated $\Delta T_{\text{th}} (=T_{\text{th}}-T_{\text{air nest}}$, where $T_{\text{air nest}}$ is the ambient air temperature at the center of the dance floor inside the nest) to provide a standardized way to compare forager thoracic temperatures.

Statistical analysis

We used JMP IN v4.0.4 statistical software to conduct analysis of variance (ANOVA). We log transformed (Zar, 1984) the following data: average T_{th} , nest visit duration, T_{range} and T_{variance} . All data met assumptions for normality as determined by residual analyses. For simplicity, we will refer to these transformed variables by their untransformed names. We used two different analysis models. First, we used standard least squares ANOVA and avoided pseudoreplication by using the average per bee for measures describing a complete nest visit (nest visit duration, average T_{th} , average ΔT_{th} , T_{range} and T_{variance}). We tested for a significant effect of colony (a random effect, EMS algorithm), sucrose concentration, feeder distance, and behavior (fixed effects). We tested the significance of all fixed-factor interactions, and then ran simplified models after removing non-significant interactions (Zar, 1984). We used Tukey–Kramer Honestly Significant Difference (HSD) tests for post-hoc analyses.

To examine the effect of time on forager temperatures, we used a one-way ANOVA repeated-measures model because temperatures were successively measured each 3 s with the same individuals. A major goal of our study was to examine thermal fluctuations in waggle dancers for comparison with data found in other studies. Thus, we analyzed the nest visits of foragers visiting the 100 m feeder providing 2.5 mol l^{-1} sucrose (the only concentration that reliably elicited waggle dancing in our study). There was substantial variation in these nest visit times (39.9 ± 34.4 s), and we therefore

calculated temperature measurement times as a percentage of total nest visit time (applying the arcsine-square root transformation to normalize this data) (Zar, 1984). All averages are expressed as means \pm s.d. Where appropriate, we applied the Sequential Bonferroni correction (Zar, 1984). Tests passing this correction are marked '*SB'.

RESULTS

In total, we analyzed the nest visits of 186 foragers from three colonies. Throughout the trials, air temperatures inside the nest ($34.8 \pm 1.2^\circ\text{C}$) and at the feeder ($17.9 \pm 2.4^\circ\text{C}$) remained relatively constant. As expected, there was a strong linear correlation between average $T_{\text{air nest}}$ and average T_{th} for each nest visit ($F_{1,184}=240.0$, $P < 0.0001$) such that T_{th} increased by 0.6°C for each 1°C increase in $T_{\text{air nest}}$ (linear regression: $y=0.60x+19.60$, $R^2=0.56$, data pooled from all trial conditions). Over all trials (pooling all colonies, distances, sucrose concentrations and behaviors), average $T_{\text{th}}=38.5 \pm 1.6^\circ\text{C}$, average $\Delta T_{\text{th}}=3.8 \pm 1.4^\circ\text{C}$, $T_{\text{range}}=1.9 \pm 1.1^\circ\text{C}$, $T_{\text{variance}}=0.4 \pm 0.7^\circ\text{C}$, and nest visit duration= 49.0 ± 37.8 s. Fig. 1B shows representative examples of temperature fluctuations in the four behavioral categories at different distances and sucrose concentrations.

Average T_{th}

We first examined effects on average T_{th} . There was a significant effect of sucrose concentration ($F_{1,178}=15.91$, $P < 0.0001$ *SB), such that average T_{th} was significantly higher for higher sucrose concentrations. There were no significant effects of distance ($F_{1,178}=0.54$, $P=0.47$) or colony ($F_{2,178}=0.24$, $P=0.79$). There were no significant interactions ($F_{2,175} \leq 0.40$, $P \geq 0.67$). There was no significant relationship between average T_{th} and nest visit duration ($F_{1,184}=0.07$, $P=0.79$). Foragers exhibiting different behaviors had a significantly different average T_{th} ($F_{3,178}=4.91$, $P=0.003$ *SB; Fig. 2A). Dancing and trembling foragers were significantly warmer than stationary foragers, but T_{th} among moving bees was not significantly different (dancing, trembling and walking, Tukey–Kramer HSD, $Q=2.59$, $P > 0.05$). We therefore pooled moving bees to calculate that for each 1 mol l^{-1} increase in sucrose concentration, there was a 1.0°C increase in average T_{th} ($F_{1,134}=19.46$, $P < 0.0001$; Fig. 2B). However, ambient air temperatures were significantly colder when foragers choose to remain stationary after returning to the nest as compared to when they moved ($F_{3,182}=8.23$, $P < 0.0001$; Tukey–Kramer HSD $Q=2.59$, $P < 0.05$; Fig. 2A).

Average ΔT_{th} ($T_{\text{th}} - T_{\text{air nest}}$)

Because of the significant differences in $T_{\text{air nest}}$ during different forager behaviors, we calculated ΔT_{th} . As with T_{th} , the following factors were not significant: sucrose concentration ($F_{1,178}=2.79$, $P=0.10$), distance ($F_{1,178}=0.38$, $P=0.54$) and colony ($F_{2,178}=0.49$, $P=0.61$). There were no significant interactions ($F_{3,169} \leq 1.51$, $P \geq 0.22$). We again found a significant effect of behavior ($F_{3,178}=5.41$, $P=0.001$ *SB). However, unlike T_{th} , a slightly different behavioral pattern emerged for ΔT_{th} . Dancing bees had a higher average ΔT_{th} than trembling or walking bees (dancing, $3.9 \pm 1.1^\circ\text{C}$; trembling, $2.8 \pm 0.6^\circ\text{C}$; walking, $2.5 \pm 1.5^\circ\text{C}$; pooled across all sucrose concentrations; Tukey–Kramer HSD $Q=2.59$, $P < 0.05$). On average, dancers were, respectively, 1.4- and 1.5-times warmer than trembling or walking bees, relative to nest ambient air temperatures (Fig. 2C). For ΔT_{th} , dancers were not significantly different from stationary bees (Fig. 2C).

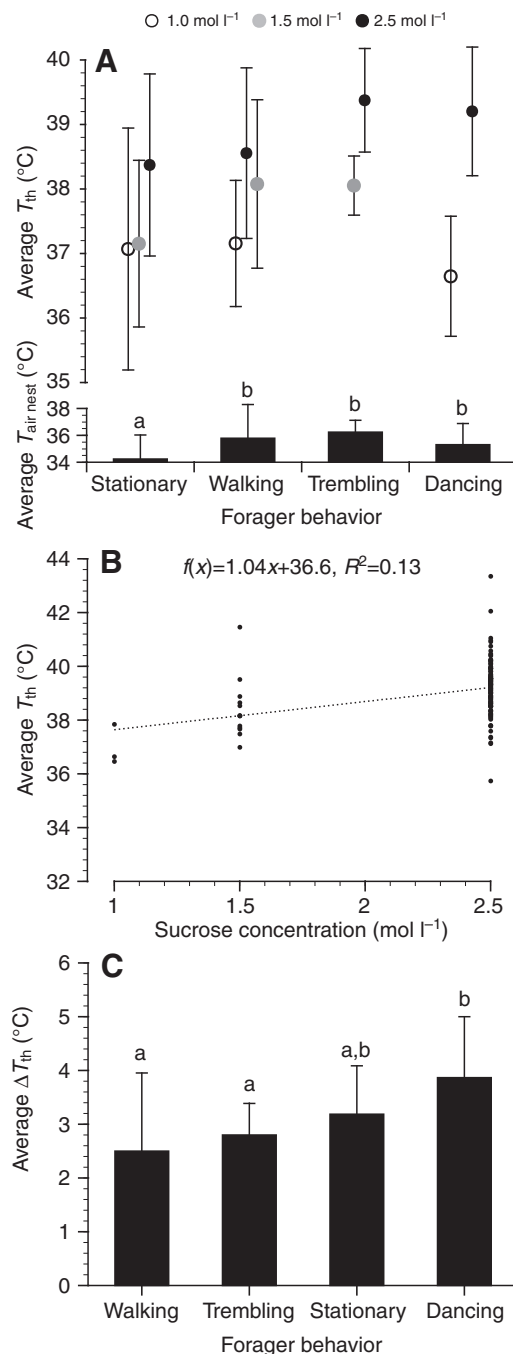


Fig. 2. Relationships between thoracic temperature and forager behavior inside the nest. Averages and standard deviations are shown. (A) Effect of sucrose concentration on T_{th} for each behavioral category, in order of increasing average temperature. All distances and colonies were pooled because there were no significant distance or colony effects. For 1.0 mol l^{-1} sucrose solution, no foragers tremble danced. During trials with 1.5 mol l^{-1} sucrose solution, no foragers danced. Average ambient air temperatures ($T_{\text{air nest}}$) are shown for each behavioral category (significant differences are indicated by different letters; Tukey–Kramer HSD test, $Q=2.59$, $P < 0.05$). (B) Effect of sucrose concentration on average T_{th} of moving bees (linear regression, $P < 0.0001$). The data for all moving bees (walking, trembling and dancing) at all distances is pooled because average T_{th} is not significantly different between these groups. (C) Relationship between average ΔT_{th} and behavior (in order of increasing temperature), with different letters indicating behaviors for which average ΔT_{th} is significantly different (all sucrose concentrations were pooled; Tukey–Kramer HSD test, $Q=2.59$, $P < 0.05$).

T_{th} fluctuation within a nest visit

The temperature range (T_{range} ; examples in Fig. 1B) during a nest visit was not significantly affected by sucrose concentration ($F_{1,177}=0.29, P=0.59$), distance ($F_{1,177}=0.37, P=0.54$), behavior ($F_{3,177}=0.17, P=0.92$) or colony ($F_{2,177}=0.05, P=0.95$). There were no significant interactions ($F_{1,170}\leq 1.76, P\geq 0.19$). However, T_{range} significantly increased with increasing nest visit duration ($F_{1,183}=44.38, P<0.0001^{*SB}$; Fig. 3A). For each 1 min increase in nest visit duration, T_{range} increased by $0.72^{\circ}C$. Similarly, the variance in T_{th} per nest visit ($T_{variance}$) during a nest visit was not significantly affected by sucrose concentration ($F_{1,177}=0.79, P=0.38$), distance ($F_{1,177}=0.25, P=0.62$), behavior ($F_{3,177}=0.50, P=0.68$) or colony ($F_{2,177}=0.08, P=0.92$). There were no significant interactions ($F_{3,177}\leq 151, P\geq 0.21$). Like T_{range} , $T_{variance}$ significantly increased with increasing nest visit duration ($F_{1,183}=6.27, P=0.006^{*SB}$; Fig. 3B). Thus, foragers (all behavioral categories) who spent longer periods inside the nest exhibited a wider T_{th} range and greater variation in T_{th} .

Waggle dancer motivation and T_{th}

On average, waggle dancers performed 6.4 ± 4.7 circuits with an average return phase of 2.3 ± 0.7 s. In waggle dancing bees (100m feeder, 2.5 mol l^{-1} sucrose), there was no significant relationship between the number of dance circuits per nest visit or return phase duration (measures of dancer motivation) and different measures of dancer thoracic temperature (average T_{th} , average ΔT_{th} , T_{range} or $T_{variance}$; $P\geq 0.12$; Table 1). There were no significant interactions ($P\geq 0.33$; see Table 1).

Changes in T_{th} and ΔT_{th} during waggle dancing

During waggle dancing, there was a slight but significant change in T_{th} over time ($F_{10,532}=3.36, P=0.0003^{*SB}$; Fig. 4A). There was also a significant effect of bee identity on T_{th} ($F_{62,532}=34.31, P<0.0001^{*SB}$). The interaction of time and bee identity was not significant ($F_{353,532}=0.83, P=0.93$). For ΔT_{th} , there was a significant effect of time ($F_{10,532}=4.53, P<0.0001^{*SB}$), such that ΔT_{th} increased slightly throughout each waggle dancer's nest visit (Fig. 4B). For ΔT_{th} , there was also a significant effect of bee identity ($F_{62,532}=29.22, P<0.0001^{*SB}$), and no significant interaction of time and bee identity ($F_{353,532}=0.86, P=0.88$). There was, therefore, significant individual variation in T_{th} and ΔT_{th} during a waggle dancer's nest visit. However, despite this individual variation, waggle dancers' thoracic temperatures increased slightly throughout their nest visit. Waggle dancer T_{th} and ΔT_{th} was estimated (from linear regression; Fig. 4) to increase by $0.18^{\circ}C$ and $0.28^{\circ}C$, respectively, halfway through a nest visit (average nest visit duration of 30.3 ± 22.6 s).

DISCUSSION

We examined T_{th} and fluctuations in T_{th} with respect to nest air temperature, time spent inside the nest, food quality, food location, and forager motivation to recruit while foragers were inside the nest between foraging trips. In general, average T_{th} was strongly

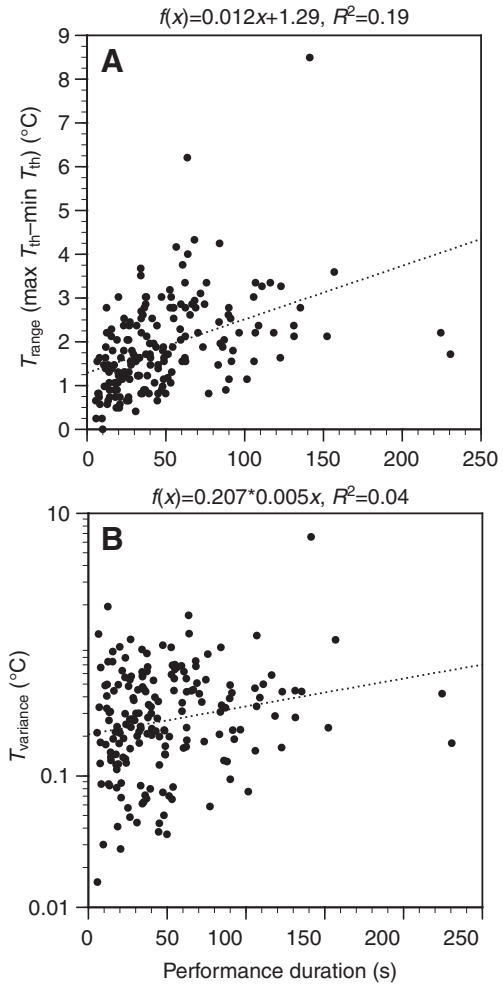


Fig. 3. Effect of nest visit duration on measures of T_{th} fluctuation per nest visit. (A) Relationship between nest visit duration and T_{range} (the difference between the maximum and minimum T_{th} within each nest visit. The dashed linear regression line, equation, and R^2 value are shown. (B) Relationship between nest visit duration and $T_{variance}$. A logarithmic regression is a better fit for this data (y -axis plotted on a log scale), and the log regression line and corresponding R^2 value are therefore shown.

correlated with $T_{air\ nest}$ (confirming previous studies) (Esch, 1960). For waggle dancers, there was a slight increase in T_{th} and ΔT_{th} over time ($0.18\text{--}0.28^{\circ}C$ after 15 s, on average) that exhibits high variation (Fig. 4) and that is significant ($P\leq 0.0003^{*SB}$). Similarly, T_{range} and $T_{variance}$ significantly increased over time for waggle dancers (Fig. 3).

As expected (Dyer and Seeley, 1987; Schmaranzer and Stabentheiner, 1988; Stabentheiner et al., 1995; Underwood, 1991), foragers had a higher average T_{th} ($2.7^{\circ}C$ higher) when returning from richer food (2.5 mol l^{-1} sucrose) than when returning from

Table 1. Effect of fine-scale waggle dancer motivation on dancer body temperature

	Average T_{th}	Average ΔT_{th}	T_{range}	$T_{variance}$
Average temperatures ($^{\circ}C$)	39.1 ± 1.2	3.7 ± 1.0	2.2 ± 1.1	0.5 ± 0.4
No. waggle dance circuits	$F_{1,26}=0.08, P=0.78$	$F_{1,26}=2.45, P=0.12$	$F_{1,26}=1.06, P=0.31$	$F_{1,26}=0.22, P=0.64$
Average return phase duration	$F_{1,26}=0.08, P=0.78$	$F_{1,26}=0.21, P=0.65$	$F_{1,26}=0.68, P=0.42$	$F_{1,26}=0.36, P=0.55$
Interaction*	$F_{1,25}=0.20, P=0.66$	$F_{1,25}=0.007, P=0.94$	$F_{1,25}=1.00, P=0.33$	$F_{1,25}=0.03, P=0.87$

This analysis uses data from a 100 m feeder providing a 2.5 mol l^{-1} sucrose solution. All P -values are non-significant.

*No. waggle dance circuits \times return phase duration.

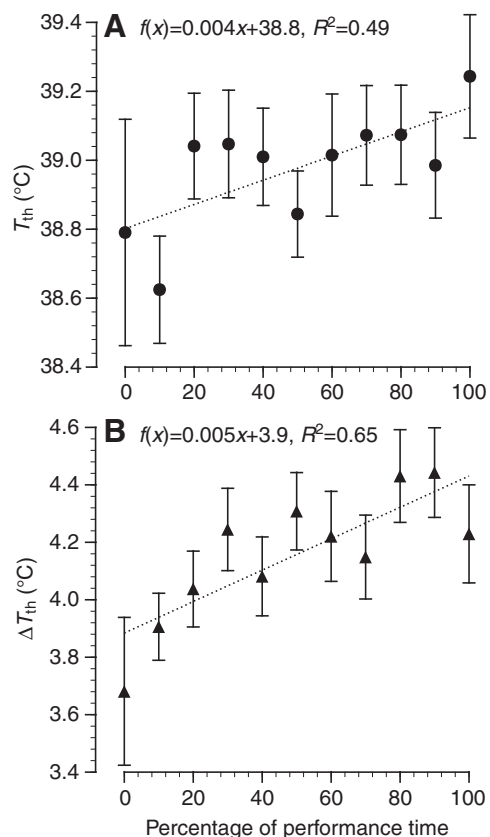


Fig. 4. Waggle dancer thoracic temperature increased slightly over time within a nest visit (averages from 63 different foragers collecting 2.5 mol l^{-1} sucrose solution, 100 m from the nest). Data from all colonies were pooled because there was no colony effect. Because of high variability due to significant individual differences, averages with standard errors are shown. To standardize waggle dancer nest visit durations, percentage of total nest visit time is shown. The relationships between percentage of nest visit duration and (A) average T_{th} (linear regression, $P=0.0003^{*SB}$), and (B) average ΔT_{th} are depicted (linear regression, $P<0.0001^{*SB}$). Linear regression lines (dashed lines), equations and R^2 values are shown for each plot.

poorer food (1.0 mol l^{-1} sucrose, Fig. 2A). For moving bees (walking, trembling and dancing), we found a T_{th} increase of 1.0°C per 1 mol l^{-1} increase in sucrose concentration (Fig. 2B), similar to the 1.5°C increase per 1 mol l^{-1} sucrose increase shown by Stabentheiner et al. [calculated from log regression of their data on waggle dancers, pooled distances, estimating change in T_{th} from $1\text{--}2 \text{ mol l}^{-1}$ sucrose (Stabentheiner et al., 1995)]. However, we found no significant effect of sucrose concentration on ΔT_{th} , perhaps because we used relatively high sucrose concentrations ($1.0\text{--}2.5 \text{ mol l}^{-1}$), which were necessary at our site and season to train bees and have them recruit for an artificial feeder. Bees generally find such sucrose concentrations quite rewarding (Balderrama et al., 1992).

At the relatively short distances used ($5\text{--}100 \text{ m}$), we did not find an effect of distance on T_{th} . This is not surprising because Stabentheiner (Stabentheiner, 1996) reported a temperature decrease of between $0.5\text{--}0.8^\circ\text{C}$ per 1000 m increase in distance (0.5 to 2.0 mol l^{-1} sucrose feeders). Using these numbers, we would expect at most a 0.08°C decrease in average T_{th} from 5 to 100 m . In general, our foragers had an average T_{th} of $38.5\pm 1.6^\circ\text{C}$ (La Jolla, CA, USA), nearly identical to the T_{th} measured for honey bees collecting nectar

from floral resources ($38.0\pm 2.2^\circ\text{C}$, Graz, Austria) (Stabentheiner, 2001).

Thoracic temperatures (T_{th} , uncorrected for nest air temperature) varied with forager behavior and were highest for recruiting foragers (dancers; Fig. 2A). Prior studies have demonstrated that the T_{th} of honey bees placed in boxes is positively correlated with activity (Stabentheiner and Crailsheim, 1999). Fuchikawa and Schimizu found that thoracic temperatures were elevated by $7\text{--}8^\circ\text{C}$ during locomotor activity (Fuchikawa and Schimizu, 2007). Like Stabentheiner et al. (Stabentheiner et al., 1995), we did not find significant average T_{th} differences between dancing and walking bees. In our experiment, there was a significant variation in dance floor air temperatures, and stationary foragers were observed when the nest air temperature was on average 1°C and 2°C below that recorded when dancing and trembling foragers were observed (Fig. 2A). Lower ambient nest air temperatures may contribute, for some unknown reason, to stationary behavior between foraging bouts.

We explored the association between behavior and thoracic temperatures by using ΔT_{th} , a measurement that corrects for different ambient air temperatures because $T_{air \text{ nest}}$ exerts a strong influence on T_{th} (linear regression $R^2=0.56$, $P<<0.0001$). Bees that were highly motivated to recruit nestmates (dancers) were significantly warmer ($1.4\text{--}1.5$ -times higher average ΔT_{th}) than trembling or walking bees who simply foraged but did not recruit during their nest stay (Fig. 2C). This higher thoracic temperature may reflect general foraging motivation, but other explanations are possible. For example, cold stress within the colony increases thoracic flight muscle heat generation (Stabentheiner et al., 2010). This could explain why stationary bees had the second highest average ΔT_{th} (Fig. 2C), although this does not account for the average elevated ΔT_{th} of dancers, which was the highest in our study. Elevated ΔT_{th} could result from maintenance of flight muscle temperature in preparation for a rapid flight back to the food source. With respect to proximate causation, elevated ΔT_{th} could be a byproduct of waggle dancing because leg muscles have origins in the thorax and are active during waggle dancing (Stabentheiner, 1996). However, dancers were significantly warmer than trembling or walking bees (ΔT_{th} , Fig. 2A) Slight wing motions occur during the waggle phase that are not as consistently present in trembling or walking bees. However, video thermography of waggle dancing did not reveal increases in T_{th} during the waggle phase (wing motion and walking) when compared with walking-only return phases immediately before and afterwards (Stabentheiner and Haggmüller, 1991). The thermal contribution of muscles due to wing motions during the waggle phase is therefore likely minimal.

It is important to consider whether T_{th} fluctuations are artifacts from optically measuring the temperature of moving foragers. If so, then temperature fluctuations should be greater for moving than for stationary bees. However, there is no significant effect of behavior (stationary or moving) on the magnitude (T_{range} or $T_{variance}$) of these fluctuations ($P\geq 0.68$). In addition, data from investigators using real-time video thermography (which allows continuous tracking of bee temperatures) clearly demonstrate similar temperature fluctuations in forager intranidal T_{th} (Stabentheiner and Haggmüller, 1991). Such T_{th} fluctuations are also exhibited by guard bees, bees investigated by guards, and workers warming the nest (Kleinhenz et al., 2003; Stabentheiner et al., 2002; Stabentheiner et al., 2007). Although we used a different method of IR thermography, our average T_{range} of $1.9\pm 1.1^\circ\text{C}$ is within the T_{range} of $2.4\pm 1.1^\circ\text{C}$ obtained with continuous thermography (calculated from graphs of 18 dancing foragers collecting $1.0\text{--}2.0 \text{ mol l}^{-1}$ sucrose at distances of $60\text{--}1750 \text{ m}$ from

the nest) (see Stabentheiner and Haggmüller, 1991; Stabentheiner et al., 1995).

In summary, we did not find significant correlations between waggle dance parameters associated with fine-scale dancer motivation (number of waggle circuits or dance tempo) and T_{th} or ΔT_{th} . Thus, the effect of dancing motivation on T_{th} is limited to a broad-scale contrast between bees that recruit and those that only tremble dance or walk around inside the nest between foraging bouts. Elevated relative T_{th} and ΔT_{th} may be related to recruitment motivation, not only to heat generated by a moving bee, because tremble dancers and walking bees were cooler than dancers. Thus, three factors: the basic physiology governing thoracic temperature maintenance, the relationship between T_{th} and flight, and forager motivation likely play an important role in regulating intranidal T_{th} .

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REFERENCES

- Baird, J. M. (1986). A field study of thermoregulation in the carpenter bee *Xylocopa virginica virginica* (Hymenoptera: Anthophoridae). *Physiol. Zool.* **59**, 157-168.
- Balderrama, N. M., de Almeida, B. L. O. and Nunez, J. A. (1992). Metabolic rate during foraging in the honeybee. *J. Comp. Physiol. B*, **162**, 440-447.
- Barron, A. B., Schulz, D. J. and Robinson, G. E. (2002). Octopamine modulates responsiveness to foraging-related stimuli in honey bees (*Apis mellifera*). *J. Comp. Physiol. A* **188**, 603-610.
- Coelho, J. R. (1991). The effect of thorax temperature on force production during tethered flight in honeybee (*Apis mellifera*) drones, workers, and queen. *Physiol. Zool.* **64**, 823-835.
- Coelho, J. R. and Ross, A. J. (1996). Body temperature and thermoregulation in two species of yellowjackets, *Vespa germanica* and *V. maculifrons*. *J. Comp. Physiol. B*, **166**, 68-76.
- De Marco, R. J. (2006). How bees tune their dancing according to their colony's nectar influx: re-examining the role of the food-receivers' "eagerness". *J. Exp. Biol.* **209**, 421-432.
- De Marco, R. J., Gil, M. and Farina, W. M. (2005). Does an increase in reward affect the precision of the encoding of directional information in the honeybee waggle dance? *J. Comp. Physiol. A* **191**, 413-419.
- Dornhaus, A. and Chittka, L. (2004). Why do honey bees dance? *Behav. Ecol. Sociobiol.* **55**, 395-401.
- Dyer, C. D. and Seeley, T. D. (1987). Interspecific comparison of endothermy in honey bees (*Apis*): deviations from the expected size-related patterns. *J. Exp. Biol.* **127**, 1-26.
- Dyer, F. C. (2002). The biology of the dance language. *Annu. Rev. Entomol.* **47**, 917-949.
- Eckles, M., Wilson, E. E., Holway, D. A. and Nieh, J. C. (2008). Yellowjackets (*Vespa pensylvanica*) thermoregulate in response to changes in protein concentration. *Naturwissenschaften* **95**, 787-792.
- Esch, H. (1960). Über die Körpertemperaturen und den Wärmehaushalt von *Apis mellifica*. *Z. Vgl. Physiol.* **43**, 305-335.
- Farina, W. M. and Wainelboim, A. J. (2001). Changes in the thoracic temperature of honeybees while receiving nectar from foragers collecting at different reward rates. *J. Exp. Biol.* **204**, 1653-1658.
- Farina, W. M. and Wainelboim, A. J. (2005). Trophallaxis within the dancing context: a behavioral and thermographic analysis in honeybees (*Apis mellifera*). *Apidologie* **36**, 43-47.
- Fuchikawa, T. and Shimizu, I. (2007). Effects of temperature on circadian rhythm in the Japanese honeybee, *Apis cerana japonica*. *J. Insect Physiol.* **53**, 1179-1187.
- Harrison, J. F. and Fewell, J. H. (2002). Environmental and genetic influences on flight metabolic rate in the honey bee, *Apis mellifera*. *Comp. Biochem. Physiol. A Mol. Int. Physiol.* **133**, 323-333.
- Heinrich, B. (1993). *The Hot-Blooded Insects: Strategies and Mechanisms of Thermoregulation*. Berlin: Springer-Verlag.
- Josephson, R. K. (2006). Comparative physiology of insect flight muscle. In *Nature's Versatile Engine: Insect Flight Muscle Inside and Out* (ed. J. Vigoreaux), pp. 35-43. New York, NY, USA: Springer Science and Business Media.
- Kammer, A. E. and Heinrich, B. (1978). Insect flight metabolism. *Adv. Insect Phys.* **30**, 511-521.
- Kearns, C. A. and Inouye, D. W. (1993). *Techniques for Pollination Biologists*. Niwot, Colorado: University Press of Colorado.
- Kearns, C. A., Inouye, D. W. and Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annu. Rev. Ecol. Syst.* **29**, 83-112.
- Kirchner, W. H. (1993). Vibrational signals in the tremble dance of the honeybee, *Apis mellifera*. *Behav. Ecol. Sociobiol.* **33**, 169-172.
- Kleinhenz, M., Bujok, B., Fuchs, S. and Tautz, J. (2003). Hot bees in empty broodnest cells: heating from within. *J. Exp. Biol.* **206**, 4217-4231.
- Mapalad, K. S., Leu, D. and Nieh, J. C. (2008). Bumble bees heat up for high quality pollen. *J. Exp. Biol.* **211**, 2239-2242.
- Mattila, H. R. and Seeley, T. D. (2007). Genetic diversity in honey bee colonies enhances productivity and fitness. *Science* **317**, 362-364.
- May, M. L. and Casey, T. M. (1983). Thermoregulation and heat exchange in euglossine bees. *Physiol. Zool.* **56**, 541-551.
- Nieh, J. C. (1993). The stop signal of honey bees: reconsidering its message. *Behav. Ecol. Sociobiol.* **33**, 51-56.
- Nieh, J. C. (2010). A negative feedback signal that is triggered by peril curbs honey bee recruitment. *Curr. Biol.* **20**, 310-315.
- Pastor, K. A. and Seeley, T. D. (2005). The brief piping signal of the honey bee: begging call or stop signal? *Ethology* **111**, 775-784.
- Reinhard, J., Srinivasan, M. V., Guez, D. and Zhang, S. W. (2004). Floral scents induce recall of navigational and visual memories in honeybees. *J. Exp. Biol.* **207**, 4371-4381.
- Roubik, D. W., Yanega, D., Aluja, S. M., Buchmann, S. L. and Inouye, D. W. (1995). On optimal nectar foraging by some tropical bees (Hymenoptera: Apidae). *Apidologie* **26**, 197-211.
- Schmaranzer, S. and Stabentheiner, A. (1988). Variability of the thermal behavior of honeybees on a feeding place. *J. Comp. Physiol. B*, **158**, 135-142.
- Seeley, T. D. (1992). The tremble dance of the honey bee: Message and meanings. *Behav. Ecol. Sociobiol.* **31**, 375-383.
- Seeley, T. D., Mikhayev, A. S. and Pagano, G. J. (2000). Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. *J. Comp. Physiol. A*, **186**, 813-819.
- Stabentheiner, A. (1996). Effect of foraging distance on the thermal behaviour of honeybees during dancing, walking and trophallaxis. *Ethology* **102**, 360-370.
- Stabentheiner, A. (2001). Thermoregulation of dancing bees: thoracic temperature of pollen and nectar foragers in relation to profitability of foraging and colony need. *J. Insect Physiol.* **47**, 385-392.
- Stabentheiner, A. and Craisheim, K. (1999). The effect of activity level and ambient temperature on thermoregulation in isolated honeybees (Hymenoptera: Apidae). *Entomologia Generalis* **24**, 13-21.
- Stabentheiner, A. and Haggmüller, K. (1991). Sweet food means hot dancing in honeybees. *Naturwissenschaften* **78**, 471-473.
- Stabentheiner, A., Kovac, H. and Haggmüller, K. (1995). Thermal behavior of round and wagtail dancing honeybees. *J. Comp. Physiol. B* **165**, 433-444.
- Stabentheiner, A., Kovac, H. and Schmaranzer, S. (2002). Honeybee nestmate recognition: The thermal behaviour of guards and their examinees. *J. Exp. Biol.* **205**, 2637-2642.
- Stabentheiner, A., Kovac, H. and Schmaranzer, S. (2007). Thermal behaviour of honeybees during aggressive interactions. *Ethology* **113**, 995-1006.
- Stabentheiner, A., Kovac, H. and Brodschneider, R. (2010). Honey bee colony thermoregulation – Regulatory mechanisms and contribution of individuals in dependence on age, location, and thermal stress. *PLoS* **5**, e8967. doi:10.1371/journal.pone.0008967.
- Stone, G. N. (1993). Thermoregulation in four species of tropical solitary bees: the roles of size, sex and altitude. *J. Comp. Physiol. B*, **163**, 317-326.
- Thom, C. (2003). The tremble dance of honey bees can be caused by hive-external foraging experience. *J. Exp. Biol.* **206**, 2111-2116.
- Underwood, B. A. (1991). Thermoregulation and energetic decision-making by the honeybees *Apis cerana*, *Apis dorsata*, and *Apis laboriosa*. *J. Exp. Biol.* **157**, 19-34.
- von Frisch, K. (1967). *The Dance Language and Orientation of Bees*. Cambridge, Massachusetts: Belknap Press.
- Woods, W. A. J., Heinrich, B. and Stevenson, R. D. (2005). Honeybee flight metabolic rate. *J. Exp. Biol.* **208**, 1161-1173.
- Zar, J. H. (1984). *Biostatistical Analysis*. Englewood Cliffs N.J.: Prentice-Hall.