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Effect of food quality, distance and height on thoracic temperature in the stingless bee *Melipona panamica*

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Summary

Stingless bees (Apidae, Meliponini) can recruit nestmates to good food sources. We present the first data showing that recruiting meliponine foragers at feeders and inside nests regulate their thoracic temperature according to net food profitability. Using infrared thermography, we found that *Melipona panamica* foragers elevated their thoracic temperature at profitable food sources (higher sucrose concentration, closer to the nest). At food sources, there is an increase of approximately 0.9°C in thoracic temperature (T_{th}) per 1 mol l^{-1} increase in sucrose concentration (at 20 m distance from nest: 1 mol l^{-1} sucrose concentration, T_{th}=36.6±0.8°C, T_a=31.3±0.5°C; 2.5 mol l^{-1} sucrose concentration, T_{th}=36.9±0.6°C, T_a=29.9±0.2°C). Inside the nest, the difference between thoracic temperature T_{th} and ambient air temperature T_a (ΔT_{nest}) decreases by 0.4°C with each 100 m increase in feeder-to-nest distance and increases by 0.1°C per 1 mol l^{-1} increase in sucrose concentration. The T_{th} of returning foragers was significantly higher at all tested sucrose concentrations (1.0–2.5 mol l^{-1} sucrose concentration) and distances (25–437 m) as compared to T_a (at 2.5 mol l^{-1} sucrose concentration: 25 m distance from nest, intranidal T_{th}=30.2±1.3°C, T_a=24.8±0.7°C; 437 m distance from nest, intranidal T_{th}=28.6±1.7°C, T_{nest}=25.4±1.4°C). For highly profitable food sources (2.5 mol l^{-1} sucrose concentration and ≤100 m from the nest), forager T_{th} was slightly higher than that of randomly chosen control bees inside the nest.

Key words: thermoregulation, thoracic temperature, foraging, recruitment, meliponine, stingless bee, *Melipona panamica*.

Introduction

Thermoregulation is widespread among large-bodied insects (Heinrich, 1993), particularly the Hymenoptera (Himmer, 1932), wasps (Coelho and Ross, 1996; Stabentheiner et al., 2004), solitary bees (Baird, 1986; Chappell, 1982; May and Casey, 1983; Nicolson and Louw, 1982; Stone, 1993a) and social bees (Bujok et al., 2002; Kleinhenz et al., 2003; Seeley et al., 2003; Stabentheiner et al., 1990; Starks and Gilley, 1999). Thermoregulation has significant ecological consequences (Corbet et al., 1993) because internal heat generation enables solitary (Stone, 1994) and social bees (Heinrich, 1993) to forage and pollinate under colder ambient conditions compared to animals that cannot actively thermoregulate.

Several studies have found evidence for thoracic temperature regulation during honeybee recruitment (Esch, 1960; Stabentheiner, 2001; Stabentheiner and Hagmüller, 1991; Waddington, 1990). Honeybees can regulate their body temperature according to food quality, exhibiting higher thoracic temperatures after feeding at richer food sources (Schmaranzer and Stabentheiner, 1988; Underwood, 1991). Thoracic temperature positively correlates with the quality of the food as perceived by sweetness (Stabentheiner and Hagmüller, 1991), proximity to the nest (Esch, 1960; Stabentheiner, 1996) and nectar flow rate (Farina and Wainselboim, 2001). Moreover, thoracic temperatures are affected by the status of the hive (amount of pollen and nectar stores) and are thus tuned to colony need (Schulz et al., 1998). Mechanistically, honeybee thoracic temperature is tied to metabolic expenditure, which increases with increasing sugar concentration and nectar flow rate (Moffatt and Nunez, 1997), and perhaps with forager motivational state (Balderrama et al., 1992). Honeybee thoracic temperature is tied to the thermal stability and the ability to generate high mechanical power output in flight (Dudley, 2000; Woods et al., 2005).

To date, no studies have examined whether stingless bees have similar thermal abilities. We therefore hypothesized that food profitability to the colony would significantly affect the temperatures of recruiting meliponine foragers at the feeder and inside the nest.

What little is known about meliponine thermoregulation largely concerns the regulation of nest temperatures, not individual thermoregulation (Kerr and Laidlaw, 1956; Kerr et al., 1967; Michener, 1974; Roubik, 1989; Wille, 1976; Zucchi and Sakagami, 1972). Preserving sufficiently high brood temperatures is vital, and temperatures can drop daily and seasonally to suboptimal levels (below 28–36°C) for

maintaining brood even in the tropical and semi-tropical regions inhabited by stingless bees (Engels et al., 1995; Roubik and Peralta, 1983). Meliponine nest thermoregulation is thus widespread. Zucchi and Sakagami (1972) measured elevated brood temperatures relative to other portions of the nest in several species (*Trigona spinipes*, *Leurotrigona mulleri*, *Frieseomelitta varia*, *Plebeia droryana*, *Scaptotrigona depilis*, *M. quadrifasciata anthidiodes* and *M. rufiventris*; species names as listed by authors). In *S. postica depilis*, nest temperatures were also largely independent of external temperatures (Rosenkranz et al., 1987). Roubik and Peralta (1983) propose that the brood area acts as a central heat source for the nest, with immature bees supplying the majority of heat and dissipating excess through fanning. Temperatures within the brood area were on average 2–3°C higher than the region immediately outside the involucrum, a resin and wax structure covering the brood area.

Stingless bees can thermoregulate by modifying their nests and generating heat. *Scaptotrigona postica* foragers close their entrance funnel during cold weather (Engels et al., 1995). Meliponines can also thicken the nest walls to improve insulation. Engels et al. (1995) observed workers gathering cerumen particles to plaster the glass covering an observation nest at the low temperature of 15°C. Interestingly, no evidence has been found that stingless bees use evaporative cooling (Fletcher and Crewe, 1981; Roubik and Peralta, 1983), a strategy used by honeybees (Lindauer, 1954) and wasps (Coelho and Ross, 1996). Ventilation appears to be the preferred strategy (Fletcher and Crewe, 1981; Roubik and Peralta, 1983; Zucchi and Sakagami, 1972) and may be sufficient to cool colonies under most circumstances, given the well-insulated nest structure (Engels et al., 1995; Rosenkranz et al., 1987). Ground-nesting African species, *Trigona denoiti* and *T. gribodoi*, decreased phases of inspiration and expiration in the night when temperatures decreased (Moritz and Crewe, 1988) and *Dactylurina staudingeri*, opens nest pores with higher temperatures during the day and closes them during the colder night (Darchen, 1973).

In addition to nest modification, bees actively generate heat. Physical activity can increase meliponine body temperature. Using an infrared thermometer, de Lourdes and Kerr (1989) reported that *Melipona compressipes fasciculata* workers had elevated thorax temperatures (1.0–3.4°C higher) while working as compared to resting. *Trigona* (*Plebeina*) *denoiti* workers increased brood area temperatures when the external temperature was dropped from 31°C to 15.4°C (Fletcher and Crewe, 1981). Such thermoregulation demonstrates that many meliponines can actively modulate their body temperature by generating heat. This raises the possibility that stingless bee and honeybee foragers share an ability to regulate their thoracic temperatures with respect to net food profitability (caloric intake minus caloric expenditure). Thus, the goal of our study was to determine whether the temperatures of recruiting meliponine foragers could be affected by sucrose concentration and location.

We focused on a species, *Melipona panamica* (previously known as *M. eburnea* and *M. fasciata*; D. W. Roubik, personal communication; Roubik, 1992), whose foraging recruitment system has been fairly well studied and is known to specify the three-dimensional location of good food sources to nestmates (Nieh, 1998a,b; Nieh and Roubik, 1995, 1998). Like honeybees, stingless bees can use optic flow to measure foraging distances (Esch et al., 2001; Hrncir et al., 2003). Using mark and recapture studies, Roubik and Aluja (1983) estimated the maximum flight range of this species to be 1.7–2.1 km on Barro Colorado Island, Panama. Foragers are intermediate in size for the genus *Melipona*, being approximately 1 cm in length, with an average wingspan of 8 mm and an average unloaded mass of 0.06 g. Roubik and Buchmann (1984) report that the average food load for *M. panamica* foraging at a 45% sugar solution was 46.2±6.7 μg (sucrose solution density calculated for 29°C; Bubnik et al., 1995). Sugar concentrations of floral nectar loads ranged from 21% to 60% in *M. panamica*, and foragers were able to collect even relatively high viscosity sucrose solutions (70%), performing better at this task than several other *Melipona* species (Roubik and Buchmann, 1984).

We performed four experiments. The first examined overall body temperature changes in response to sucrose concentration at the feeder and in the nest. The second examined the effect of sucrose concentration on thoracic temperature in detail, and the third and fourth examined the effect of food location (feeder distance and height) on thoracic temperature.

Materials and methods

Study site and colonies

We conducted our experiments in a native habitat, Barro Colorado Island, Panama, during the rainy season (June–July) and the beginning of the dry season (November–December) of 2003. Three wild colonies were used, named D, E and F, to continue the sequence published in Nieh and Roubik (1998). All colonies were collected in Colón Province, from the Santa Rita Ridge region approximately 15 km southwest of Portobello, Panama; 9°33′00′′N, 79°39′00′′W. Colony D (approximately 2000 workers) was housed in an observation nest inside a laboratory building connected to the exterior by a 1.5 cm vinyl tube, and had been at this location for over 5 years. The room was open to the outside during experiments and thus maintained at ambient external air temperature and humidity (verified using a weather meter; Kestrel 4000, Boothwyn, PA, USA). Nest temperatures were generally 2–3°C cooler than external air temperatures in the food unloading area (away from the brood chamber) because of bee-built insulation that prevented an immediate rise from the cooler evening temperatures (also observed in *Tetragonisca angustula* by Proni and Hebling, 1996). Colonies E and F (approximately 600 workers each) were kept inside their natural log nests and placed on the landing outside the lab (9°9.923′N, 79°50.193′W), where they had been for 3 years. Only one colony was used at any given time, with the other two sealed by inserting a wire into the nest entrance.

Feeders and training

We trained individually marked *M. panamica* foragers to a grooved-plate feeder (Nieh et al., 2003) containing a scented sucrose solution (100 μl anise extract/liter solution; McCormick & Co. Inc., Hunt Valley, MD, USA). Bees were trained using an anise-scented 0.5 mol l^{-1} sucrose solution to which they did not recruit. During the experiments, we used sucrose solutions ranging in concentration from 1.0–2.5 mol l^{-1} (von Frisch, 1967) mounted on a 1 m high tripod. We marked each visiting bee with an individual combination of paint marks on the distal tip of the abdomen. At the beginning of experiments on each day, we used the first marked foragers to arrive (Nieh et al., 2003). Foragers were trained to feeder locations south of the nest, including the 40 m high Lutz canopy tower (Nieh and Roubik, 1995) located 437 m from the nest. All recruited nestmates were captured in aspirators until the end of each experimental day (Nieh et al., 2003), marked on the abdomen, and then released. The identity of all foragers was verified by viewing their return to the colony entrance (E and F) or inside the colony (D). Each day, we used a different set of foragers that had been recruited and verified on the previous days. Foragers were counted each 15 min and excess foragers were captured in aspirators and released at the end of the day. Germ et al. (1997) recommends that honeybee thermal studies be avoided in the early morning or later afternoon to reduce daily climactic variability. Sunrise and sunset times at our field site were approximately 06:00 h and 18:30 h, respectively, throughout our field seasons, and we typically conducted experiments between 10:00 h and 15:00 h. All feeders were kept in the shade, as is normal for foraging in the forest understory. On a few days, rain limited data acquisition.

Temperature measurements

We measured the temperature of the thorax (T_{th}), the ambient air temperature at the feeder (T_a), and the ambient air temperature inside the nest (T_{nest}). To determine thermal conspicuousness, we calculated the difference between the thorax temperature and the ambient air temperature at the feeder (ΔT_a; Stone, 1993b) and inside the nest (ΔT_{nest}). We also calculated ΔT_{ctrl}, the difference between the temperature of the trained forager and a randomly chosen bee within 5 cm of the trained forager. For controls, we only chose bees that were not actively foraging or engaging in trophallaxis while we measured trained forager T_{th}.

We used infrared thermography to measure forager temperatures (method of Stabentheiner and Schmaranzer, 1987). To measure forager temperatures on the feeder, we recorded bee temperatures 10 s after they had begun feeding on the feeder or 10 s after they had returned to the nest. During our observations, all foragers found nestmates to unload their food to within 10 s. From June through July 2003, we used a Raytek PhotoTemp MX6 (close-focus model, supplier FLW Inc., San Diego, California, USA) photographic infrared (IR) thermometer equipped with True Spot laser sighting to precisely delineate the measured area (spot measurement size adjustable to the diameter of a *M. panamica* thorax). From November through December 2003, we used a Raytek ThermoView Ti30 infrared imager (FLW Inc.). PhotoTemp MX6 values were directly entered into a Macintosh iBook computer (supplier UCSD Bookstore, La Jolla, CA, USA) running Microsoft Excel v.X, and ThermoView Ti30 images were downloaded onto a Sony Vaio laptop PCGTR1A (Amazon.com, USA), running InsideIR v2.0.2. Each time we made a thermographic measurement, we measured air temperature inside the nest (T_{nest}) or at the feeder (T_a) using a Mastech MAS-345 meter (100 cm long type K thermocouple, copper–constantan, 0.3 mm diameter; Amazon.com, USA) placed 1 cm above the nest or feeder substrate and within 4 cm of the returning foragers. Thermocouple air temperature measurements were highly stable.

Calibrations

To calibrate our IR sensors, we waited until the internal and external surface temperatures of a dead bee had equilibrated, inserted a type K thermocouple into the bee, and then recorded its dorsal thoracic IR temperature through IR transparent plastic film (BCU Plastics, San Diego, CA, USA; Polyolefin FDA grade 75 gauge film, catalog #LS-2475; protocol of Stabentheiner and Hagmüller, 1991). This film is optically transparent, reduced disturbances to the nest, and facilitates more normal colony thermoregulation. Equipment emissivity values were then adjusted until both thermocouple and infrared temperature readings matched. Comparisons of calibrated readings from the PhotoTemp MX6 and the ThermoView Ti30 showed no differences in the temperatures measured by these two devices to the limit of equipment readings (0.1°C). Both sensors were highly stable and, although tested at a variety of different temperature and humidity levels in the field and in the lab at the beginning and end of the experiment, exhibited no need for recalibration.

Experiment 1: Individual thermal profiles

At 11:00 h on 4 days (over 2 weeks), we randomly selected five individuals from colony D and recorded their temperatures at the feeder and in the nest for a period of 1 h using thermographic scans. The feeder was placed 276 m south of the nest and 1 m above the ground. The same individuals were recorded at the feeder and the nest during consecutive 1 h intervals (with a break of 15 min to allow equipment transport). We then switched to a different sucrose concentration and repeated the procedure. The order of low and high sucrose concentration presentation and the order of first recording at the feeder or in the nest were alternated each day to control for potential time effects and new individuals were chosen each day. Ambient air temperatures were measured as previously described. We used InsideIR v2.0.2 software to measure the longitudinal thermal profile along the forager's midline, calculating the average temperature of each body part (head, thorax, abdomen) for statistical comparisons.

Experiment 2: Effect of sucrose concentration on thoracic temperature at the food source

We examined the effect of sucrose concentration in detail at

a feeder placed 20 m south of the nests, using seven trained foragers per day (6 total trials, one trial per day). We used all seven sucrose concentrations (presented in random order) on each day and a new set of foragers each day. We consecutively used all three colonies in this experiment (four trials per colony), measuring thoracic temperatures on the feeder with the PhotoTemp MX6.

Experiment 3: Effect of distance and sucrose concentration on intranidal thoracic temperature

We trained foragers from colony D to feeders placed 25 m, 50 m, 100 m, 150 m, 276 m and 437 m south of the nest over a period of 31 days. Depending on weather conditions (frequency and duration of rain), we were able to train the same set of bees to three or four different locations per day. Each day, we used a new set of five foragers. We measured forager temperatures inside the nest with the ThermoView Ti30 and report the thoracic temperature.

Experiment 4: Effect of height on intranidal thoracic temperature

We trained foragers from colony D to either the base (1 m high) or the top (41 m high) of the Lutz canopy tower. We used a different set of five foragers per trial and conducted one trial per day for a total of 15 trials at the tower top and seven at the base (fewer trials due to rain). We did not use the lower 1.0 mol l^{-1} sucrose concentration in this experiment because the bees would not feed at the 437 m feeder for such a low concentration, a common effect encountered when using distant feeders (Jarau et al., 2000; Nieh, 2004).

Statistical analyses

We used JMP IN v4.0.4 software for multiple regression, ANOVA, *t*-tests and Tukey–Kramer HSD tests for pairwise comparisons (Wilkinson, 1996; Zar, 1984). We used Statview v5.0.1 to conduct Sign tests, presenting the results as the ratio of the number of observations greater than zero to the number of observations less than zero. Where appropriate, we applied the sequential Bonferroni correction (Zar, 1984). Averages are presented as mean ± 1 S.D.

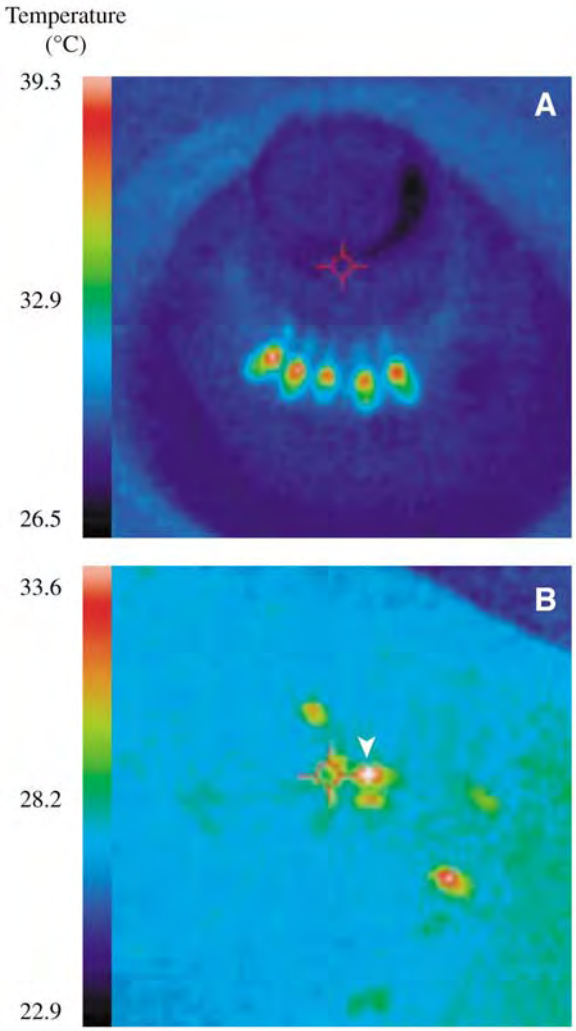

Fig. 1. Typical thermograms of (A) foragers feeding at 2.5 mol l^{-1} sucrose solution on a feeder 20 m from the subject colony (thermal reflections off the glass are visible by the heads) and (B) of a forager returning from this feeder inside the nest (white arrowhead indicates the forager; three bees unloading their food are just barely visible to her left). In all cases, the thorax is the hottest region of each forager. The cursor mark shown in red is a reference point that corresponds to the laser target emitted during recording.

Results

The average recruitment rate for all distances per seven experienced foragers was 1.0±1.4 newcomers per hour for 1.0 mol l^{-1} sucrose solution (N=94 hourly measurements) and 8.1±7.9 newcomers per hour for 2.5 mol l^{-1} sucrose solution (N=94 hourly measurements).

Experiment 1: Individual thermal profiles

Thermograms reveal that foragers can be much hotter than either the background (Fig. 1) or other bees inside the nest (Fig. 1B). As Fig. 2 shows, foragers are hotter at the head and thorax than at the abdomen, at higher ambient air temperatures, for 2.5 mol l^{-1} than for 1.0 mol l^{-1}, and at the feeder than in the nest. These four factors (in order of decreasing effect: body section, air temperature, sucrose concentration and measurement location) play a significant role in forager temperatures at the feeder and in the nest. Forager identity (bee no.) has no significant (NS) effect (ANOVA overall model $F_{6,233}$=335.6.1, P<0.0001, r^2=0.90; body section: $F_{2,233}$=189.3, P<0.0001; sucrose concentration: $F_{1,233}$=52.6, P<0.0001; air temperature: $F_{1,233}$=128.1, P<0.0001; measurement location: $F_{1,233}$=39.6, P<0.0001; bee no.: $F_{1,233}$=0.7, P=0.40; interactions NS). The effect of measurement location is not surprising given the cooler temperatures inside the nest.

At both sucrose concentrations and locations, there are significant differences between the temperatures of different body sections (ANOVA: $F_{2,57}\geqslant$22.3, P<0.0001, interactions NS). The thorax is hotter than the head and the abdomen in all

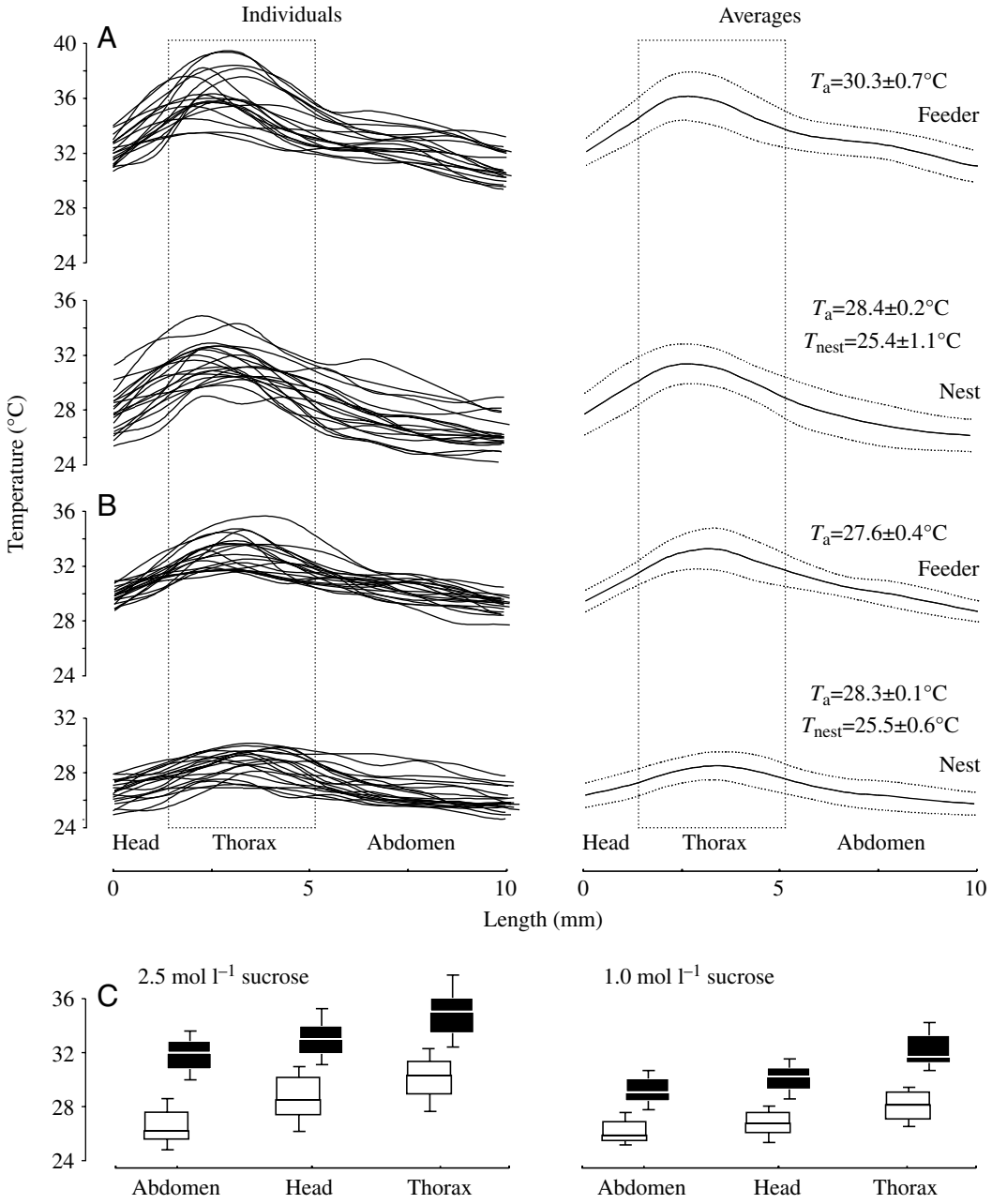

Fig. 2. Longitudinal thermal profiles of foragers at the feeder and in the nest. Midline profiles for 20 different randomly chosen bees foraging at (A) 2.5 mol l^{-1} and (B) 1.0 mol l^{-1} sucrose solutions. Average thermal profiles are shown at right; broken lines indicate ± 1 S.D. Broken rectangles indicate thoracic regions. (C) Temperature distributions of different body parts for both sucrose concentrations inside the nest (open boxes) and at the feeder (filled boxes). Box plots show the 10th, 25th, 50th, 75th and 90th percentiles of the distribution plotted. The feeder was placed 276 m south of the nest and 1 m above the ground.

pairwise comparisons under all conditions (Tukey–Kramer HSD, q^*=2.5062, P<0.05). The head is significantly cooler than the thorax and hotter than the abdomen in all pairwise comparisons in all conditions (Tukey–Kramer HSD, q^*=2.5062, P<0.05) except when measured in the nest after returning from 1.0 mol l^{-1} sucrose solution (no difference between head and abdomen; Tukey–Kramer HSD, q^*=2.5062, NS). Comparing the distal (painted) tip of the abdomen with the proximal end of the abdomen reveals no significant difference (t-test, t_{79}=0.207, P=0.84). Each body section was 32.2±1.1°C (thorax), 30.1±0.7°C (head) and 29.2±0.7°C (abdomen, N=20) while the bee was on the feeder.

We therefore focused on thoracic temperatures. There is a significant effect of ambient air temperature on thoracic temperature at the feeder (ANOVA: $F_{1,497}$=61.1, P<0.0001) and inside the nest (ANOVA: $F_{1,2628}$=962.2, P<0.0001, Fig. 3).

Experiment 2: Effect of sucrose concentration on thoracic temperature at the food source

(1) We first examined the effect of sucrose concentration and air temperature on T_{th} at the food source (Table 1). The overall model incorporating both of these factors accounts for 23% of the variance in T_{th} (ANOVA: $F_{2,495}$=74.0, P<0.0001), and both factors explain a significant portion of variance in T_{th} (ANOVA: air temperature, $F_{1,495}$=110.9, P<0.0001; sucrose concentration, $F_{1,495}$=78.8, P<0.0001; NS interactions and NS colony effect, leading to the simplified two factor model). At the average T_a during the experiment (30.0±1.5°C, N=499),

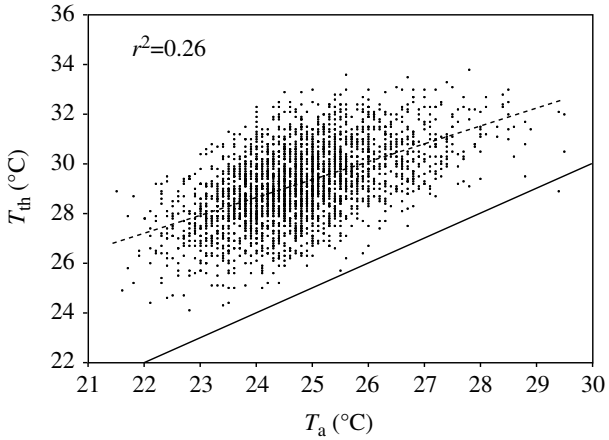

Fig. 3. Effect of ambient air temperature T_a on forager thoracic temperature T_{th} inside the nest. Pooled data from all sucrose concentrations and distances. The regression line is shown as a broken line. The solid line shows how a one-to-one correspondence between T_a and T_{th} would appear.

this corresponds to an increase in feeder T_{th} of 0.9°C per 1 mol l^{-1} increase in sucrose concentration.

(2) Sucrose concentration has a significant positive effect on ΔT_a (ANOVA: $F_{1,496}$=253.6, P<0.0001, r^2=0.21). This corresponds to a rise of 1.4°C in ΔT_a per 1 mol l^{-1} increase in sucrose concentration at the food source (Fig. 4). Sucrose concentration explains 80% of the variance in average ΔT_a.

Experiment 3: Effect of distance and sucrose concentration on intranidal thoracic temperature

(1) Location and sucrose concentration are significantly correlated with individual thoracic temperature inside the nest (Table 2). The overall three-factor model accounts for 36% of the variance in T_{th} (ANOVA: $F_{3,2145}$=410.9, P<0.0001), and each factor is significant (ANOVA: air temperature, $F_{1,2145}$=1125.1, P<0.0001; distance, $F_{1,2145}$=281.3, P<0.0001; sucrose concentration, $F_{1,2145}$=13.6, P=0.0002, interactions NS and thus three-factor model used). The multiple regression fit yields the following parameters: a decrease of 0.4°C in ΔT_{air} with each 100 m of distance, an increase of 0.1°C per 1 mol l^{-1} increase in sucrose concentration, and an increase of 0.8°C per 1°C increase in T_{nest}. The effect of distance on T_{th} is approximately 20 times greater than that of sucrose concentration.

(2) Both distance and sucrose have significant positive effects on ΔT_{nest} (ANOVA: overall model, $F_{2,2146}$=170.4, P<0.0001, r^2=0.14, sequential Bonferroni correction applied) with each factor significant (ANOVA: distance, $F_{1,2146}$=340.8, P<0.0001; sucrose concentration, $F_{1,2146}$=11.2, P<0.0001, interaction NS and thus two-factor model used, sequential Bonferroni correction applied). Model fit yields a decrease of 0.4°C in ΔT_{nest} with each 100 m of distance and an increase of 0.1°C per 1 mol l^{-1} increase in sucrose concentration (Fig. 5). The effect of distance on ΔT_{nest} is approximately 30 times greater than that of sucrose concentration. Sucrose concentration explains 86% of the variance in average ΔT_{nest}.

Table 1. *Effect of sucrose concentration*

Sucrose concentration (mol l^{-1})	T_{th} (°C)	T_a (°C)	N
1.00 (34%)	36.6±0.8	31.3±0.5	48
1.25 (43%)	35.5±0.7	30.3±0.3	45
1.50 (51%)	35.0±0.7	30.1±0.7	55
1.75 (60%)	36.5±0.7	30.7±0.3	53
2.00 (68%)	35.2±0.7	29.1±1.9	101
2.25 (77%)	36.6±1.4	29.6±1.9	132
2.50 (86%)	36.9±0.6	29.9±0.2	64

Forager thoracic temperatures T_{th} and ambient air temperatures T_a measured at a feeder offering different sucrose concentrations and located 20 m away from the colonies.

Equivalent percentage sucrose concentrations by weight are also shown (Bubnik et al., 1995).

Values are means ± S.D. (data pooled from all colonies); N = no. of measurements; 42 total bees used.

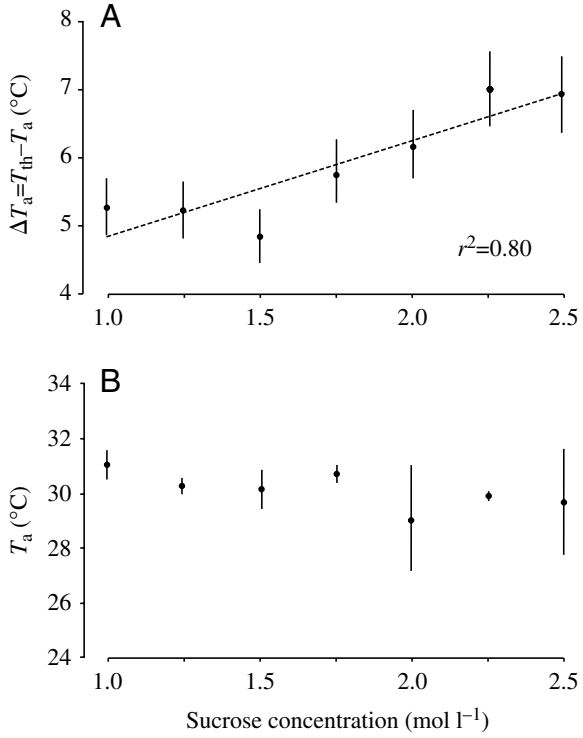

Fig. 4. Effect of sucrose solution concentration on forager thoracic temperatures T_{th} at a feeder located 20 m from nest. (A) The average increase in T_{th} over ambient air temperature T_a (ΔT_a) with broken linear regression line; (B) the corresponding average T_a values. Values are means ± S.D. Sample size given in Table 1.

A closer examination of Fig. 5 suggests a steeper drop in ΔT_{nest} at distances greater than 150 m. We therefore divided this data into two sets, 25–100 m and 150–437 m, focusing upon the 2.5 mol l^{-1} data because this was collected for the largest range of distances. At the short distances (25–100 m),

Table 2. *Effect of distance*

Distance (m)	T_{th} in nest (°C)	T_{nest} (°C)	*N*
25	30.2±1.3	24.8±0.7	198
50	28.8±1.2	23.9±1.0	198
100	29.9±1.3	25.1±0.7	195
150	29.6±1.4	24.5±0.7	198
276	28.7±1.3	24.7±1.1	212
437	28.6±1.7	25.4±1.4	524

Forager thoracic temperatures T_{th} and ambient air temperatures in the nest T_{nest} for a feeder containing 2.5 mol l^{-1} sucrose located at different distances from the nest.

Values are means ± S.D. *N* = no. of measurements; 155 total bees used.

Data from the canopy tower base and top (437 m from the nest) are pooled as there was no significant effect of height on T_{th}.

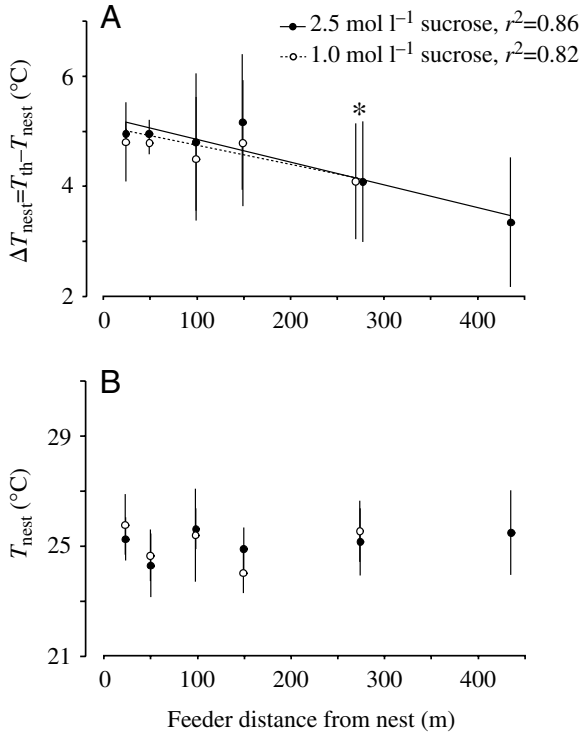

Fig. 5. Effect of distance and sucrose concentration on forager thoracic temperatures in the nest. (A) The average increase in thoracic temperature over ambient air temperature at the nest (ΔT_{nest}). Linear regression lines shown. (B) The corresponding average ambient air temperatures (T_{nest}) for each sucrose concentration are shown. Values are means ± S.D. Sample sizes given in Table 2. Asterisk indicates that symbols are displaced to either side of the 276 m coordinate to avoid obscuring values. Open symbols, 1.0 mol l^{-1} sucrose; closed symbols, 2.5 mol l^{-1} sucrose.

there is a very slight, but significant negative correlation between distance and ΔT_{nest} (linear regression, r^2=0.04, slope=–0.008, $F_{1,589}$=26.3, P<0.0001, sequential Bonferroni correction applied). At greater distances (150–437 m), there is also a significant but slight negative correlation between distance and ΔT_{nest} (linear regression, r^2=0.27, slope=–0.006, $F_{1,558}$=203.1, P<0.0001, sequential Bonferroni correction applied). The slopes are small for both distance ranges, but distance accounts for a far larger portion of the variance in ΔT_{nest} at the greater distances. This is perhaps not surprising given that the distance range spanned by the greater distances (Δ287 m) is 3.8 times larger than the distance range spanned by the short distances (Δ75 m).

(3) With respect to thermal conspicuousness inside the nest, foragers were individually hotter than the ambient air temperature in the nest (ΔT_{nest}) at all sucrose concentrations and distances (see effect of distance and sucrose on ΔT_{nest} in previous analysis). At distances up to 150 m from the nest, (ΔT_{nest}=5.1±1.2°C, N=789 for 2.5 mol l^{-1} sucrose solution and ΔT_{nest}=4.8±1.4°C, N=787 for 1.0 mol l^{-1}sucrose solution) and thus there was a slight difference between ΔT_{nest} at the different sucrose concentrations up to 150 m (ANOVA $F_{1,1574}$=15.8, P<0.0001). When the feeder was placed 276 m from the nest, there was no difference between the average ΔT_{nest} at the different sucrose concentrations (Fig. 5A, ΔT_{nest}=4.0°C at both concentrations).

On average, trained foragers were slightly but significantly hotter than the control bees at distances close to the nest (Table 3). Overall, there was high variance in ΔT_{ctrl}, with maximum positive and negative differences of 11.2°C and –7.2°C, respectively (taken from all distances at both sucrose concentrations). At 2.5 mol l^{-1} sucrose solution, there were significant differences up to 100 m, but at 1.0 mol l^{-1} sucrose solution the only significant difference was at 50 m. There was no significant effect of sucrose concentration on ΔT_{ctrl} at distances up to 150 m (ANOVA $F_{1,1574}$=0.23, P=0.63). The potential trend of decreasing ΔT_{ctrl} with increasing distance did not hold for the base of the canopy tower, located 437 m from the nest (Table 3).

Experiment 4: Effect of food height on intranidal thoracic temperature

(1) There is no significant effect of feeder height above the ground on intranidal thoracic temperature. In the overall model, only nest air temperature (T_{nest}) is a significant factor (ANOVA: overall model, $F_{2,521}$=304.9, P<0.0001; effect tests: T_{nest}, $F_{1,521}$=569.8, P<0.0001; height above ground, $F_{1,521}$=0.1, P=0.70, interactions NS and thus two-factor model used). (2) There is also no significant effect of height on ΔT_{nest} (ANOVA, $F_{1,629}$=2.0, P=0.12, r^2=0.003). (3) However, foragers returning from both the top and the base of the forest canopy were significantly hotter than the ambient air temperature (T_{nest}; P<0.0001) and as compared to control bees inside the nest (P≤0.01, Table 3).

Discussion

Passive thermoregulatory traits such as stingless bee coloration and body size play a role in the ability to forage at different ambient temperatures and thereby contribute to temporal niche differentiation (Pereboom and Biesmeijer,

Table 3. *Thermal conspicuousness of returning foragers inside the nest*

(A)		1.0 mol l^{-1} sucrose				2.5 mol l^{-1} sucrose			
	Distance (m)	ΔT_{ctrl} (°C)	*N*	Sign test	*P*	ΔT_{ctrl} (°C)	*N*	Sign test	*P*
	25	0.1±1.4	198	102/88	0.346	0.4±1.3	198	121/66	<0.0001
	50	0.7±1.9	198	141/50	<0.0001	0.4±1.2	198	111/74	0.008
	100	0.2±1.2	194	100/83	0.237	0.2±1.4	195	111/77	0.016
	150	–0.2±1.7	197	86/106	0.170	–0.3±1.7	198	83/103	0.164
	276	–0.1±1.4	211	99/104	0.779	–0.04±1.8	212	98/105	0.674
(B)									
	Height (m)	ΔT_{nest}	*N*	Sign test	*P*	ΔT_{ctrl}	*N*	Sign test	*P*
	1	3.3±1.3	150	149/1	<0.0001	0.3±1.5	150	93/51	0.0006
	41	3.3±1.1	374	374/0	<0.0001	0.2±1.6	374	205/155	0.01

Measurements were taken for foragers returning from food sources at (A) different distances from the nest and at different sucrose concentrations and (B) at different heights above ground.

ΔT_{ctrl}, difference between thoracic temperature of a trained forager and a randomly chosen control (see Materials and methods); ΔT_{nest}, difference between thoracic temperature of a trained forager and the ambient air temperature inside the nest.

Values are means ± S.D.; *N* = no. of measurements; 155 total bees used.

Sign test values give the ratio of the number of cases where ΔT is greater than 0 to the number of cases when it is less than 0. The number of ties can be calculated from the difference between *N* and the sum of these two values.

In (B) values are for 437 m distance because the canopy tower is 437 m from the colony.

2003). Thus the ability of *M. panamica* foragers to regulate their body temperatures above ambient air temperatures at the feeder and inside the nest, using the thorax as the primary heat source (Figs 1 and 2), may enhance foraging ability in cold conditions, particularly when exploiting rich food sources. There is a significant positive effect of sucrose concentration (Fig. 4) and a significant negative effect of distance on forager body temperature (Fig. 5) such that food sources providing less net energetic value to the colony are correlated with lower thoracic temperatures. There was no effect of height on forager thoracic temperature. Our data demonstrate that a stingless bee, *M. panamica,* can regulate thoracic temperature based upon food quality and location.

Effect of ambient air temperature

As expected for a heterotherm, ambient air temperature had a significant effect upon forager body temperature at the feeder and inside the nest (Figs 2 and 3), as it does in honeybees (Schmaranzer and Stabentheiner, 1988), bumblebees (Heinrich, 1993) and wasps (Kovac and Stabentheiner, 1999). The relationship between forager thorax temperature (T_{th}) and T_{air} is approximately linear in the range of air temperatures that occurred during our experiments (21.5–29.5°C; Fig. 3). It is possible that *M. panamica* foragers regulate relatively lower and more stable T_{th} at higher air temperatures, as suggested by the slight reduction in T_{th} values below the regression line at T_a>28°C (Fig. 3). Further studies at higher T_a are needed to clarify this point.

Sucrose effect

In general, floral nectars contain from 5% to 80% sugar (Baker and Baker, 1983), corresponding to a range of 0.15 mol l^{-1} to 2.3 mol l^{-1} sucrose concentration (Bubnik et al., 1995). Roubik and Buchmann (1984) report that sucrose concentrations of nectar collected by four species of *Melipona* in central Panama during the dry season (including *M. panamica* colonies studied on Barro Colorado Island) ranged from 0.6 mol l^{-1} (21%) to 1.8 mol l^{-1} (60%). We used sucrose concentrations ranging from 1.0 mol l^{-1} to 2.5 mol l^{-1}, with 1.0 mol l^{-1} as the lowest concentration for which bees reliably foraged up to 276 m from the nest. Due to competition from natural food sources, relatively high sucrose concentrations are required to elicit consistent foraging at artificial feeders, even during periods of relative food dearth (Nieh, 2004).

Although we focused on thoracic temperature measurements, it is clear that foraging at higher sucrose concentrations resulted in elevated thoracic, head and abdominal temperatures at the feeder and inside the nest (Fig. 2). With regards to measurement technique, painting surfaces for improved thermographic measurements is a standard practice (Wolfe and Zissis, 1985), and the thin layer of paint applied to the distal tip of the abdomen did not interfere with temperature measurements (no significant temperature differences between painted and unpainted abdominal sections). At the feeder, forager thoracic temperatures were on average higher by 2.1°C than the head and by 3.0°C than the abdomen. Higher thorax temperatures relative to the head and abdomen are reported for *M. compressipes fasciculata* (de Lourdes and Kerr, 1989), foraging honeybees (Schmaranzer and Stabentheiner, 1988), bumblebees (Heinrich, 1993) and wasps (Kovac and Stabentheiner, 1999) and are thus common, if not universal, in flying heterothermic insects (Heinrich, 1993).

Melipona panamica foragers likely shiver their thoracic flight muscles to regulate temperature (Fig. 2). Respiratory metabolism (oxygen consumption) increased with temperature

in the meliponines *Scaptotrigona postica* (Silva, 1981), *T. a. fiebrigi* and *T. a. angustula* (Proni and Hebling, 1996). Recently, Hrncir et al. (2004) have shown that thoracic vibrations produced by recruiting *M. seminigra* foragers increase in duration with increasing food quality. Such vibrations may also have an effect upon thoracic temperature. The mechanism of heat production has not been elucidated in stingless bees, but in all endothermic insects investigated, muscle warm-up occurs through contractions of opposing sets of thoracic flight muscles (shivering) or *via* substrate cycling of a pair of enzymes (Newsholme and Crabtree, 1973; Stone and Willmer, 1989). In bumblebees and honeybees, close relatives of stingless bees (Cameron and Mardulyn, 2001), contractions of thoracic flight muscles, particularly the dorsoventral muscle fibers, were most associated with flight warm-up (Esch and Goller, 1991).

Location effect

There is no significant effect of height on T_{th} inside the nest. However, we found a significant effect of distance on T_{th} that is 20–30 times greater than that of sucrose concentration. Thus T_{th} decreases rapidly with increasing distance of the food source from the nest. A similar result is reported for honeybees (Stabentheiner, 2001). At distances greater than 150 m (the maximum distance at which a significant difference was found between 1.0 mol l^{-1} and 2.5 mol l^{-1} sucrose source), there is evidently little effect of sucrose concentration on *M. panamica* ΔT_{th} (Fig. 5A). The flight range of *M. panamica* on Barro Colorado Island, Panama, is approximately 2.1–2.4 km (Roubik and Aluja, 1983).

Conspicuousness and potential signalling

In honeybees, it remains unclear whether thoracic temperature regulation acts as signal. Germ et al. (1997) reported finding no correlation between honeybee recruitment rates and dancing temperature and thus concluded that thermal information was unlikely to be a primary source of information about food quality. Seeley and Towne (1992) found no evidence that recruiters dancing for a better food source attracted more dance followers than those dancing for a poorer food source. Moreover, the variation in temperature can be quite significant, particularly given the ambient temperature, and even for a fixed food quality in honeybees (Schmaranzer and Stabentheiner, 1988).

Stingless bees can detect changes in nest temperature, as shown by the heating experiments of Engels et al. (1995) and observations of foragers closing and opening nest pores in response to changing air temperatures (Darchen, 1973). The thermal sensitivity of stingless bees has not been measured, but may be similar to that of honeybees, which is approximately 0.25°C (true sensitivity may be higher; Heran, 1952). Our foragers were hotter than nest air temperatures in the food unloading area at all distances tested. All foragers, whether returning from 2.5 mol l^{-1} or 1.0 mol l^{-1} food, were hotter than ambient air temperatures (Fig. 5A). However, evidence for their thermal conspicuousness relative to control bees was limited (average ΔT_{ctrl} no greater than 0.7°C and then only for distances close to the nest, <150 m). At 150 m and 276 m, ΔT_{ctrl} was negative (Table 3). There is high variance in ΔT_{ctrl} (average of 0.1±2°C), as expected given that bees were randomly chosen. This may account for the higher than expected ΔT_{ctrl} at 437 m. Control bees, although inactive foragers at the time of temperature measurement, may have just completed foraging at good natural food sources during those trials. Thus, we found thermal differences based upon the net food quality (Figs 4 and 5), but these differences seem unlikely to play a signaling role given their low level of conspicuousness. Based upon these data, *M. panamica* forager temperature would only provide information about recruiter proximity if nestmates were quite sensitive to small differences in temperature, and then only for high quality food sources close to the nest.

The phenomenon of increasing thoracic temperature with increasing sucrose concentration is thus widespread among the Hymenoptera. For example, the wasp *Paravespula vulgaris* also exhibits significantly higher thorax temperature for higher sucrose solution concentrations (Kovac and Stabentheiner, 1999). This ability may be linked to flight physiology, because the large flight muscles can serve as excellent heat generators through shivering thermogenesis and because these muscles must attain a minimum temperature to achieve flight (Coelho, 1991; Dudley, 2000; Esch and Goller, 1991; Harrison and Fewell, 2002; Woods et al., 2005). Thus, one function of increasing thoracic temperature may be to maintain readiness for high mechanical power production in immediate take-off. We therefore predict that all members of the Apidae will exhibit a similar response of increased thoracic temperature when feeding at increasingly concentrated sucrose solutions.

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