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Bumble bees heat up for high quality pollen

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SUMMARY

Thermoregulation plays a key role in bee foraging, allowing some species to forage in suboptimal temperatures. Recently, bumble bee thoracic temperature (T_{th}) has been shown to increase with nectar carbohydrate content. However, pollen is also vital to bees and exhibits a greater than 20-fold range in protein quality. We provide the first demonstration that bee T_{th} is also correlated with pollen quality. We allowed bumble bee, *Bombus impatiens*, foragers from two colonies to collect pollen varying in quality (25%, 50%, 75% and 100% by mass mixed with indigestible α-cellulose). We used infrared thermography to measure surface T_{th} when a forager finished collecting feeder pollen and when she returned to the nest. Foragers significantly elevated their T_{th} over ambient air temperature while collecting pollen and maintained this elevated T_{th} upon returning to the nest. On average, foragers increased T_{th} over ambient by 0.4°C per 25% increase in pollen protein content. Bumble bees can therefore adjust their thoracic temperature according to pollen quality.

Key words: thermoregulation, foraging, pollen quality, endothermy, *Bombus*.

INTRODUCTION

The power output of insect flight muscles is proportional to muscle temperature up to certain limits (Coelho, 1991; Esch, 1976), and thus thermoregulation plays an important role in the carbohydrate and protein foraging of flying insects (Heinrich, 1993). For example, bumble bees require a minimum flight muscle temperature of 30°C to achieve flight (Heinrich, 1979), and honey bee flight force production is positively correlated with internal thoracic temperature up to 38°C (Woods et al., 2005). Thus, the capacity to modulate body temperature has allowed some insects (such as the arctic bumble bee, *Bombus polaris*) to forage in suboptimal thermal conditions (Heinrich, 1993). Recently, bumble bee (*Bombus wilmattae*) foragers have been shown to increase their flight muscle temperature (measured as thoracic temperature, T_{th}) with increased sugar concentration of a food source. For each 1 mol l^{-1} increase in feeder sucrose concentration, foragers increased their thoracic temperature, on average, by 1.2–2.4°C (Nieh et al., 2006).

However, carbohydrate foraging provides only part of the colony's needs. Protein foraging is also essential to colony growth (Sagili and Pankiw, 2007). Increased pollen intake can increase brood production and enhance colony fitness. Pollen is a protein source for developing larvae, newly emerged workers and the queen (Haydak, 1970; Crailsheim, 1992). Thus, it is not surprising that bumble bees prefer pollen with a higher protein concentration. For example, *Mimulus guttatus* flowers can produce large quantities of pollen grains with no cytoplasm and no nutritive value for bees. In field studies, Robertson and colleagues demonstrated that bumble bees (*B. pratorum*, *B. pascuorum*, *B. lucorum*, *B. hortorum*, *B. lapidarius*, *B. ruderatu* and *B. terrestris*) can discriminate between *Mimulus guttatus* (monkey flowers) that are polymorphic for pollen quality (Robertson et al., 1999). When given the choice between low and high quality pollen patches, foragers visited the higher quality patch more often and probed more flowers within that patch.

Stabentheiner (Stabentheiner, 2001) found that honey bees foraging on natural pollen sources (which may also have provided nectar) had elevated T_{th} that correlated with colony brood level and thus colony need. However, it is not known whether any bee can adjust its thorax temperature according to pollen quality when pollen foraging. Pollen collected by corbiculate bees ranges from 2.5% to 61% protein content [by dry mass, 377 plant species from 93 plant families (Roulston et al., 2000)], and honey bees will forage preferentially for higher quality pollen when given a choice (Cook et al., 2003). We therefore experimentally manipulated pollen quality and measured pollen forager surface T_{th} at a pollen food source and when she returned to the nest. Elevated T_{th} would provide the first evidence that bees can regulate thoracic temperature according to the quality of pollen alone and maintain this elevated temperature on their return to the nest, with potential implications for flight efficiency and the activation of nestmate foraging.

MATERIALS AND METHODS

Study site and bees

We conducted our experiments at the campus of the University of California San Diego, La Jolla, California, USA in a temperature-controlled room (N09°09.890′, W79°50.201′). The ambient temperature was 29.7±1.7°C (Fig. 1A), on average slightly below the minimum flight muscle temperature threshold for bumble bee flight (Heinrich, 1979). Experiments occurred between 08:00 and 12:00 h on each experimental day from September 2005 to August 2006. We consecutively used two lab-reared *Bombus impatiens* Cresson colonies (obtained from Koppert Biological Systems in Romulus, MI, USA). *Bombus impatiens* is a native of North America and ranges across Ontario, Maine, Florida, Michigan, Illinois, Kansas and Mississippi (Heinrich, 1979). We housed each colony in a 44 cm×27 cm×15 cm wooden box covered with an infrared-transparent clear plastic film (Polyolefin FDA grade 75 gauge film, catalog no. LS-2475; BCU

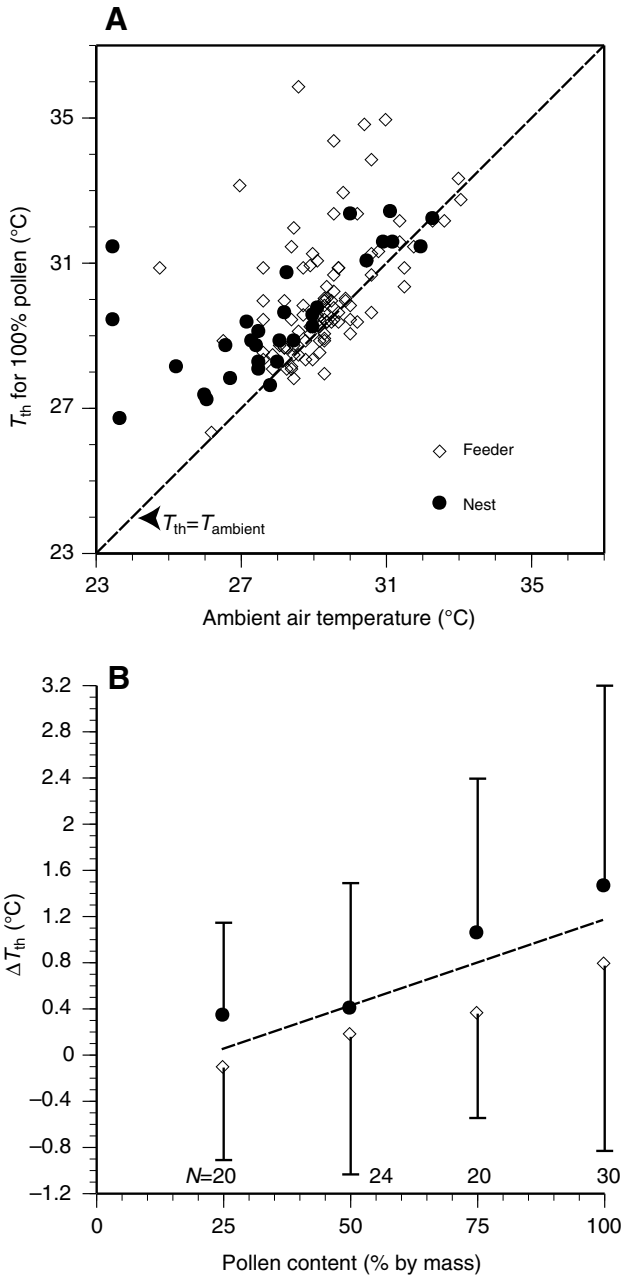

Fig. 1. (A) Relationship between forager thoracic temperature (T_{th}) and ambient air temperature ($T_{ambient}$) at the feeder and inside the nest for 100% pollen ($T_{th}=T_{ambient}$ is shown by the broken line). Ambient air temperature corresponds to the feeder or the nest, as appropriate for the T_{th} measurement. (B) Effect of pollen concentration on ΔT_{th} (calculated using ambient air temperature measured at the feeder or the nest, as appropriate). Open diamonds correspond to ΔT_{th} at the feeder (ambient feeder air temperature, 29.7±1.7°C) and filled circles to nest ΔT_{th} (ambient nest air temperature, 29.1±3.0°C). For clarity, only one tail of the standard deviation error bars is shown. The broken regression line for nest and feeder data combined is shown because there was no significant location. Sample sizes (*N*) are given at the bottom of the plot.

Plastics, San Diego, CA, USA). We fed each colony with sugar solution (2.0 mol l^{-1} unscented sucrose solution) *ad libitum* inside its nest. A clear plastic tube (4 cm diameter, 13 cm long) connected the nest box to a 78 cm×30 cm×33 cm plastic foraging arena covered with the infrared-transparent film.

We labeled all bees in the colony with thoracic plastic tags (each 0.2 mm thick, 2.5 mm diameter, 2 mg; Bee Works, Orillia, ON, Canada) attached with cyanoacrylate adhesive. To determine whether the plastic bee tags affected T_{th} measurements, we randomly removed eight foragers from one colony, excised their thoraces, dried out and removed all internal material, filled each thorax with silver thermoconductive adhesive (thermal conductivity >7.5 W mK^{-1}; Arctic Silver, Visalia, CA, USA), and attached each thorax to a solid copper plate (11 cm×8 cm). We attached bee tags with cyanoacrylate adhesive to four of the thoraces and simultaneously measured the temperature of all eight thoraces with a ThermoView Ti30 thermal imager (Raytek Corporation, Santa Cruz, CA, USA) during heating and cooling on a PCR machine (21–40°C at 1°C s^{-1}). There was no measurable temperature difference (to ±1% of the measured temperature) between tagged and untagged thoraces within a 1 s time window. Thus, the bee tags did not measurably alter T_{th} values within a biologically relevant temperature range (21–40°C), even at a rapid rate of thermal change (1°C s^{-1}).

Foragers collected pollen inside the foraging arena from a circular plastic dish (35 mm diameter, 10 mm high), 64 cm from the arena entrance. We conducted one trial per day and randomly chose the pollen content for each trial. For consistency, we used the same lot of frozen pollen for all experiments. We presented 25%, 50%, 75% or 100% pollen by mass in a dish containing 1 g of total material distributed as a uniform layer. Pollen quality was reduced with powdered α-cellulose (Sigma, EC 232-674-9, St Louis, MO, USA), an odorless, inert, indigestible compound used to vary the protein nutrient value of pollen for foraging honey bees (Pernal and Currie, 2001; Waddington et al., 1998) and caterpillars (Lee et al., 2004). We ground the mixture of α-cellulose and pollen together with a mortar and pestle to achieve even consistency and texture. To ensure freshness, we ground the frozen pollen 10 min before each trial, a period that allowed the sample to come to room temperature.

We used a Raytek PhotoTemp MX-6 (close-focus model; Raytek Corp., Santa Cruz, CA, USA) photographic infrared thermometer equipped with True Spot laser sighting to record temperature data (accuracy of 1% of measured temperature), and measured the thoracic pile surface temperature through infrared-transparent plastic film. Ambient air temperature was measured with a Mastech MAS-345 meter (100 cm long type K thermocouple, copper–constantan, 0.3 mm diameter, supplier www.amazon.com) suspended 1 cm from the feeder or suspended inside the center of the nest. Measurements were made by randomly selecting a focal forager and recording T_{th} and ambient air temperature ($T_{ambient}$), (1) as she finished collecting pollen at the feeder and (2) as she entered the nest. We calculated $\Delta T_{th}=T_{th}-T_{ambient}$ (feeder or nest as appropriate). To avoid pseudoreplication, we collected only one set of measurements for each focal forager.

Statistical analyses

We conducted all statistical analyses with JMP IN v4.0.4 software. Residuals were normally and homogeneously distributed, and thus we performed parametric mixed-model analysis (using restricted maximum likelihood to estimate the proportion of model variance explained by colony as a random effect). We used the second colony to replicate the experiment conducted on the first colony. Pollen content was treated as a continuous variable and location (feeder or nest temperature measurements) as a fixed effect.

RESULTS

Foragers walked and flew through the foraging arena to the pollen feeder, and 75% of foragers spent some time flying while traveling

to the feeder and returning to the nest. Upon arriving at the feeder, they typically dug their mandibles into the pollen for 1–2 s before collecting pollen on their legs by compacting it into their corbiculae (specially modified hairs that hold pollen on the metathoracic legs). There was variation in T_{th}, but the majority of foragers collecting 100% pollen had elevated T_{th} over ambient air temperature at the feeder and inside the nest (Fig. 1A, Table 1).

Table 1. Thoracic and air temperatures at the feeder and inside the nest

Pollen content (by weight)	ΔT_{th} feeder (°C)	Ambient feeder (°C)	ΔT_{th} nest (°C)	Ambient nest (°C)	*N*
25%	–0.1±0.9	30.3±1.1	0.3±0.8	30.9±3.5	20
50%	0.6±1.2	29.1±1.5	0.4±1.1	29.2±3.5	24
75%	0.7±0.9	30.4±2.5	1.0±1.4	30.2±1.4	20
100%	0.8±1.4	29.3±1.3	1.4±1.8	28.7±1.3	30

Means ± s.d. and sample sizes (*N*=number of individuals) are shown.

In the full four-factor model, there was no significant interaction between pollen content and location ($F_{1,283}$=0.8, P=0.37). In the reduced three-factor model, pollen content was the only significant predictor of ΔT_{th} (pollen content effect: $F_{1,283}$=18.6, P<0.0001). There was no significant effect of colony (REML variance component estimate, 5.6%) or location ($F_{1,283}$=3.1, P=0.08). The three-factor model accounts for 12.1% of variance, and yields a regression slope estimate of 1.41 with a standard error of 0.33 for pollen content ranging from 0.25 to 1.00. Thus, foragers heated up while collecting higher quality pollen and maintained this higher temperature upon returning inside the nest (Fig. 1B).

DISCUSSION

Bombus impatiens foragers were significantly hotter, maintaining higher thoracic temperatures, when foraging for pollen with a higher protein content than with a lower protein content. We used a 4-fold range of protein content (by mass), falling within the natural 20-fold range of pollen protein content (2.5–61% dry mass) in bee-pollinated plants (Roulston et al., 2000). At the feeder and when she returned to the nest, a forager had elevated T_{th} over ambient air temperature by 0.4°C per 25% increase in pollen protein content. Thus, they evidently maintained elevated thoracic temperatures on their trip back from the feeder to the nest.

Gustation may play a role in determining pollen quality because foragers investigated the pollen with their mouthparts as soon as they arrived at the feeder. In our study, olfaction may also have played a role in quality determination because bumble bees have an excellent ability to detect and discriminate odors (Heinrich, 2005). The lower pollen content food that we provided had a less pronounced pollen odor because they were mixed with inert α-cellulose. In field studies, Robertson and colleagues demonstrated that several bumble bee species can distinguish between patches of *Mimulus guttatus* (monkey flowers) offering naturally varying qualities of pollen, and visit high quality patches more often than low quality patches (Robertson et al., 1999).

There are potential benefits to increasing T_{th}. Outside the nest, elevating T_{th} with pollen quality may reflect a strategy of increasing thoracic temperature to facilitate flight. After leaving the feeder, foragers walked and flew around the foraging arena, eventually returning to the nest, with some foragers continuing to explore the arena for up to 20 min after collecting pollen. This was particularly true of bees collecting lower quality pollen. Relatively few foragers collected 25% or 50% pollen, even when it was the only available pollen source. These foragers spent long periods roaming around the foraging arena before returning to the nest. Differences between foraging in a limited arena *versus* an unconstrained open habitat may also have led to this exploration behavior. Field studies will probably be required to test the hypothesis that elevated T_{th} increases flight speed and decreases return time. However, given the low level of interest exhibited for 25–50% pollen, foragers would be unlikely to collect such pollen when they have access to natural pollen sources in the field.

Inside the nest, elevated T_{th} for higher quality pollen may also increase colony foraging activity, although this remains to be shown. In our *B. impatiens* colonies, we observed some returning pollen foragers making irregular runs and jostling-type contacts with other bees inside the nest, behavior similar to the foraging activation behavior reported in *B. terrestris* (Dornhaus et al., 2001). Elevated T_{th}, as experienced through jostling contact, may therefore provide a foraging activation cue. Whether higher quality pollen elicits an increase in bumble bee foraging activation compared with lower quality pollen deserves future study. Honey bee foragers round danced at a higher rate (increasing the number of 180° turns per minute) and would thus recruit more nestmates for pure pollen compared with a 50% v/v dilution of pollen with α-cellulose (Waddington et al., 1998). Moreover, honey bee foragers dancing for natural pollen and nectar sources increased their surface T_{th} with increasing colony brood levels and thus with an increasing need for food [37.4±1.6°C for pollen foragers (Stabentheiner, 2001)].

Average values of ΔT_{th} were somewhat higher inside the nest than at the feeder for each pollen content level (Table 1, Fig. 1B). However, there was no significant difference between nest and feeder ΔT_{th}. This is shown in the relatively large standard deviation values for ΔT_{th} at each pollen content level (Fig. 1B). Similarly large T_{th} standard deviations have been reported for perched honey bees (Waddington, 1990) and bumble bees (Nieh et al., 2006) collecting sucrose solution. Pollen content level accounts for only 12% of variance in ΔT_{th} after controlling for colony and location. Thus, the overall effect of pollen content level on ΔT_{th} is relatively small. Sugar concentration has a somewhat stronger effect on ΔT_{th} in the bumble bee *B. wilmattae* (Nieh et al., 2006). In this species, T_{th} increased by 1.2–2.4°C for each 34% increase in sucrose concentration, and sucrose concentration accounted for 11–31% of variation in ΔT_{th} (for 16–65% sucrose solutions by mass).

Elevation of thoracic temperature with sugar forage quality occurs in bumble bees (Nieh et al., 2006), stingless bees (Nieh and Sanchez, 2005), honey bees (Stabentheiner and Hagmüller, 1991; Waddington, 1990), wasps (Kovac and Stabentheiner, 1999) and solitary bees (Chappell, 1982). Our results demonstrate that at least one species of bumble bee, *B. impatiens*, is similarly affected by pollen protein content alone. We therefore speculate that other social corbiculate bees (honey bees and stingless bees) and perhaps even solitary bees will respond to protein quality in the same way. Recently, Eckles and colleagues demonstrated that ΔT_{th} of wasp foragers (*Vespula pensylvanica*) is positively correlated with the protein content level of collected meat baits (Eckles et al., 2008). We hypothesize that tuning flight muscle temperature to food carbohydrate and protein quality is a widespread strategy in flying social insects and has evolved to enable efficient foraging because the power of insect flight muscle is proportional to its temperature,

within certain biological limits (Woods et al., 2005). Whether social corbiculate bees can use elevated T_{th} as a cue for food quality through tactile contacts remains an intriguing question.

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