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Thermal investigations of a nest of the stingless bee *Tetragonisca angustula* Illiger in Colombia

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Abstract

Thermal investigations were performed in Colombia on a colony of the stingless bee *Tetragonisca angustula* by means of thermometry and direct calorimetry. To this end, a nest with approximate 5000 individuals was collected in the field and transferred to a twin calorimeter, where the bees continued their normal life and development. Two camping boxes (Poor Man's Calorimeter, PMC) with Peltier elements as cooling systems were used as a differential system. Each box had a volume of 8 L and a sensitivity of 19.2 mV W^{-1}. The PMCs were modified to give forager bees a free access to the environment. The thermal situation within the colony was monitored calorimetrically and thermometrically. 10 kΩ of NTC resistors were distributed at special points of the brood combs, storage pots and involucrum. The thermometric and calorimetrical signals were recorded continuously over 3 months by a four-channel data logger.

Keywords: Isothermal calorimetry; Honeybees; Metabolism; Stingless bees; *Tetragonisca angustula*; Thermometry

1. Introduction

Stingless bees live in all tropical and subtropical regions of the world between approximately 30° north and south latitudes. These bees are characterized by a reduced sting, weak wing venation and bared eyes (except the genus *Trichotrigona* Camargo and Moure 1983). *Tetragonisca angustula*, Syn. *Trigona angustula*, known in Colombia as “angelita” (little angel), is one of the most common stingless bee species in South America, distributed from Argentina to Mexico from sea level to 1800 m height. Honey from this bee is highly appreciated for its pleasant flavour and used for the treatment of respiratory and eye infections (anticataract properties) [1]. Some calorimetric experiments on the antimicrobial action of stingless bee honey were performed recently by the present authors [2–4].

Due to their effectiveness as a pollinating vector, these stingless bees have great importance for the pollination of native tropical plant species. According to the investigations of Kerr et al. [5] the bees are pollinators of 40–90% of plant species, and are thus closely related to their conservation. About 500 species of stingless bees exist in the world; 300 of them live in both Americas, about 120 are found in Colombia.

Unfortunately, these species (discovered or still undiscovered) are suffering under severe human intervention, and may be counted as endangered organisms. For this reason, the necessity was emphasized some years ago, to increase the knowledge of wild bees, to propose and execute actions for their protection and conservation, to collect information about their particular relations with the endemic vegetation, and to underline their importance as pollinators of wild and cultivated plants [6]. Studies made up to now in Colombia concentrated on taxonomy, description of bee nests and of bee behaviour, but no investigations have been performed about the metabolism of these insects as individuals or colonies.

Here we present for the first time calorimetric data for *Tetragonisca angustula*. Moreover, this simple calorimetric method was never before applied for stingless bees in general.

2. Experimental

2.1. *Tetragonisca angustula nest*

The experiments were performed from 22 February to 15 May 2006, 13 km from the university town Pamplona in Norte

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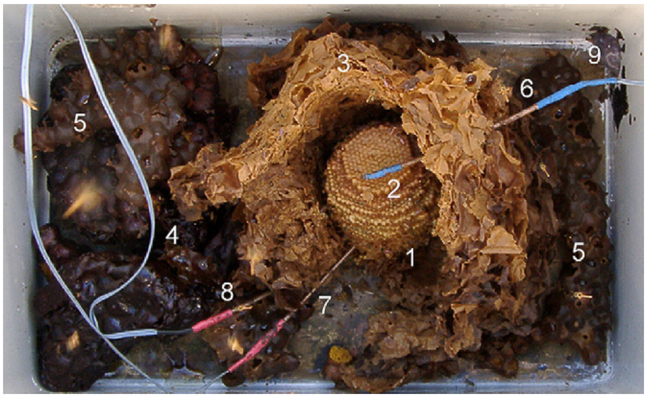

Fig. 1. View of the nest inside the calorimeter box showing the brood area (1) with the pile of combs (2), the involucrum (3), the storage area (4 and 5), the top (6), middle (7) and bottom sensor (8), and the entrance duct (9).

de Santander, Northern Colombia (7°27′N; 72°38′W) at an altitude of 1600 m and a yearly mean temperature of 19.3 °C (15.2–25.6 °C). February counts as dry and March–May as wet season, however rains were strong but sporadic in 2006, the climate of this season was more dry than rainy.

A *Tetragonisca angustula* nest, found in a guamo tree (*Inga spectabilis*) approximately 90 cm above the ground, was transferred into the calorimeter. To ensure that all colony members were collected after the transfer of the nest into the box we kept the calorimeter in the place of the nest and sticked pieces of wax in the entrance, closed it at night and only then transported the nest to its new place in the laboratory. The measurements started 8 days later after an equilibration time to let the bees repair their nest and continue their normal life. The nest of 304 g contained one laying queen, about 5000 workers, an involucrum (an insulating, multilayered envelope around the brood area; see below and Fig. 1) of 11.5 cm height and 15 combs (8 cm high) and 143 storage pots, 82 with honey, 50 with pollen and 11 empty. We assumed that the nest was of cylindrical shape and estimated a total volume of 1 L in the beginning so that an air space of 7 L remained in the calorimeter. The bees accepted the translocation and continued to develop their nest. At the end of the experimental period the nest filled the box completely. All the time, the bees had free access to the outside by means of a tunnel of about 40 cm length, long enough to limit cold draughts and to ensure a stable baseline. The reference box beside the experimental box had a similar tunnel but was closed with a grid.

Between February and May, the foraging bees visited – among others – the following plants in an area 500 m around the nest and collected honey and pollen there: lemon trees (*Citrus sinensis*), guajava trees (*Psidium* spp.), mandarin orange trees (*Citrus reticulate*), peach trees (*Prunus persica*), and pineapple guave trees (*Acca sellowiana*).

2.2. Instrumentation

2.2.1. Direct calorimetry

Two electric camping cold boxes of 8 L volume each were used as a differential twin calorimeter. They were bought already equipped with Peltier units at their bottoms, incorporated as coolers or heaters depending upon dc current polarity. Here they served as heat flux sensors (Seebeck effect). This mobile set-up was used as a simple and cheap twin calorimeter called the "Poor Man's Calorimeter" (PMC) earlier [7,8]. The boxes were placed in a room where the temperatures showed only small variations between 18 and 20 °C. To prevent condensation of water inside the calorimeter, the boxes were ventilated two times during the experiment by opening the cover.

The boxes were slightly modified for measuring purposes. Copper foil was glued to the walls and the bottom of the boxes to improve heat conduction to the Peltier elements. Entrance ducts (diameter, 4 cm) were drilled into the calorimeter boxes and connected to exit holes in nearby windows with transparent plastic tunnels (length, 40 cm). In this twin-arrangement, one box served as a measuring chamber containing the nest, the other empty one as the control unit. The calorimeter was electrically calibrated by the Joule effect for all modifications of the interior design of the chamber and at different ambient temperatures. The sensitivity of the calorimeter always amounted to $19.2\,\mathrm{mV\,W^{-1}}$.

The calorimetrical signal was recorded continuously with a four-channel data logger (UNIDAN PLUS, ESYS, Berlin, Germany) with variable amplifications between 1- and 128-fold and a resolution of 0.1 mV in these experiments. Up to 4 MB of experimental data could be stored with repetition rates between 1 s and 24 h. The data were further processed with Microsoft Excel. The obtained heat production rates were integrated and regarded as the average daily heat production rate. In addition, the stage of nest-building was noticed and the weight of the stingless bee colony evaluated. For this purpose the entrance ducts were closed, and the complete measuring chamber was weighed, always at the same time of the day (7 p.m.), using a mechanical balance. This procedure was repeated only once in 2 weeks because it disturbed the colony. We assumed constant weight of the colony for the time of the experiment, as we observed no significant variations. Dividing the heat flux by the total mass of the bees provided the specific heat production rates for this period.

2.2.2. Temperature measurement

Nest temperatures could be monitored by means of 10 kΩ NTC resistors distributed at special points throughout the brood combs (suspended and separated by connectives and pillars), storage pots and the involucrum. One resistor determined the temperature inside the reference chamber (Figs. 1 and 2). The resistors were connected to one-channel data loggers (HOBO Temp, Series 01, Onset Computer Corporation, Pocasset, MA, USA) and a four-channel version for ambient temperature, humidity, light intensity and a further external signal, e.g. a second temperature (HOBO RH, Temp, Light, External, Series 08). Both worked in a temperature range from −20 to +70 °C with an accuracy of ±0.7 K at 20 °C and a resolution of 0.1 K. The first HOBO could store up to 1800 data points (2 kB), the second one 8000 data points (8 kB). The stored data were transferred to a PC with a special software (BoxCar 3.5) and imported into Microsoft Excel for further treatment.

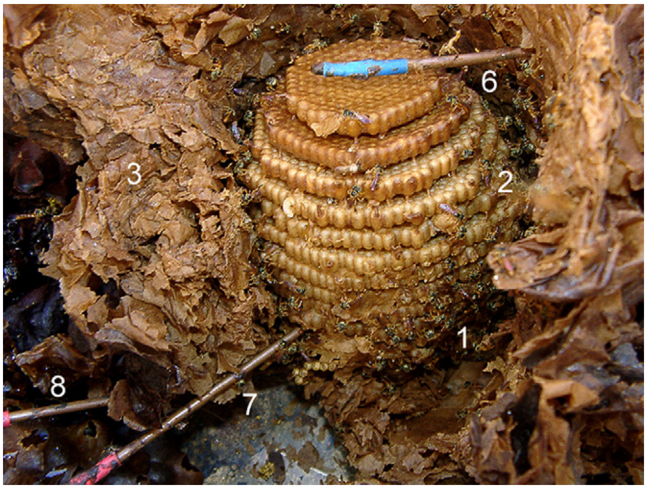

Fig. 2. Enlarged view to the brood area (1) with the combs (2), the involucrum (3) and the temperature sensors (6–8). Explanations are as in Fig. 1.

3. Results

The bees accepted their new environment after translocation and continued their normal development from February to May. The resistors placed in different parts of the brood area showed highest temperatures in the centre part of the nest (30.3 ± 0.4 °C) and lowest values in the lower part (25.4 ± 0.6 °C), in contact with the calorimeter bottom and thus the Peltier element. The storage area for pollen and nectar outside the brood area kept a rather constant mean temperature of 19.6 ± 0.3 °C ($n = 191$; 1 point per hour during 1 week; Fig. 3) with fluctuations that are only half of those found at the bottom of the nest.

Fig. 3 depicts the temperature courses during a whole week from the 9th to the 16th of April 2006. The strong daily temperature fluctuations of the outside air between 14 and 24 °C (lowest curve) influenced all the other signals. The least impact is seen in the temperature inside the honey pots (second curve from below) supposedly because of the higher heat capacity of their content. It becomes clear from Fig. 3 that the maximum temperature of the outside air (lowest curve) always occurs 4–5 h before those determined for different nest parts inside the calorimeter. Therefore, the latter curves were shifted in such a way that their maxima coincided with that of the air and that the attenuation of the signal could be determined. The correlation coefficient was 0.43 for the brood area temperature indicating that the temperature fluctuations are damped to about 40% of those in air. The regression factor of $R^2 = 0.6629$ is not very high due to the irregularities mainly in the outside temperature. Without the time shift the regression factor was only $R^2 = 0.14$. The honey pots showed a corresponding factor $R^2 = 0.67$ as the brood area, but a damping to less than 20%. With $R^2 = 0.44$ the temperature at the top of the nest was more loosely coupled to the air (data not shown).

The strong fluctuations seen in Fig. 3 give the impression that the whole system is thermally rather labile. This is mainly due to the way of representation. The outside air fluctuates around 18.2 °C with a standard deviation of ±2.1 °C (11.7%) and maximum and minimum values of 24.4 and 14.5 °C, respectively. All the other deviations are essentially smaller: the brood area with 25.5 ± 1.2 °C (4.6%), the top of the nest with 29.2 ± 0.91 °C (3.1%) and the honey pots with 19.7 ± 0.51 °C (2.6%). The temperature difference between the brood area and the air is 7.4 ± 2.0 °C over the week. The impression that there is a steady decrease in all temperatures is also not correct; linear trend lines for the temperatures and their differences show correlation coefficients around −0.1 or significantly smaller.

Plotting the heat production rate together with the ambient air temperature as functions of the time during the experiment, gives slopes that appear perfectly reversed horizontally: heat flow minima and temperature maxima occur at the early afternoon (Fig. 4). Minima of the heat production rate are more pronounced than the smeared maxima. Minima occur in the afternoon between 12 a.m. and 6 p.m. with a mean at

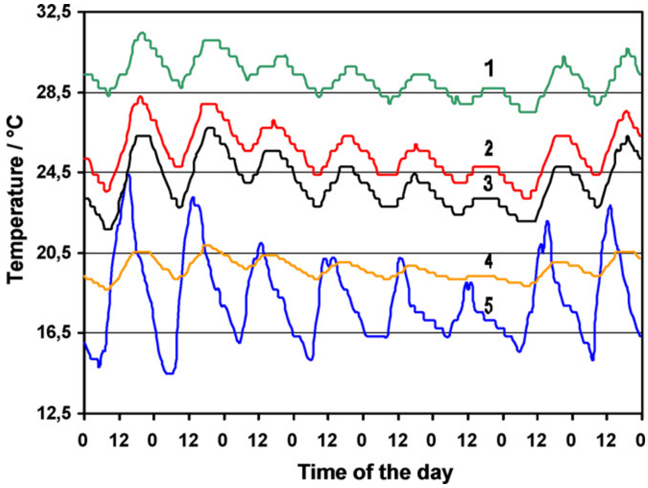

Fig. 3. Temperature course of main components connected with the *Tetragonisca* nest: top part of the nest (1); centre of the nest (brood area) (2); bottom of the nest (3); honey pots (4); outside air (5). “0” and “12” indicate midnight and noon of 9th till to 16th March 2006.

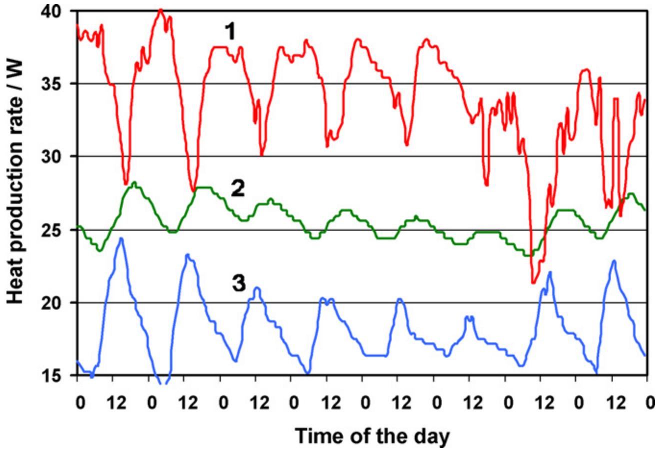

Fig. 4. Time course of the heat production rate (1), the brood area temperature (2) and the air temperature (3) during the experimental period. Temperature is given in °C. The heat production (W) was multiplied by 100 to have corresponding ordinate values.

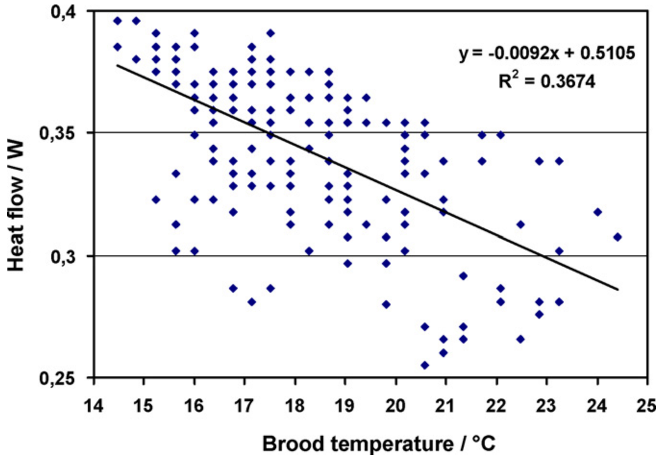

Fig. 5. Heat flow vs. brood area temperature for the experimental period from April 9 to April 16. The negative correlation as sign for thermoregulation is clearly indicated. The values below 0.25 W at the last day but one are excluded as experimental artefacts.

3 p.m. ± 2 h. Maxima appear at the end of the day or in the early morning between 5 and 8 a.m. Because of the 4 h time shift between outside and inside temperatures and because of the biological reasoning it is more appropriate to plot the heat production rate as a function of the brood area temperature (Fig. 5). Increasing heat production rates at decreasing temperatures underline a non-ectothermic behaviour of the bees and thus an active thermoregulation. The correlation coefficient of -0.0092 provokes a rate increase of the heat production by 32% when the temperature drops by 10 °C from 24.5 to 14.5 °C. This result is significant with $p < 0.001$.

A different presentation of these data is given in Fig. 6. The observed hysteresis – typical for thermoregulating systems – starts at point “a” (9 April 2006; 11 a.m.) and ends about 36 h later at point “z” (10 April 2006; 6 p.m.) with time proceeding in a counter-clockwise manner. These results will be discussed below.

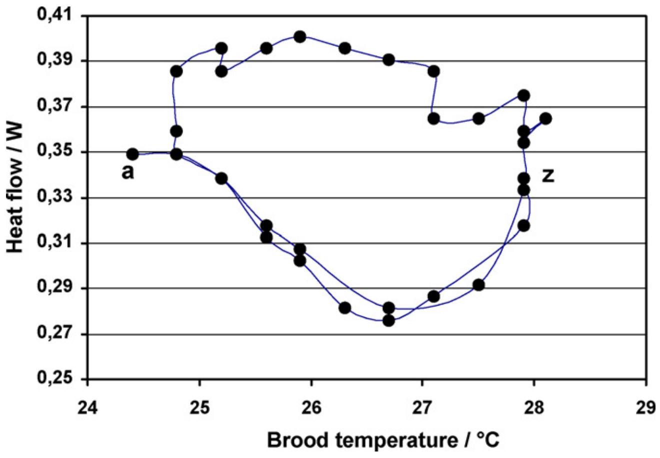

Fig. 6. Hysteresis structure for the dependence between heat flow and brood temperature. The time window starts at “a” (9 April 2006; 11 a.m.) and ends at “z” (10 April 2006; 6 p.m.). The points are 1 h apart each. The direction of progressing time is counter-clockwise.

4. Discussion

Stingless bees are only found in tropical and subtropical regions, mainly of South and Meso-America. The essential reason why stingless bees did not spread into temperate zones is assumed to be their lack of tolerance to low temperatures. Amano discovered that *T. carbonaria*, used as a pollinator of greenhouse crops in Japan, is not able to control the hive temperature easily, especially when the temperature drops considerably [10]. However, the tolerance of adult *T. carbonaria* workers to low temperatures is not as poor as that of some *Apis* honeybees [10]. Moreover, they tolerate much higher temperatures than *Apis* species and are thus interesting pollinators for horticulture and agriculture.

Apis colonies as a whole do possess an elaborate system of thermoregulation and are able to maintain the temperature of the brood at 34–36 °C throughout the year. They passively raise the nest temperature with their own body heat by shivering the wing muscles. If necessary, they lower the temperature by fanning with their wings at the nest entrance to transport warmer air out and cooler air into the nest. Moreover, foragers collect water, shed it in the entrance of the nest or spread it over the combs and take advantage of the high enthalpy of evaporation [11].

The brood area forms the thermal core in stingless bee nests, where heat loss is damped by the concentric cerumen layers of the involucrum. It has long been speculated that its layers surrounding the brood keep the heat there and passively act in thermoregulation [12,13]. Recent studies with *Tetragonisca weyrauchi* showed that this species actively controls the nest temperature within the brood area [14]. The internal temperatures of the inhabited nest were compared to those of an empty nest. Both values followed the environment fluctuations. The calculated regression value for the occupied nest is much higher ($R^2 = 0.98$) than the value determined in our nest. This is supposedly due to the fact that their temperatures were measured at two times during the day and not continuously as in this paper. The correlation factor is similar to that obtained here for the brood area. The results suggest that the physical structure of the nest would be responsible for the retention of a small part of the heat energy [14].

Thermometric investigations of a nest of the stingless bee *Trigona carbonaria* showed similar temperature slopes as presented in Fig. 3 [10]. Strong fluctuations of 14.7 °C in the air were damped to 3.5 °C (24%) in the brood area and a time lack of 3.9 h similar to that in the *Tetragonisca angustula* are evident.

The maximum temperature difference between the brood centre and the storage area is about 8 °C with a mean value of 5.8 during the experimental interval. Unlike this strategy of *Tetragonisca* to separate brood and nests, hives of *Apis* show a tight connection between brood, nectar and pollen cells all at approximately the same temperature [8]. The distinct temperature difference found in the stingless bee nest might be favourable for the conservation of the nutritional reserves. The *Tetragonisca* honey is more diluted than that of *Apis* bees and thus more easily prone to microbial deterioration. On the other hand, a smaller comb area with effective insulation by the involucrum enables an effective thermoregulation, but with the drawback of a reduced heat capacity in the combs and thus an increased temperature fluctuation.

The marked hysteresis in Fig. 6 indicates a slow regulatory response of the heat production by the bees on temperature changes in their immediate environment. This time latency leads to the fact that, for instance, two heating levels of 0.28 and 0.39 W exist at the same brood temperature of 26.7 °C. Correspondingly, a flow rate of 0.35 W is found at 24.4 and 27.8 °C. One can estimate from the hysteresis that it takes 4–5 h between the onset of active temperature regulation. Such heat production hystereses were intensively investigated by Seymour et al. in connection with the thermoregulation in a few species of thermogenic plants [15,16]. Well-known for their regulation are *Philodendron selloum*, the sacred lotus (*Nelumbo nucifera*) and the Eastern skunk cabbage (*Symplocarpus foetidus*) that keep their flower temperatures rather constant over hours, days or even weeks [15]. While some intermediate metabolic regulators, among them supposedly the alternative oxidase, are active in the plant systems, thermoregulation proceeds actively in the beehive on the level of the individual worker bee.

The heat production rate exhibits a clear periodic behaviour. Until now it is an open question if this is a diurnal regulation triggered by the light/dark cycle (which is always 12 h/12 h in the tropics) or the daily temperature fluctuations. As long as no calorimetric experiments with constant brood area temperatures have been performed, this question is difficult to answer. Proni and Dias Macieira made respiratory investigations with isolated stingless bees (*T. a. angustula* and *T. a. fiebrigi*) and observed a circadian pattern of the metabolic rhythm that is in a significant contrast to the present calorimetric results. They showed a larger consumption of oxygen during the day, with a peak between 10 a.m. and 3 p.m. At night the consumption decreased, reaching the minimum levels between 2 and 3 p.m. [9].

Diurnal rhythm could also mean that many foragers leave the nest during the day, so that the heat production inside the nest is low. At night all bees are at home and at rest; thus, the mass specific heat production is low, but the number of bees high and therefore the product of rate × number also high. The inverse is correct for the day. Moreover, the bees would try to keep the nest warm during cooler nights. However, the food storage area shows such a constant temperature line that it could be (passively) the same with the brood area.

True energetic measurements about stingless bees are scarce so that is it difficult to compare the present data with values from the literature. Proni and Dias Macieira determined the respiration rates of three stingless bee species, among them *T. angustula* [9]. They found specific values between 2.5 and 6.0 μL O_2 mg^{-1} h^{-1} that transform to 15 and 35 W kg^{-1} of bees, assuming a pure carbohydrate metabolism. From the present calorimetric data with a mean heat production of 0.34 W for the whole nest and an estimated number of 5000 bees one arrives at 68 μW per bee. The mean mass of 6 mg per bee transforms the result to 11 W kg^{-1}, a value that fits to the Proni data [9].

Investigations with a similar PMC calorimeter were performed on whole bumblebee and hornet colonies. The nest of the bumblebees (*Bombus lapidarius*) showed heat production rates of 0.9–1.6 W in the high season when the nest was inhabited by 20–50 adults, otherwise 0.3–1.4 W [17]. The largest contribution came from workers (∼105 mg wet weight) with 111 W kg^{-1}. The young queens (∼320 mg) had mass specific rates of 27 W kg^{-1}, the males (∼120 mg) 76 W kg^{-1}. The heat flow of the nest showed a sinus like fluctuation over many days. The significantly heavier hornets (*Vespa crabro* L.) contributed 49 W kg^{-1} (workers, ∼450 mg) and 36 W kg^{-1} (drones, ∼350 mg) to the heat output of 1.8 W of the nest at maximum biomass (23.8–26.8 °C), otherwise 1.3 W [8,18]. European honeybee nests (*Apis mellifera*) were never investigated with a PMC. Results of other authors for *Apis* nests show a median of 55 W kg^{-1} (16.3–126.5 W kg^{-1}) during daytime and are discussed in [19]. *Apis* foragers (∼80 mg) had high heat production rates of 280 W kg^{-1}, the drones (∼210 mg) much smaller ones with only 118 mW kg^{-1} [20].

Table 1
Characteristic data of the colony

Size of the nest	
Diameter (cm)	3.1–5.3
Height (cm)	8
Total mass (g)	∼300
Nest mass (g)	60.6
Involucrum mass (g)	47.4
Nest parameters	
Amount of honey stored (82 pots) (g)	142.6
Amount of pollen stored (50 pots) (g)	35.4
Number of worker bees	5000
Bee parameters	
Length of a worker bee (mm)	4
Mass of a worker bee (mg)	6
Life-span of a worker bee (day)	30–50
Length of the queen (mm)	13
Mass of the queen (mg)	50
Life-span of the queen (year)	2–5
Thermal data	
Heat production rate of the nest (mean) (mW)	340
Heat production rate of the nest (max) (mW)	400
Heat production rate of the nest (min) (mW)	210
Heat production rate of a worker bee (mW)	0.068

The data collected in this work show that *Tetragonisca angustula* bees perform social thermoregulation and that they are able to keep the brood temperature constant at 25.5 ± 1.2 °C. It might be interesting to study some species of those stingless bees that do not build an involucrum. The results would help to know more about the mechanisms used by both types of stingless bees to maintain the temperature of the brood constant. There are no previous thermal data on *Tetragonisca angustula* in the literature that could allow us to compare the obtained specific heat production rates with those in our experiments (Table 1). As stingless bees have a great importance in the South American apiculture and folk medicine in general and in Colombia specially, we think that further experiments on all aspects of stingless bees are not only scientifically interesting, but economically justified and necessary.

Acknowledgement

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