Journal of Thermal Analysis and Calorimetry, Vol. 95 (2009) 3, 737–741

THERMAL INVESTIGATIONS OF A NEST OF THE STINGLESS BEE *TRIGONA* (*FRIESEOMELITTA*) *NIGRA PAUPERA* PROVANCHER IN COLOMBIA

A. Torres[1*], *W. Hoffmann*[2] *and I. Lamprecht*[3]

[1]Universidad de Pamplona, Departamento de Biología-Química, Grupo de Biocalorimetría, Km 1 Vía Bucaramanga-Pamplona Pamplona, Colombia
[2]Universidad de Pamplona, Grupo de Biocalorimetría, Km 1 Vía Bucaramanga-Pamplona, Pamplona, Colombia
[3]Free University of Berlin, Institute for Biology, Königin-Luise-Strasse 1-3, 14195 Berlin, Germany

Thermal investigations were performed on a colony of the Colombian stingless bee *Trigona* (*Frieseomelitta*) *nigra paupera* by means of thermometry and direct calorimetry.

A nest with about 2000 individuals was 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 colony was monitored calorimetrically and thermometrically with resistors distributed in the nest. Signals were recorded continuously by a four-channel data logger.

Keywords: *calorimetry, Frieseomelitta nigra paupera, thermometry, thermoregulation*

Introduction

The tribe Meliponini belongs to the group of corbiculate bees (subfamily Apinae) and encompasses all the bees known as ‘stingless bees’, found throughout the tropical and subtropical areas of the world [1]. They are the major visitors and native pollinators of flowering plants in the tropics, comprising a large group of small to medium sized bees (from 2 mm up to 1.5 cm) with a level of social organization comparable to that of the common honeybee *Apis mellifera* [2]. Stingless bees are considered of vital importance for ecosystems, due to their efficiency as agents pollinators. According to Roubik [3], effectiveness in the reproductive cycle of the majority of the native plant species in the tropical regions is strongly connected with the great variability in size of these bees. Moreover, bee pollination may help reducing the process of habitat fragmentation or at least overcoming its problems for rainforest plant species.

According to historical references, the Muiscas Indians of the highlands of Eastern Colombia cultivated stingles bees and added their honey to sweeten ‘chicha’, a fermented corn drink used for ritual purposes and consumed in large quantities during religious festivals. They also applied the wax in goldsmith workshops. Another group of Indians, the Tayrona, aborigines of the Sierra Nevada de Santa Marta (Colombian Caribbean coast), were experts in cultivating stingless bees directly in their houses or surroundings and used the honey in food as well as the wax in adornment production [4].

Frieseomelitta is a stingless bee subgenus (Meliponinae, Trigona), exclusively Neotropical, with wide geographical distribution, occurring from Southwest of Mexico to the Southeast Region of Brazil. In Colombia, there are only two *Frieseomelitta* species: *Trigona* (*Frieseomelitta*) *varia* and *Trigona* (*Frieseomelitta*) *nigra paupera*. *Frieseomelitta* is one of the stingless bees which do not build brood cells in regular combs, but in a cluster arrangement. The area of the brood-cluster with the new cells turns spirally clockwise around the cluster. In this way the new cells replace old ones from which bees had emerged. The cluster as a whole is linked with the hive walls by means of pillars thicker than those interconnecting the brood cells. As is characteristic for most cluster builders, there is no involucrum (a canopy of wax around the nest for insulation and protection) surrounding the brood. However, wax plates are built between the major supporting pillars [5]. It seems likely that a spiral brood comb efficiently conserves heat generated by the brood, whereas the flat matrix-style of brood cell construction facilitates heat dissipation [6, 7].

Until now, no studies on biology, behaviour and metabolism of these insects, neither as individuals nor as colonies, have been performed in Colombia. This is astonishing as these bees are of great importance

---

[*] Author for correspondence: datorres@unipamplona.edu.co

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

for pollination in local ecosystems due to their biodiversity and great abundance in tropical forests.

Here we present for the first time thermometric and calorimetric data for *Trigona* (*Frieseomelitta*) *nigra paupera* obtained with an 8 L-twin calorimeter constructed from commercial cool/heat-camping boxes. This simple calorimetric method has been successfully applied for bumblebees, wasps and hornets [8, 9], but nearly never for bees, except for the winter cluster of *Apis mellifera* honeybees [9] and for the stingless bee *Tetragonisca angustula* [10].

Experimental

Trigona (Frieseomelitta) nigra paupera nest

The experiments were performed from April 17 to June 2, 2008, 13 km away from the university town Pamplona in Norte de Santander, Northern Colombia (7°54 N; 72°63 W) at an altitude of 1200 m and with a yearly mean temperature of 19.2°C (16.6–22.3°C). The chosen period of the year counts as the wet season with strong rainfall.

2004 a *Frieseomelitta* swarm made its home in an empty wooden box used already as a stingless bee hive before. This new nest was transferred into the calorimeter in April 2008. To ensure that all colony members were collected, the calorimeter remained in the original place during the following hours with open entrance marked by pieces of wax. At night the entrance was closed and the whole ensemble transported to its new place in a laboratory. There, the calorimeter entrance was opened again after some hours. The measurements started after an equilibration time of 20 days to let the bees repair their displaced nest and continue their normal life. This nest of 202 g contained one laying queen and about 2000 workers with a mean mass of 0.012 g and a body size of 6 mm. The brood cells were in the typical cluster arrangement and 19 cm high (Fig. 1) and with 42 pots in the storage area, 19 for honey, 5 with pollen and 18 empty. We estimated a total nest volume of 1.4 L in the beginning assuming a cylindrical shape. Thus, the bees had free space of 6.6 L in the 8 L-calorimeter.

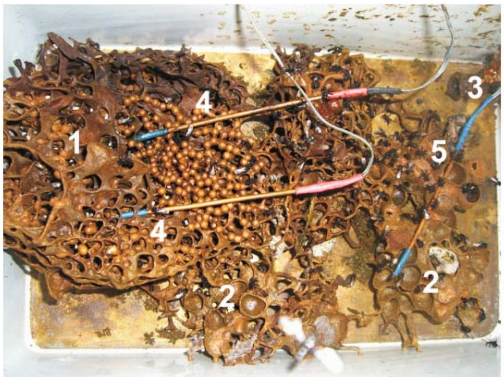

Fig. 1 View of the nest inside the calorimeter box showing the 1 – brood area, 2 – storage area, 3 – entrance duct, 4 – two brood area sensors and 5 – one storage area sensor

Periodical visual observation showed that the bees accepted the translocation but needed time to repair the nest. No significant changes in the growth of the nest were seen during the following six weeks of the experiment. All the time, the bees had free access to the outside by means of a tunnel of about 15 cm length, long enough to limit cold draughts and to ensure a stable baseline of the calorimeter. The identical reference box beside the experimental box had a similar tunnel but closed with a grid. During the experiment, the foraging bees collected honey and pollen in an area 500 m around the nest mainly at the following plants: lemon trees (*Citrus sinensis*), guajava trees (*Psidium* spp.) and mandarin orange trees (*Citrus reticulate*).

Instrumentation

Direct calorimetry

Two commercial electric camping cold boxes of 8 L volume (length×depth×height=32×22×28 cm) were used in a differential twin calorimeter mode. 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 [9, 11]. The boxes were placed in a room with small temperature variations between 20 and 22°C. To prevent condensation of water inside the calorimeter, the boxes were ventilated two times during the experiment by opening the cover.

These boxes were slightly modified for measuring purposes. Copper foil was glued to the inner walls of the boxes to improve heat conduction to the Peltier elements. Entrance ducts (inner diameter 4 cm) were drilled into the calorimeter boxes and connected by transparent plastic tunnels (length, 15 cm) to exit holes in nearby windows. In the twin-arrangement, one box served as a measuring chamber containing the nest, the other empty one as the control unit. Electrical calibration of the calorimeter showed a sensitivity of 19.2 mV W^{-1} at different ambient temperatures. 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 during a chosen period of time were integrated, divided by this time and taken as the average heat production rate. In addition, the stage of nest-building was noted and the mass of the stingless bee colony evaluated. For this purpose the entrance duct was closed and the complete measuring chamber 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. As we observed no significant variations, we assumed constant mass of the colony for the time of the experiment. Dividing the heat flux by the total mass of the bees provided the specific heat production rates for this period.

Temperature measurement

Nest temperatures were monitored by means of 10 kΩ NTC resistors placed at special points in the brood and the storage area. A further resistor determined the temperature inside the reference chamber. The sensors 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). All 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 group could store up to 1800 data points (2 kB), the second one 8000 data points (8 kB). The stored data were read out, transferred to a PC with special software (BoxCar 3.5, Onset Computer Corporation, Pocasset, MA, USA) and imported into Microsoft Excel for further treatment. The observed course of temperatures is shown in Fig. 2.

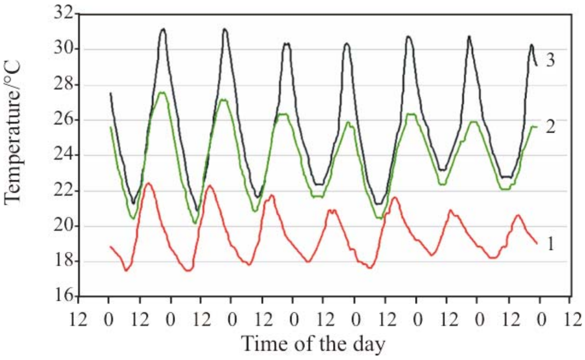

Fig. 2 Temperature course of the 1 – outside air, 2 – food storage area and 3 – brood area of the *Frieseomelitta* nest. '0' and '12' indicate midnight and noon during the period May 13 to May 19, 2008

Results and discussion

After the translocation of the nest the bees had enough time to return to usual behaviour. Temperature data were taken in the brood and the storage areas and in the outside air. The monitored values were very similar to those of *Tetragonisca angustula* in the first investigation [10]. The two resistors installed in the brood area showed the highest temperatures in the nest (24.2±2.6°C). The temperature variations in the storage area (23.8±1.8°C) are very close to those of the brood area. The results shown in Fig. 2 correspond to one week (May 13 to May 19) with data taken every hour. The fluctuations in the air temperature were between 22 and 17°C (lowest curve). From Fig. 2 it is obvious that the ambient temperature variations influence both brood and storage area temperatures and that the maximum temperatures for the storage area occur about 6 h later than the maximum of the ambient temperature. The largest fluctuations occur in the brood area with 25.4±2.9°C (11.3%) and the storage area with 23.8±1.9°C (8.0%). The outside air temperature varies around 19.5°C with a standard deviation of ±1.3°C (6.4%) and maximum and minimum values of 22.5 and 17.5°C, respectively.

The heat production rate oscillated in the same period between 0.172 and 0.026 W with a mean value of 0.077±0.043 W decreasing slightly during this time. Plotting the heat production rate together with air and brood temperatures over time gives a rather asymmetric slope: heat flow minima occur in the afternoon around 2 p.m., maxima at night between 9 and 10 p.m. (Fig. 3). Such an asymmetry is already indicated in the air and consequently also in the brood temperature.

Increasing heat production rates at decreasing temperatures emphasize a non-ectothermic behaviour and active thermoregulation of a biological system. Figure 4 demonstrates such a response of the bee nest.

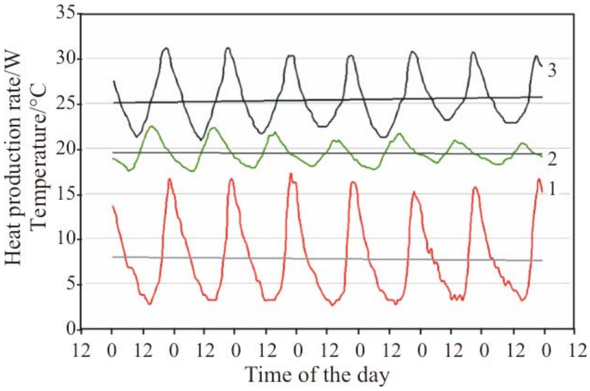

Fig. 3 Time course of the 1 – heat flow, 2 – air temperature and 3 – brood area temperature during the experimental period. Temperature in °C, heat production multiplied by 100 to have corresponding ordinate values in Watt

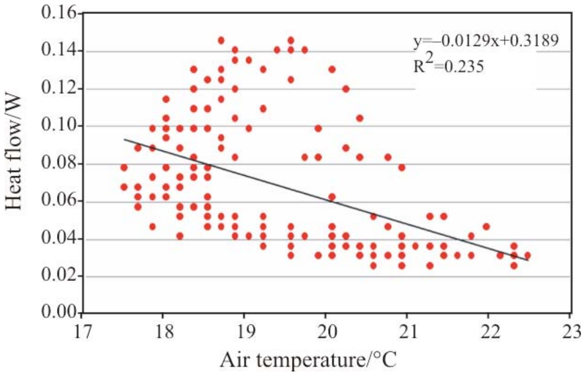

Fig. 4 Heat flow *vs.* air temperature for the experimental period. The negative correlation as sign for thermoregulation is clearly indicated

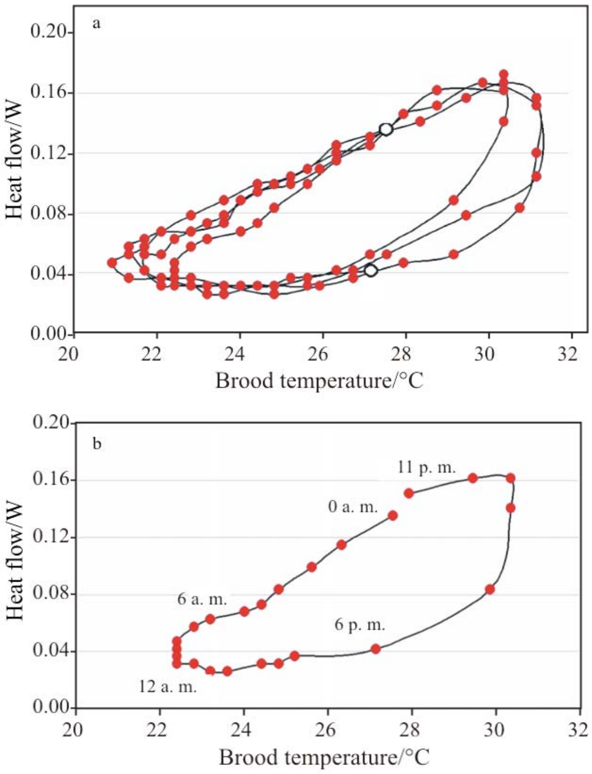

Fig. 5 Hysteresis structure for the dependence between heat flow and brood temperature. a – The time window starts at 'a' (May 13, 2008; 0 a.m.) and ends at 'z' (May 16, 2008; 6 p.m.); b – first day of this period from 0 a.m. till 11 p.m. The points are 1 h apart. The direction of progressing time is counter-clockwise

The correlation coefficient of –0.0129 provokes a rate increase with a factor of 2.84 when the temperature drops by 5°C from 22.5 to 17.5°C.

Figures 5a and b display the heat production rate of the bee nest as function of the brood area temperature, in Fig. 5a for nearly four days from May 13 (0 a.m.) till May 16, 2008 (6 pm). The rather stable thermoregulating regime is clearly indicated in this graph. Figure 5b helps to discuss the thermoregulation in more detail. The slope starts at midnight of May 13. The following early morning period is coined by a strong decrease of heat production with dropping temperatures, even higher than expected after the Q_{10} rule (van't Hoff law). The final minimum is reached in the later morning at about 11 a.m. and lasts till to the early evening. During this time, brood experiences an increasing temperature at a special rate. When this rate slows down, brood becomes active to keep their comfort till to their maximum of 0.17 W at 10 p.m. Here a new cycle starts with nearly the same slope as the day before. But Fig. 5a indicates that the heating phase occurs already at lower temperatures in the following days.

Social insects (all termites and ants, some wasps and bees) have at least some ability to regulate their nest temperatures within specific, sometimes very narrow, boundaries, despite extremes in ambient temperature. Honey bee colonies, for example, are able to maintain brood nest temperatures within the range of 33–36°C, even at ambient temperature ranges from well below freezing to above 45°C [12].

Selecting an appropriate cavity appears to be the primary mechanism for many stingless bee species to keep nest temperatures optimal, despite of high ambient values. Many stingless bee species build nests insulated by three main layers. First, the cavity is lined with batumen (a Portugese word meaning 'wall') made of propolis (plant gums, saps or resins collected outside the hive) or a mixture of wax and propolis and sometimes vegetable matter and mud [13]. The batumen seals up the nest cavity, except for the entrance and, in some species, for ventilating holes. The batumen layer is also used to separate cavities that are too large to be included in the nest. Second, often a layer of storage pots for pollen and honey made of cerumen (a mixture of wax and propolis) is found immediately within the batumen lining. Third, inside the layer of storage pots there is an involucrum of thin vertical leaves of cerumen joined to each other and to the pots. Usually, the involucrum encloses the brood comb completely [13]. In many stingless bee species the involucrum section of the nest is very important for heat conservation because it traps much of the heat produced by brood metabolism. Other stingless bee species, such as *Leurotrigona muelleri* and *Frieseomelitta*, do not build an involucrum or closely packed brood combs, but construct brood cells in a loosely-joined matrix.

The only other calorimetric experiment on stingless bees was performed with the same equipment from April 9 to April 16 in 2006 [10]. Although air temperatures at that time were between 14.5 and 24.4°C instead of 17.5 to 22.5°C during the present investigations, we will try to compare the results. The similarity between the former temperature slopes of *Tetragonisca angustula* [10] and those in Fig. 2 are evident as are the fluctuations in the brood area and the time lack of 6 h between the maximum values for air and brood. Average temperatures in the brood area of *Frieseomelitta nigra paupera* and *Tetragonisca angustula* nests oscillate between 24.2±2.6°C and 25.5±1.2°C, respectively. These differences may be due to the varying nest architecture in both species as discussed above.

Temperatures of the storage area were calculated for the *Frieseomelitta* nest to 23.8±1.8°C and for the *T. angustula* nest to 19.7±0.51°C. These data are again in agreement with the structure of the nests: *Frieseomelitta* values are higher because the insulating involucrum is missing separating brood and storage area thus allowing easy heat transfer. In the same sense the *Tetragosnisca* temperature is closer to the ambient one in contrast to *Frieseomelitta* that is nearer to the brood value.

The marked hysteresis in Fig. 5 indicates a slow regulatory response of the bees' heat production rate on temperature changes in their immediate environment. This time latency leads to the fact that, for instance, two heating levels of 0.026 and 0.099 W exist at the same temperature of 24.8°C. Correspondingly, a flow rate of 0.12 W is found at 26.3 and 31.1°C. One can estimate from the hysteresis that both a passive Q_{10} and an active biological factor are engaged in thermoregulation of the brood. Similar heat production hystereses were found in *Tetragonisca angustula* nests [10]. The data collected in this work show that *Frieseomelitta nigra paupera* bees perform social thermoregulation and that they are able to keep the brood temperature constant at 24.2±2.6°C. Thus it is possible to compare the thermal data obtained for two stingless bee species with and without an involucrum and different means of thermoregulation.

Table 1 Characteristic data of the colony

Size of the nest	
Diameter/cm	6–12
Height/cm	19
Total mass/g	~440
Nest mass/g	202
Nest parameters	
Amount of honey stored (19 pots)/g	71.4
Amount of pollen stored (5 pots)/g	6.4
Amount of empty pots (18 pots)/g	3.5
Number of worker bees/	2000
Bee parameters	
Length of a worker bee/mm	6
Mass of a worker bee/mg	12
Life-span of a worker bee/day	30–50
Length of the queen/mm	15
Mass of the queen/mg	60
Life-span of the queen/year	2–5
Thermal data	
Heat flow of the nest (mean)/mW	71.9
Heat flow of the nest (max)/mW	213
Heat flow of the nest (min)/mW	5.2
Heat flow of a worker bee/mW	0.0359

There are no previous thermal data on *Frieseomelitta nigra paupera* in the literature that could allow us to check the published specific heat production rates against those in our experiments (Table 1). Further experiments on all aspects of stingless bees are necessary and have great importance as they will contribute to the knowledge and the conservation of this essential pollinating vector and the native plants it visits.

Acknowledgements

A. T. thanks the Universidad de Pamplona, Colombia for the financial support.

References

1 G. Nates-Parra, Biota Colombiana, 2 (2001) 235.
2 T. A. Heard, Annu. Rev. Entomol., 44 (1999) 183.
3 D. W. Roubik, J. Kansas Entomol. Soc., 52 (1979) 443.
4 G. Nates-Parra, Abejas corbiculadas de Colombia. Universidad Nacional de Colombia, (2006) 57.
5 M. J. Sommeijer, J. L. Houtekamer and W. Bos, Insects Sociaux, 31 (1984) 199.
6 D. J. C. Fletcher and R. M. Crewe, J. Entomol. Soc. South Africa., 44 (1981) 183.
7 W. Engels, P. Rosenkranz and E. Engels, Stud. Neotrop. Fauna Environ., 30 (1995) 193.
8 P. Schultze-Motel, Thermochim. Acta, 193 (1991) 57.
9 E. Schmolz, I. Lamprecht and B. Schricker, Thermochim. Acta, 251 (1995) 293.
10 A. Torres, W. Hoffmann and I. Lamprecht, Thermochim. Acta, 458 (2007) 118.
11 T.Wesolowski, B. Schaarschmidt and I. Lamprecht, J. Thermal Anal., 30 (1985) 1403.
12 L. Fahrenholz, I. Lamprecht and B. Schricker, J. Comp. Physiol. B, 159 (1989) 551.
13 A. Wille and C. D. Michener, Rev. Biol. Trop., 21 (1973) 1.

DOI: 10.1007/s10973-008-9466-4