

Thermal investigations associated with the behaviour patterns of resting workers of *Bombus atratus* (Hymenoptera: Apidae)

L. Vega · A. Torres · W. Hoffmann ·
I. Lamprecht

ISBCXVI Special Issue
© Akadémiai Kiadó, Budapest, Hungary 2011

Abstract The Neotropical bumblebee *Bombus (Fervidobombus) atratus* Franklin is widely distributed in South America ranging from tropical and subtropical lowlands to high altitudes in the Andes. Thermal investigations of the Neotropical bumblebee *Bombus atratus* at rest were performed by means of thermometry. Most social insects are diurnal foragers. When they are inside their nests during the night, they carry out some tasks such as cleaning the nest, brood care or nest thermoregulation. However, forager bees generally pause at night in a sleep-like state. A bumblebee nest was transferred to a brood chamber which was maintained at approximately constant temperature (28 ± 1 °C) to prevent the workers from building the protecting involucrum so that one could mark them at birth. To provide a temperature gradient, two chambers were installed adjacent to the brood chamber, so that the workers had the chance to choose between 3 temperatures: the first one kept at 22 ± 1 °C, the second at 19 ± 1 °C and 28 ± 1 °C. The temperatures of the chambers were recorded continuously by a four channel data logger. Three categories of tasks performed by workers were registered in this investigation: warming brood (task 1), feeding brood (task 2) and foragers (task 3). During the experiment 357

workers were born and 252 (71%) of them showed sleep-like behaviour. The total sleep time in hours according to the work done by the workers was task 1 (240.6 h) > task 3 (236.0 h) > task 2 (227.3 h). The workers who performed tasks 1 and 2 preferred to sleep at the highest temperature (28 ± 1 °C) and the forager workers at 22 ± 1 °C.

Keywords *Bombus atratus* · Bumblebees · Sleep-like behaviour · Thermal investigations

Introduction

Insects are the largest group of animals on Earth. About 750,000 species have been described, 2% of which are considered eusocial with a division of labour between males, queens and workers. Yet, this 2% contribute more than half of the total biomass of all insects [1]. The eusociality in insects involves many processes including heat production and many patterns of behaviour that ensure the survival of the colony.

Bumblebees belonging to the genus *Bombus* are eusocial; there are about 250 species distributed worldwide, most species are in temperate zones of North America, Europe and Asia and less in the tropics. Nine of these species have been reported for Colombia [2].

The Neotropical bumblebee *Bombus (Fervidobombus) atratus* (Franklin) is widely distributed in South America, from tropical and subtropical areas up to 3400 m, suggesting a good ability to adapt to different environmental conditions [3]. *Bombus atratus* belongs to the “pocket maker” bumblebees: they provide food for their larvae in “pockets” of wax built next to the larval cells [4]. There are a few studies of the nine species of the genus *Bombus*

A. Torres (✉)
Departamento de Biología y Química, Universidad de Pamplona,
Grupo de Biocalorimetría, km 1 Vía Bucaramanga-Pamplona,
Pamplona, Colombia
e-mail: datorres@gmx.net

L. Vega · W. Hoffmann
Universidad de Pamplona, Grupo de Biocalorimetría, km 1 Vía
Bucaramanga-Pamplona, Pamplona, Colombia

I. Lamprecht
Institute for Biology, Free University of Berlin,
Königin-Luise-Straße 1-3, D-14195 Berlin, Germany

reported for Colombia, including description of their biology and ecology [5] and even less about their use as pollinators [6].

The phenomenon of a sleep-like behaviour (in the following called “sleep”) is not restricted to mammals, but also found in other vertebrates such as birds, turtles and even in invertebrates [7]. Thus, it is not unexpected that it exists in bees also. First indications for it in honeybees (*Apis mellifera carnica* L.) came from electrophysiological, long-term recordings on individual neurons of the visual system in the brain of this insect [8]. Kaiser found for honeybees that the state of antennal immobility was an important sign for the depth of the sleep process in this social insect [9, 10]. Sauer presented a newly developed video and computer technique for continuous contact-free recording of position and movements of the antennae allowing comparing the dynamics of honeybee’s sleep with the time-course of the nightly ventilatory activity [11].

Sleep of bees was also explained taking into account the necessity to conserve energy during inactive phases. Schmolz et al. [12] investigated the preferred ambient temperatures for sleep as well as the heat production rates of sleeping honeybees at ambient temperatures between 20 and 35 °C. They found that during the night, the heat production rates of honeybees followed an ectothermic pattern, which means a deviation from their thermoregulatory behaviour during day time. Nevertheless, the bees did not choose the lowest ambient temperature to sleep, but a medium value of 28 °C [12]. The authors concluded that the reduced thermoregulatory behaviour of sleeping honeybees seems to be a compromise between energy conservation and feasibility of regenerative processes.

In this article, the authors show for the first time a sleep-like behaviour for bumblebees connected with the age and tasks of workers and the preferred temperature to sleep.

Experimental

Preferred temperatures in insects are often investigated with temperature organs or gradient chambers. As such a temperature gradient chamber (TGC) the authors built two wooden boxes, which were placed one above the other. The lower chamber was divided into two compartments by a wooden wall. The first compartment housed a resistor that was connected to a thermostat (Biotherm 2000, Dohse Aquaristik KG, Germany), the second compartment was left empty. A wire mesh was installed on top of this compartment to allow the heat transfer to the upper chamber. Moreover, the upper chamber was also divided into two compartments connected by a transparent plastic tube; the top was covered with a lid of 2 mm glass for observations (Fig. 1).

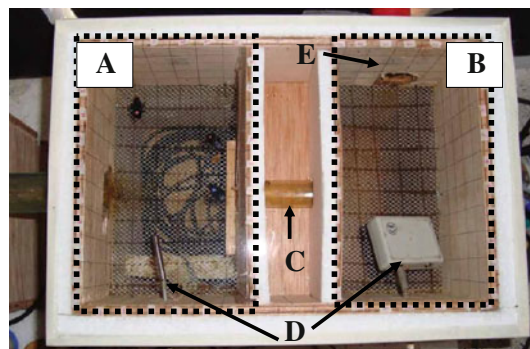


Fig. 1 Temperature gradient chamber/TGC. Compartment 1 (a), compartment 2 (b), connection between the two compartments (c), temperature sensors (d) and entrance duct (e)

The bumblebee nest was translocated in the second period on January 28, 2009 to the brood chamber (BC). The brood chamber consisted of two wooden boxes, one above the other, and previously separated by a wire mesh. A resistor was installed in the lower chamber connected to a thermostat. It should keep the temperature of the nest approximately constant (28 °C ± 1) preventing the workers to build the protecting involucrum. BC and TGC were connected by a transparent plastic tube (Fig. 2).

The temperatures of the gradient chamber and the brood chamber were registered using temperature sensors (HOBO Data Logger, Onset Computer Corporation 311155 Series). The obtained data were transferred to a computer with the software BoxCar 3.5 (Onset Computer Corporation) and then imported into Microsoft Excel for further processing.

The age of each bumblebee worker was registered by marking the thorax with numbered “Opalithplättchen” at birth. The authors established three categories of workers according to their task: warming brood (task 1), feeding brood (task 2) and foragers (task 3). The measuring period started on April 1, when the workers began to sleep, and endured until June 30, 2009. Their thermal preferences were registered every 30 min from 6:00 pm in the evening until 6:00 a.m. the next morning.

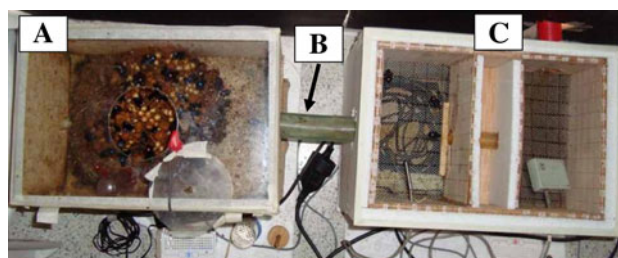


Fig. 2 Brood chamber (a), transparent plastic tube (b), temperature gradient chamber (c)

Results and discussion

After a paper of Gonzales et al. [5] the production of queens in *Bombus atratus* is bimodal, one period between April and August, the other between November and January. The authors chose the second period for the investigations. At the time of translocation (January 28, 2009), the nest had a population of one queen, 86 workers, 68 cocoons, 4 larval cells and 7 egg cells. 287 workers were borne in the time until April 1. The number of workers borne per week fluctuated considerably with time and without a clear trend, the mean was 31.8 ± 19.6 ($n = 20$) for the period after translocation, 36.2 ± 19.6 ($n = 11$) for the period with sleep. The first median was 30, the second 33 workers borne per week. During this measuring period of 91 days 430 workers were borne, 252 of them (58.6%) showed sleep. They located their antennae forward and down and did not perform movements with their wings and legs during the night.

Registered tasks included activities inside and outside the nest; they were: warming brood (task 1), feeding brood (task 2) and foragers (task 3). Most of the workers were engaged in task 1, followed by task 2 and task 3. The average age when workers began to sleep was 11 ± 6.1 days for the full investigative period.

The average life-span of workers with sleep was determined by searching for dead individuals in the nest, chambers 1 and 2 and an area of 200 m² outside the laboratory (found $n = 135$). Other dead bees of the colony were not included in this evaluation, values and percentages given in the text are not for the total colony, but just for numbered individuals. Corresponding to the number on the thoraces of the deceased sleepers, they were divided among their former tasks. Their life-spans did not vary significantly: the longest was observed for workers performing task 3 (55 ± 8.4 days), followed by task 2 (53 ± 8.4 days) and the lowest value for task 1 (52 ± 8.4). The average life-span of all investigated *Bombus atratus* workers was 53.3 days, a value differing considerably from the reported value of 24.3 days for Brazil [14]. It was highest compared with other species such as *Bombus terricola* (13.2 days for Canada) [15], 34.1 days for *Bombus fervidus* from Canada [16] and 41.3 days for the Neotropical species *Bombus morio* from Brazil [17].

The total time of sleep was evaluated in 252 workers and ordered for the tasks they performed: warming brood (240.6 ± 58.2 h), foragers (236.0 ± 55.4 h) and feeding brood (227.3 ± 57.7 h). Workers of tasks 1 and 2 preferred to sleep in the nest (28 °C), while workers of task 3 liked to stay in the first temperature gradient chamber of 22 °C (Tables 1 and 2).

Nearly 50% of all workers were engaged in task 3 (foraging), 30% in task 2 (feeding) and slightly more than

Table 1 Preferences of sleep temperature depending on tasks performed by workers

Task	Sleeping h/%	Temperature/°C
1	52.0	28
	18.4	22
	3.8	19
2	52.1	28
	21.9	22
	3.6	19
3	10.8	28
	51.4	22
	15.8	19

Table 2 Workers by task, life-span and total time of sleep

Workers by task/ $n = 252$			
Task	1	2	3
Number of workers	57	75	120
Percentage	23%	30%	47%
Life-span of workers/days/ $n = 135$			
Task	1	2	3
Number of workers	32	46	57
$x \pm SD$	53 ± 8.4	52 ± 8.4	55 ± 8.4
Total time of sleep per worker/ $n = 252$			
Task	1	2	3
Hours/ $x \pm SD$	240.6 ± 58.2	227.3 ± 57.7	236.0 ± 55.4

20% in task 1 (warming). This is understandable since tasks 1 and 2 are transitions to the final and long lasting state of foraging. With a mean life-span of about 53.3 days and a mean sleep of 234.6 h per worker, it gives just 4.4 h per worker and day (18%), an astonishing low value.

It might not only be interesting to know the life-span of bumblebees in general, but at which age they started their sleep also. Unfortunately, no other experiments on bumblebees were reported. Moreover, we even could not find corresponding data for *Apis mellifera*. Figure 3 shows the age in days when a chosen worker (indicated by its number on the thorax) started to sleep. The average age was 11 ± 7.2 days for the full investigative period. But it presents two well-defined behaviours with respect of age and sleep: The first part of the figure ($n = 1-100$) demonstrates that the age of starting to sleep (y) decreased with an increasing number of workers (x), following the equation: $y = -0.23x + 29.6$, $R^2 = 0.969$. Beyond worker 100 the starting age of sleep remains approximately constant (6.3 ± 1.3 days, $n = 289$). Such behaviour could be easily explained. Considering the eusocial phase in the beginning, the number of workers is low and the requirements of the colony demand more time to perform different tasks; there is seldom or even never time to sleep. As the population

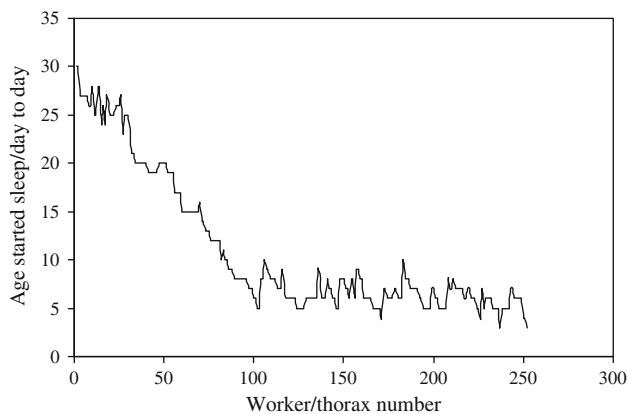


Fig. 3 Age in days when each worker began to sleep/ $n = 252$

grows, more division of tasks is possible and thus the start of sleep.

Figure 4 shows the total hours of sleep (y) versus the age (x) for 135 workers ($y = 4.86x - 1.63$; $R^2 = 0.59$). The additive term -1.63 may be neglected for hours of sleep between 100 and 400. That means that the equation changes to a straight line through the origin with a coefficient of 4.86 and bumblebees slept 4.86 h per day (20% of time) independent of their age. The value is comparable with the 4.4 calculated above. This behaviour differs from recent data of Klein et al. [17], who studied the dependence of diurnal and nocturnal periods of sleep for honeybee castes of *Apis mellifera* and found that young bees (cleaning cells) slept longer.

Figure 5 shows the relationship between total sleep time divided by the number of workers for $n = 100$ to $n = 252$. The highest value is presented in week 2 just after the switch point. It was rainy weather with about 15 °C when more bumblebees remained in the nest. After this week the ratio decreases and does not change so largely (30.0 ± 8.2 total sleep time per worker), although the number of sleeping workers per week and therefore the number of total hours of sleep increases. This could indicate that there may be a mechanism controlled by bees in a way about the total number of workers who sleep and the amount of time they do it. It would be interesting to conduct studies that will clarify whether this activity at rest is also regulated by some mechanism.

Figure 6 shows for 3 workers (124 task 1, 135 task 2 and 110 task 3) the thermal preferences depending on the task they perform. It exhibits a strong relationship between temperature and task at hand. The workers performing task 1 and 2 slept most time during life inside the nest (28 °C); forager worker of task 3 (foragers) preferred to sleep inside compartment 1 of the temperature gradient chamber (22 °C). Only a few workers of all tasks were found in compartment 2 (19 °C).

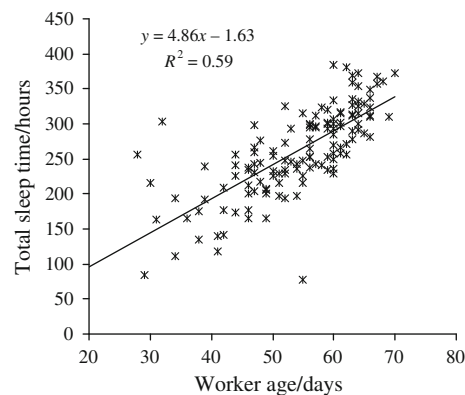


Fig. 4 Total time of sleep in hours versus their age

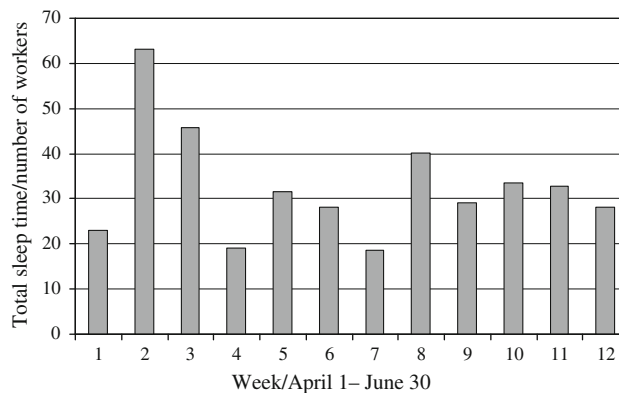


Fig. 5 Total time of sleep/number of workers for the measuring period

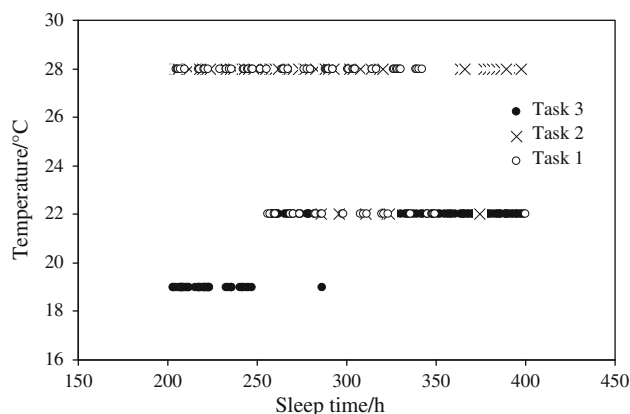


Fig. 6 Thermal preferences of *B. atratus* workers during periods of sleep

Results of Schmolz et al. [12] on the thermal preferences of foragers of *Apis mellifera* showed that forager bees prefer to sleep at 28 °C in a temperature gradient from 20 to 35 °C. It is a compromise between a reduction of metabolism down to 25% and sufficiently high rates of recreation processes. Bumblebees engaged in tasks 1 and 2 behave comparable to honeybees, while foragers prefer

lower temperatures for sleep. It would be interesting to investigate this behaviour for a larger number of bumblebees and more intensively from an energetic viewpoint.

Conclusions

The average age at which workers of a bumblebee colony begin to sleep is 11 ± 6.1 days for the whole collective. But the age of an individual worker beginning to sleep shows two well-defined different behaviours: (i) as the number of workers increased from 0 to 100, the age of starting to sleep decreased linearly from about 30 to 6 days ($R^2 = 0.9709$), (ii) when the colony reached a “switch point” around 100, the age of starting to sleep remained approximately constant at 6.3 days. Moreover, workers performing tasks inside the nest as warming the brood (task 1) or feeding it (task 2) preferred to sleep inside the nest at a temperature of 28 °C, while foragers (task 3) outside the nest liked to sleep at a lower temperature (22 °C). This seems to indicate a close relationship between the task temperature and the preferred temperature to sleep. The lowest temperature available (19 °C) was rather few times chosen. For all three tasks the main choice was significant with more than 50%.

Acknowledgements The authors wish to thank Colciencias (Project 112145221228) and the Universidad de Pamplona for the economical support.

References

- Hölldobler B, Wilson EOA. Journey to the Ants. Cambridge Massachusetts: Harvard University; 1994.
- Abrahamovich AH, Díaz NB. Bumble bees of the neotropical region (*Hymenoptera: Apidae*). Biota Colombiana. 2002;3: 199–214.
- Liévano A, Ospina R, Nates-Parra G. Distribución altitudinal del género *bombus* en Colombia (*Hymenoptera: Apidae*). Invertebrados. 1991;4:541–50.
- Michener CD. The bees of the world. Baltimore: Johns Hopkins University; 2000.
- González VH, Mejía A, Rasmussen C. Ecology and nesting behavior of *Bombus atratus* Franklin in Andean highlands (*Hymenoptera: Apidae*). J Hymenoptera Res. 2004;13:234–42.
- Almanza MT. Management of *Bombus pauloensis* bumblebees to pollinate Lulo (*Solanum quitoense* L), a native fruit from Andes of Colombia. Ecology and Development Series No 50. ZEF Bonn. 2007.
- Tobler I, Neuner-Jehle M. 24-h variation of vigilance in the cockroach *Blaberus giganteus*. J Sleep Res. 1992;1:231–9.
- Kaiser W, Steiner-Kaiser J. Neuronal correlates of sleep, wakefulness and arousal in a diurnal insect. Nature. 1983;301:707–9.
- Kaiser W. Busy bees need rest, too. J Comp Physiol A. 1988; 163:565–84.
- Kaiser W. Sleep in insects. World Fed Sleep Res Soc Newsletter Online 8, issue. 2000. http://www.wfsrs.org/newsletters/NewsLetter8_1/inside/images.html. Accessed 1 Apr 2009.
- Sauer S, Kinkelin M, Herrmann E, Kaiser W. The dynamics of sleep-like behaviour in honey bees. J Comp Physiol A. 2003; 189:599–607.
- Schmolz E, Hoffmeister D, Lamprecht I. Calorimetric investigations on metabolic rates and thermoregulation of sleeping honeybees (*Apis mellifera carnica*). Thermochimica Acta. 2002; 382:221–7.
- da Silva-Matos EV, Garófalo CA. Observations on the development of queenless colonies of *Bombus atratus* (*Hymenoptera, Apidae*). J Apicult Res. 1995;34:177–85.
- Rodd FH, Plowright RC, Owen RE. Mortality rates of adult bumble bee workers (*Hymenoptera, Apidae*). Can J Zool. 1980; 58:1718–21.
- Goldblat JW, Fell RD. Adult longevity of workers of the bumble bees *Bombus fervidus* (F.) and *Bombus pennsylvanicus* (De Geer) (*Hymenoptera: Apidae*). Can J Zool. 1987;65:2349–53.
- Garófalo CA. Evolução do comportamento social visualizada através da ecologia de *Bombus morio* (*Hymenoptera, Bombinae*). PhD thesis, Universidade de São Paulo, Ribeirão Preto, Brasil, 1976.
- Klein BA, Olzowy KM, Klein A, Saunders KM, Seeley TD. Caste-dependent sleep of worker honey bees. J Exp Biol. 2008; 211:3028–40.