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The rate of brood cell production in the stingless bee *Melipona bicolor* fluctuates with nest box temperature

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The nesting sites of stingless bees probably offer the possibility of maintaining quite a stable temperature for the developing brood. For laboratory studies, the nests of stingless bees are often placed in observation hives, which are made of wood and have glass lid on top. In this paper, we report on the effect of glass plate removal on brood cell production in *Melipona bicolor* and demonstrate the impact of temperature fluctuations on the brood cell activity of this species. The production of new brood cells was observed in a polygynous colony which was experimentally subject to different regimes of cold and warm episodes. These results were compared with data obtained simultaneously from three other colonies, each in its own incubator box. It showed that the rate of brood cell production during cold episodes was only 50-60% that during the warmer ones. The observations lead to several recommendations for improving conditions under which colonies of stingless bees are kept and studied.

Index terms: Thermoregulation. Brood cell production. Stingless bees. Melipona bicolor.

A taxa de produção de células de cria na abelha indígena *Melipona bicolor* flutua com a temperatura da caixa do ninho. Os locais de nidificação das abelhas sem ferrão provavelmente oferecem possibilidades de manutenção de temperatura estável para a cria em desenvolvimento. Para estudos em laboratório, os ninhos de meliponíneos são freqüentemente colocados em colmeias de observação, as quais são feitas de madeira e têm tampas de vidro. Neste trabalho, tratamos do efeito da remoção da tampa de vidro na produção de células de cria em *Melipona bicolor* e demonstramos o impacto das flutuações de temperatura na atividade de construção de células de cria desta espécie. A produção de novas células de cria foi observada numa colônia poligínica que esteve sujeita experimentalmente a diferentes regimes de episódios de frio e calor. Estes resultados foram comparados com dados obtidos simultaneamente a partir de três outras colônias, cada uma delas na sua caixa incubadora. Foi demonstrado que a taxa de produção de células de cria durante os episódios frios foi 50-60% da observada nos episódios mais quentes. As observações permitem várias recomendações sob a manutenção de colônias de abelhas indígenas em condições de laboratório.

Descritores: Termorregulação. Produção de cria. Abelhas indígenas. Melipona bicolor.

In many social bees, such as bumblebees, honeybees and stingless bees, the architectural aspects of the nest indicate the importance of thermoregulation: the nest may be surrounded by several layers of involucrum or, in its absence, the bees may assemble around the comb to form a protective insulating layer. The bees may also engage more actively in thermoregulation: cooling results from water evaporation and heat can be generated by contractions of the thoracic flight muscles (Heinrich, 1993; Seeley & Heinrich, 1981).

The nests of some species of bees are maintained at a rather constant temperature, while large fluctuations are seen in others.

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Fluctuating temperatures have been noted inside the nests of some of the smaller stingless bees, especially of those species that live in hollows of bamboo or thin branches and thus are subject to changing ambient temperatures for which the bees cannot compensate. Zucchi and Sakagami (1972) demonstrated this and noted that species with more populous colonies, such as *Trigona spinipes* and *Scaptotrigona depilis*, and colonies of larger bees of the genus *Melipona* were able to maintain their brood nest temperature within a few degrees Celsius. Darchen (1973) studied termoregulation in African stingless bees; he showed that minute stingless bees, as *Hypotrigona*, control very efficiently internal nest temperature when externally temperature suffer great fluctuations. Engels, Rozenkranz, and Engels (1995) confirmed this result for *Scaptotrigona postica* and argued that this species' nesting inside the trunks of large trees already provided a rather stable environment. *Melipona* spp. also live inside tree trunks of often considerable diameter. Although nest temperature and its fluctuations have never been measured under natural conditions, such a nesting site probably offers the possibility of maintaining quite a stable temperature for the developing brood. Tree trunk-inhabitating species keep their brood nest temperature at 30.0°C (*S. depilis, M. quadrifasciata*), 32.0°C (*S. postica, M. rufiventris*), and 34-35°C in the large arboreal nests of *T. spinipes,* (Engels et al., 1995; Zucchi & Sakagami, 1972) (data obtained under laboratory conditions).

For laboratory studies, the nests of stingless bees are often placed in observation hives, which are made of wood and have a glass lid on top. This glass lid enables the researcher to inspect the nest without disturbing the bees, but has the disadvantage of being a poor insulator. On relatively cold days, for example, the colony may lose quite a lot of energy through the glass.

Sakagami (1966) developed a method to keep the temperature inside the observation hive rather constant. He made an outer box, that was provided with a heating spiral and a thermostat. This simple incubator was also covered with a glass lid, so that the colony remained visible. This model incubator has been improved and modified since, but has not been changed in its principal features.

Due to the reflection caused by the glass plates, the upper glass panel is generally removed for detailed behavioral observations as well as for filming thus interrupting heat conservation. In this paper, we report on the effect of glass plate removal on brood cell production in *Melipona bicolor* and demonstrate the impact of temperature fluctuations on the activity of this species.

Methods

The first set of data was collected from a polygynous colony of *M. bicolor bicolor* Lepeletier during october and november 1998. The observations concerned brood cell production during 4 periods, which differed in the way cold and warm episodes alternated.

Behavioural observations were made during the first period. To accomplish this, the heating system of the incubating box was switched off during the day and the glass lid removed. This occurred for periods of 6-9 hrs on 9 consecutive days. To improve the observations, part of the colony was illuminated with a Leitz cold light source.

During the subsequent period of 10 days, the heating system of the incubator box was switched off during the night, thus reversing the cycle of warm and cold episodes during the 24-hr. The glass lid of the incubator was covered day and night with a black sheet as well as a layer of styrofoam. The duration of the cold night episodes varied from 7 to 18 hrs.

The third period, the following 6 days, was characterized by the alternation of cold and warm episodes independent of the 24-hr cycle. The duration of both cold and warm episodes varied from 10 to 28 hrs. Again, the colony was permanently darkened.

Finally, during the last period of 11 days, the heating system was permanently on and the colony was kept in the dark.

Temperatures were measured with a max/min thermometer, placed in the incubator box. The number of new brood cells produced was recorded for each warm and cold episode.

These different regimes made it possible to distinguish the effects stemming from the temperature changes, the light-dark cycles related to daytime, and the circadian activity cycles that may occur under the influence of the natural light-dark cycles and which are experienced by the colony or, at least, by its foragers.

The results of this experiment were compared with data obtained simultaneously from three other colonies, each in its own incubator box, to eliminate the possibility that other factors, not included in our analysis, caused the fluctuations observed in the brood production of the polygynous colony. Two colonies, numbered 1 and 3, were left undisturbed, while the brood production in colony 2 was experimentally reduced during the second of the three periods during which brood cell construction was averaged. To do this, the youngest comb, once it had between 10 and 20 cells, was removed. This was performed three times and concerned a total of about 50 cells. Since young combs are small and new cells are added at the periphery of the comb, this procedure limited cell production during the middle phase of the experiment.

Chi-square test was used for statistical comparisons.

Results

During the episodes when the heating system of the incubator was on the maximum temperature in the incubating box of the polygynous colony was 30-32°C. When the heating system was switched off and the glass lid removed, the minimum temperature was, of course, dependent on the ambient temperature, whether it was a cold or warm day or night. On most nights, the minimum was between 18 and 22°C. During daytime, it was generally between

Table 1. The rate of brood cell production of the experimental colony in relation to time of day, illumination during daytime (period 1), and temperature.

Period		summed duration	number of cells	rate of production	p value
1:	cold during day	72.5 hrs	65	0.90	<0.001
	warm at night	143.5 hrs	229	1.60	
2:	cold at night	88 hrs	74	0.84	<0.001
	warm during day	77 hrs	109	1.42	
3:	cold	53 hrs	42	0.79	<0.001
	warm	67 hrs	107	1.60	
4:	continuously warm	262 hrs	357	1.36	

20 and 23°C. On two of the nights that were expected to be "cold," the temperature did not go below 25°C; for this reason, these two nights were discarded from our analysis. Similarly, when the electricity supply to the laboratory was interrupted on two weekends, the data obtained were not incorporated.

A summary of measurements is given in Table 1. The table shows that an average of 0.8-0.9 brood cells was finished per hour during the cold episodes, while this ranged between 1.4 and 1.6 cells per hour during the warm episodes (on average 1.55). In all cases, fewer eggs were produced per hour during a cold episode than during the following warm episode (n = 9 pairs of episodes for period 1, n = 8 for period 2, n = 3 for period 3). During the last period, when the nest was kept continuously warm, the cell production averaged 1.36 cells per hour. Statistically, this is not different from the 1.55 found for the warm phases when temperature fluctuated.

The comparison with the other 3 colonies is presented in Table 2. This table shows that brood cell production in colonies 1 and 3 was rather constant over a period of more than a month, while the values obtained in colony 2 varied like those in the experimental colony with warm and cold episodes.

Discussion

Our results demonstrate, that the temperature in the incubator box has an impact on the rate at which brood cells are produced: the rate of production during the cold episodes reached only 50-60 percent of the rate observed during the warmer ones. The fact that this did not depend on the moment (during the day) the cold episode occurred suggests that a circadian cycle of activity, if present at all, or the presence of light during observations are not responsible for this result.

It is of interest to note that several cells were often ready to be provisioned by the bees at the end of a cold episode, while this number was usually rather small at the end of a warm period. This suggests that the two phases in the process of brood rearing, i.e. cell construction and the subsequent filling of the cell with food, are different in their dependence on temperature. Since the queen initiates the provisioning process (Sakagami & Zucchi, 1963), we are inclined to suggest that the queen is more sensitive to lower temperatures than the workers who construct the cell.

With regard to cell production rate, the experimental colony was able to follow the temperature fluctuations in the incubator box within a period of a day. New cells in *Melipona* species are generally produced at the margins of the combs. In the initial phase of a new comb, new cells are also still added to the previous comb. However, with increasing comb diameter, once the old comb attains the size of the previous one below it, this number declines rapidly. At the same time, the small periphery of a young comb offers only limited space for the addition of new cells. By taking away the young comb, in colony 2, we induced a decrease in cell construction over a

Table 2. Brood cell production in three monogynous colonies compared to the polygynous colony of Table I. See text.

Colony	periods measured	average n. cells/day
1	19/10- 6/11	18.5
	7/11-16/11	16.6
	17/11-25/11	17.3
2	24/10- 5/11	20.0
	18/11-25/11	26.4
3	28/10- 6/11	30.0
	7/11-16/11	29.7
	17/11-27/11	31.5
Experimental colony from Table 1	1	32.7
	2	26.6
	3	29.8
	4	32.5

longer time period (12 days) (Table 2), and were able to see whether there would be compensation later on. This compensation did indeed happen: not only did the cell production rate increase from 20.0 to 26.4 ($p < 0.01$), but the increased production remained at the higher level for the 8 days the colony was observed (period 3). The cell production in the other colonies did not change in a significantly different manner during these time periods, suggesting that the reduced possibility for cell construction during the second time period of colony 2 was indeed related to the increased production during the third time period. There was probably an accumulation of young workers during the period of restricted production, which enabled a response of such duration.

It appears unlikely that such a compensation also occurred in the polygynous colony, where the changes in nest temperature occurred with smaller time intervals: the differences in the rate of production during a cold or a warm episode during periods 1-3 do not reflect the relative time lengths of these episodes. Furthermore, the average rate of production during the warm episodes in periods 1-3 did not differ significantly from the rate of production found for period 4 (Table 1).

These data show that the rate of brood cell construction is a stable character of a colony, that expresses its homeostatic nature rather well. Brief periods of lower temperature appear to have little effect on the long-term rate of brood production. However, when behavioral data related to reproduction are to be collected, the drastic reduction in the rate of reproduction during those brief periods of lower nest temperatures has its consequences.

These observations lead to three recommendations. First, it is often advised that the thermostat of the incubator be kept at about 28°C in studies on stingless bees. Taking into account possible inaccuracies of the measuring and regulation devices, this is 3-5 °C below the temperatures we maintained. Given the unexpectedly large effect of reduced temperatures, we suggest that the temperature in the incubator box be raised.

Second, observations on natural behavior inside the nest should preferably be conducted in an environment where temperature has no negative impact on that behavior. A room, with its temperature adjusted to the preferences of the bees, rather than to those of the observer, is advisable. Finally, we advise that a study on nest temperatures under natural conditions be made. This could lead to a further improvement of the conditions under which colonies of stingless bees are kept and studied.

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