The nest of the Brazilian stingless bee
Melipona quinquefasciata

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The neotropical genus Melipona, found from Mexico to Argentina, contains about 40 species. Most species live in woodland, some in savannas. Nesting sites are found inside cavities within the trunk and branches of trees. M. quinquefasciata is exceptional in that it lives underground, where it occupies abandoned nest cavities dug by other animals. We describe details of the nest and discuss the impact of the secluded nesting site for the physical conditions inside the nest.

Keywords: Melipona quinquefasciata, nest, regulation of physical conditions

The stingless bee genus Melipona is neotropical and comprises about 40 species (Michener 2000), 36 of which are found in Brazil (Silveira et al. 2002). These species typically nest in cavities in the trunk or branches of living trees. Within the nest, a brood area and a storage area can be distinguished. Brood cells, arranged in horizontal combs, are within an involucrum which, in most species, is multilayered. Outside this involucrum are aggregated food pots of extraordinary size. Some contain honey, while others harbour pollen. From within the cavity a long and narrow tube leads to the single one-bee-wide nest entrance on the outside of the tree.

We assume that within a cavity of a tall living tree the temperature is fairly constant, due to the water flow through the xylem, from the roots towards the leaves. The cavity, therefore, is a kind of incubator, where apart from a stabilised temperature the relative humidity will be constant and high. When such a cavity is selected as a nesting site, the bees cover its inner side-wall with a resin-like layer and separate the nest space from additional space in the cavity by heavy batumen plates. Such constructions suggest that the physical conditions inside the nest are well isolated from thermal and humidity fluctuations in the outside world. This interpretation finds additional support in the long and narrow internal entrance tube, which precludes air streams, and only allows a slow gas exchange to occur by way of diffusion.
Within the genus, *Melipona quinquefasciata* Lepeletier 1836 is exceptional in that it nests in the soil, utilising nest cavities made and abandoned later by ants. The species is found on the plateaus of the Brazilian interior characterised by a savannah type of vegetation. The lack of trees of sufficient diameter in its biotope may have contributed to the peculiar evolutionary shift towards a subterranean nesting site. The considerable temperature differences between day and night, and between the hot dry season and the cooler wet season, could also have played a part.

The species has been reported from the Southern and Central states of Brazil, ranging from Rio Grande do Sul to Mato Grosso, including Paraná, São Paulo, Minas Gerais and Goiás. Due to the expansion of agriculture, however, at many places the species has disappeared or has become endangered. Recently, its occurrence was reported in the NE Brazilian states of Pernambuco and Ceará (Lima-Verde & Freitas 2002). Interestingly, the inhabitants of this region probably do know this bee already for centuries, long before its presence was established scientifically. They excavate nests to harvest approximately 1 litre of honey per nest, the pollen and the wax, a practise that kills the colonies discovered. Given the expansion of the human population and the reforms in agricultural activities, these excavations imply that this bee species is threatened in this area as well. Attempts are made to develop a more sustainable exploitation by means of a method for keeping and cultivating it in hive boxes. We report here our observations on the nests we encountered and excavated during a recent field trip. Characteristics of the nest site, nest construction, nest temperature and the temperature gradient in the soil are described.

**MATERIALS AND METHODS**

The field trip was made to the Chapada do Araripe. This plateau constitutes the physical boundary between NW-Pernambuco and S-Ceará and reaches an altitude of 900 m (Lima-Verde & Freitas 2002). We visited the area during the first week of December 2006. In this period of the year the transition from the dry to the wet season takes place. After the first rains excessive blooming occurs, and soon thereafter the nests contain the largest quantity of honey. This is the period of the year local people excavate the nests they had located earlier. We planned our trip just prior to such excavations.

Three nests were excavated in the vicinity of Moreilandia (PE), a locality not far from Santana de Cariri (CE). Rural residents, which had been met and instructed already during a previous visit, showed us the localities of the nests and performed the digging. For each nest dug out, the temperature of the soil was measured at several depths, using a digital thermometer, until the nest cavity was disclosed. From a number of sealed honey pots the moisture content of the honey was determined with a refractometer.
RESULTS

The architecture of the nest

On the horizontal soil surface the presence of a nest was indicated by a small turret made of soil particles, about 2 cm high. In the circular central opening, 8 mm in diameter, a guard bee could be present (Fig. 1a). About 5 cm below the entrance the tunnel widens to a diameter of 1.9 cm, thus allowing the departing and returning bees to pass each other. This part is not necessarily circular; we noted ellipsoid cross sections. Over its entire length the wall of the entrance tunnel was coated with a cerumen rich in resin, which may prevent sand grains to loosen and drop. The tunnels had small curvatures along its course and in one instance even ran horizontally over small distances. Upon reaching the cavity in the soil where the nest is located it continued as a more fortified tunnel, extending into the cavity. It ended at the involucrum of the nest, at 1/2-2/3 from the top of the round-shaped nest.
The nest cavities of the three nests we excavated were located at 30 cm (nest 2), 70 cm (nest 1) and 100 cm (nest 3) deep, respectively. They were of different shapes. One nest cavity was spheroid, the two other cavities ovoid with their longest axis horizontal. In one instance at least one empty cavity of undetermined size, neighboring the nest cavity, was present. The nests proper did not fully occupy the cavities, there was an air space of up to 10 cm above and at the sides of the nest. The nests, composed of the involucrum and two groupings of food pots at the sides, had a diameter of about 30 cm. The involucrum is made of several layers, and is rather brittle at the exterior but the innermost layer is soft and flexible. At the top of the nest the involucrum is much thinner compared to at its side and bottom parts.

The nest contents
The nest encountered at 70 cm depth did not contain bees any more, and only a single, empty brood cell was present. The turret was closed by a roof with a number of rather small openings, apparently constructed by the bees (Fig. 1b). Within the nest two ants were seen, suggesting that the colony had died because of ants preying on the adults and brood. Interestingly, outside the involucrum sealed food pots were present that contained honey as well as pollen.

The other two colonies contained 7 and 8 brood combs, respectively (Fig. 1c), and young bees emerged from three combs in each colony. The combs were irregular in shape, with a longest axis of 11-13 cm. At two opposite sides the involucrum had groups of food pots; in one colony at least 50 pots were present (Fig. 1d). From 30 of them, with honey, the volume and the water content of the honey was measured. The volumes ranged 3-7 ml and the water content varied from 26.2-31.6%. The other 20 pots contained pollen.

Temperature aspects
The temperature of the air, measured at 1 m above the ground during the excavations, was 26.3-30.0°C at 9.30 h, depending on the wind (nest 1); 28.8°C at 11 h (nest 2) and 34.5°C at 12.40 h (nest 3). In the morning hours the relative humidity of the air was around 40%; it dropped to 23% at 15.00 h. We were told night temperatures could be as low as 15°C.

In Figure 2 the temperature gradients in the soil are given. At the location of the empty nest 1 the soil temperatures were 2.5-3.5 degrees centigrade lower than at the other two nests. At the other two localities the soil temperature at 10 cm from the surface was above 35°C, but at 30 cm deep it was found to be 30°C, and at 1 m depth it was only a further 0.7°C lower. The temperature inside the nest, in between the brood combs, was found to be 32.7°C (nest 2) and 31.6°C (nest 3), respectively; i.e. 2.1 and 2.2°C above the temperature of the surrounding soil. The pots of nest 2 had a temperature of 31.1°C, in between the values for the brood and the soil surrounding the nest.
Like most other species of its genus, *Melipona quinquefasciata* builds its nest in a well-insulated place. Unlike the honeybees, which either nest in the open (the *Apis dorsata* and *A. florea* groups of species), or occupy well-accessible cavities that have a wide entrance (e.g. *A. mellifera* and *A. cerana*), this bee can only reach its nest through a long and narrow tunnel. A single tunnel of these dimensions excludes a regulatory system based on air currents, because for air currents to occur separate in- and outgoing flows are needed. There is, however, just one such tunnel leading to the nest. It means that the air humidity and the carbon dioxide and oxygen concentrations within the nest can change only by diffusion. As a consequence of the metabolic activities of the bees the concentrations of these gases change, and along with it the temperature is raised. Any difference with the surrounding soil will be equalised during a slow process of dissipation. We found the nests at 30-100 cm below the surface. These are depths nearer to the surface than is reported in the literature (1.5-3 m, Lima-Verde & Freitas 2002, Nogueira-Neto 1997; and up to 5 m, B.M. Freitas, pers. comm.). Our excavators reported that most nests are at about 1.5 m depth, in rare cases at 4 m. Before starting the excavation, they blow air into the entrance and then listen whether they can hear the bees’ buzzing. If not, the nest is deep, and perhaps not worth the efforts of digging.

In as far as the regulation of temperature within the nest is concerned, we infer that the *Melipona* bees could easily increase the temperature of the brood nest by contractions of their flight muscles, in the same way as honeybees do. Reducing the temperature, however, is quite a problem. Honeybees combine wing movements with the exposure of diluted nectar or water inside the nest to
reduce the nest temperature, as the wide nest opening permits an efficient gas exchange with the exterior of the nest. In the *Melipona* nest, however, wing fluttering will only cause the air to circulate within the nest; any evaporated water will necessarily condense nearby, and therefore, such activity will only raise the temperature inside the nest instead of reduce it. Active reduction of temperature within the nest appears impossible, so the only remedy to increasing temperature seems to be reducing the activity level. We may suppose that this is a limiting factor for the size a colony can reach ultimately.

In this context it is of interest that the temperature within the nest of *M. quinquefasciata* was found to be only two degrees centigrade above the temperature of the sand surrounding the nest. Dissipation of heat towards the lateral sides is obstructed by the presence of several layers of food pots; perhaps such lateral dissipation is promoted by the concentration of these pots at only about half of the perimeter of the nest, leaving parts of the side wall available for exchange. However, the best dissipation may occur at the top of the nest, where the involucrum is rather thin, above which there is an air space. The remaining air space in the nest cavity might be of importance. The greater this space, the larger the inner wall of the cavity that conducts excess heat.

Stability in the physical aspects of the nest is favourable for the development of the brood. The optimal temperature for *Melipona* is unknown, but probably does not differ much from that of *Apis mellifera* and *A. cerana*, where the brood-nest is maintained at approximately 35°C (Kraus et al. 1998). At slightly lower temperatures the development time for the brood increases, while at slightly higher temperatures there is no reduction in the time needed to complete development. Just a few centigrades above the optimum the temperature becomes harmful and lethal (Velthuis & Kraus 2002). Because the relative humidity changes with temperature, a stable temperature makes it easier to maintain a preferred level of humidity in the nest.

Not much is known about the temperatures of the brood nests of *Melipona* species. In *M. scutellaris* nests, the combs had an average temperature of 30.7°C, varying from 26.6 to 34.6°C (n=58, Cortopassi, unpubl.). Occasional measurements in nests of the Amazonian species *M. seminigra* and *M. rufiventris* provided values of 31.7°C and 31.3°C, respectively, while in a nest of *M. quadrifasciata* in southeastern Brazil 29.3°C was measured (Cortopassi & Nogueira-Neto, unpubl.). These temperatures are similar or just below our values.

However, not all species of *Melipona* nest in a well-protected cavity with a high degree of temperature stability. For instance, *M. subnitida* and *M. asilvai* often have their nest in the relatively thin branches or stems of shrubs. As species of savannah-type vegetations with occasionally high day-time temperatures and contrastingly low temperatures at night, considerable fluctuations in nest temperature should be expected. This was indeed found in some nests of *M. subnitida* and, interestingly, relative humidity turned out to be more constant.
(Cortopassi-Laurino, unpublished). There is, however, no detailed study on such fluctuations in temperature and humidity within the natural nest, and neither are there data on the impact of temperature differences on the development of immature stingless bees.

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