Scientific note: behavioral dynamics of pollen storage in Melipona quadrifasciata

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Short Report

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Abstract

Stingless bees are important eusocial insects found in tropical and subtropical regions. They store nectar and pollen from flowers to survive periods of scarcity. During storage, nectar and pollen undergo fermentation processes, resulting in honey and fermented pollen. However, the fermentation process of pollen collected by stingless bees is not well understood. Understanding these processes not only clarifies pollen fermentation in different stingless bee species but also sheds light on the transformation process of pollen. This knowledge can be applied to produce fermented feeds using specific microorganisms. Therefore, this study aimed to observe pollen storage behavior within colonies to understand the fermentation process and aid in the development of fermented feeds for bees. A Melipona quadrifasciata colony from the Experimental Meliponary of the Universidade Federal de São Paulo, Brazil, was observed for 30 days. Daily observations were conducted in the morning and night, and pollen pots were marked with random colors to differentiate them and collect data on worker bee dynamics. Observations showed no consistent patterns in the opening and closing of the pots, with worker bees frequently entering and exiting the open pollen pots. This suggests an ongoing fermentation process involving gas exchange and nutrient flow. The lack of regularity in handling pollen storage in M. quadrifasciata indicates its noteworthy variability. These findings are crucial for advancing meliponiculture techniques and developing fermented protein supplements globally and locally.

Full Text

Stingless bees (Apidae, Meliponini) make up an important group of eusocial insects occurring in tropical and subtropical areas of the planet (Costa and Venturieri 2009). To survive periods of scarcity, these bees store nectar and pollen collected from floral resources inside cerumen pots inside the colony (Roubik 1982). Both nectar and pollen undergo fermentation processes during storage, turning into honey and fermented pollen, respectively (Nogueira-Neto 1997).

Although stingless bees share many similarities with *Apis mellifera*, this diverse group (Roubik 1992) still conceals many particularities that have not yet been explored (Menezes et al. 2013). The characteristics of the pollen, such as flavor, odor, color, and texture, change considerably after being stored and vary among bee species (Camargo et al. 1992; Souza et al. 2004). A few bee species, such as *Tetragonisca angustula* and *Frieseomelitta varia*, produce dry and relatively sweet fermented pollen. However, other meliponines, such as bees from the genus *Melipona* and *Scaptotrigona*, produce and store moist and sour pot-pollen (Menezes et al. 2013). It is clear that the dynamics of pollen storage are still unclear, and knowing this dynamic is essential for the investigation of the fermentation process, clarifying questions about fermentation time, moment of inoculation of microorganisms, and consumption of fermented pollen by bees. This study aimed to investigate pollen storage behavior within the colonies of the bee species *M. quadrifasciata* to obtain information that can aid in understanding its fermentation process.

Observations were carried out for 30 days between November and December 2021. An observation colony of *M. quadrifasciata* from the Experimental Meliponary of the Federal University of São Paulo was used on the Diadema campus (23° 43' 10"). S 46° 37' 39" W).

The colony used for observation had a glass top to facilitate internal visualization and, consequently, study data collection. Before the beginning of the observations, the pollen pots in the colony were removed, leaving only one pot to supply the bees' needs with protein and mainly to stimulate the search for external resources by the forager bees to build new pollen pots. During the 30 days of the experiment, observations were made in the morning and at night. In the morning, the pots were observed between 7 am and 10 am (UTC -3), and in the night, between 7 pm and 9 pm (UTC -3). The active pollen pots that had pollen entry and/or exit observed were marked daily with a non-toxic, water-based pen (Posca®), facilitating the observations of the pots and the work dynamics performed by the worker bees (Fig. 1). Thus, each time a new pot was observed with pollen inside (Start), it was randomly marked with a new combination of colors. To differentiate active pollen pots (marked with random colors) from honey pots and empty pots (not considered in this experiment), a yellow marking was used (in these particular pots). In addition to the observations regarding pollen storage dynamics, the pollen emptying process (Finish) was also monitored. For this, each time a pollen pot was emptied, the observation date of what happened was noted (Table 1).

According to the results of Graph 1, we see that the pollen pots analyzed during the experiment did not remain closed for long periods. In addition, it was noted in the active pots that they were most of the time open during the morning period. Another important fact to be reported is that after thirty days, the presence of 14 active pots was observed, and at the beginning of the experiment, only one active pot had been left. This fact may have occurred to guarantee the constant supply of essential nutrients provided only by pollen. Stingless bees, like honeybees, adjust their foraging activities (food search) to maximize food intake when floral resources are unavailable (Biesmeijer and de Vries 2001; Maia-Silva et al. 2015). These adjustments, such as the search for more resources such as pollen, are a reaction to changes in external environmental factors and the colony's needs (Eltz et al. 2001; Hofstede and Sommeijer 2006). Since the observations were carried out during spring in Brazil, the increased input of floral resources, mainly pollen (the study's objective), justifies the appropriate period for the study.

According to the results, there was no standardization regarding the dynamics of opening and closing the pollen pots of the observed colony (Fig. 2), a fact that happens with *A. mellifera* (Detry et al. 2020). Honeybees progressively fill the wax alveoli with pollen grains brought by foraging bees, practically in their entirety of the storage space. After filling the alveoli, they receive a layer of honey for a certain period and then are used for consumption by worker bees (Seeley 1982). From the observations of this study, it was evident that there is no regularity between having pots open until filled with pollen brought in from the field by foragers and closing after the pot is at or near full capacity. In addition, as shown in Fig. 2, when we compare two different pots in the same period, we notice that the opening times and the frequency of the work dynamics in the pots differ from one to the other. Our data go opposite to those reported by Nogueira-Neto and Menezes (Nogueira-Neto 1997; Menezes et al. 2013), who indicate that stingless bees close the pots when complete, storing them for approximately two weeks before consumption.

As stingless bees are a diverse group, understanding how worker bees work to store pollen inside cerumen pots is highly relevant information for understanding the storage time and transformation of food through fermentation processes and later consumption by individuals of the colony. Few studies report the fermentation dynamics of pollen stored by stingless bees (Menezes et al. 2013). As shown in Fig. 2, the behavior of the workers who supply the pollen pots does not maintain regularity in how they perform the task, as studies show that it occurs in *A. mellifera* (vanEngelsdorp et al. 2009). In the case of this behavior, for *M. quadrifasciata*, it was observed that several pots are opened and closed throughout the day or night, regardless of the amount of pollen inside. It can be verified through Graph 1; Table 1, that 18 pots were observed throughout the experiment. Of these, some could be observed over several days, such as pot number two (36 observations - remaining open for 58.4% of the time and 41.6% of the time closed); four (38 observations - remaining open for 89.4% of the time and 10.6% closed) and five (34 observations - remaining for 64.7% of the open time and 35.3% of the closed time).

Thus, an interesting and recurrent data in the observations is the way the foraging bees bring pollen and how it is treated, as it is known that during this conditioning, workers add secretions that are assumed to contain beneficial microorganisms (Teixeira et al. 2003). These microorganisms may be involved in the stored food's metabolic conversion, fermentation, and preservation. The transformation of pollen to bee bread has often been postulated to result from microbial action, mainly lactic acid fermentation caused by bacteria and yeasts (Haydak 1958).

New observational studies need to be carried out about the pollen storage of stingless bees, but the dynamics of opening and closing the pollen pots may indicate a continuity of the fermentation process where gas exchanges may be occurring and even maintenance of nutrient flow by adding more content between closing and opening pots, as noted.

Thus, information on how the workers of stingless bee species work, store and ferment their food is of great value within the scenario of understanding the importance of the microbiota associated with this process to produce alternative sources of food during periods of scarcity.

Our observations on the behavior of storage and handling of pollen in the species *M. quadrifasciata* led us to conclude that there is a lack of regularity in treating this food, contrary to the behavior of *A. mellifera*. The pollen pots are opened and closed daily, and bee entry and exit behavior are frequent. Studies on the behavioral dynamics of stingless bees in food pots are scarce in the literature. Therefore, this work highlights the importance of data that explain how food storage occurs within colonies. Such data are paramount for advancing techniques that improve meliponiculture nationally and internationally, such as developing fermented protein supplementation. Understanding the storage time and natural occurrence within the colonies is essential for the fermentation of an artificial diet that mimics the natural characteristics of this important food source.

Declarations

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Table

Table 1 is available in the Supplementary Files section.

Graph

Graph 1 is available in the Supplementary Files section.

Figures

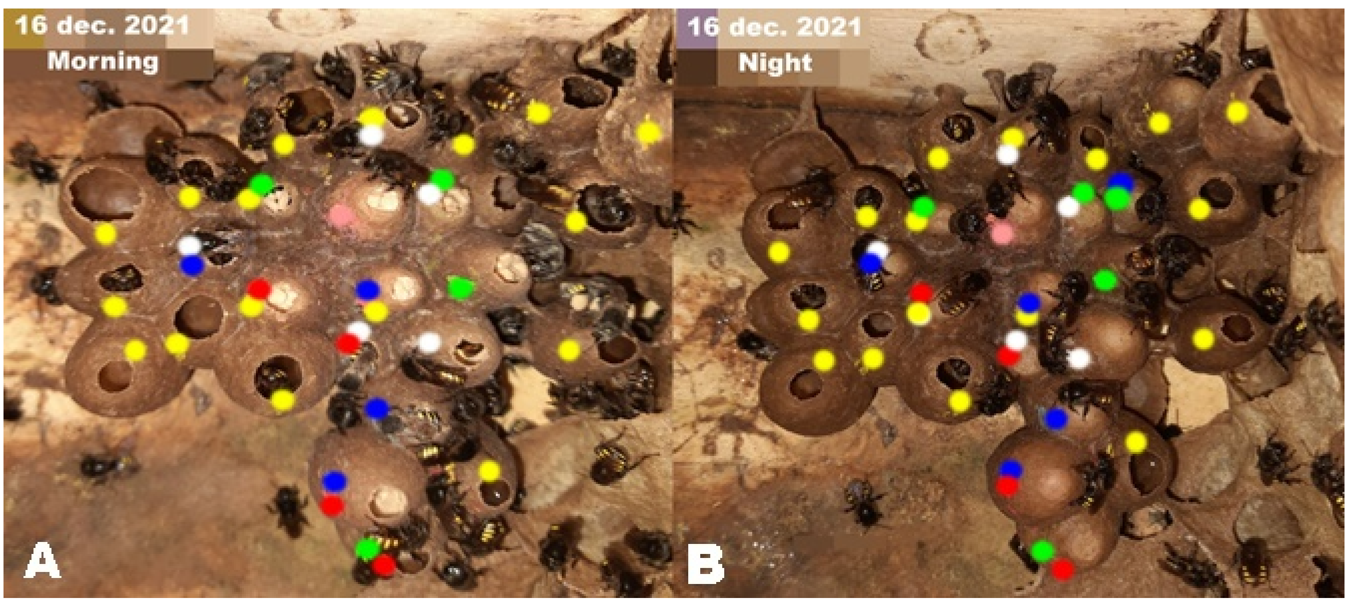

Figure 1

Marking of the food pots with a non-toxic Posca® pen using random colors for the active pollen pots and for the honey pots and empty pots, marking in yellow was used. (A) visualization of pollen pots in the morning (B) visualization of pollen pots in the night

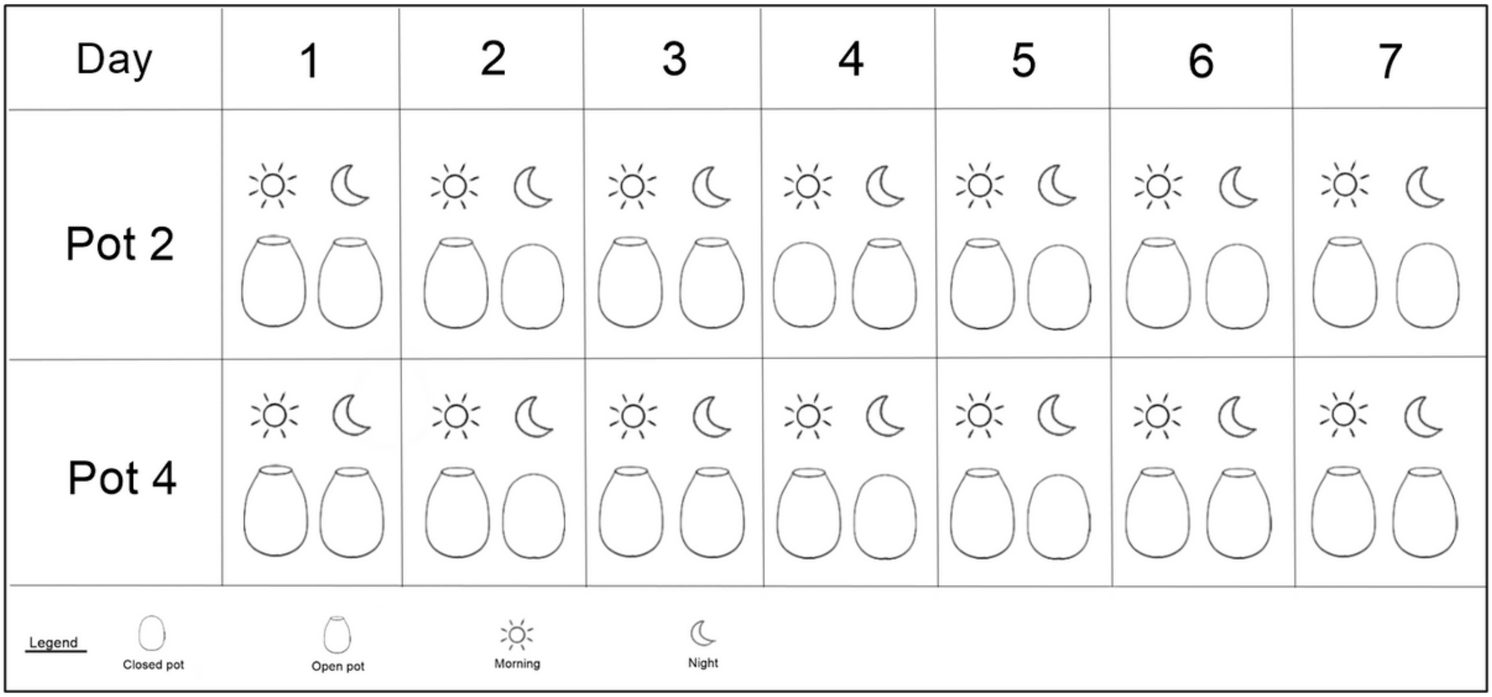

Figure 2

Representation of the opening and closing dynamics of pollen pots two and four, respectively, observed during the same week in the morning and night periods

Supplementary Files

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- Table1.docx
- floatimage3.png