NON-OVIPOSITING NURSE WORKERS WITH DEVELOPED OVARIES IN *Trigona cilipes cilipes* (HYMENOPTERA, MELIPONINI)

Eunice Vieira da Silva-Matos[1], Fernando Barbosa Noll[2], Sidnei Mateus[1] and Ronaldo Zucchi[1]

[1]Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP, Brazil [2]Department of Zoology and Botany, Institute of Biosciences, Letters and Exact Sciences, São Paulo State University (UNESP), São José do Rio Preto, SP, Brazil.

ABSTRACT

Numerous species of tropical stingless bees (Hymenoptera, Meliponini) have workers that lay eggs even in the presence of a queen, i.e., queenright colonies. These worker eggs, which are laid during cell provisioning and oviposition process (POP), are eaten mainly by the queen. In this work, we studied POP in a colony of *Trigona cilipes* in the queenright and queenless stages. POP behavior was observed on 67 occasions. In addition, the ovaries of different aged workers in both stages were analyzed. Although workers in both stages of the colony possessed developed ovaries, they never oviposited, despite showing behavior similar to that seen in species with workers which lay eggs. This form of sterility is outstanding among social insects and is comparable only to other meliponines that have fully sterile workers.

Key words: Meliponini, nurse workers, social regulation, *Trigona cilipes*

INTRODUCTION

In stingless bees, the cell provisioning and oviposition process (POP) shows impressive diversity [10] that may be used to understand the evolution of social organization, the reproductive monopoly of queens and other aspects. Features such as explicit (sometimes agonistic) or ritualized caste interactions, cyclic patterns of cell construction, the single or one-time use of brood cells, and the mass provisioning of brood cells ensure that POP is compact, cohesive, sequential and rhythmic [21]. These highly interrelated events mean that POP progresses according to periods (interoviposition and oviposition), stages (cell preprovisioning, provisioning, postprovisioning, ovipositioning by the queen and cell sealing), and substages (rotation and sidework) [21]. The main stages proceed under conspicuous inter-caste excitement (or agitation, according to Sakagami and Zucchi [17]) that includes local (cellular) and general agitation (reviewed in [10]).

The workers of some stingless bees show ovarian development in queenright colonies and, depending upon the taxon, they may produce either trophic or reproductive (viable) eggs, or both types simultaneously. However, neither the development of worker ovaries nor the production of both types of eggs is evenly distributed. In this work, we investigated the bionomics of *Trigona cilipes*, a stingless bee with workers that develop ovaries but never lay eggs.

MATERIAL AND METHODS

The bees used in this study were from a mature colony of *Trigona cilipes cilipes* (Fab. 1804) collected (April 3, 1996) in Nova Xavantina (S 14° 41", W 52° 21"), Mato Grosso State, Brazil. Although *T. cilipes* colonies are usually found cohabiting with ants and termites [8], the colony studied here was a rare instance of an association with paper wasps [12] since the colony was found inside a recently abandoned wasp nest hanging 5 m high on the outer branches of a mango tree. Although the wasps were absent, the nest structure clearly corresponded to the swarm-founding wasp *Brachygastra lecheguana* (Hymenoptera; Polistinae). The fairly well preserved nest architecture indicated that the bees and wasps in this case had apparently coexisted for some time. The host nest containing the bee colony was transported to the laboratory at the University of São Paulo in Ribeirão Preto, São Paulo State (ca. 900 km southeast of the collection site). The entire bee colony was transferred to a double-boxed, temperature-controlled (28°C) observation hive [17], with a plastic tube that allowed the bees to leave the hive. Sugar

Correspondence to: Dr. Fernando Barbosa Noll
Departamento de Zoologia e Botânica, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista (UNESP), Rua Cristóvão Colombo, 2265, CEP 15054-000, São José do Rio Preto, SP, Brazil. Tel: (55) (17) 3221-2373, Fax: (55) (17) 3221-2374. E-mail: noll@ibilce.unesp.br

cane syrup and honey bee pollen moistened with syrup were supplied *ad libitum*.

Behavioral observations

The POP and related aspects were monitored starting immediately after the workers became involved in regular foraging activities (June 5, 1996). Data were collected by the direct observation of behavior and the use of a video-recorder. Cold white light was used to illuminate the inside of the colony. Behavioral observations took place from April 10 to October 10, 1996. The colony was observed regularly for 75 days. Video-recorded behavioral observations in the queenright colony were done from April 10 to May 30. Thirty-two POPs were recorded and 79 cells were monitored. Since there were no significant changes after this period (May 30), the colony was observed directly in order to obtain qualitative data. Direct observations continued until July 31, when the queen was removed. During orphanhood, the colony was video-recorded for 37 days, from August 1 to October 10, at which point the entire colony was fixed in ethanol. Thirty-five POPs were observed, mainly involving attempts by workers to lay eggs.

Ovarian development in workers

To assess the development of ovaries in nurse bees involved in POP, 51 workers were collected during the preprovisioning stage in the queenright colony, and 63 workers were collected during agitation in front of structurally complete cells in the orphan colony (Table 1). The workers were collected and fixed in Dietrich's solution for 24 h and then stored in 70% ethanol. Because nurse workers were never seen laying eggs (see Results), 30 workers were collected when they mounted the cell and simulated ovipositioning. Dissections were done using a stereomicroscope and the ovaries were classified as: A - devoid of oocytes, B - incipient oocytes, C - medium-sized (or resorption) oocytes and D - fully grown oocytes (Fig. 1). The largest oocyte from each worker examined was used to assess whether there was any difference between the queenright and orphan stages (based on Student´s *t*-test done using Statistica software).

Table 1. Frequency of oviposition mimicking by six *T. cilipes* workers in a cell before ovipositioning by the queen.

Worker number	Frequency of mimicking
1	10
2	5
3	0
4	1
5	2
6	1

The relative age of the bees was determined using four color patterns related to the progressive pigmentation of the transverse apodeme across the hidden base of each sternum; these colors ranged from pale-yellow (younger bees) to dark-brown (older bees).

RESULTS

General aspects of POP in queenright and queenless states

A comprehensive analysis of *T. cilipes* POP will be given elsewhere, but a summary of important aspects is provided here. The number of oviposited cells per POP ranged from one to nine. The sequence of POP was facultatively batched, with cell construction and cell provisioning being successive, i.e., each cell was treated as a unit. Preprovisioning and provisioning were peculiar because the queen stood at a considerable distance from the cell being attended by the nurse workers. During post-provisioning, one of the attendant workers mounted the provisioned cell and mimicked an oviposition act, although no egg was ever laid; during this act, the queen rushed to the same cell to lay her egg. Thirty-two POP events were observed and recorded on video in the queenright state. Because each POP event involved 1-9 cells, the behavioral details were studied in a total of 79 oviposited cells. The data consistently showed that despite frequent simulations of ovipositioning by workers, not a single egg was laid, even though all of the workers examined (n=30) had fully developed oocytes. However, this worker behavior always immediately attracted the queen to the provisioned cell.

During provisioning and post-provisioning, the workers showed conspicuous agitation and strongly disputed cell attendance, especially at the end of the post-provisioning phase. The number of workers mimicking oviposition in the same cell varied from 1 to 6 (Table 1). However, the workers immediately left the area as soon as the queen reached the cell. Before ovipositioning, the queen regularly inspected the cell and occasionally ingested some brood-food. The number of cells under construction and the number of collared cells (8-27 and 2-9, respectively) indicated that the queenright colony was functioning normally since these values were within the range for colonies in the wild.

Following removal of the queen from the hive, the pattern of cell construction was initially the same as in the queenright state and remained unchanged until

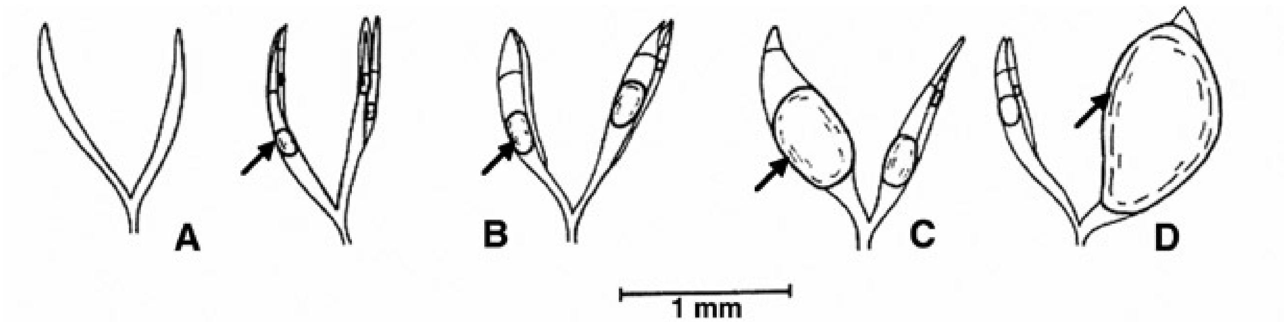

Figure 1. Ovarian development in *T. cilipes* workers. **A -** Absence of oocytes, **B** - Some oocytes at an initial stage of development (incipient oocytes), **C** - Medium-sized (or resorption) oocytes and **D** - Bulky, fully developed oocyte. **Arrows** indicate the different stages of oocyte development seen in this study.

the eighth day of orphanage, when cell construction ceased. Beyond this point, some cells were sealed (n = 14) but contained only liquid and not the food mixture seen in the queenright colony. Cell sealing in the queenless period was abnormal and was done without rotation, with the workers often opening the sealed cells and ingesting their contents. In contrast, some unsealed provisioned cells remained untouched for up to three days, although several of these cells (n = 4) were destroyed during this period.

In the queenless state, cell provisioning often involved irregular behavioral sequences in which some cells were provisioned with less food (pure honey, as mentioned before) than usual. These and other behavioral disruptions associated with POP and the occurrence of abnormal traits characterize queenlessness in stingless bees, as reviewed elsewhere [10]. Of the 38 cells constructed (8 on the day of queen removal and 30 in the entire orphan period), 20 were destroyed before provisioning. Entire or partial destruction of the cells (provisioned or not) involved the deposition of excess wax and an abnormal increase in the cell collar (n = 4) that included the addition of thread-like waxy connections (probably prospective pillars). Except for the 35th to 40th day when no more cells were constructed, these uncommon traits appeared throughout the orphan period, with the observations ending on the 71st day after being orphaned. The appearance of a new cycle of cell construction, provisioning, sealing and destruction close to the 71st day suggested that the entire cycle could have been repeated if new cohorts of younger workers had been present to support related activities.

The remaining nest population was fixed on the 78th day of the orphan period, when there were no

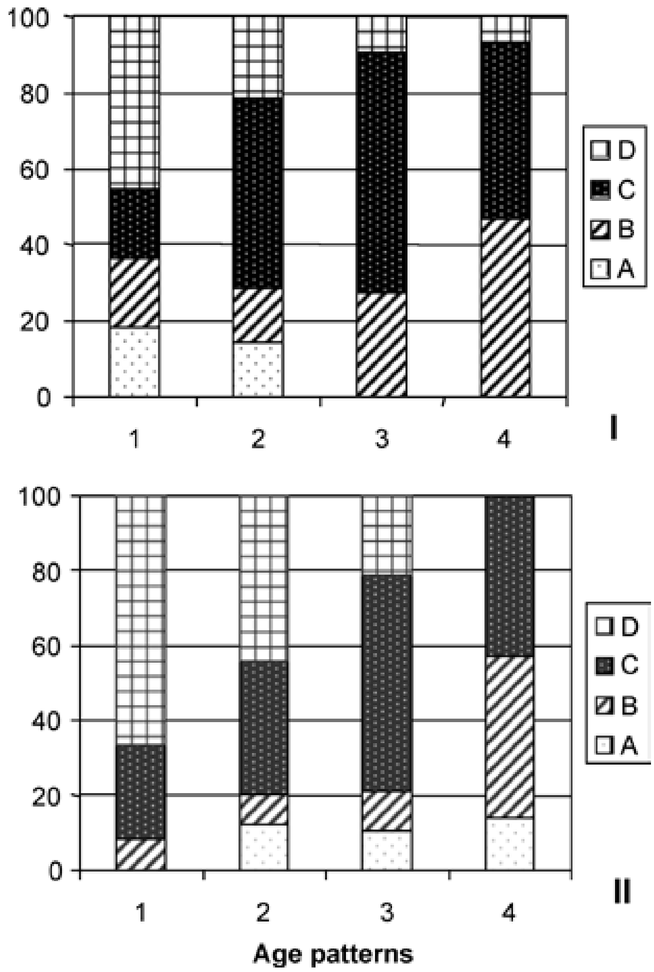

Figure 2. Age-dependent ovarian development in *T. cilipes* workers. **I -** Queenright colony and **II -** Queenless colony. The predominant body colors (light-yellow, dark-yellow, light-brown and dark-brown) associated with increasing age are identified as 1, 2, 3 and 4, respectively. Ovarian patterns are as illustrated in Figure 1, where: **A** - Absence of oocytes, **B** - Some oocytes at an initial stage of development (or resorption), **C** - Medium-sized (or resorption) oocytes and **D**- Bulky, fully developed oocyte.

cells present in the nest. At this point, the workers (n = 342) had a body color ranging from dark-brown to completely black, indicating their advanced age. Despite the continuous presence of workers with fully developed ovaries (Fig. 2II), none of these workers ever layed eggs during the orphan period, as confirmed by direct observation, video recording of the events within the cells, or by indirect analysis of the contents of the sealed cells.

Ovarian development of nurse workers in queenright and queenless states

Although no ovipositioning by workers was ever seen in the queenright and queenless states, nurse-workers of *T. cilipes* always showed age-related ovarian development identical to that of species with workers which regularly lay trophic eggs. As in other stingless bees that produce trophic eggs [1,5,13], *T. cilipes* workers with developed ovaries were younger individuals. In addition, fully grown (mature) *T. cilipes* oocytes were larger ($t = 3.24$, $p<0.05$) in the queenright state (0.86 ± 0.32; $n = 27$) than in the queenless state (0.65 ± 0.11; $n = 27$). Figure 2 shows the age-related classification of cohorts of workers (Table 2), with all four ovarian patterns being found in the queenright (Fig. 2I) and queenless (Fig. 2II) states.

Table 2. Sampling dates and sample size of *T. cilipes* workers collected in different conditions.

	Queenright colony	Queenless colony
June 11	24	--
July 14	28	--
August 8	--	13
August 19	--	21
October 17	--	30
Total	52	64

DISCUSSION

As shown here, *T. cilipes* workers never lay eggs, despite their well-developed ovaries. However, the workers´ behavior in mimicking oviposition may stimulate the queen to move to the cell and lay an egg, although the mechanism by which the queen perceives this signal remains unclear. Since visual cues are of little use inside the dark hive, one alternative may be the odors released during the worker's attempt to oviposit. Since the bees that mimicked ovipositioning often had fully developed ovaries, it appears reasonable to suggest that odors originating from the worker's reproductive apparatus are involved in the queen´s response. This could explain why worker ovaries continue to develop even though the eggs remain in the ovaries until resorption. Another possibility is that specific substrate vibrations related to the mimicking of oviposition may alert the queen that there is a cell ready to receive her egg. The case of *T. cilipes* highlights patterns that can result in full monopoly of the reproductive process by the queen. The presence of different categories of workers with functional ovaries, especially those involved in producing reproductive eggs, suggests that there may be various levels of reproductive conflict [20]. Fully sterile workers occur in queenright colonies of several *Frieseomelitta* (reviewed in [21]), including *Duckeola ghilianii* [16], *Tetragonula laeviceps* [14] and *T. minangkabau* [19]. In these species, worker oocytes never develop.

Comparison of the main characteristics of unlaid worker eggs with the classification of egg types described above showed that the fully developed eggs of *T. cilipes* workers were exclusively of the trophic type, i.e., type C (large bulky eggs). The frequent resorption of eggs indicated that ovarian development proceeded as in other congeneric taxa, with oocyte resorption occurring in the absence of oviposition. In the Meliponini, worker eggs may differ from queen eggs in their (a) size, with worker eggs usually being smaller than queen eggs (*Melipona* spp.) despite their chorionic and micropylar peculiarities (*M. rufiventris paraensis*; [9,18]), (b) color (milky in *Paratrigona* and *Tetragonisca*, and yellowish in *Nannotrigona*), with this type of worker egg being suggestive of a derived state and an exclusively nutritional role, either because the egg nucleus degenerates before laying [1] or because the egg laid is immature [6], and (c) shape, with worker eggs being bulky and exclusively trophic or viable and shaped like queen eggs (*Scaptotrigona* spp. [2,3,15]). In this case, although the queen eats both types of eggs if they are laid close to the cell's rim, the smaller eggs are potentially male-producing because of their prevalent seasonal distribution [2]. During male production, viable eggs produced by workers are laid at different times and positions during cell provisioning: they are laid on top of the brood food during cell sealing and not at the cell rim during the post-provisioning stage [2,3]. Curiously, such caste divergence is rare among eusocial Hymenoptera. Indeed, there are only a few other examples of full worker sterility, e.g., the swarm-founding Epiponini wasps [11] and some ants (reviewed in [4,7]). The development of worker ovaries under queenright conditions apparently provides the colonies with additional bionomic plasticity [10]. The presence of functional, fully sterile workers [10] in *T. cilipes* represents one of the most derived systems detected among eusocial insects to date.

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