



RESEARCH ARTICLE - BEES

Ovary Activation in Virgin Queens of *Plebeia lucii* Moure (Hymenoptera: Meliponini)

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Abstract

Stingless bees (Meliponini) are commonly used in meliponiculture and pollination. In colonies of *Plebeia lucii* Moure (Hymenoptera: Meliponini), a single queen undergoes changes in hormone profiles, as well as physiological, behavioral, transcriptional, and morphological modifications, particularly after copulation. Among these changes, some bee species exhibit ovary activation in virgin queens before mating. However, few studies have described the reproductive system of virgin queens of Meliponini. The colony's dynamics depend on the queen, making an understanding of its reproductive biology essential for effective management of this species. This study aimed to determine whether ovary activation occurs before mating in virgin queens of *P. lucii*. Three virgin queens of *P. lucii* were collected from royal chambers of different colonies, and their ovaries were analyzed using light microscopy. Each virgin queen possessed two ovaries, each composed of four meroistic polytrophic ovarioles containing a terminal filament, a germarium, and a vitellarium. Oocytes in early developmental stages, spherical in shape and with well-developed nurse cells, were observed in these ovarioles, along with nurse and oocytic chambers in the vitellarium. Additionally, vitellogenesis was observed in the vitellarium, characterized by the formation of elliptical oocytes, yolk accumulation, and the degeneration of adjacent nurse cells. These findings indicate that ovary activation occurs before mating in virgin queens of *P. lucii*.

Introduction

Social bees are characterized by a structured reproductive division of labor, cooperative brood care among colony members, and generational overlap (Danforth et al., 2019). Among them, Bombini, Halictinae, and Xylocopinae include species considered primitively eusocial bees, displaying simpler social organization. At the same time, Apini and Meliponini are classified as highly eusocial bees, exhibiting complex social organization with permanent colonies and well-defined castes (Michener, 2007). Meliponini, commonly referred to as the stingless bee group, comprises

corbiculate bees characterized by the absence of a functional sting and reduced wing venation (Grüter, 2020). Stingless bees have a wide distribution across tropical and subtropical regions, with their highest abundance in the Neotropical region (Bueno et al., 2023b). The high abundance and species richness of these bees are associated with their active participation in pollinating native vegetation, making them the primary group of pollinators in tropical regions (Michener, 2007; Marques et al., 2020). This group is managed for the production of beekeeping products and the pollination of commercial crops (Slaa et al., 2006; Bueno et al., 2023b; Macêdo et al., 2023). Products of stingless bees,



such as honey, cerumen, pollen, and propolis, have been managed and used by different populations, including for food and traditional folk medicine (Barbiéri & Franco, 2020; Engel et al., 2023; Rossa et al., 2025). The genus *Plebeia* Schwarz, comprising approximately 40 species, is the second-largest genus of stingless bees and one of the main groups managed in Brazil (Melo & Costa, 2009; Santos et al., 2021). Species of this genus range from two to seven millimeters in body length, have a shiny integument with yellow or white maculation, and are found in tropical and subtropical regions of the Americas, ranging from Argentina to Mexico (Michener, 2007; Engel et al., 2023). Among them is *Plebeia lucii* Moure (Hymenoptera: Meliponini), recorded in eastern Brazil, with workers measuring approximately 3 mm in body length, building small nests with a short and narrow entrance tube and brood clusters without an involucre (Moure, 2004; Gonçalves & Brandão, 2008; Marques et al., 2020; Grüter, 2020).

A single physogastric queen has been reported in most Meliponini species (Toledo-Hernández et al., 2022; Bueno et al., 2023b). The emergence rate of queens in Meliponini colonies is significantly lower than that of workers and drones (Bueno et al., 2023a), and newly emerged queens may be eliminated or confined in royal chambers until a new physogastric queen is required (Imperatriz-Fonseca & Zucchi, 1995), as frequently observed in colonies of *P. lucii*. Nervous stimuli and chemical signals in the reproductive tract, triggered by nutritional, endocrine, and social factors or by mating, initiate ovary maturation in queen bees and regulate various pre- and post-copulation reproductive strategies (Souza et al., 2007; Kapheim, 2017). While the ovary activation in virgin and fertilized queens of *Apis mellifera* Linnaeus (Hymenoptera: Apini) has been well described (Gutzeit et al., 1993; Patrício & Cruz-Landim, 2002; Berger & Cruz-Landim, 2012), in Meliponini queens it remains insufficiently studied (Cruz-Landim, 2000; Melo et al., 2001; Ronnau et al., 2016). The only study on *Plebeia* species describes variations in the number of ovarioles in queens of *Plebeia remota* Holmberg, and *Plebeia* sp. (Hymenoptera: Meliponini) (Cruz-Landim et al., 1997).

Changes in the hormonal profiles of Meliponini queens lead to physiological, behavioral, transcriptional, and morphological modifications (Slessor et al., 1990; Kocher et al., 2008; Niño et al., 2013), including ovary activation, which generally occurs after mating (Patrício & Cruz-Landim, 2002; Martins & Serrão, 2004). However, ovary activation before mating has been observed in *A. mellifera* virgin queens (Patrício & Cruz-Landim, 2002), as well as in *Melipona quadrifasciata antidioides* Lepeletier (Hymenoptera: Meliponini), which possess small ovarian follicles with nurse cells (Martins & Serrão, 2004). This early activation accelerates the oviposition process in these and other bee species, potentially providing an evolutionary advantage (van Eeckhoven & Duncan, 2020). Although stingless bee rearing is common among different populations due to their valuable

products and ecological importance (Santos et al., 2016; Barbiéri & Franco, 2020), improving productivity, colony dynamics, and propagation of new Meliponini colonies largely depends on strategies such as virgin queen selection and controlled mating (Menezes et al., 2013; Baptistella et al., 2014). Understanding the reproductive biology of queens is crucial for optimizing meliponiculture practices and ensuring sustainable colony management (Santos et al., 2016). Here, this study aimed to determine whether ovary activation occurs before copulation in virgin queens of *P. lucii*.

Material and Methods

Specimens

Three virgin queens of *P. lucii* were collected from different colonies during the spring of 2018 at the Central Apiary of the Federal University of Viçosa in Viçosa, Minas Gerais, Brazil. All three virgin queens were found alone in royal chambers, structures built by workers for their confinement.

Light microscopy

The virgin queens of *P. lucii* were cryoanesthetized at -5 °C for 10 minutes and their ovaries were dissected in 0.1 M sodium cacodylate buffer at pH 7.6, transferred to Zamboni's fixative solution (Stefanini et al., 1967) for 24 hours, dehydrated in a graded ethanol series (50, 70, 90, and 95%) and embedded in JB-4 historesin. These ovaries were sectioned into 2 µm slices using a glass knife on a rotary microtome (Leica RM2255), stained with hematoxylin and eosin, and analyzed using an Olympus BX53F light microscope with an Olympus MX10 camera and CellSens Imaging software.

Results

The ovaries of virgin queens of *P. lucii* consisted of four elongated ovarioles, each surrounded by a thin peritoneal sheath. They were composed of terminal filaments, a germarium where oocyte differentiation begins (Fig 1A), and a vitellarium containing oocytic and nurse chambers surrounded by follicular cells (Fig 1B). In virgin queens of *P. lucii*, oocytes in the early stages of development are spherical, with acidophilic and reduced cytoplasm (Fig 1B). In contrast, the nurse cells are well developed, forming cytoplasmic bridges (fusomes) and containing nuclei rich in decondensed chromatin (Fig 1C). As the oocytes grow, they move toward the proximal region of the vitellarium, accumulating yolk in the cytoplasm and displaying a prominent germinal vesicle (nucleus; Fig 1D). At this developmental stage, the follicular cells surrounding the oocytes are cuboidal, with enlarged intercellular spaces (Fig 1D), while the nurse cells begin to degenerate.

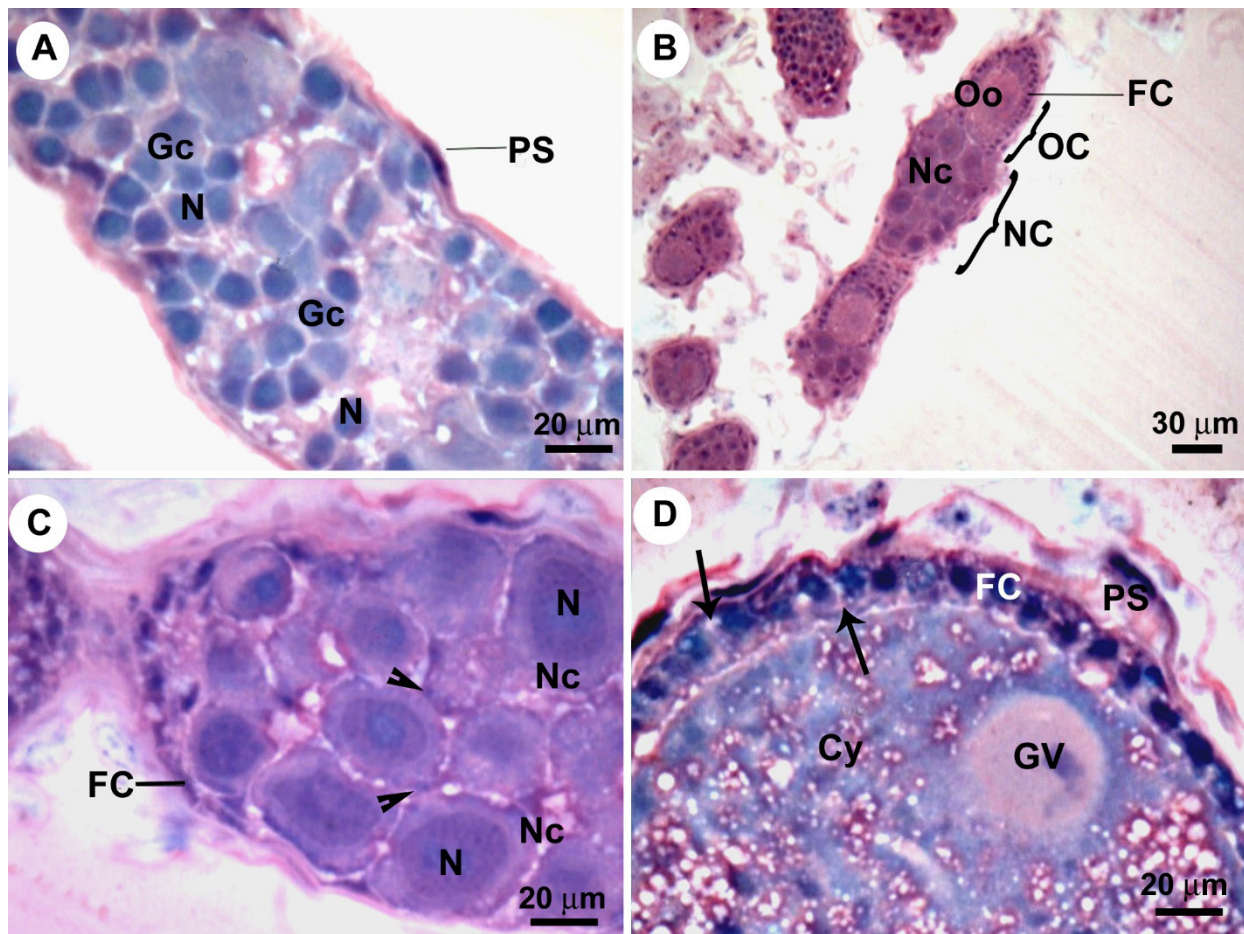


Fig 1. Light micrographs of the ovary of *Plebeia lucii* virgin queens. A) Longitudinal section of the germarium showing a narrow peritoneal sheath (PS) and young germ cells (Gc) with a developed nucleus (N). B) Distal portion of the vitellarium showing a small oocytic chamber (OC) with a small oocyte (Oo) lined by cuboidal follicular cells (FC) and a nurse chamber (NC) with well-developed nurse cells (Nc). C) NC lined by flattened FC filled with a Nc forming cytoplasmic bridges (arrowheads) and with N rich in decondensed chromatin. D) Detail of oocyte with cytoplasm (Cy) showing yolk storage and the well-developed germinal vesicle (GV). Note cuboidal FC with enlarged intercellular space (arrows).

Discussion

In virgin queens of *P. lucii*, the four long ovarioles, formed by terminal filaments and the germarium, are similar to those observed in *Melipona bicolor bicolor* Lepeletier, *Melipona quadrifasciata anthidioides* Lepeletier, *Paratrigona subnuda* Moure, *Plebeia* sp., *Scaptotrigona postica* Latreille, and *Tetragonisca angustula* Latreille (Hymenoptera: Meliponini). However, they differ from those observed in virgin queens of *P. remota*, *Schwarziana quadripunctata* Lepeletier, *Nannotrigona testaceicornis* Lepeletier, and *Trigona spinipes* Fabricius (Hymenoptera: Meliponini), which possess, respectively, four to eight, 10 to 15, and 10 to 12 ovarioles (Cruz-Landim et al., 1997). The evolutionary dynamics of ovariole number in insects are characterized by shifts between states of variable and invariant ovariole numbers among species. Within a single species, the variation in ovariole number has been attributed to differences in the number of precursor cells of the terminal filament (Church et al., 2021).

The germarium with oocyte differentiation is common among Meliponini species, including *P. lucii*, where ovarian development progresses to the stage of differentiated oocytes, but without follicular formation (Cruz-Landim et al., 2006). This differentiation in the meroistic polytrophic ovariole type, as observed in bees, suggests that ovarian activation is regulated by a combination of hormones, genetics, and environmental factors (Tanaka & Hartfelder, 2004; Roy et al., 2018; Aamidor et al., 2022), in addition to mating stimuli.

The vitellarium in virgin queens of *P. lucii*, formed by nurse and oocytic chambers surrounded by follicular cells, is similar to that reported for *M. quadrifasciata anthidioides* (Martins & Serrão, 2004). However, it differs from that of virgin queens of *A. mellifera*, *M. bicolor bicolor*, *M. quadrifasciata anthidioides*, *N. testaceicornis*, *P. subnuda*, *Plebeia* sp., *P. remota*, *S. postica*, *S. quadripunctata*, *T. angustula*, and *T. spinipes*, in which ovarian activation is suspended before oocytes reach the vitellarium (Cruz-Landim et al., 1997; Patrício & Cruz-Landim, 2002; Santos & Cruz-Landim, 2002).

The spherical morphology of oocytes, characterized by acidophil cytoplasm, a prominent nucleolus, reduced cytoplasm, and well-developed nurse cells in the early stages of ovarian development, confirms ovarian activation before copulation (Cruz-Landim et al., 2006) in virgin queens of *P. lucii*. Cytoplasmic bridges (fusomes) formed by nurse cells in virgin queens of *P. lucii* characterize the formation of mature oocytes through the transfer of cytoplasmic material to the oocytes during pre-vitellogenesis (Büning, 1994; Souza et al., 2007; Ronnau et al., 2016). As oocyte development progresses, elliptical oocytes, surrounded by cuboidal follicular cells, in the vitellogenesis phase in virgin queens of *P. lucii* are associated with the transfer of yolk across the follicular cells (Dohanik et al., 2018; Assis et al., 2019). This yolk deposition in the oocyte cytoplasm within the vitellarium is similar to that reported in mated queens of *A. mellifera* and *M. quadrifasciata* (Hymenoptera: Meliponini) (Souza et al., 2007; Ronnau et al., 2016). In addition, the onset of nurse cell degeneration confirms the final maturation phase of the oocyte during vitellogenesis (Aamidor et al., 2022). Finally, this process of advanced vitellogenesis, along with the regression of nurse chambers, was reported in mated queens of *M. quadrifasciata* starting from the 10th day post-copulation (Martins & Serrão, 2004).

Taken together, the ovaries of virgin queens of *P. lucii*, consisting of four long ovarioles formed by terminal filaments, germarium, vitellarium, and mature oocytes, are activated before copulation. This pre-copulation activation of ovaries with mature oocytes in *P. lucii* suggests the potential for accelerating the oviposition process after mating, providing an evolutionary advantage for this bee, which frequently keeps virgin queens in royal chambers. The suspension of ovarian activation at different stages is related to the nuptial flight of virgin queens, optimizing resource use before mating (Cullen et al., 2024). Although no study has observed the nuptial flight distance in *P. lucii* species, virgin queens of *Plebeia droryana* Friese, for example, have an estimated nuptial flight distance of 433 meters from the colony based on their intertegular distance (Bueno et al., 2023b). Since queen bees adjust their flight behavior to cope with the physical demands of flight (Hayworth et al., 2009; Duell et al., 2022), we suggest that *P. lucii* virgin queens may exhibit similar flight adaptations, such as a reduction in nuptial flight distance potentially associated with their more advanced ovarian activation, in contrast to other virgin queens in which ovarian development is suspended before oocytes reach the vitellarium stage (Cruz-Landim et al., 1997; Patrício & Cruz-Landim, 2002; Santos & Cruz-Landim, 2002). Although virgin queens are produced throughout the year in stingless bee colonies (Bueno et al., 2023b), the imprisonment of these queens in *P. lucii* is a strategy for rapid queen replacement to prevent colony collapse in case the physogastric queen needs to be replaced or has died (Imperatriz-Fonseca & Zucchi, 1995).

Overall, our data show that the ovaries of virgin queens of *P. lucii*, consisting of four long ovarioles formed by terminal filaments, germarium, vitellarium, and mature oocytes, are activated before copulation.

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Author's Contribution

All authors contributed equally to the conception of this paper.

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