ECOLOGY, BEHAVIOR AND BIONOMICS

Age Polyethism in *Plebeia emerina* (Friese) (Hymenoptera: Apidae) Colonies Related to Propolis Handling

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ABSTRACT - Stingless bees collect plant resins and make it into propolis, although they have a wider range of use for this material than do honey bees (*Apis* spp.). *Plebeia* spp. workers employ propolis mixed with wax (cerumen) for constructing and sealing nest structures, while they use viscous (sticky) propolis for defense by applying it onto their enemies. Isolated viscous propolis deposits are permanently maintained at the interior of their colonies, as also seen in other Meliponini species. Newly-emerged *Plebeia emerina* (Friese) workers were observed stuck to and unable to escape these viscous propolis stores. We examined the division of labor involved in propolis manipulation, by observing marked bees of known age in four colonies of *P. emerina* from southern Brazil. Activities on brood combs, the nest involucrum and food pots were observed from the first day of life of the marked bees. However, work on viscous propolis deposits did not begin until the 13th day of age and continued until the 56th day (maximum lifespan in our sample). Although worker bees begin to manipulate cerumen early, they seem to be unable to handle viscous propolis till they become older.

KEY WORDS: Propolis deposit, colony defense, division of labor, task partitioning, Meliponini

Eusocial insect colonies present remarkable and sophisticated cognitive capacities in individual interactions, which are related to their division of labor. Two patterns in division of labor can be observed in permanent bee colonies: among individuals of different castes and individuals in the same caste (Beshers *et al* 1999, Huang & Robinson 1999, Beshers & Fewell 2001). The queen is in charge of laying eggs while worker bees are in charge of tasks concerning to colony construction and maintenance (Michener 1974, 2000, Roubik 1989).

The division of labor related to age polyethism was first studied in Apini and Meliponini (Michener 1974). Age polyethism studies in *Apis mellifera* L showed that worker bees perform different activities according to their age. Generally, worker bees begin performing tasks inside the nest, such as cleaning honeycombs, caring for offspring, comb construction and ventilation, as well as external guarding and foraging activities (Rösch 1927, Lindauer 1953, Ribbands 1953, Michener 1974, 2000, Winston 1987). Similarly, age polyethism patterns have been observed in stingless bee species and alterations may also occur according to the internal needs of the colony (Kerr & Santos Neto 1956, Hebling *et al* 1964, Sommeijer 1984, Simões & Bego 1991, Van Benthem *et al* 1995, Giannini & Bego 1998).

External activities performed by worker bees include gathering food and other materials, such as plant resin, called propolis (Nogueira Neto 1997). Field bees gather resin from flower sprouts, leaves and damaged tree trunks. This material is gathered with the mandibles and transferred to the legs, stored in the corbiculae, and then transported to the colonies (Meyer 1956, Kamazawa *et al* 2003, Teixeira *et al* 2005). In stingless bees, worker behavior is similar to that of honey bees (Bassindale 1955, Sakagami & Camargo 1964).

In *A. mellifera* colonies, the same bees seen using and handling resin were also seen collecting resin in the same day, indicating the inexistence of task partitioning between propolis collectors and users (Nakamura & Seeley 2006). Resin unloading in honey bees is a little different from what was observed in *Trigona* (*Hypotrigona*) *gribodoi* Magretti, as in the first resin users can bit off pieces of resin from the collector's corbiculae, while in the second propolis is unloaded directly against the nest cavity wall (Bassindale 1955, Nakamura & Seeley 2006).

The primary use of resins by honey bees is for caulking cracks and crevices in the walls of nest cavities, for adding it to brood combs, for embalming intruders and for disinfection (Michener 1974, Winston 1987, Roubik 1989). In stingless bees, propolis is also important for colony defense, and it is kept in deposits as a sticky material. In colonies of *Tetragonisca angustula* (Latreille) and species of *Plebeia*,

Nogueira-Neto (1970, 1997) reported isolated viscous propolis clusters throughout the interior of the colonies. The bees use these substances in threatening situations, for gluing small enemies, as observed in *Plebeia saiqui* (Pick & Blochtein 2002). When mixed with the product of wax glands, propolis will form the cerumen, which is stored mainly in the involucrum, on the food pot walls (Sakagami & Camargo 1964), and on the nest entrances (Roubik 2006).

Plebeia emerina (Friese) occurs in São Paulo, Paraná, Santa Catarina, Rio Grande do Sul states in Brazil and in Paraguay (Hoffmann & Wittmann 1990, Alves dos Santos 1999, Silveira *et al* 2002, Steiner *et al* 2006, Camargo & Pedro 2007), including urban areas. We studied division of labor dealing with propolis, mainly focusing on activities involving cerumen and the isolated viscous propolis deposits. Observations of newly-emerged *P. emerina* workers, made by us in 2004 in a pilot experiment, showed that these bees, when placed on isolated viscous propolis deposits, remain attached to them and are not able to free themselves, differently from mature bees. The observed age polymorphism of the cephalic glands of *P. emerina* workers suggest that the higher gland activity of 20-30 d-old bees could explain their abilities to manipulate the viscous propolis (Santos *et al* 2009). Therefore, in order to understand the tasks related to propolis handling in *P. emerina*, this study aimed to identify which age group of worker bees performs direct propolis-related activities inside the colonies.

Material and Methods

Sampling. Four colonies (C1, C2, C3 and C4) of *P. emerina*, established in standard wooden boxes (37 x 24 x 18 cm), were kept at the Central PUCRS Campus (30°03'38.64"S, 051°10'33.18"W), in Porto Alegre, Rio Grande do Sul state, Brazil, from September 2004 to January 2005. The mean diameter of two mature combs and the involucrum volume of all four colonies were evaluated on a monthly basis, during the study period, using a digital Mitutoyo® caliper. The involucrum volume was estimated assuming a half-ellipsoid shape, which was given by $V = \pi(a.b.c)/3$, where a, b, and c are the maximum length, height and width dimensions.

Brood combs close to emergence were isolated in wooden hive boxes (15 x 15 x 4 cm) with nurse bees; the emerging worker bees were marked on the thorax with Revell Color® paint. A color code was used to identify the bee group according to emergence date. Once the paint was dry, the worker bees were returned to their original colonies C1 (n = 279), C2 (n = 145), C3 (n = 222) and C4 (n = 338). The involucrum lamellae were partially removed in order that marked worker bees could be observed on the brood combs. Visual inspections (scans, 12 repetitions of 60 seconds, per colony) were made twice a week, in the morning and in the afternoon, by sweeping the entire colony from above, counting marked worker bees and recording propolis-related tasks throughout the lifespan of the bees following Giannini (1997). The activities were grouped as those involving the cerumen (construction, modeling and repair) in 1) brood combs, 2) comb involucrum and 3) food pots, and in the direct handling of the viscous propolis (propolis gathering, storage and maceration) in the 4) isolated viscous propolis deposits.

Statistical analysis. Comb diameters from all four colonies were compared with ANOVA with repeated measures and a correlation analysis was used for comparisons of nest involucrum volume. These analyses were performed using GraphPad Prim 5.0 software (Motulsky 1999). The association of age of worker bees with propolis activities (brood combs, involucrum, feeding pots and isolated propolis deposits) was examined using the chi-square test and then ranked using a correspondence analysis. These statistical tests were run with Sigma Stat 3.5 and Statistica software. The amplitude variation of worker bee mean age for each activity was determined by using the average age equation ($\bar{I} = \Sigma I.F/\Sigma F$). With the exception of the multivariate tests, α was set at 0.05.

Results

Internal conditions of the colonies. The comb diameters of the *P. emerina* colonies did not vary significantly during the study (ANOVA Repeated Measures, $P = 0.2398$). They were significantly correlated with involucrum volume ($F = 4.636$, $P = 0.0492$), indicating that this measure could be used for determining internal conditions, without the need of removing the involucrum (Fig 1).

Propolis-related division of labor. We found that *P. emerina* workers exhibited age polyethism for propolis-related tasks. Age appeared to be a limiting factor for the development of propolis-related activities. Bees were observed (n = 59) working on the combs (Fig 2a and b) from their first day of life until 28 days of age (Fig 3); they did so during six to 12 days. Bees were observed (n = 2126) on the involucrum (Fig 2a) from their first day of life to 63 days of age (Fig 3). The age of worker bees involved in this activity ranged from 19 to 25 days. *Plebeia emerina* workers were observed (n = 1597) in food pots (Fig 2c) up to 62 days of age (Fig 3). The most

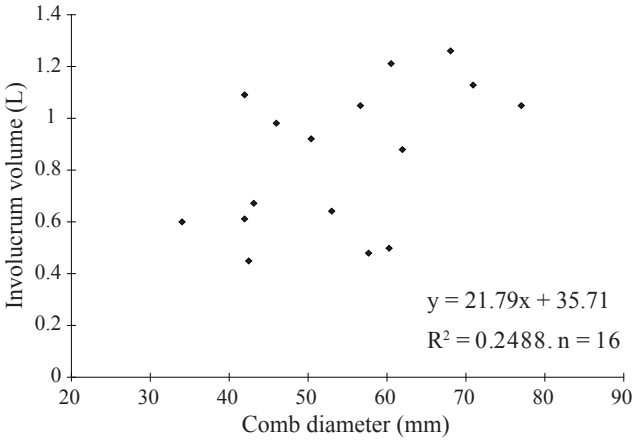

Fig 1 Relation between comb diameter and involucrum volume in *Plebeia emerina* colonies, from September/2004 to January/2005, in Porto Alegre, Rio Grande do Sul State, Brazil.

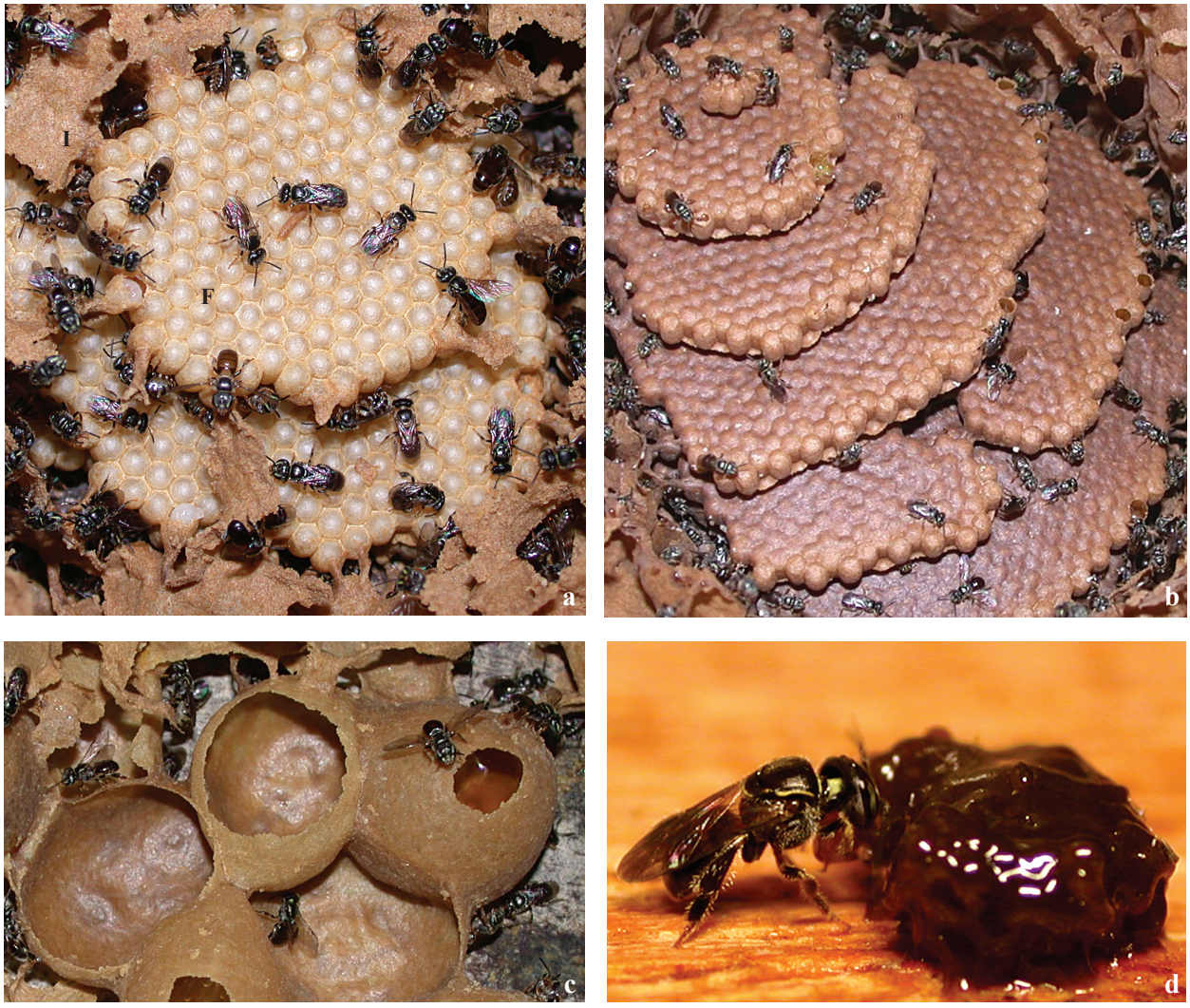

Fig 2 a) Overwiew of a *P. emerina* nest focused on brood combs (F) and involucrum (I); b) bees working on the brood combs; c) set of food pots; d) worker bee handling on viscous própolis deposit with her mandibles.

frequent age group among the bees observed in this type of activity was from 27 to 29 days of age.

Plebeia emerina worker bees were found working on viscous propolis deposits (Fig 2d) from their 13th to 56th day of age (Fig 3). The mean age (based in n = 142 observations) of workers performing this function in three colonies was 27 days and in one colony was 33 days. Worker bees were frequently found at these propolis deposits macerating propolis with their mandibles.

A behavioral rhythm was observed for *P. emerina* worker bees based on their task execution frequency, with intense activity peaks for groups of bees that were 11 to 15, 26 to 30 and 41 to 45 d-old (Fig 3).

We also found that *P. emerina* worker bees are capable of executing wax-related activities during their entire lifespan. There is a significant relation between the worker bees' age and their activities in the colony ($\chi^2 = 616.24$; df = 15; $P < 0.001$). Correlation analysis confirmed that young worker bees are linked to comb activities and older bees are linked to propolis deposit activities (Fig 4). However, the bees demonstrated some flexibility in age polyethism during their lifespan.

Discussion

The four colonies of *P. emerina* had similar comb diameters, minimizing the possibility of behavioral pattern differences related to the state of the colony. Additionally, comb diameters have a significant correlation with involucrum volume, which may also help determine internal nest conditions. However, the internal architecture of these colonies differed, specially the area and disposition of the involucrum which may have affected the number of worker bees identified during visual inspections of behavior. The few records of worker bee activities, especially for colony 1, could be due to the internal disposition of the colony structure, since the involucrum covered the pots and limited the observation area. On the other hand, colony 3 presented more activity related to propolis, and workers were often seen

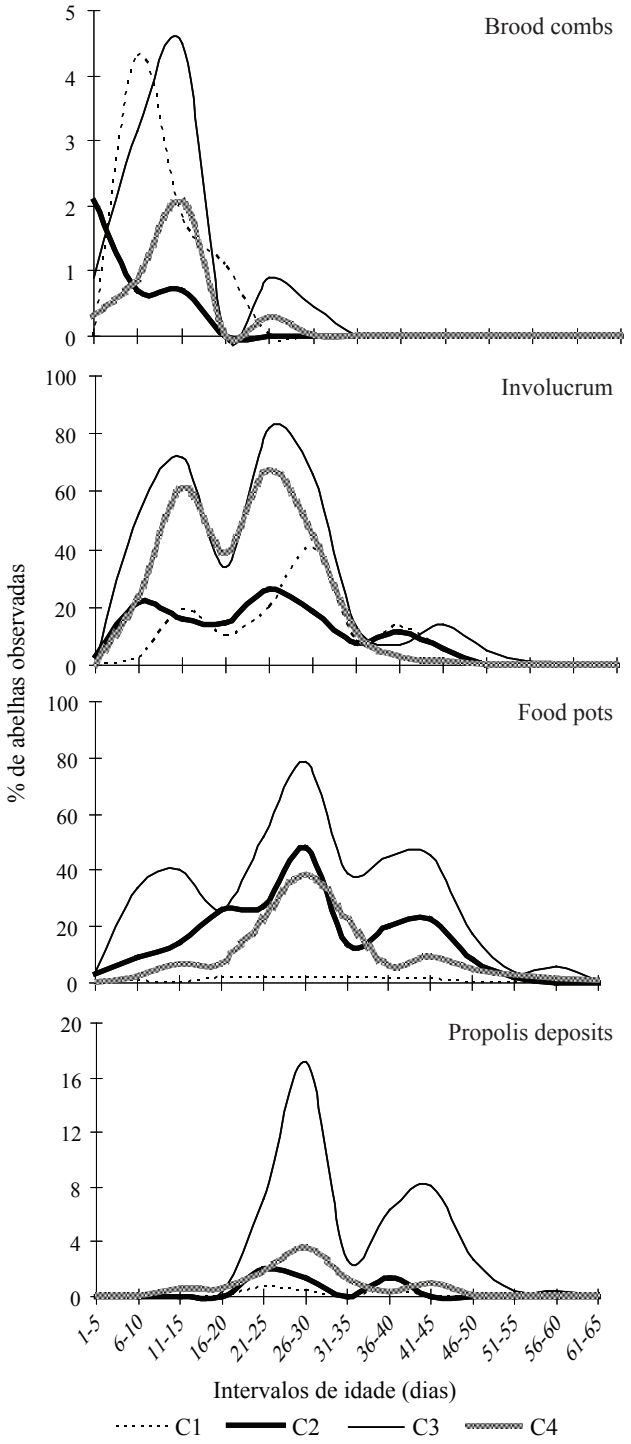

Fig 3 Frequency of marked *Plebeia emerina* worker bees in propolis-related activities, in four colonies, from September 29th 2004 to January 5th of 2005.

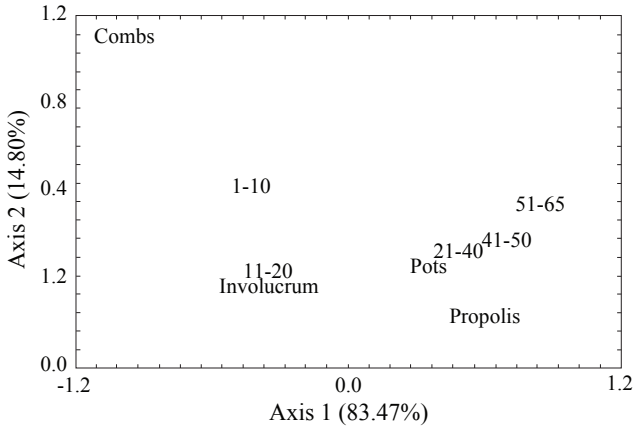

Fig 4 Sorting diagram (correspondence analysis) of worker bee activities (combs, involucrum, feeding pots and propolis deposits) inside colonies of *Plebeia emerina*, concerning different age groups.

on the propolis deposits. In the studied colonies, records of worker bee activities on combs were relatively scarce due to superposition of the horizontal combs.

We observed three age peaks of propolis-related activities in *P. emerina* workers bees, in terms of the frequency of workers manipulating propolis deposits, similarly to the ontogenic rhythm associated with the behavioral development of *A. mellifera* worker bees (Moore *et al* 1998).

Some studies have joined comb, involucrum and food pot activities as common activities, defining them as cerumen activities for various species of stingless bees. In *P. emerina*, we observed cerumen activities in worker bees from their first day of age until a maximum age of 63 days. In the other species that have been studied, worker bees began these activities during their first day of age and extended them for variable periods (Kerr & Santos Neto 1956, Bego 1983, Simões & Bego 1991, Giannini 1997, Giannini & Bego 1998).

Considering the mean age variation amplitude for worker bees performing the three activities involving cerumen, we observed a temporal sequence in the execution of these activities for *P. emerina*, although they may also occur simultaneously. Terada *et al* (1975), while studying age polyethism related to cerumen activities of *P. droryana* (Friese) worker bees, also indicated comb activities followed by involucrum and food pot construction activities, similar to what was reported for *P. emerina*.

The beginning of activities of *P. emerina* worker bees on viscous propolis deposits was observed after their 13th day of age, close to what is observed in *A. mellifera* (Nakamura & Seeley 2006), but later than in *Trigona* (*Geotrigona*) sp. workers (5th day) (Russo 1976), and much earlier than *Trigona* (*Scaptotrigona*) *xanthotricha* workers (32nd day) (Hebling *et al* 1964). Worker bees of these species do work with propolis, but viscous propolis deposits are not reported for *T.* (*Scaptotrigona*) *xanthotricha* (Nogueira-Neto 1970, 1997).

Differently from cerumen activities, which were observed from the beginning of the *P. emerina* worker bees' life, work at isolated viscous propolis deposits only occurred at later ages. When comparing the mean ages during which worker bees are involved in different activities with cerumen and propolis, viscous propolis activities are the last in the temporal sequence of tasks. Although many tasks may occur simultaneously, task overlapping did not occur for viscous propolis and cerumen activities during the first two weeks

of age. Considering the spatial disposition of combs, the involucrum, food pots and propolis deposits in the nest, we can conclude that worker bees move away from the brood emergence area according to their tasks, which are related to their age.

In *P. emerina* colonies, the propolis in viscous deposits is adhesive. This physical property may be a limiting factor for their handling by young worker bees, unable to work with this material. Apparently, the production of glandular substances by worker bees enables them after a certain age to handle propolis without facing adherence hazards.

Although we did not observe task partitioning related to propolis manipulation by workers, we registered propolis unloading from their corbiculae by other workers, seldom described in Meliponini. Foragers arrived in the nest with propolis in their corbiculae and pressed the propolis against the wall of the food pot. The propolis stayed adhered on the cerumen of the pot with the forager leaving the pot, as observed by for *T.* (*Hypotrigona*) *gribodoi* (Bassindale 1955). Other workers arrived and contacted the propolis with their mandibles. In *A. mellifera*, Nakamura & Seeley (2006) described that the forager carrying resin in the corbiculae needed help of other workers in order to unload the propolis.

We identified the worker bee age groups executing cerumen and isolated viscous propolis deposit activities in *P. emerina*, and provided information to strength the hypothesis that worker bees perform cerumen activities in the beginning of their lifespan, but are not able to handle viscous propolis until they are older. Possible factors related to worker bee capacity improvements, such as gland development, which could enable them to handle viscous propolis, need to be defined and investigated.

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