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AGE-DEPENDENT MASS VARIATION IN THE STINGLESS BEE *Melipona quadrifasciata* (APIDAE, MELIPONINI)[1]

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ABSTRACT

The relationship between worker body mass and age in stingless bees is an important aspect of morphological development that is poorly understood. In this work, we examined the body mass-age relationship in workers of the stingless bee *Melipona quadrifasciata*. Newly emerged workers (n=151) were marked and weighed and then returned to their nest, after which body mass was monitored for 45 days. *Melipona quadrifasciata* workers showed a substantial increase in body mass during the first five days of life in the nest ($F_{5,190}$=146.91, P<0.001) that most likely reflected the extensive glandular and ovarian development during this period. From the 6th to the 24th day, there was a gradual decrease in body mass ($F_{12,183}$=10.32, P<0.001) that continued after the 25th day ($F_{13,327}$=5.94, P<0.001) before eventually stabilizing ($F_{17,127}$=0.35, P=0.99). The decrease in body mass with age probably reflected the greater participation of workers in processes associated with provisioning and oviposition, as well as the preference of workers to donate rather than receive food during trophallaxis and at the beginning of foraging activity.

Key words: Mass, *Melipona,* stingless bee, weight, workers

INTRODUCTION

In eusocial hymenopterans, body size variation in worker bees is related to task specialization at the individual level and to the variability of tasks performed within the colony [6]. In the stingless bee *Melipona quadrifasciata*, the within-nest body mass and size variation of workers is related to the efficiency of foraging [see 7, for discussion], with weak colonies of *M. quadrifasciata* producing small workers that can carry larger loads of pollen per weight unit than workers from strong colonies. Allometric size variation of the corbiculae partially explains the decrease in the load carrying capacity of large workers in this species [7].

In contrast, little is known about the relationship between body mass and age in *Melipona* spp. In this group, as in other eusocial bees, there is an age-related division of labor in which young workers preferentially perform tasks within the nest whereas old workers perform activities outside the nest, e.g., foraging [Ceccato S, Masters dissertation, University of São Paulo, São Paulo, Brazil;8]. In this work, we examined the variation in the body mass of workers in a colony of *M. quadrifasciata anthidiodes*, one of the most common stingless bees in southern Brazil [13].

MATERIALS AND METHODS

The experiments were done from March to May 2001 in the Bee Laboratory at the University of São Paulo, in São Paulo, Brazil. The colony used was kept in a box from which the bees could leave to forage through a vinyl tube that led to the outside. The colony was considered strong based on its brood comb size, number of pollen and honey pots and population size (approximately 800-900 individuals). To obtain workers for our experiment, two combs were removed from the colony and placed in an incubator at 28°C and any bees that emerged were marked using coded paper tags and and then weighed on a precision balance (OHAUS Explorer). The paper tags were weighed before the experiment, so we could exclude their influence on our results**.** The bees were returned to the nest after being marked and weighed. The bees were marked over a period of 10 days. Each day, at the same time (9:00 a.m.), the nest was opened and during a 5 min period we captured all of the marked

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bees that were observed. The captured bees were then weighed to determine their new mass. To avoid the influence of nectar or pollen loads on the measurements, only workers that were not participating in foraging activities were weighed. To capture as many bees as possible, this procedure was repeated three times at 5 min intervals for a total capture period of 20 min, with all bees being subsequently released back inside the nest. Only bees with readable tags were weighed. This entire procedure of catching, weighing and releasing tagged bees was done daily for 45 days. Male bees and gynes were also weighed, but since in this genus gynes are normally killed when very young [8,15] and males are difficult to observe inside the nest, only the mass variation of workers was analyzed.

Statistical analysis

The weights of newly emerged workers, gynes and males of *M. q. anthidiodes* were compared using the Kruskal-Wallis test. When significant differences were found, a multiple comparisons test [MCT test, 16] was used to determine which groups differed significantly. ANOVA was used to compare the changes in worker weight during the experiment [16].

RESULTS

One hundred and fifty-one workers, 21 males and 12 gynes were marked and weighed. At emergence, workers (6.83 ± 0.44 mg) were significantly heavier than males (6.09 ± 0.65 mg) and gynes (5.10 ± 0.36 mg), with males also being heavier than gynes (K-W: $H = 54.89$, $P < 0.0001$; multiple comparison test: [MCT test, 16] differences among all groups: $P < 0.001$).

The variation in worker weight for up to 45 days after emergence is shown in Figure 1. During first five days of life, there was a significant increase in worker weight compared to the weight at emergence ($F_{5,190} = 146.91$, $P < 0.001$). After the 5th day and up to the 25th day of life, worker mass decreased compared to the peak on the fifth day ($F_{12,183} = 10.32$, $P < 0.001$). Beyond the 25th day, worker weight continued to decrease compared to the previous age-class ($F_{13,327} = 5.94$, P

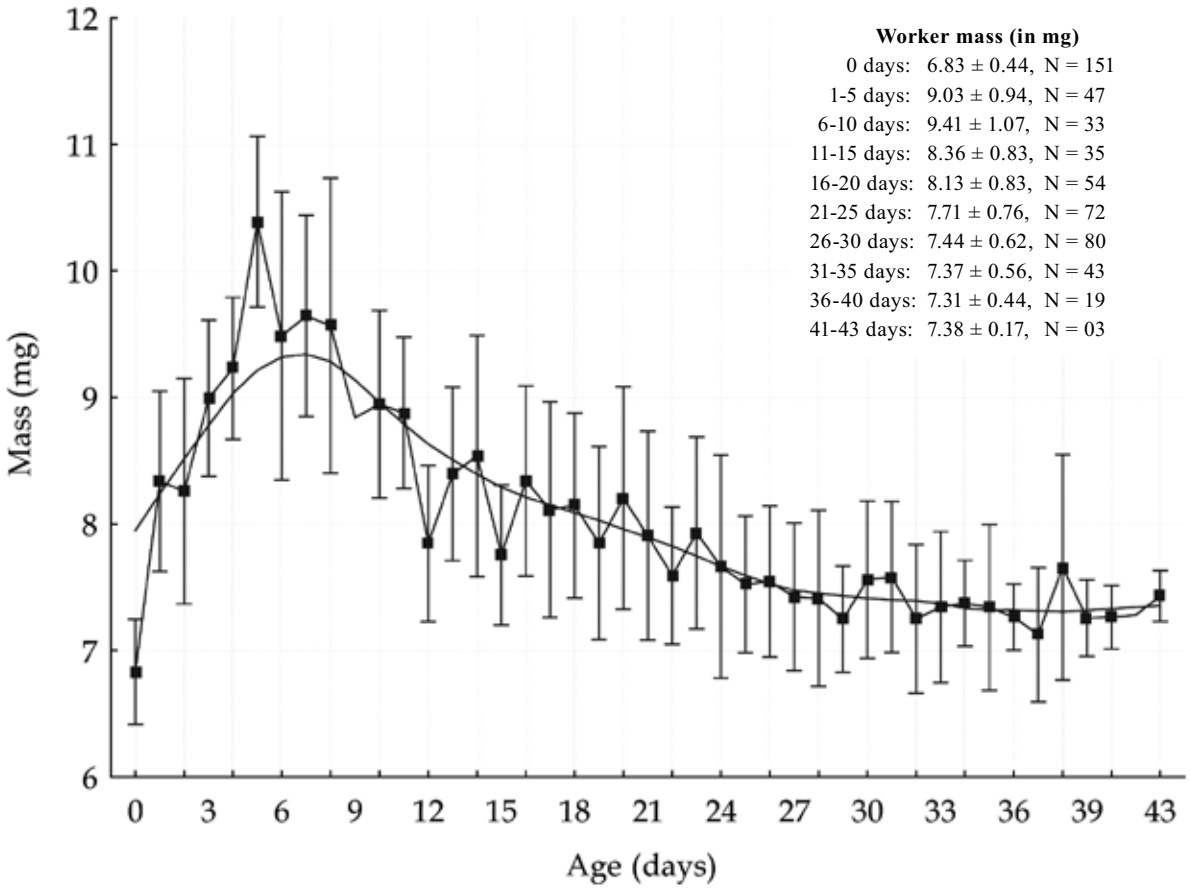

Figure 1. Variation in body mass versus age in *Melipona quadrifasciata anthidiodes* workers showing the best fit regression line. The values (mean±SEM) in the table show the changes in body mass for 5-day classes.

< 0.001), but became stable, with no further changes ($F_{17,127}$ = 0.35, P = 0.99).

DISCUSSION

In the first five days of life and for a period thereafter, *Melipona* sp. workers remain within the nest, where they work mainly with wax and participate in the Provisioning and Ovipositing Process (POP) [8]. The increase in body weight seen in *M. q. anthidiodes* during this period was probably associated with the maturation of internal body organs since, in contrast to honeybees, *Melipona* workers can develop ovaries and lay trophic and/or reproductive eggs [8,10]. Maturation soon after emergence has also been observed in other bees. In *Apis mellifera*, most morpho-physiological changes are completed 3-5 days after emergence [12, p.48] and workers of the Asian stingless bee *Trigona minangkabau* spend most of the time feeding during this post-emergence phase of maturation [5].

A significant amount of the food necessary for this increase in weight may be obtained by trophallaxis. Few studies have examined the trophallactic behavior of stingless bees and in only one species of *Melipona* (*M. favosa*) has this phenomenon been studied. *Melipona favosa* workers up to ~13 days old preferentially receive food from other nestmates during trophallactic contact and rarely take pollen directly from the pots [14]. Such feeding could account for the increase in the body weight of *M. q. anthidiodes* workers.

In *M. favosa*, bees more than five days old begin to participate more actively in POP by depositing larval food in cells where the queen will oviposit and by laying trophic and reproductive eggs [14]. The decrease in the body weight of *M. q. anthidiodes* workers more than five days old was probably related to the energy expenditure associated with POP and their activity in oviposition.

The decrease in the body mass of older workers probably reflected their increasing involvement in foraging activity and the regression in ovarian development [8]. Foraging is a risky activity that consumes much energy, accelerates senescence and death [2,11], and requires the bees to donate food during trophallaxis [14]. In addition, during this phase of life in stingless bee workers there is marked regression in glandular and ovarian development [1,4,8,9]. All workers of *M. bicolor bicolor* more than 26 days old show partial or total ovarian degeneration [1]. The decrease in the body weight of *M. q. anthidiodes* workers most probably resulted from a combination of diminished food intake, higher energy expenditure and the regression of glandular and ovarian structures.

The state of glandular and ovarian development in *M. q. anthidiodes* workers was not assessed because sacrificing the bees would have affected the results of the experiment by reducing the number of bees available for weighing. In addition, there were no behavioral observations of food exchange (trophallaxis) between marked workers because this would have required removal of the wax involucrum surrounding the combs [8]. Such removal would have stimulated wax production by the workers, thereby influencing their morphological state. Other experiments are necessary to assess the relationship between glandular and ovarian development, age and the changes in body weight. A better understanding of the variations in body weight in stingless bees could provide valuable information on the trophic relationships among nestmates, and on their development and maturation.

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