



Beyond temporal-polyethism: division of labor in the eusocial bee *Melipona marginata*

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Abstract

Division of labor plays a fundamental role in colony organization in social insects. In many species, division of labor is based on temporal behavioral castes, whereby workers change tasks as they age. However, division of labor remains relatively poorly understood in the large and diverse group of stingless bees (Meliponini), particularly in the largest and economically important genus *Melipona*. Recent research suggests that stingless bees can differ considerably from other eusocial bees in their division of labor. Here, we studied the lifetime task performance of individually marked workers of the Brazilian species *Melipona marginata*. We found that colony organization in *M. marginata* is characterized by temporal castes and a tendency for elitism, i.e. positive performance correlations across the major tasks. Additionally, we also found that individual workers differ considerably in their work profiles and overall effort. A cluster analysis found evidence for a group of workers that are particularly active in wax manipulation and cell building, two behaviors that are linked to the provisioning and oviposition process (POP). Remarkably, the majority of bees (59%) were never seen foraging and non-foragers were characterized by fewer trophallaxes and less grooming during their lifetime. Bees that did forage often specialized in collecting particular resources, e.g. pollen and mud collectors. In summary, our results suggest that the colony organization in *M. marginata* is complex and includes temporal castes, elitism across some tasks and specialization in others.

Keywords Meliponini · *Melipona marginata* · Task-partitioning · Division of labor · Age polyethism · Sub tasks

Introduction

One of the hallmarks of highly eusocial insect colonies is division of labor, which is often based on the age of workers (temporal polyethism). A general pattern found in ants, bees, and wasps is that tasks performed inside the nest, such as care for offspring or nest building, are performed

by younger workers, whereas tasks performed outside the nest, mainly food collection and defense, are performed by older workers (Lindauer 1952; Robinson 1992; Beshers and Fewell 2001; Yerushalmi et al. 2006; Hölldobler and Wilson 2009; Shorter and Tibbetts 2009; Hammel et al. 2016). This sequence is thought to be adaptive at the colony level because it ensures that workers that are closest to the end of their lives perform the most risky tasks, whereas workers with a greater life expectancy perform safer tasks and are, thus, more likely to be available to the colony for longer (Hölldobler and Wilson 2009). A proximate explanation for temporal polyethism is that workers emerge in the brood area and, therefore, are more likely to encounter brood stimuli. In other words, they are born into the first task (Tofts and Franks 1992). However, the behavioral sequence is not fixed and can be changed in some cases, for example, if the conditions and the needs of colonies require a different worker allocation (Robinson 1992; Beshers and Fewell 2001). For example, if a honeybee colony loses most of its foragers, younger nurse-aged workers will take over foraging duties (Robinson 1992).

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Individual marking of workers has led to the discovery of work patterns beyond temporal polyethism. For instance, many studies show that the workload is very unevenly distributed among workers, both within and across tasks. Some workers consistently perform more work than others (Hurd et al. 2003; Pinter-Wollman et al. 2012; Robson and Traniello 1999; Tenczar et al. 2014; Charbonneau and Dornhaus 2015; Hammel et al. 2016; Inoue et al. 1996). The phenomenon that some workers perform a disproportionate amount of work across various tasks has also been called “elitism” (Oster and Wilson 1978; Robson and Traniello 1999; Pinter-Wollman et al. 2012; Hammel et al. 2016) and can be recognized by positive performance correlations across major tasks. Workers performing a disproportionate amount of work in a single task, or a small number of related tasks, suggests specialization (Pinter-Wollman et al. 2012). Since specialization in one task often means that workers spend less time performing other tasks, negative correlations between major tasks result from this.

Stingless bees are a large and diverse group of highly eusocial bees that play important functions as pollinators in tropical and sub-tropical habitats (Heard 1999; Rasmussen and Cameron 2010). They remain relatively understudied compared to some groups of bees, as the honeybees (*Apini*) and the bumblebees (*Bombini*). *Apis mellifera*, in particular, has become an important model system for the study of various aspects of division of labor (Robinson 1992; Ben-Shahar et al. 2002; Whitfield et al. 2006; Ament et al. 2012; Johnson and Frost 2012), while work in bumblebees has found that body size, rather than age can play a key role in the organization of work in colonies (Goulson et al. 2002; Jandt and Dornhaus 2009). Early studies on the division of labor in stingless bees have confirmed that age is linked to behavior in the typical fashion described above: younger workers mainly perform tasks inside the nest, whereas older workers guard the nest entrance or forage for resources (Bassindale 1955; Kerr and Santos Neto 1956; Darchen 1969). On the other hand, more recent studies have also shown that division of labor can be more complex in stingless bees and involve the existence of behavioral (Inoue et al. 1996) and morphological sub-castes (Grüter et al. 2012; Grüter et al. 2017; Segers et al. 2015; Segers et al. 2016). In the Neotropical species *Tetragonisca angustula*, for example, workers performing defensive tasks are larger than their foraging nestmates. *T. angustula* also shows age-based division of labor (Grosso and Bego 2002), but larger bees work harder and proceed faster from one task to the next (Hammel et al. 2016).

With more than 70 recognized species, *Melipona* is the largest stingless bee genus (Camargo and Pedro 2013), consisting mainly of large bodied species. Even though many species are of economic importance due to their valued honey, only a small number of studies on their division of

labor exists (Kerr and Santos Neto 1956; Sommeijer 1984; Giannini 1997; Bustamante 2006). These studies (and most other studies on division of labor in stingless bees) have analyzed division of labor at the level of age-cohorts, which has left open the question of how much individual worker bees differ from each other in their overall work effort and in the kinds of tasks they perform throughout their lives. Here, we studied division of labor and lifetime task performance of individually marked *Melipona marginata* workers. First, we asked if this species shows age-related division of labor. We then asked whether we find evidence for specialization in the context of two complex tasks, foraging and the provisioning and oviposition process (POP) (Sakagami 1982; Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995). By this we mean that workers involved in either foraging or the POP show behavioral profiles that differ from the behavioral profiles of workers that never performed foraging or tasks related to the POP. The POP involves complex interactions between the queen and workers, the mass-provisioning of the cell after oviposition and the constant construction of new cells due to the fact that stingless bees use brood cells only once (Sakagami 1982). Workers of some species produce trophic and reproductive eggs, which are often consumed by the queen during POP. This complex sequence of behaviors could keep some workers involved in POP activities for an extended period of time, while other workers might perform different activities. We also explored more generally, whether behavioral specialization or elitism characterizes work organization in *M. marginata*, as has been found in other social insects (e.g. Pinter-Wollman et al. 2012; Hammel et al. 2016).

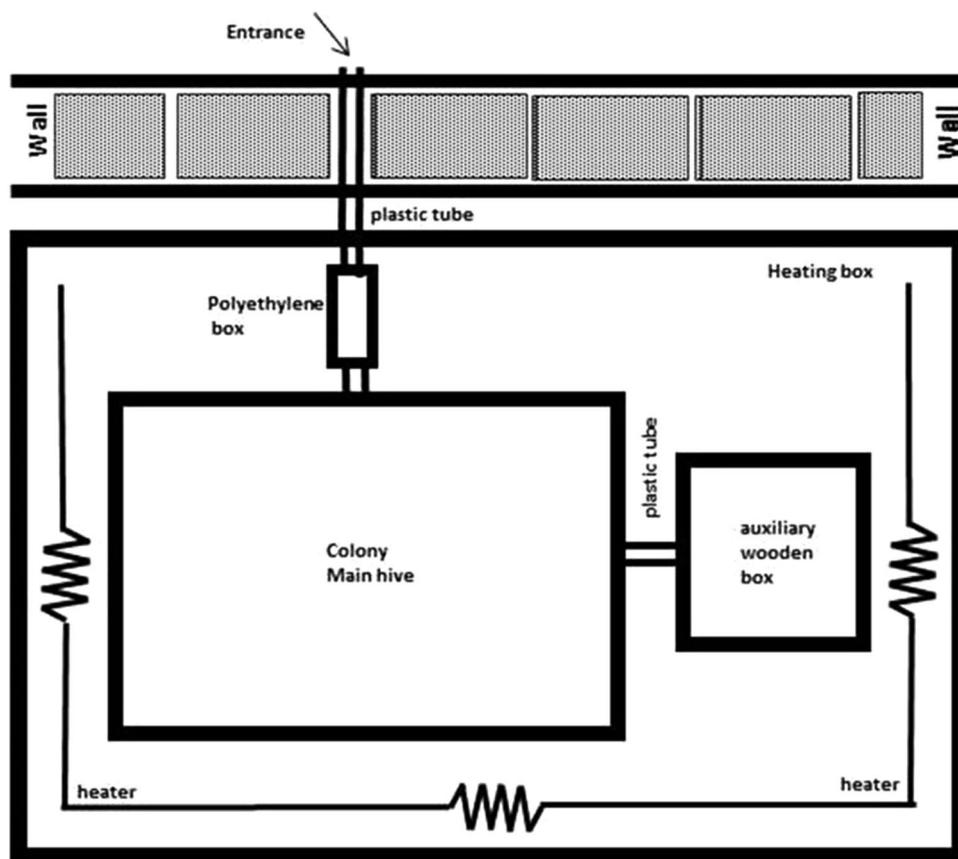
Materials and methods

Study species and study site

Two colonies of *Melipona marginata* containing approximately 350 workers, food stores and a queen were used in this study, both colonies originating from the municipality of Cunha, São Paulo state. This species naturally builds nests in tree cavities in cerrado vegetation and Atlantic rain forests in São Paulo. Like many other species, it builds a brood nest that consists of horizontal brood combs, surrounded by the involucre (e.g. Roubik 2006). As building material, *M. marginata* workers use cerumen, which mainly consists of wax, resin and mud.

In the laboratory, the hives were kept in rectangular wood boxes (29 × 19 × 10 cm), and a glass plate was placed between the lid and the box (Fig. 1) to allow filming and direct observation of internal structures and the bees' behavior. For individual marking, the older brood combs from which bees were ready to emerge were removed from the

Fig. 1 Schematic representation of the heating box with the heater, the main hive, the auxiliary wooden box, the polyethylene box and the connecting plastic tubes, the wall and the colony entrance



hive and placed in an auxiliary wooden box with a glass lid connected to the main hive by a plastic tube to allow the free flow between the main hive and the auxiliary wooden box (Fig. 1). The newly emerged bees (callows) were removed from the auxiliary box and individually marked on the thorax using a color code with nontoxic paint. After marking, they were quickly returned to the auxiliary box. A small rectangular polyethylene box with a glass lid was connected to the entrance tube near the wall to aid in the observation of exits and returns of the foragers and the other behaviors performed in the entrance tunnel. The temperature was maintained at 28 °C using a heating box with internal heater (Fig. 1). To make sure colonies remained healthy under laboratory conditions, we occasionally fed them with 50% sugar and water solution and pollen.

In colony I, 95 workers were marked, and observations took place from October to January (summer). In colony II, 149 workers were marked, and observations took place from April to July (autumn/winter). Daily video recordings of worker activities were taken for 2 h in the morning and 2 h in the afternoon. In addition, every day direct observations were made inside the hive in locations where filming was not possible. From the video analyses and the direct observations, a behavioral catalogue of the tasks performed by the workers was created (Table 1). We excluded behaviors that

were performed less than 20 times in all observations combined, which left us with 29 behaviors. We also excluded bees that were observed performing fewer than 5 behavioral acts (12% of all workers) as these seem to have died at a very young age. As a result, we followed 215 individual bees throughout their lives, i.e. ~ 4 h of daytime observation per day, 81 in colony I and 134 in colony II.

Data analysis

All statistical tests were done in R 3.3 (R Development Core Team 2016). Since, we found no difference in activity levels between the two colonies (Mann–Whitney–*U* test for non-parametric data; see “Results”), we combined the data from the two colonies to explore correlation coefficients between behaviors. For the correlations (Spearman’s rank correlations) and the cluster-analysis, we excluded rare tasks and unspecific behaviors like “grooming”, “inactive” or “trophalaxis”. In total, 9 tasks were used for these two analyses (see “Results”). Due to the large number of correlations (36) we corrected for a false-discovery rate (FDR) (Benjamini and Hochberg 1995) and adjusted *P* values accordingly. We also explored if the activity (sum of all recorded behavioral acts) when bees were young (< 10 days) predicted the activity of the same bees when they were older (10–20

Table 1 A list of the different behaviors that were analyzed, including their frequency and the average age of bees performing these behaviors for both colony I and II (age is given as mean \pm SD)

Behavior description	Total acts	Age in Colony I	Age in Colony II
Chewing wax	1797	7.4 \pm 2.9	13.4 \pm 3.9
Grooming	1620	29.0 \pm 15.0	35.9 \pm 14.9
Walking on floor	1010	12.6 \pm 10.3	35.1 \pm 14.6
Trophallaxis	891	30.9 \pm 15.0	38.2 \pm 14.4
Inactive	854	17.1 \pm 15.4	40.6 \pm 11.6
Walking near storage pots	766	14.4 \pm 10.5	24.4 \pm 12.1
Building involucre	577	6.5 \pm 2.9	15 \pm 8.4
Leaving/entering colony	526	33.3 \pm 10.8	46.7 \pm 8.3
Chewing cerumen pots	488	11.7 \pm 5.6	17.5 \pm 8.2
Chewing cerumen	356	18.3 \pm 4.4	18.9 \pm 10.5
Cell building	326	8.5 \pm 2.0	14.1 \pm 3.2
Working with mud	307	26.6 \pm 8.0	38.9 \pm 6.5
Walking on comb	266	7.1 \pm 3.0	14.7 \pm 6.7
Pollen foraging	243	45.1 \pm 14.0	51.0 \pm 8.3
Eat from honey pot	224	16.5 \pm 7.3	23.7 \pm 12.2
Dehydrating nectar	179	21.8 \pm 7.9	33.5 \pm 7.7
Making waste balls	155	17.3 \pm 6.7	33.0 \pm 8.8
Enter/eat/leave pollen pot	142	11.7 \pm 6.5	14.6 \pm 9.7
Carrying waste outside	118	25.3 \pm 1.8	40.9 \pm 5.5
Scrape bottom of box	95	15.1 \pm 5.0	20.2 \pm 12.9
Discharging larval food	78	10.3 \pm 4.1	14.3 \pm 2.9
Inspect cell during POP	70	8.3 \pm 1.9	12.8 \pm 2.6
Carrying waste inside box	69	13.2 \pm 3.6	37.0 \pm 9.2
Resin foraging	61	25.7 \pm 3.3	46.1 \pm 8.1
Remove wax from abdomen	60	9.4 \pm 7.6	10.2 \pm 5.4
Construct food pots	50		19.4 \pm 7.1
Carrying dead body	37	20.9 \pm 13.7	31.5 \pm 7.9
Liquid foraging	24		47.2 \pm 7.5
Mud foraging	20		39.7 \pm 7.6

days of age or 21–30 days of age) using linear mixed effects models (LME's) with a Gaussian distribution and colony as a random effect (nlme package). To test whether activity patterns showed significant skew, we used T-tests and the method proposed by Crawley (2007, p. 285). For the cluster analysis, we used the package "pvclust". Significant clusters ($P < 0.05$) were identified following 10,000 bootstrap replications, using Euclidian distance and Ward's linkage method (Suzuki and Shimodaira 2006). Ward's linkage method is based on a sum-of-squares criterion, producing groups that minimize within-group dispersion at each binary fusion (Ward 1963; Murtagh and Legendre 2014). While the cluster analysis helps to identify clusters without any prior knowledge of their existence, a principal components analysis (PCA) can test global differences in work profiles among particular groups that are hypothesized to differ (e.g. Hammel et al. 2016). We used standard PCA (Venables and Ripley 2002) to explore differences between foragers and non-foragers (i.e. bees that were never observed foraging) and between POP-workers and non-POP-workers. This was

done either with MANOVA (Multivariate analysis of variance), when multiple principle components were included or with ANOVA (Analysis of variance), when testing single PC's as response variables. The behavioral variables included in the PCA (see results) were centered (mean = 0) and scaled to have identical variance (Manly 1994).

Results

The role of age in division of labor

Table 1 shows how frequently behaviors were performed. Colonies I and II did not differ in how many behavioral acts workers were seen performing during our observations (55.7 \pm 36.0 vs. 51.2 \pm 33.3, Mann–Whitney U test including "inactive": $W = 5724.5$, $P = 0.50$; excluding "inactive": $W = 6083$, $P = 0.14$). The total work effort (number of behavioral acts performed by a bee during her lifetime) showed a left skew in both colonies, i.e. the majority of workers

performed less than the average amount of work (*t* test for significant skew: colony I, *t* value = 3.95, *N* = 81, *P* < 0.0001; colony II, *t*-value = 4.89, *N* = 134, *P* < 0.0001) (Fig. 2). For example, the most industrious 10% performed as much work as the less active half of the workforce combined (~2500 behavioral acts in total in both groups).

The bees of colony I died at a younger age than the bees of colony II (32.2 ± 11.4 [max. = 69] vs. 50.0 ± 11.0 [max. = 70] days; Mann–Whitney *U* test: *W* = 1291.5, *P* < 0.0001). Accordingly, the average age of bees performing the different behaviors was lower in Colony I for all 29 behaviors (Table 1), suggesting an accelerated behavioral maturation of workers in colony I (summer colony). However, despite these differences the overall pattern of the task–age relationship was remarkably similar in the two colonies. First, activity levels for behaviors were positively correlated between colonies, i.e. behaviors that were performed more often in one colony were also performed more often in the other colony (Spearman rank correlation: colony 1 vs. 2: *r* = 0.64, *N* = 29 tasks, *P* < 0.001). Second, the age at which different behaviors were performed were highly correlated (Spearman rank correlation: colony 1 vs. 2: *r* = 0.86, *N* = 26 tasks, *P* < 0.001). To visualize general patterns of age polyethism for some of the most important behaviors, we combined the two colonies (Fig. 3). The data show that building tasks, e.g. building cells or involucrum and the provisioning of larval food, were performed by young workers. Another activity seen in young workers was the production of wax. The mean age of workers secreting wax from the epithelial abdominal glands (constituted by specialized cells with secretory activity) was 9.41 ± 7.55 days in colony I and 10.20 ± 5.35 days in colony II. The waste material produced inside the colony (feces, pieces of corpses and old cocoons) was first processed and formed into waste balls by young workers

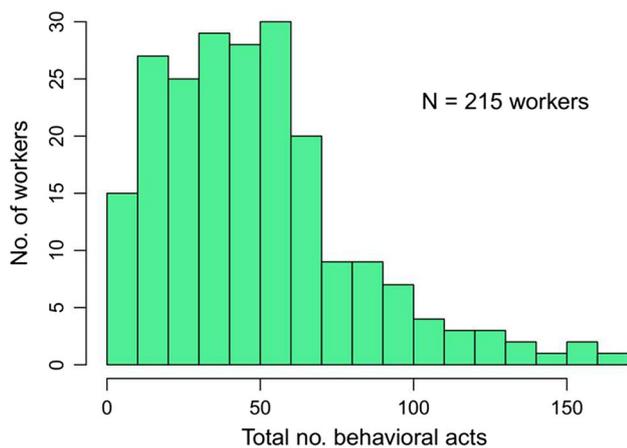


Fig. 2 Frequency distribution of the lifetime work performance, i.e. the total number of behavioral acts performed by a bee during her lifetime (both colonies combined, “inactive” was excluded)

(Table 1). Interestingly, the balls were then carried outside by older workers (Table 1; Fig. 3). Thus, waste removal is a partitioned task. Middle-aged bees performed tasks such as dehydrating nectar or handling of mud (Fig. 3). Foraging started at an age of ~25 days and peaked around 50 days of age (Table 1; Fig. 3).

Correlated activities

We tested whether the activity of 9 key tasks (see Table 1) correlated significantly to explore signs of specialization, elitism or idiosyncrasy (i.e. no pattern of positive or negative correlations). After correction for false discovery rate (FDR), 15 of 36 correlations were significant (Fig. 4). The strongest positive correlations involved the building of involucrum, cell building, nectar dehydration and wax chewing, all tasks performed by young and middle-aged bees. On the other hand, working with mud correlated negatively with cerumen manipulations (Fig. 4).

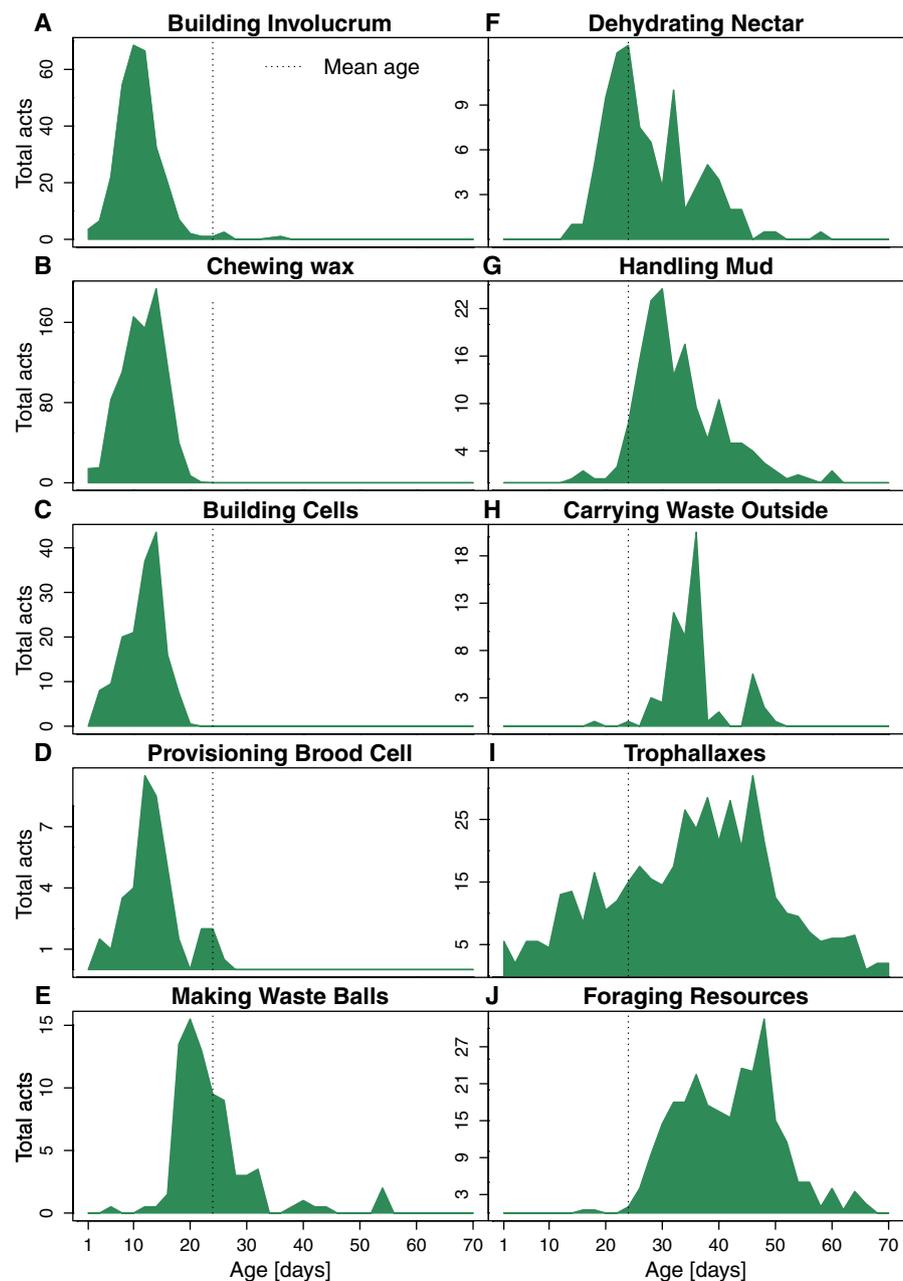
We tested whether activity is consistent during the life of a worker bee. We analyzed all workers, which lived at least 30 days (176 of 215 or 81.9%) and compared their activity from days 0–10, days 10–20 and days 20–30. We found that activity levels (sum of recorded behavioral acts) significantly correlated between days 0–10 and days 10–20 (LME: *t* value = 3.74, *N* = 176 bees, *P* < 0.001) and between days 10–20 and days 20–30 (*t* value = 4.75, *N* = 176 bees, *P* < 0.001). Finally, we found that activity from days 0–10 predicted the activity at days 20–30 (*t* value, *t* = 2.72, *N* = 176 bees, *P* = 0.0073).

Hierarchical cluster analysis

The cluster analysis identified two significant clusters, a small cluster containing 14 bees from both colonies and a large cluster containing all remaining bees (Fig. 5). The small cluster consisted of bees that were particularly active in chewing wax and building cells. This finding is in accordance with our pair-wise correlations where these two tasks showed the strongest positive correlation (Fig. 4).

We performed a separate cluster analysis that only included foraging tasks, including only those workers that had been observed performing foraging tasks at least once (Fig. 5b). This analysis suggested that there is a considerable degree of specialization regarding the resources that are collected. Active foragers would mostly collect just one resource. For example, most resin was collected by just a handful of bees, and 16 of 20 observed mud collection trips were performed by a single bee. However, there were also some cases of alternation, e.g. foragers that collected both pollen and resin and foragers that collected mainly pollen and liquids. The two largest clusters represented bees that

Fig. 3 Temporal polyethism in *Melipona marginata*. Panels show the number of acts performed by all bees for 10 different behaviors. The dotted line represents the mean age bees had when they were seen performing one of the behaviors. Workers in colony 1 were on average ~10 days younger than bees in colony 2 (Table 1) when performing a task. For visualization in this figure, we corrected for these colony differences by adding 5 days to the age of bees performing a task in colony 1 and subtracted 5 days to the age of bees performing work in colony 2. Using the original data provides very similar patterns for the different behaviours (not shown)



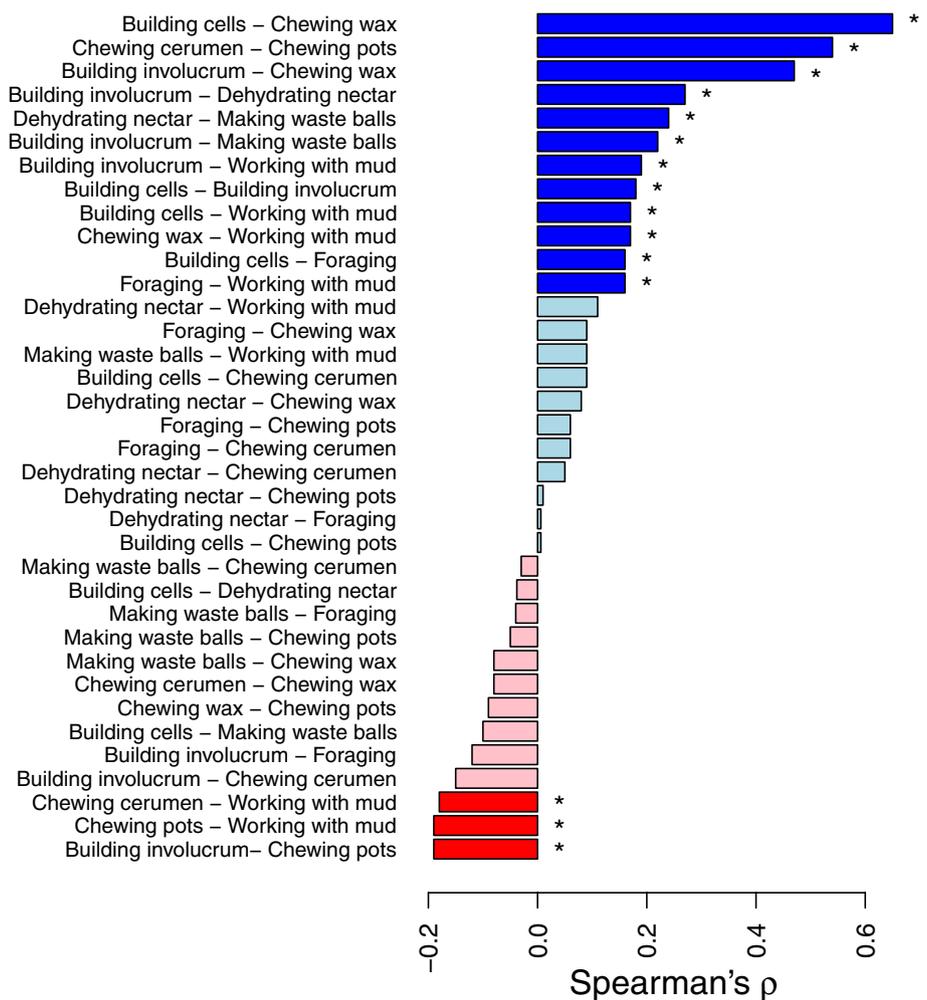
seemed to be occasional foragers, mostly without a particular preference for a resource (Fig. 4b).

PCA of foragers and POP workers

A substantial proportion of workers were never seen foraging (59%), even though they reached typical foraging age (39.2 ± 14.2 days). We tested whether foragers and non-foragers have different work profiles for non-foraging tasks. For the PCA analysis, we included 25 non-foraging tasks and excluded workers that died before day 30, because these bees died before reaching the typical foraging age (Table 1; Fig. 3). The remaining 93 non-foragers reached an average

age of 45.5 ± 10.7 days. The first six principal components explained 60% of the variation and were used in a MANOVA to test for differences in work profiles between worker types (foragers vs. non-foragers) and colonies. There was a significant effect of both worker-type and colony (MANOVA, worker-type: $F_{6,162} = 6.2$, $p < 0.001$; colony: $F_{6,162} = 46.8$, $p < 0.001$). Examining the PCs separately shows significant effects of worker-type on PC1 and PC4 (Fig. 6a; Table S1). PC1 (explained 17.3% of the variation) was most strongly affected by grooming, trophallaxis, walking on the floor and walking near storage pots (Fig. 6a). Foragers showed on average higher values in all these tasks (Fig. 6a). For example, foragers were observed performing 97% more

Fig. 4 Correlations among key tasks. Bar size represents the Spearman's ρ coefficients for the task pair indicated on the left. Statistically significant correlations after correction for false discovery rate (FDR) are indicated as: $* < 0.05$. Positive correlations are in blue (light blue for non-significant correlations); negative correlations are indicated in red (light red for non-significant correlations)



trophallaxes (6.5 ± 5.9 vs. 3.3 ± 3.3) and 81% more grooming events (11.4 ± 10.1 vs. 6.3 ± 5.2) than non-foragers during their lifetime.

The tasks involved in the POP—cell inspection, food discharge into the cells, laying of trophic eggs and cell sealing—were all performed by young workers (Table 1; trophic egg laying was only observed 9 times (9 bees, 12.8 ± 3.7 days) and was not included in Table 1). Preliminary observations suggested that workers involved in the POP may behave differently compared to other workers (S. Mateus, personal observation). Therefore, we combined POP related tasks (beating wings during POP, cell inspections, closing cells, laying of trophic eggs, regurgitate larval food) and divided workers into POP ($N=63$) and non-POP ($N=152$) workers. The latter were never observed performing any of these tasks. We restricted our PCA to 23 non-POP related behaviors. The first six PC's explained 62% of the variation and were again used in a MANOVA. We found a significant effect of both worker-type and colony (MANOVA, worker-type: $F_{6,207} = 18.7$, $p < 0.0001$; colony: $F_{6,207} = 47.4$, $p < 0.0001$). Examining the PCs separately shows

significant effects of worker-type on PC2, PC3 and PC5 (Table S1; Fig. 6b). PC2 (explained 13.9% of the variation) was most strongly affected by cell building, working with wax and walking on the combs (Fig. 6b). Workers involved in the POP process showed higher values in all these tasks (Fig. 6b).

Discussion

We found that *Melipona marginata* workers from two observation hives performed tasks close to the brood combs during the first ~2 weeks of adulthood, before moving on to handling and processing nectar, mud and waste. Foraging was performed by older bees, between approximately weeks 4 and 9. This temporal sequence from inside to outside tasks is common in social insects, including honeybees (Lindauer 1952; Robinson 1992). While some behaviors, e.g. building brood cells, are restricted to a narrow age-range, other behaviors are performed by workers of variable age, e.g. mud handling or trophallaxis. One difference between

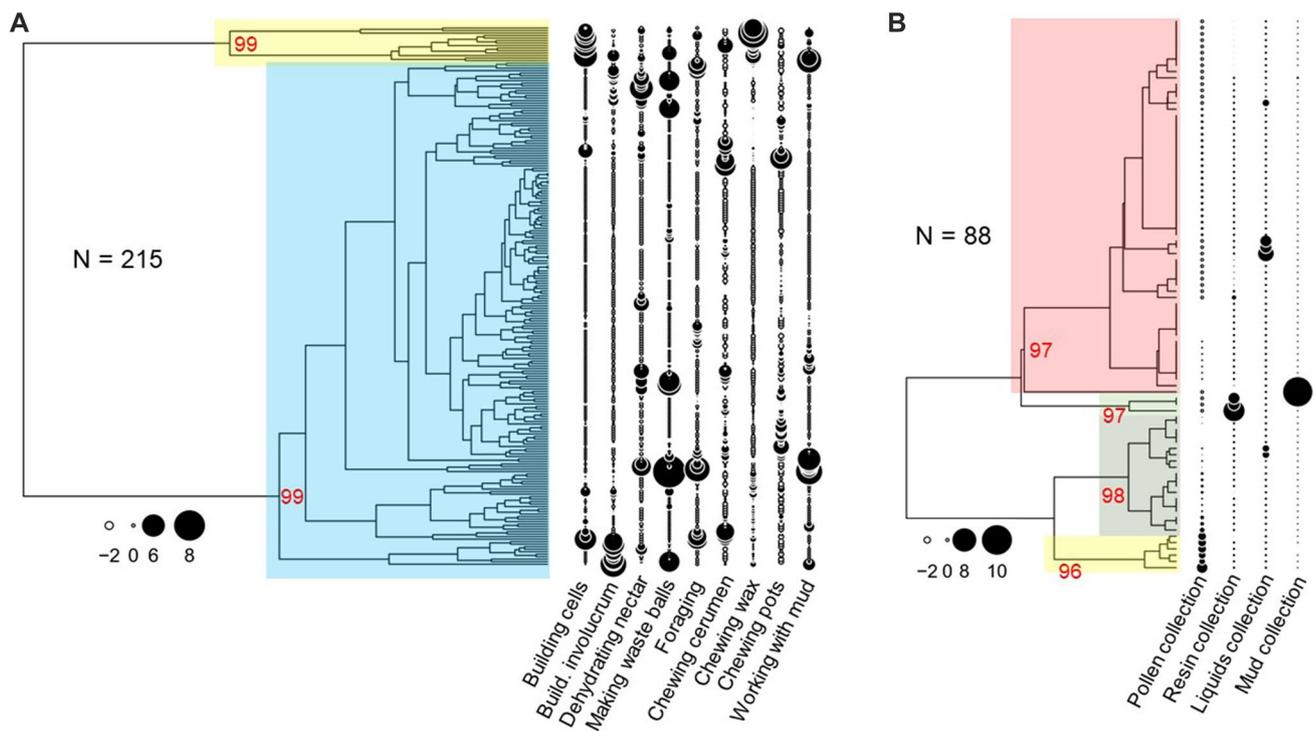


Fig. 5 Clustering of activity among nine key tasks (a) and among the four types of resources collected (b). Each row represents a bee. Significant clusters (numbers ≥ 95 ; 95 corresponds to a p value of 0.05)

are indicated by different colours. The size of the circles indicate how often this behavior has been performed by a bee

stingless bees and honeybees is that in stingless bees each brood cell is dismantled after the emergence of the bee and, therefore, new cells have to be constructed again cell by cell for the next generation (Engels and Imperatriz-Fonseca 1990; Roubik 2006). Indeed, this task is performed by young bees (Fig. 3c). Additionally, stingless bees build enveloping structures around the colony (“batumen”) and the brood nest (“involucrum”), which means that, depending on the species, workers collect large amounts of resin, plant material, mud and even little stones, which they combine with wax in various ways (Wille and Michener 1973; Roubik 2006). *Melipona marginata* uses mud mixed with resin (also called “geopropolis”) to build, repair and modify the entrance. As a result of the constant building, many behaviors seen by very young *M. marginata* workers involve the manipulation of building materials or building itself. At that age, wax secretion is also common. The activity periods of wax production varies according to the species. In the stingless bee *Friesella schrotkyi* and in *Apis mellifera*, the period of wax production lasts approximately 15 days, but it stops at a younger age in *F. schrotkyi* (Justino et al. 2018).

Despite this general sequence of tasks, which parallels the sequence found in other stingless bee species (Bassindale 1955; Kerr and Santos Neto 1956; Hebling et al. 1964; Darchen 1969; Sommeijer 1984; Giannini 1997; Bustamante 2006; Hammel et al. 2016), we found considerable variation

among individual bees in their work effort and behavioral profiles. For instance, we found that a surprisingly large number of workers (59%) never foraged for resources. This cannot be explained by bees dying at a young age, as most non-foragers reached relatively old age. Given that some bees were observed to forage on dozens of occasions it is also unlikely that the lack of foraging in many bees is simply a result of our sampling method. Instead, the data suggest that a large proportion of *M. marginata* workers never forage or forage very little. Equally surprising was the observation that 70.7% of workers were never seen contributing to the provisioning and oviposition process, which is a cluster of behaviors that is mainly performed by young workers. These findings could be partly explained by “lazy” workers, i.e. workers that are consistently less active than others. Inactivity would make it less likely that these workers are observed performing tasks related to the POP or foraging (reviewed in Charbonneau and Dornhaus 2015). In accordance with this, we found that the lifetime work effort showed a left-skewed frequency distribution (Fig. 2), suggesting that a disproportionately large number of workers work only a little. Inoue et al. (1996), studying the Sumatran species *Tetragonula minangkabau*, found similar patterns. Their observations suggested four different groups of workers, including a group of inactive bees and a group specialized in foraging. Several hypotheses have been put forward to explain the existence

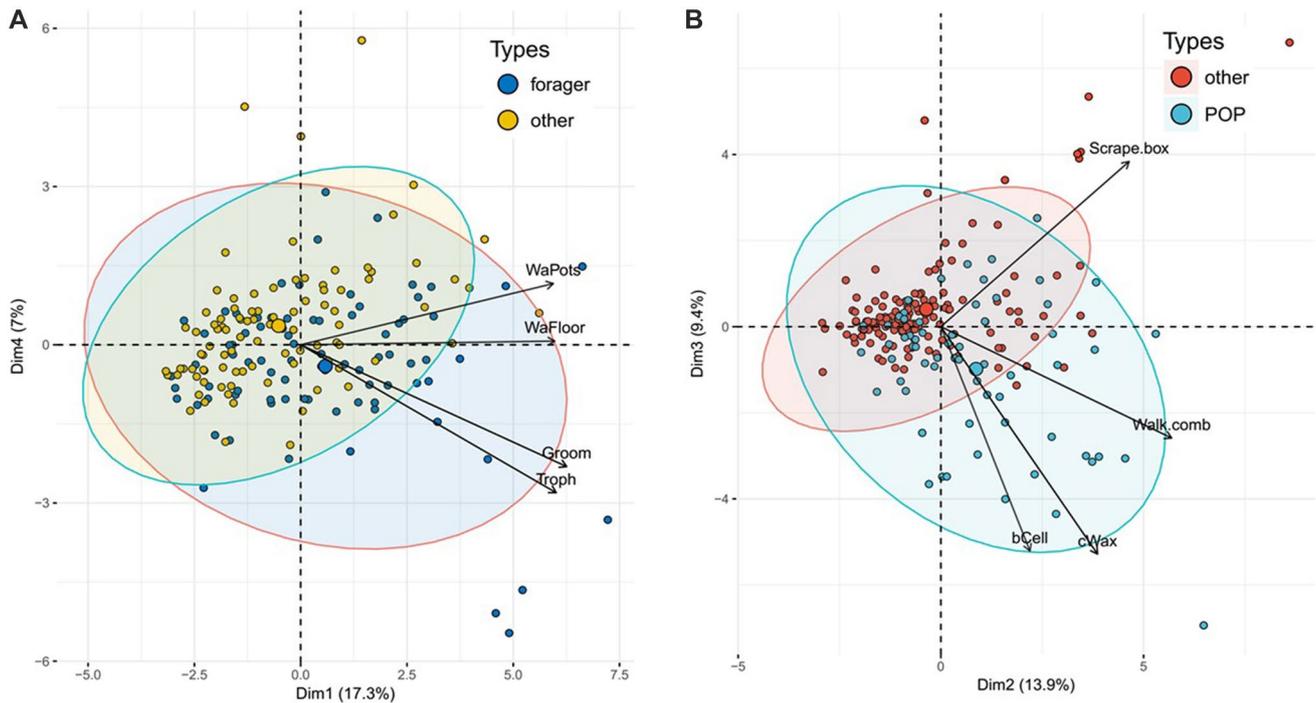


Fig. 6 Principal components analysis (PCA) that compares **(a)** the overall activity patterns of foragers vs. non-foragers (25 non-foraging behaviors) and **(b)** POP-workers vs. non-POP-workers (23 non-POP behaviors). Ellipses include 95% of all bees belonging to the corresponding group. **a** Principal component 1 (Dim1) and principal component 4 (Dim4) showed significant differences among the two worker types (see text). The biplot shows the four behaviors with

the strongest effect (largest loadings): walking on pots (WaPots), walking on the floor (WaFloor), grooming (Groom) and trophallaxis (Troph). The direction and length of the arrows shows the direction and strength of the effect. **b** Principal components 2 and 3 separated the two worker types. The four behaviors with the strongest contribution were building cells (bCell), chewing wax (cWax), walking on the combs (Walk.comb) and scaping the box walls (scape.box)

of consistently less active workers, including the idea that inactive workers are “reserve-workers” that become active when the workload suddenly increases (Kolmes 1986; Charbonneau et al. 2017), selfish workers trying to avoid risky or energetically costly behaviors (Jandt and Dornhaus 2011; Mattila et al. 2012) or that these workers perform functions that are difficult to observe, e.g. processing of food or secretion of communicative substances. However, the evidence remains contradictory (Charbonneau and Dornhaus 2015) and we lack a satisfactory explanation for the left-skewed worker activity in *M. marginata*.

We found that workers that were more active as young bees were also more active as middle-aged and older bees, suggesting consistency in work effort. This finding combined with the fact that many correlation coefficients for key tasks were significantly positive is consistent with some degree of elitism, *i.e.* some bees consistently work more than others do across tasks (the “flip side of the coin” of “lazy workers”) (Oster and Wilson 1978; Robson and Traniello 1999; Hammel et al. 2016). The strongest positive associations were found between the chewing of wax and cerumen, building cells and building involucrem. The same association between cell building and chewing of wax was also

found in our cluster analysis. This suggests that the same bees that build brood combs and the protective sheets surrounding the brood combs are shaping and processing these building materials. Interestingly, the processing of other materials, mainly nectar, mud and waste, is performed by bees of different age in sequential fashion, also called task-partitioning (Ratnieks and Anderson 1999). For example, young bees prepare waste for further processing by making waste balls, which are then carried outside by older bees (see also Medina et al. 2014).

Not all tasks were positively correlated. Working with mud, in particular, correlated negatively with the handling of cerumen and working at food pots. This negative correlation could be explained by the need to have specialists for mud handling, e.g. because it requires skill and/or time, or because mud handling could lead to the contamination of food and other nest structures with soil-based bacteria or fungi. Specialization also seems to occur in foraging. The most active foragers mainly collected one resource, especially in the case of resin and mud. For example, 16 of the 20 observed mud collection trips were performed by just one bee. Foraging for pollen and nectar was more distributed, but pollen collection showed strong clustering

(Fig. 5b). On the other hand, a large number of foragers seem to forage only occasionally. These occasional foragers appear to be less specialized to collect a particular type of resource.

Since foraging is a cognitively demanding task due to the need to constantly learn about the foraging environment and the features of food sources (Menzel 1990; Dukas and Visscher 1994), we explored whether foragers are behaviorally different from non-foragers. Our principal components analysis (PCA) revealed significant differences and foraging was characterized by more grooming, trophallaxis, walking on the floor and walking near storage pots. Trophallaxis might be more common because stingless bee foragers perform frequent trophalactic interactions, either receiving or donating liquids in-between foraging trips (Hart and Ratnieks 2002; Hrncir et al. 2006; Farina and Grüter 2009). Likewise, increased walking on the floor and near storage pots could be consequences of the collection of resources. Thus, foraging, trophallaxis, walking on the floor and spending time near the storage pots are functionally linked. Grooming, on the other hand, could be common in temporarily inactive foragers or in workers that have been exposed to the outside world.

Bees involved in the POP also showed some differences compared to bees not performing these behaviors. Our PCA revealed that POP workers showed elevated levels of cell building, working with wax and walking on combs (highest contribution to the significant principal components) (Fig. 6). Interestingly, two of these behaviors clustered together in our cluster analysis, further suggesting that POP activities are performed by a particular group of bees.

Even though we studied only two cohorts of workers in two observation colonies, our results suggest that there are general patterns in task sequences, but also highlight that these patterns leave room for considerable individual differences. Depending on the tasks, both elitism and specialization can be found in colonies. Open questions that should be addressed by future research include how seasonal factors or the social environment (e.g. amount of brood, colony size, variation in body size) affect division of labor in *M. marginalata*. The social, endocrine, neurochemical and molecular basis of task performance and behavioral maturation have been studied extensively in honeybees (Ben-Shahar et al. 2002; Herb et al. 2012; Leoncini et al. 2004; Robinson 1992, 2002; Schulz et al. 2002; Whitfield et al. 2006), but surprisingly little is known about the proximate causes of behavioral maturation in stingless bees. For example, juvenile hormone (JH) plays an important role in the behavioral maturation of honeybees (Schulz et al. 2002; Sullivan et al. 2000), but little is known about the role of JH for division of labor in stingless bees. Interestingly, a recent study found that *Melipona scutellaris* foragers had lower JH titers than nurses, which is the opposite of what is found in honeybees (Cardoso-Júnior et al. 2017). Stingless bees remain a largely

untapped resource to understand the proximate causes of age polyethism.

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