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## Soldiers in a Stingless Bee: Work Rate and Task Repertoire Suggest They Are an Elite Force

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**ABSTRACT:** The differentiation of workers into morphological subcastes (e.g., soldiers) represents an important evolutionary transition and is thought to improve division of labor in social insects. Soldiers occur in many ant and termite species, where they make up a small proportion of the workforce. A common assumption of worker caste evolution is that soldiers are behavioral specialists. Here, we report the first test of the “rare specialist” hypothesis in a eusocial bee. Colonies of the stingless bee *Tetragonisca angustula* are defended by a small group of morphologically differentiated soldiers. Contrary to the rare specialist hypothesis, we found that soldiers worked more (+34%–41%) and performed a greater variety of tasks (+23%–34%) than other workers, particularly early in life. Our results suggest a “rare elite” function of soldiers in *T. angustula*, that is, that they perform a disproportionately large amount of the work. Division of labor was based on a combination of temporal and physical castes, but soldiers transitioned faster from one task to the next. We discuss why the rare specialist assumption might not hold in species with a moderate degree of worker differentiation.

**Keywords:** division of labor, *Tetragonisca angustula*, age polyethism, Meliponini.

### Introduction

Division of labor is a central feature of insect societies and is thought to be important for their ecological success (Oster and Wilson 1978; Robinson 1992; Beshers and Fewell 2001; Hölldobler and Wilson 2009). Growing interest in the evolutionary consequences of individual differences and plasticity (Dingemanse et al. 2010; Dall et al. 2012; Jandt et al. 2014; Jeanson and Weidenmüller 2014) and the availability of

new molecular tools to study these differences (Whitfield et al. 2006; Lucas and Sokolowski 2009) have led to a renewed interest in the study of the division of labor and the general patterns of how activity is distributed among the workers of a colony (Johnson 2008; Smith et al. 2008; Johnson and Frost 2012; Pinter-Wollman et al. 2012; Jandt and Dornhaus 2014; Jandt et al. 2014; Jeanson and Weidenmüller 2014). It is well known that there is a link between worker age and division of labor in many social insect species: workers temporarily specialize in certain tasks before moving on to other tasks (age polyethism or temporal castes; Robinson 1992; Beshers and Fewell 2001; Hölldobler and Wilson 2009; Giraldo and Traniello 2014). In some species, workers go through a relatively well-defined sequence of tasks (Robinson 1992; Hölldobler and Wilson 2009; Mersch et al. 2013), whereas in others, it is the size of the task repertoire rather than the type of task that changes with age (Seid and Traniello 2006; Camargo et al. 2007). The most spectacular cases of division of labor can be found in species with physical worker subcastes (or physical castes): similarly to the differentiated cell types in multicellular organisms, morphologically distinct worker types perform different tasks in a colony (Wilson 1953; Oster and Wilson 1978; Bourke and Franks 1995; Hölldobler and Wilson 2009; Molet et al. 2012; Tian and Zhou 2014). For example, in some ant and termite species, colony defense is performed by a soldier subcaste (also called “majors” in ants)—workers that are morphologically adapted for defensive tasks (Oster and Wilson 1978; Hölldobler and Wilson 2009; Tian and Zhou 2014). It is thought that the evolution of task-related worker differentiation improves the efficiency of division of labor (Oster and Wilson 1978; Hölldobler and Wilson 2009).

Until recently, it was assumed that physical castes were absent in social bees (Oster and Wilson 1978; Wheeler 1986, 1991; Hölldobler and Wilson 2009). In honeybees,

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worker size variation is small, and division of labor is based on temporal castes (Seeley 1982; Robinson 1992; Johnson 2003). Bumblebee workers (Bombini) show considerable intracolony size variation, and body size is often linked to particular tasks (Goulson et al. 2002; Yerushalmi et al. 2006; Couvillon and Dornhaus 2009; Jandt and Dornhaus 2009), but overall behavioral specialization is relatively weak (Cameron 1989; Jandt et al. 2009), and physical castes are absent (Goulson et al. 2002). In stingless bees (Meliponini), the largest group of eusocial bees (>500 described and many undescribed species; Rasmussen and Cameron 2010), temporal polyethism is thought to be the rule (Sakagami 1982): workers first perform nurse duties before directing their attention to general housekeeping duties. Finally, they perform the outside tasks of guarding and foraging (Sommeijer 1984; Grosso and Bego 2002). However, division of labor is not well studied in the Meliponini, and recently the first case of a stingless bee with physical castes was reported—in *Tetragonisca angustula*, among which colony defense is performed by a specialist soldier subcaste (or majors). Soldiers are both larger and of different shape than foragers (minors) and represent 1%–6% of the colony population (Grüter et al. 2012; Segers et al. 2015).

Division of labor is often assumed to be the outcome of workers of different age or morphology having different internal thresholds for responding to task-specific stimuli, such as brood or alarm pheromones (Robinson 1992; Gordon 1996; for other models of division of labor, see Beshers and Fewell 2001). Even though a variety of factors can affect response thresholds (e.g., genetic background: Jones et al. 2004; Julian and Fewell 2004; environmental conditions: Weidenmüller et al. 2009; social interactions: Schneider and Lewis 2004; experience: Ravary et al. 2007; Pinter-Wollman et al. 2012), it has been suggested that there is a general pattern of how response thresholds and activity patterns are distributed in insect species with physical castes: soldiers have a relatively small behavioral repertoire compared to minors (small workers) and are more specialized, that is, they have a high response threshold for most tasks (Wilson 1980; Bourke and Franks 1995; Beshers and Fewell 2001; Hölldobler and Wilson 2009). Wilson (1980), for example, found that majors overall work less in *Atta sexdens*, while minors have a larger behavioral repertoire in *Pheidole* (Calabi et al. 1983; Wilson 1984; Sempo and Detrain 2004). As a consequence, it is commonly accepted that soldiers are rare specialists, whereas minors are a more common generalist worker type (Bourke and Franks 1995; Beshers and Fewell 2001; Hölldobler and Wilson 2009). The degree of specialization of majors is thought to determine the proportion of majors in a colony: the more specialized a worker type, the fewer of them are needed to perform a task (Wilson 1984; Hölldobler and Wilson 2009). Until now, only ant species

have been studied in detail, and these studies have been performed in the laboratory, with colonies being removed from their natural environment (e.g., Wilson 1980, 1984; Calabi et al. 1983; Beshers and Traniello 1996; Sempo and Detrain 2004; Seid and Traniello 2006; Camargo et al. 2007). Interactions with the natural environment have a strong influence on task performance, task sequence (Gordon 1996; Gordon et al. 2005), and life span (Giraldo and Traniello 2014), all of which affect lifetime work performance and task repertoire. As a consequence, information about the lifetime task performance of ant workers under natural conditions is still scarce (Robson and Traniello 1999; Giraldo and Traniello 2014). Importantly, information for nonant species with physical caste systems has been absent.

We performed the first test of this “rare specialist” hypothesis in a bee, *T. angustula*. Unlike past ant studies, we used observation colonies connected to the natural environment. This allowed bees to forage for natural food sources and to defend their nest against natural enemies such as spiders, ants, or other bees. We studied the lifetime work effort and task repertoire of individually marked workers of known size. According to the rare specialist hypothesis, majors in *T. angustula* were expected to work less and have a smaller behavioral repertoire than minors.

## Methods

Observations were conducted between March and June 2013 on four similarly sized colonies of *Tetragonisca angustula* kept at the University of São Paulo (lat. 21°9.4'S, long. 47°51.3'W; Ribeirão Preto, São Paulo, Brazil). All colonies contained stored food, a brood, and a queen. Additional pollen was supplemented to complement the natural pollen collection. *Tetragonisca angustula* is a common species and occurs from Mexico to Argentina (Michener 2007). *Tetragonisca angustula* is unusual in that it has two different groups of soldiers (we follow Hölldobler and Wilson 2009 in using “soldiers” and “majors” synonymously for the large workers and “minors” for the small workers) defending the nest entrance: hovering soldiers hover near the entrance tube and mainly intercept heterospecific intruders (Wittmann 1985; van Zweden et al. 2011), while standing soldiers stand on or near the entrance tube and also attack conspecific intruders (Kärcher and Ratnieks 2009; Jones et al. 2012; Couvillon et al. 2013). Both types of soldiers are larger and of different shape than foragers (minors), and size overlap between the soldier and the minor subcaste is small (Grüter et al. 2012). Soldier determination is most likely based on nutritional rather than genetic factors (Segers et al. 2015). Colonies nest in cavities in trees or buildings and can contain several thousand bees. Colonies were kept in wooden observation hives (30 × 19 × 13 cm, covered with a glass window) in the laboratory and connected

to the outside environment by a transparent plastic entrance tube.

The oldest brood comb and a small number of young workers were removed from each colony, placed in a medium-sized petri dish (150 × 25 mm), and kept in an incubator at 28.5°C. The head width of newly emerged bees (1 day old) was measured by using a scaled binocular (Wild Heerbrugg, Wild MB, Switzerland). We divided emerging bees into two size classes, minor and major bees. To determine these size classes, we captured six guarding soldiers and six foraging minors of each colony (see Grüter et al. 2012 for methods). We then averaged the mean head width of majors and minors separately for each colony. Emerging bees that were larger than this resulting mean (mean was 1.87 mm in all colonies) were considered majors, while bees that were smaller than or equal to the mean ( $\leq 1.87$  mm) were considered minors (the exoskeleton does not grow after emergence in Hymenoptera). Five different colors (enamel paint) were used to mark the bees individually on their thorax using a three-point code. Marked bees were placed back into their colonies of origin. In total, 206 bees (152 minors and 54 majors) were marked and reintroduced (57 in colony 1, 52 in colony 2, 44 in colony 3, and 53 in colony 4).

Observations were conducted twice per day (morning and afternoon) for 20 min on each colony using an area scan sampling method (Seid and Traniello 2006; Johnson and Frost 2012): the observer scanned the entire nest from left to right, starting at the distant corner and working toward the near corner. Every time a marked bee was encountered, its behavior was recorded. After the first marked bee was observed leaving the hive (day 8), observations were extended to also include the nest entrance (5 min of observation per sampling immediately after the in-nest observations, 25 min in total). Based on pilot observations and previously published results (Grosso and Bego 2002), we included 28 behaviors for our analysis (for examples, see fig. A1a; video 1; figs. A1, A2 and video 1 available online). The brood combs in *T. angustula* are protected by thin layers of cerumen (a mix of wax and resin), which together form the involucre (Michener 2007). We removed parts of the involucre in order to be able to observe behaviors related to brood rearing and cell building. However, it was possible for marked bees to remain unaccounted for during a scan sampling. For example, bees could be covered by involucre, wax sheets, or brood combs; positioned upside down; or inspecting a pot. In particular, newly emerged bees would often remain inactive, covered by involucre during the first few days (fig. A2). The experiment was externally ended by a fight between different colonies of *T. angustula* after 37 days, by which time marked bees were only rarely seen (figs. 1, A1b). *Tetragonisca angustula* is a short-lived bee (in Grosso and Bego 2002, the average life



**Video 1:** Individually marked bees perform some of the behaviors included in the division of labor study (video 1, available online).

span was ~20 days; ~3% survived for 40 days). Thus, we deem it unlikely that this incident affected our results.

#### Statistical Analysis

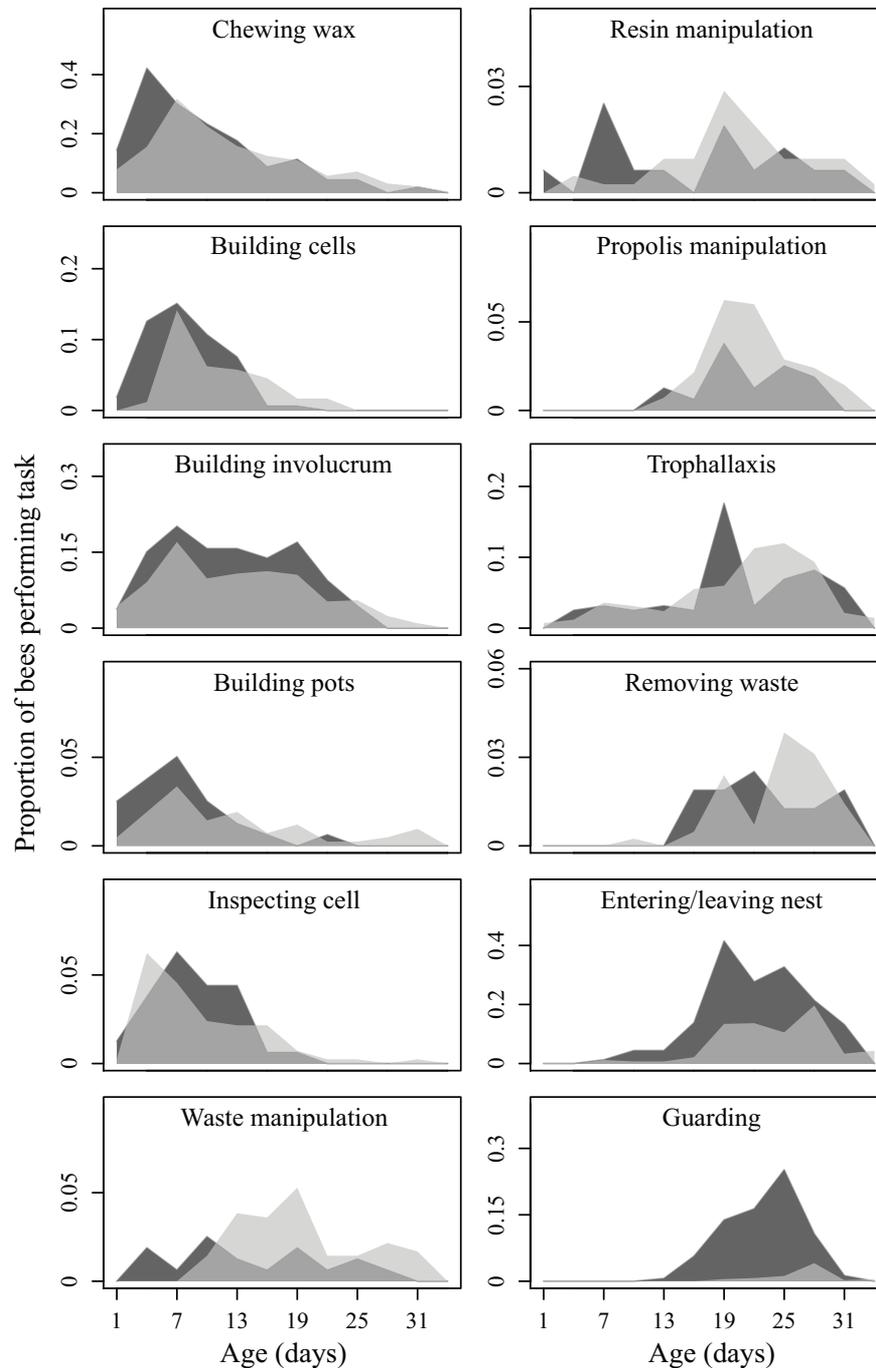
We used R 3.0.2 (R Core Team 2013) for all statistical tests. We used general linear mixed effects (LME) and generalized mixed effects models (GLMM) to control for the non-independence of data from the same colony. LMEs were used for normally distributed response variables; GLMMs were used for response variables with Poisson distribution (Zuur et al. 2009). We visually inspected the residuals to check the distribution. Colony was included as a random effect. Bee was included as a random effect nested within colony when comparing the age of bees performing different tasks. Before testing the significance of predictors (fixed effects), we compared random intercept models with random intercept and slope models (Zuur et al. 2009). After deciding on the appropriate random-effects structure based on likelihood ratio tests (LRT), we tested the significance of fixed effects (Zuur et al. 2009).

To compare the work profiles of minors and majors, we performed a standard principal component analysis (PCA; Venables and Ripley 2002). For the PCA, we used the 10 most frequently performed behaviors that were not clearly linked to either guarding or foraging (building cells, building involucre, building pots, chewing wax, trophallaxis, self-grooming, inspecting cells, ventilation, walking, entering/leaving nest). This selection minimizes the problems caused by zero-inflated data (many tasks were performed by a small number of workers). For the same reason, we excluded bees that did not survive the first week ( $N = 8$ ) for the PCA. Behavioral variables were centered for the PCA (mean = 0; Manly 1994).

## Results

In total, we observed 192 bees (139 minors, 53 majors) 6,607 times as they performed one of the behaviors from our list (fig. A1a; data underlying fig. 1 are deposited in

the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.5p1tg> [Hammel et al. 2015]). Overall, workers showed a pattern of temporal polyethism (fig. 1). Early in adult life, workers perform tasks near the brood area, such as building or inspecting brood cells. Subsequently, workers



**Figure 1:** Temporal polyethism in *Tetragonisca angustula*. Panels show the daily proportion of majors (dark gray) and minors (light gray) performing a particular behavior during worker lifetime. Twelve behaviors are shown.

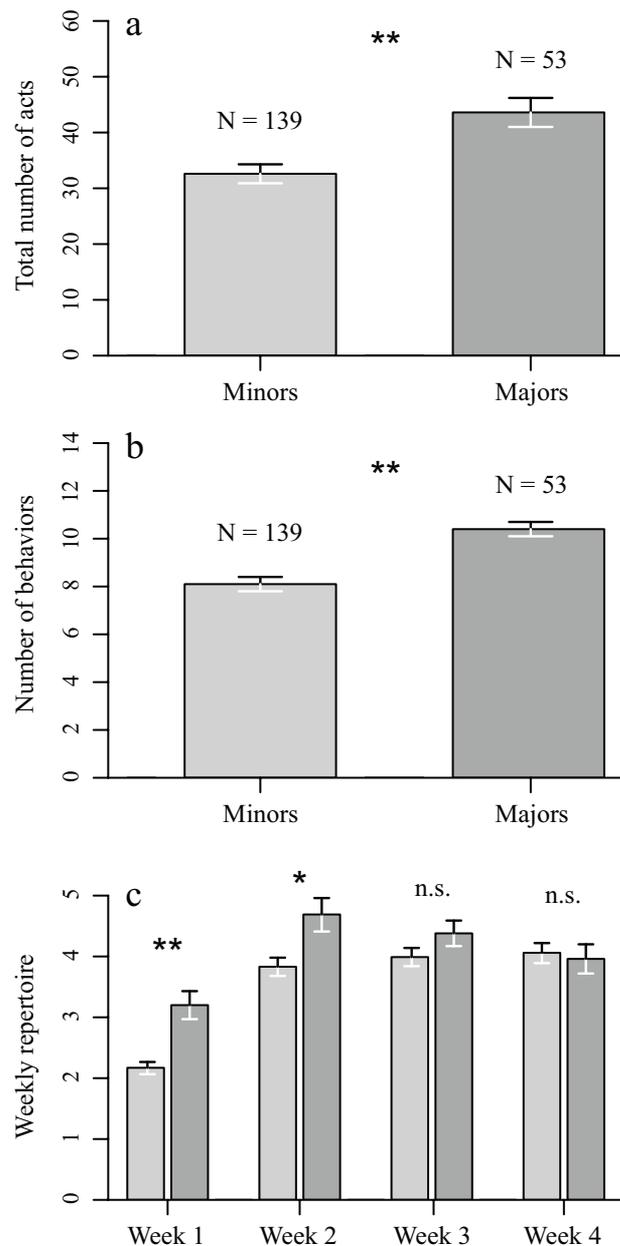
perform tasks outside the involucre, such as the manipulation of waste and resin piles. For example, the age of bees building cells was  $11.8 \pm 3.8$  days, but bees were on average  $22.6 \pm 5.8$  days old when manipulating the waste material (LME:  $t = 18.3$ ,  $P < .0001$ ). When workers are  $\sim 2$ – $3$  weeks old, they can frequently be seen walking in the entrance tube or leaving the nest to perform the outside tasks of guarding or foraging. By the time the observations ended (day 37), marked workers were seen only very rarely (fig. A1*b*), suggesting that most had died.

There was no difference in final age (last day a bee was seen in the hive) between the two size classes (majors:  $27.8 \pm 5.6$  days; minors:  $27.0 \pm 8.4$  days; LME:  $t = 0.6$ ,  $P = .55$ ). However, we found that majors performed overall 34% more behavioral acts (i.e., one of the 28 behaviors) during the entire observation period ( $42.2 \pm 18.2$  vs.  $31.5 \pm 19.4$ ; LME:  $t = 3.4$ ,  $P = .0008$ ; fig. 2*a*). We also found that majors have a larger behavioral repertoire, that is, they performed on average 1.74 behaviors more than minors ( $9.24 \pm 2.9$  vs.  $7.5 \pm 3.6$ ; LME:  $t = 2.81$ ,  $P = .0055$ ; fig. 2*b*). It is possible that these size effects are the result of guarding-related behaviors being more easily observed than foraging-related behaviors. We therefore removed behaviors that are clearly linked to either guarding or foraging (four behaviors) to re-analyze the data but again found that majors performed more acts (LME:  $t = 2.76$ ,  $P = .0064$ ) and had a larger task repertoire (LME:  $t = 2.28$ ,  $P = .024$ ).

Not all majors were observed guarding during our scan samplings, so we tested whether the actual guarding workers (bees that guarded at least once) performed more tasks than nonguarding workers. Guarding workers performed 40.7% more acts than nonguarding workers (majors and minors combined) during the entire observation period ( $45.6 \pm 17.6$  vs.  $32.4 \pm 19.4$ ; LME:  $t = 3.31$ ,  $P = .001$ ) and had a 34% larger behavioral repertoire ( $10.2 \pm 2.1$  vs.  $7.6 \pm 3.5$ ; LME:  $t = 3.85$ ,  $P = .0002$ ).

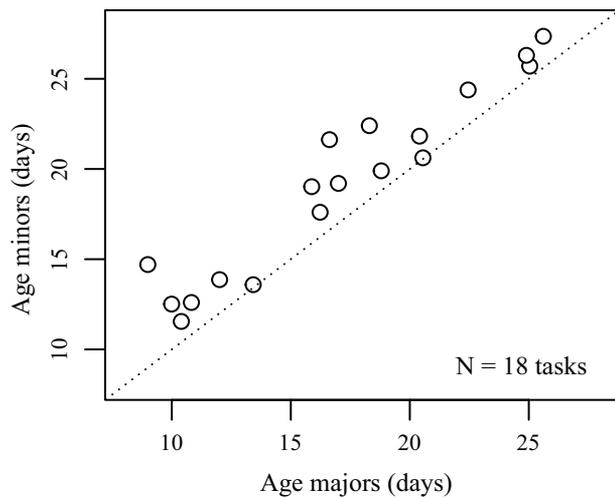
To test whether the behavioral repertoire increases with age, we determined how many different behaviors workers perform per week during the first 4 weeks (fig. 2*c*). We found a significant interaction between our two predictors size class and week (GLMM, Poisson distribution: LRT = 10.97,  $df = 3$ ,  $P = .012$ ; fig. 2*c*), suggesting that the differences between the size classes depend on the week. Figure 2*c* suggests that the change in the size of the behavioral repertoire happens during the first 2 weeks. Thus, we first analyzed the first 2 weeks separately. Both size class and week were highly significant (size class:  $z = 4.3$ ,  $P < .0001$ ; week:  $z = 8.1$ ,  $P < .0001$ ; interaction: LRT = 2.2,  $P = .14$ ). When analyzing the last 2 weeks, we did not find a significant effect of size class or week (size class:  $z = 0.39$ ,  $P = .7$ ; week:  $z = -0.37$ ,  $P = .71$ ; interaction: LRT = 0.98,  $P = .32$ ; fig. 2*c*).

Overall, the age at which minors and majors perform a particular task is highly correlated (18 tasks performed



**Figure 2:** *a*, Total number of behavioral acts (per capita) performed by majors (dark gray) and minors (light gray) during worker lifetime. Majors perform significantly more acts (linear mixed effects to control for colony effects,  $P < .01$ ; see text). Bars show mean  $\pm$  SE of all bees. The numbers above bars give the number of bees. *b*, Number of different behaviors performed during worker lifetime. *c*, Total number of all different behaviors performed per week during the first 4 weeks ( $P < .05$ ; see text).

by a minimum of 20 bees; Spearman's rank correlation:  $\rho = 0.93$ ,  $P < .0001$ ; fig. 3). However, majors are on average  $2.1 \pm 1.5$  days younger than minors when they perform a task (paired  $t$ -test:  $N = 18$  tasks,  $P < .0001$ ; fig. 3).



**Figure 3:** Mean age of majors and minors when performing behaviors that were performed by at least 20 bees. The age at which the two types of workers perform a behavior is highly correlated, but majors are younger when performing a task (see text). The dotted line represents the equal age of both majors and minors.

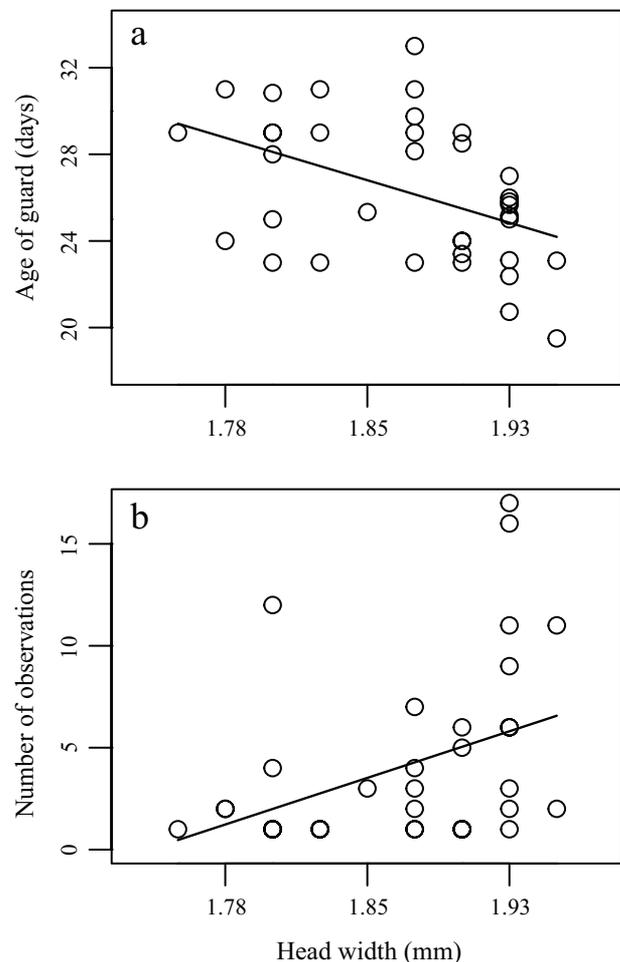
The first two principal components of the PCA explained 78.6% of the variation and were used for further analysis (fig. A2). Majors and minors significantly differed in both principal component 1 (PC1) values (LME:  $t = -2.07$ ,  $P = .04$ ) and principal component 2 (PC2) values ( $t = -3.81$ ,  $P = .0002$ ). Walking contributed most strongly to PC1 (loading = 0.95) and was associated with majors (fig. A2). Additionally, chewing wax ( $-0.22$ ) and building involucre ( $-0.18$ ) affected PC1. Entering and leaving the hive had the biggest effect on PC2 (loading =  $-0.95$ ) and was associated with majors. Again, chewing wax (loading = 0.23) and building involucre (loading = 0.18) were the second and third biggest contributors to PC2.

We found that the average age of guarding workers was  $26.3 \pm 3.3$  days, and bees started guarding at  $\sim 20$  days of age (fig. 1). The average guarding duration was estimated to be  $5.4 \pm 1.5$  days (time between first and last observation of guarding behavior of a bee). Guarding stopped before the end of the observation period, as guards disappeared after  $\sim 32$  days of age (fig. 1) and were not seen performing other tasks. Majors were significantly more likely to guard at least once during their life than minors (GLMM, binomial distribution:  $z = 3.43$ ,  $P = .0006$ ; fig. 1). In accordance with the general pattern of majors performing tasks at a younger age, we found a negative relationship between the size of a guarding worker and the average age of a bee when guarding (LME:  $t = -2.38$ ,  $P = .023$ ; fig. 4a). On the other hand, we found a positive relationship between the size of a guarding worker and how often the bee was observed guarding (GLMM:  $z = 2.6$ ,  $P = .0095$ ; fig. 4b). As figure 1 shows, we also observed minors (9.3%, or 13

of 139) occasionally performing guarding duties. When we checked the sizes of these minors, we found that there was a tendency for them to be larger than the workers of this size class that were never seen guarding (LME:  $t = 1.8$ ,  $P = .075$ ). However, the statistical power of this test is low due to the small sample size of forager-sized bees that performed guarding duties.

### Discussion

Our results suggest that soldiers (or majors) perform more behavioral acts during their lifetime and have a larger behavioral repertoire than minor workers (fig. 2). This was also the case when guarding- and foraging-related tasks (tasks performed by older bees) were excluded. The overall differences between majors and minors were driven by



**Figure 4:** Relationship between guard size and guarding behavior of all bees that performed guarding behavior at least once. *a*, There was a negative relationship between the head width of guards and their average age when guarding. *b*, Guards with a larger head width were observed more often performing guarding behavior.

an increased activity and behavioral repertoire of majors when workers were young (fig. 3c). These results demonstrate that majors are not necessarily more specialized than minors. As in ants and termites with physical castes, soldiers in *Tetragonisca angustula* represent only a small proportion of the total workforce, ~1%–6% (Grüter et al. 2012; Segers et al. 2015). They represent the main defensive force of the colony and fiercely attack potential intruders like ants or bees from other nests that approach the colony entrance (Wittmann 1985; van Zweden et al. 2011; Grüter et al. 2012). But despite being rare, soldiers were active in a number of tasks, which some authors call “elitism” (Oster and Wilson 1978; Robson and Traniello 1999; Pinter-Wollman et al. 2012). Others have defined elitism as a high activity in one particular task (e.g., Hurd et al. 2003; Rocha et al. 2014). We follow Oster and Wilson (1978, p. 152) and consider the latter case an example of behavioral specialization. Elitism is used here to describe active generalists (Robson and Traniello 1999; Pinter-Wollman et al. 2012). We can only speculate why our results differ from previous studies on ants with polymorphic workers. Some ants have evolved extreme caste differences, with soldiers in some species (e.g., in *Atta* or *Pheidole*) being dozens of times larger than the smallest workers of the colony (Hölldobler and Wilson 2009). This degree of morphological specialization might reduce the ability of these ants to perform tasks for which they are not adapted (Oster and Wilson 1978). In *Pheidole morrisi*, for example, majors are less efficient at brood care than minors (Brown and Traniello 1998). The morphological differences between majors and minors in *T. angustula* are relatively small (Grüter et al. 2012; Segers et al., forthcoming) compared to *Pheidole* or *Atta*, and majors might still be as efficient as or even more efficient than smaller bees. However, while larger *T. angustula* workers seem to be more efficient at colony defense (Grüter et al. 2012), it is currently unknown whether body size affects the efficiency for other tasks as well. In particular, it remains unknown whether the smaller bees that are mainly responsible for foraging (Grüter et al. 2012; Segers et al., forthcoming) are also more efficient at this task. The link between task efficiency and behavioral specialization might not be straightforward (Dornhaus 2008). In bumblebees, for example, larger workers seem to be more efficient at performing several tasks even if tasks are normally performed more often by smaller bees (Cnaani and Hefetz 1994). It is possible that the relationship between the degree of differentiation (major vs. minor) and task repertoire is complex: positive initially, as majors are only slightly larger than minors, but negative if extreme morphological adaptations for defense decrease the efficiency of majors for many other tasks. This hypothesis could be tested using ant species that vary in the degree of worker differentiation.

Both majors and minors showed a pattern of temporal polyethism (see also Grosso and Bego 2002; fig. 1). Such a combination of temporal and physical castes has commonly been found in other species with physical castes (Wilson 1980; Calabi et al. 1983; Camargo et al. 2007). *Tetragonisca angustula* workers first perform tasks near the brood area, such as building or inspecting brood cells. Subsequently, workers perform tasks outside the involucrum, such as manipulation of waste and resin. When workers are ~2–3 weeks old, they start to leave the nest for guarding or foraging. The age at which both types of bees perform a particular task is highly correlated, but majors transitioned faster as they were, on average, ~2 days younger when they performed a task (fig. 3). Similar size effects on the pace of behavioral transitions have been documented in both bumblebees (Yerushalmi et al. 2006) and honeybees (Kerr and Hebling 1964). It is possible that body size affects juvenile hormone production in the corpora allata. Juvenile hormone, on the other hand, affects the pace of behavioral development in other social insects (Sullivan et al. 2000; Giray et al. 2005).

Guarding was mainly performed by majors (figs. 1, 4b) and for an average duration of 5.4 days. Interestingly, we again observed the two main patterns that emerged from our study: larger bees were, on average, younger when performing this task (fig. 4a), and larger guarding workers were observed more often performing guarding. A substantial proportion of majors (70%) was never observed hovering near or standing on the wax entrance tube, the typical guarding behavior (Wittmann 1985; Kärcher and Ratnieks 2009; Grüter et al. 2011, 2012; van Zweden et al. 2011). It is possible that (i) some guarding majors were simply not detected during our scan sampling or that (ii) guarding took place at a different location. Many majors are positioned inside the tube (e.g., fig. 1 in van Zweden et al. 2011), and guarding duties seem to entail patrolling the entrance tube. Consistent with this is that we observed 75% of all majors inside the entrance tube during the typical guarding age (entering/leaving nest; fig. 1). Finally, some majors might never become guards.

There seem to be clear differences in what tasks are performed toward the end of life, with guarding being performed by majors and foraging by minors (Grüter et al. 2012; Segers et al., forthcoming). Our PCA (using the 10 most frequently performed behaviors) suggests that work profiles between majors and minors also differ somewhat earlier in life (fig. A2): PC1 and PC2 differed significantly between majors and minors. The behaviors walking, entering/leaving the hive, chewing wax, and building involucrum were amongst the biggest contributors to PC1 and PC2, and the latter two behaviors are mainly performed by young bees. Thus, apart from foraging and guarding, majors and minors seem to differ mostly in these four behaviors (performed more frequently by majors). An increased tendency

of majors to walk around in the nest could help to explain why majors perform more work than minors: walking activity is likely to affect the probability that a worker encounters task-related stimuli and will start a new task. Inoue et al. (1996) suggest that different groups of workers perform different sets of tasks in the Asian stingless bee *Tetragonula minangkabau*, but it is not known whether task performance is linked to size in this bee.

Stingless bees are particularly suited for the study of colony organization and division of labor. Workers are relatively large and have short life spans, meaning that it is feasible to mark them individually and observe lifetime work performance. Colonies can be kept in observation hives while connected to their natural environment. Because most of the more than 500 described species of this ecologically and morphologically diverse group are poorly studied (Rasmussen and Cameron 2010), they represent a large and untapped resource for future research on division of labor in social insects.

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Top left, *Tetragonisca angustula* soldier (large bee) and minor (small bee) shortly after emerging from their cell (photo credit: Christoph Grüter). Top right, brood nest with an individually marked worker (photo credit: Benedikt Hammel). Bottom, *T. angustula* nest entrance with soldiers. “Standing soldiers” are on the wax entrance tube, whereas “hovering soldiers” monitor the flight corridor in front of the entrance hole (photo credit: Christoph Grüter).