



Original Article

Changing of the guard: mixed specialization and flexibility in nest defense (*Tetragonisca angustula*)

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Task allocation is a central challenge of collective behavior in a variety of group-living species, and this is particularly the case for the allocation of social insect workers for group defense. In social insects, both benefits and considerable costs are associated with the production of specialized soldiers. We asked whether colonies mitigate costs of production of specialized soldiers by simultaneously employing behavioral flexibility in nonspecialist workers that can augment defense capabilities at short time scales. We studied colonies of the stingless bee *Tetragonisca angustula*, a species that has 2 discrete nest-guarding tasks typically performed by majors: hovering guarding and standing guarding. Majors showed age polyethism across nest-guarding tasks, first hovering and then changing to the task of standing guarding after 1 week. Colonies were also able to reassign minors to guarding tasks when majors were experimentally removed. Replacement guards persisted in nest defense tasks until colonies produced enough majors to return to their initial state. *Tetragonisca angustula* colonies thus employed a coordinated set of specialization strategies in nest defense: morphologically specialized soldiers, age polyethism among soldiers within specific guarding tasks, and rapid flexible reallocation of nonspecialists to guarding during soldier loss. This mixed strategy achieves the benefits of a highly specialized defensive force while maintaining the potential for rapid reinforcement when soldiers are lost or colonies face unexpectedly intense attack.

Key words: abejas angelitas, caste, division of labor, jataí, task allocation, temporal polyethism.

INTRODUCTION

Social groups from humans to insects face a common problem of effectively dividing essential work among individuals through task allocation. The utility of strategies with flexible switching versus specialization among task assignments is often dependent on multiple simultaneously acting factors. Insight into mixed allocation solutions to these problems can be gained by studying social insect colony dynamics. Among highly social insects, task specialization

can benefit colony function by enhanced efficiency via reduced task-switching delays (Jeanne 1986; Leighton et al. 2017). Although specialization is expected to be most beneficial under reliably consistent conditions, task flexibility is expected to be advantageous in responding to sudden environmental changes by reallocating individuals to new tasks most immediately in need (Calabi 1988; Gordon 1989; Dornhaus 2008; Overington et al. 2008; Jongepier and Foitzik 2016). Group defense is a ubiquitous demand of sociality because gathering individuals and resources into one place creates a high-value target. The degree to which colony members are committed to nest defense should also be subject to this flexibility-versus-specialization trade-off. Here, we present an

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in-depth case study of how colonies use multiple defense allocation strategies that can mitigate the costs of either flexibility or specialization, relative to a single strategy in isolation.

In some colonial animals, defense is a task specific to a morphological type, a soldier caste (Tian and Zhou 2014). Although, in social insects, morphological specialization among nonreproductive (worker) subcastes is generally rare, large-bodied soldiers are the most commonly evolved form of worker size polymorphism (Wilson 1968; Hölldobler and Wilson 1990; Korb and Thorne 2017). Morphologically distinct soldiers are typically more effective colony defenders (Wilson 1968; Oster and Wilson 1978; Gordon 1996; Grüter et al. 2012), but they are also less likely to flexibly perform tasks outside of defense (Bourke et al. 1995; Beshers and Fewell 2001; Hölldobler and Wilson 2009) and are more energetically expensive to produce relative to smaller workers (Gordon 1996; Liu et al. 2005). As such, even when present in a colony, morphologically specialized soldiers typically compose only a small minority of workers (Wilson 1968; Busher et al. 1985; Franks 1985; Grüter et al. 2012). However, strict reliance on a small number of specialists can also create a challenge if the colony loses many or all specialists in a crisis (Wheeler 1991; Robinson 1992). New morphologically specialized soldiers can require days to weeks to replace, necessitating an interim defense strategy in such cases (Gordon 1996).

Age polyethism is another important form of division of labor common among various eusocial species (O'Donnell and Jeanne 1993; Traniello and Rosengaus 1997; Hinze and Leuthold 1999; Biedermann and Taborsky 2011; Bernadine et al. 2015), particularly eusocial bees (Seeley 1982; Robinson 1987; Calderone and Page 1996; Hammel et al. 2016; Kaspar et al. 2018). In such species, young individuals often perform tasks within the nest, moving toward more dangerous tasks outside as they age (Beshers and Fewell 2001; Nascimento et al. 2005). There is also a tendency for task repertoires to expand with age (Seid and Traniello 2006). These schedules can be either dependent on environmental factors (Robinson 1992; Seid and Traniello 2006) or can be relatively fixed (Calderone and Page 1996). Mechanical wear of essential task-related body parts can also drive age-based changes in task performance, such as mandibular wear stimulating a switch from leaf cutting to carrying in leaf-cutting ants (Schofield et al. 2011). However, whether morphologically specialized soldiers also adjust their task repertoire depending on aging-related wear is not well known. We asked to what extent these morphological and aging-related selective forces are at play in shaping task allocation to discrete guarding tasks typically performed by morphologically distinct soldiers.

Morphological specialization among bees is rare, but large-bodied soldiers have evolved several times in the stingless bee subfamily Meliponini, under the selective pressure of nest robbing by other stingless bee colonies (Grüter et al. 2017a). The larger body size and increased antennal sensitivity of soldier bees (majors) in *Tetragonisca angustula* makes them more effective than their smaller nestmates (minors) at detecting and immobilizing nest intruders (van Zweden et al. 2011; Grüter et al. 2012, 2017b; Jones et al. 2012; Shackleton et al. 2015), and so colonies facing higher threats of robbing produce greater proportions of majors (Segers et al. 2016). Guarding by *T. angustula* majors is subdivided into 2 distinct tasks: hovering and standing guarding. Majors hovering in aerial formations near the nest entrance tube primarily intercept heterospecific nest invaders using visual and volatile chemical cues, whereas those standing on the nest entrance tube also detect and

intercept conspecific nonnestmates using close-range olfaction via antennal contact (Wittmann 1985; Wittmann et al. 1990; Bowden et al. 1994; Kärcher and Ratnieks 2009; Grüter et al. 2011; van Zweden et al. 2011; Jones et al. 2012; Shackleton et al. 2018). Agent-based models suggest that the presence of these 2 guard types may minimize colony losses in environments with both high conspecific and heterospecific robbers (Strickland et al. 2018). Although it has been previously shown that majors in this species typically perform both hovering and standing guarding (Grüter et al. 2011), how majors are allocated between these subtasks and the extent to which minors can assist in nest defense have remained open questions. We investigated the degree to which majors specialize on discrete guarding tasks, the extent to which bee age and wear was associated with task allocation, the length of time it takes colonies to replace lost majors, and the role minors play in defense when majors are absent. By addressing these topics, we present a study of how multiple defense substrategies can interplay to form adaptive colony-level protection.

METHODS

Field sites

The data we present here were predominantly collected in our Panamanian field site. However, guard replacement data are the result of complementary studies performed in both naturally occurring and managed colonies of *T. angustula* from 2 distinct populations. Agreements between these 2 data sets (see results) indicate the generalizability of the patterns we present, and so we report both together here. We studied naturally occurring *T. angustula* nests located in and around Gamboa, Colón Province, Panama (9.117°N, 79.696°W), and managed colonies of *T. angustula* kept in wooden hives at Fazenda Aretuzina, near the town of São Simão, São Paulo State, Brazil. Experimental work with managed colonies in Brazil was conducted in February and March of 2011. Field work with natural colonies in Panama was conducted in January and June of 2018.

Testing for behavioral specialization in guarding task

We examined task fidelity among guarding and foraging bees of naturally occurring nests in Panama by marking bees from 3 colonies according to task performed on day 0 and then observing tasks performed on subsequent days. Bees were observed for 20 s each. If a bee spent the entirety of this 20 s flying in a static aerial formation while facing toward the flyway of the nest entrance, it was considered a hovering guard. If a bee was standing motionless on the nest entrance tube facing toward the entrance of the tube for the entirety of this 20 s, it was considered a standing guard. Guards were collected immediately following observations. Foragers were collected as they left the nest and immediately took flight away from the nest entrance (unlike guards); they were distinguishable from waste removal workers by the lack of carried waste. On day 0, bees were collected for marking in this manner for 10 min every 2 h between 08:00 and 18:00. Collected bees were placed in a -20 °C freezer for increments of 15 s until immobilized. Those immobilized bees were then marked on the thorax with 1 of 3 colors (oil-based Sharpie® paint pen) corresponding to the task performed at time of collection: foraging, hovering guarding, or standing guarding. Bees were then immediately returned to the nest entrance once normal motion was regained. On average, 173

bees were marked per colony. Bees were marked according to the first task they were found performing on day 0; only 3 of 538 total marked bees across all colonies were observed performing more than one of the monitored tasks on day 0. We observed numbers and markings of foragers, hovering guards, and standing guards at the start of diurnal peak activity (11:00) on days 1–3. We used Fisher's exact tests to measure how well the task a bee performed on day 0 predicted task performance on days 1–3. This was done separately for guarding-versus-foraging task fidelity and for hovering-guarding-versus-standing-guarding task fidelity. We used Bonferroni corrections to account for multiple comparisons. All analyses were performed in R version 3.4.4 (R Core Team 2018) unless otherwise noted.

Testing for age polyethism

To test for age effects on task performance in majors, we repeated the previously described task-marking methods but with a longer observation window. We used one colony for this test. Our subject colony was a previously unused, naturally occurring colony of *T. angustula* in the Panamanian field site. From this colony, we collected and marked 24 hovering and 39 standing guards over the course of 2 days, observing the frequencies of marked bees in each guarding task across the 10 days after completion of marking.

Factors such as precipitation and nest disturbance can affect the relative numbers of guards at nest entrance tubes over short time intervals (van Zweden et al. 2011; Jernigan et al. 2018). Additionally, although a single bee can perform a shift of guarding continuously for over an hour at a time (Grüter et al. 2011, 2012), all bees typically return to the nest at night, often closing the nest entrance tube and emerging in the morning to return to guarding tasks anew (Roubik 2006). To maximize number of bees observed at a single time point and to eliminate potential repeated observations of the same task performance of a single bee on the same day, we observed the colony 2–4 times each day between 08:00 and 17:00, but we then used only the observation that yielded maximum guard bees at the entrance (regardless of marking) in the analysis. Both larger and smaller bees are similarly short lived, with few of either caste surviving longer than 37 days (Hammel et al. 2016). Large bees age into guarding tasks for approximately the last 1–2 weeks of life (Hammel et al. 2016), making 10 days an adequate window of time to check for age-related task switching between standing and hovering guards.

We predicted that if age-biased switching was not occurring (H_0), day-0 marked standing guards would comprise the majority of marked bees performing standing tasks on subsequent days, and that day-0 marked hovering guards would comprise the majority of marked bees performing hovering tasks on all subsequent days. Under this null hypothesis, we also predicted that both number and proportion of marked bees would decrease in each task gradually across days due to mortality of older bees. If bees were switching tasks with age, this would, however, generate a temporary increase in the ratio of bees in one paint-marking group as the ratio in the other group declined. We analyzed these data using a generalized linear model (GLM) with Poisson distribution that included bee number as a response variable and predictor variables of day, task-marking group (a combined factor of current task and day-0 task), and the interaction between day and task marking. A likelihood ratio (type II) test was used to test the significance of this interaction term. A significant difference in time slopes (slope of bee number vs. day), and opposite signs (positive versus negative) of these slopes across groups would indicate directional task switching over time.

Wing wear

In bees, wing collisions can cause wing deterioration over time (Foster and Cartar 2011), which may influence individual performance of hovering-versus-standing guarding. Such wing-damaging collisions might occur while guards aggressively defend the nest entrance from invaders. We measured and compared wing wear among the studied behavioral task groups (hovering guards, standing guards, and foragers) to test the hypothesis that the observed age polyethism of major bees from hovering guard to standing guard over the last 2 weeks of life (see results) is caused by age-related loss of flight ability due to wing wear in standing guards. A total of 120 bees from 4 naturally occurring Panamanian colonies (10 foragers, 10 hovering guards, and 10 standing guards per colony) were used. We measured wing wear as the total number of wing tears observed along the apical and trailing edge of either forewing (Hayes and Wall 1999). Wings were each viewed under $\times 30$ magnification. A pair of forceps was run along the underside of each wing margin to check for especially thin tears which could have been otherwise hidden from view. Wing wear score was compared across task groups by fitting a generalized linear mixed model (GLMM) with Poisson distribution and colony ID as a random factor. We used a likelihood ratio test (Type II Wald chi-square test) to test for task-group (standing, hovering, or foraging) differences in wing wear.

Morphological specialization and guard removal

Until now, we have examined flexibility within a morphologically distinct worker subcaste. Here, we investigated flexibility across morphologically distinct subcastes. Standing and hovering guards represent different ages of the same morphological caste, as evidenced by similarity in size and shape (Grüter et al. 2012; Supplementary Figure S1), and our results on age polyethism (see Results). We, therefore, refer to hovering and standing guards collectively as “guards” in the following 2 removal experiments.

To study the short-term replacement of guards across morphotypes, we conducted a guard removal experiment using one large and naturally occurring colony from the Panamanian site. Starting at 09:50 and continuing until 11:10, we removed all visible guards from the exterior of the nest every 10 min (to allow for calming of colony alarm in the interim). A total of 88 guards were removed in this timeframe. We then waited 5 h while holding all removed guards in the lab. From 16:00 to 16:30, replacement guards were then similarly collected. All collected bees (88 guards, 28 foragers, 58 replacement guards) were immobilized by 60 s exposure to -20 °C and were weighed live on a microbalance. Preremoval and postremoval guard live mass were compared with each other and to the masses of simultaneously collected foragers using an Anova and post hoc Tukey HSD test.

To test the long-term recovery of colonies from guard removal, we also conducted a guard removal experiment using managed Brazilian colonies. The effects of removal on both average guard size and average guard number were measured. We removed all guards of 4 colonies (treatment colonies) during 2 consecutive days using an aspirator (day 1 and day 2). The weight of these guards was measured on both days. We counted the number of guards 1 day before removal (day -1 in Figure 2C) and on subsequent days, 4 times per day, between 10:00 and 10:15, 11:00 and 11:15, 12:00 and 12:15, and 14:00 and 14:15 h. The other 4 colonies were used as control colonies. We removed 34.3 ± 13.6 guards per treatment colony on day 1 and day 2. We analyzed

these data in R 3.0 (R Core Team 2013) using general linear mixed-effect models (LME) with a Gaussian error distribution and included colony ID as a random effect. After deciding on the appropriate random-effects structure based on likelihood ratio tests, we tested the significance of fixed effects (Zuur et al. 2009). Fixed effects were day for the body size comparison (Figure 2B) and treatment (control versus removal) for guard number (Figure 2C). Benjamini–Hochberg corrections (Benjamini and Hochberg 1995) were used to account for false discovery rates associated with multiple testing across days.

RESULTS

Behavioral specialization and age polyethism

Observed patterns in days 0–3 task-marking data indicated a significant division of labor between guarding and foraging bees as well as between standing guards and hovering guards on day 1 (Table 1). High task fidelity was observed for guarding and foraging across 1-, 2-, and 3-day time intervals (Fisher's exact; all $P < 0.001$; Bonferroni corrected $\alpha = 0.017$), with rare instances of switching from foraging to guarding and no instances of switching from guarding to foraging. There was also significant task fidelity between hovering and standing guards over a single day ($P = 0.013$; Bonferroni corrected $\alpha = 0.017$), but task fidelity diminished across longer time intervals and was nonsignificant over periods of 2 days ($P = 0.052$; Bonferroni corrected $\alpha = 0.017$) and 3 days ($P = 0.050$; Bonferroni corrected $\alpha = 0.017$). The general direction of switching was from hovering to standing (Table 1). This raised questions about age-dependent task allocation which were then explored in the following 10-day marking test.

Ten-day task-marking observations revealed evidence of age polyethism between hovering and standing guards (Figure 1). Time slopes differed across tasks relative to day-0 markings (Type II analysis: $X^2 = 13.955$, $df = 3$, $P = 0.003$), supporting an age-dependent model for task allocation. Day-0 marked hovering guards

disappeared over time from the task of hovering and increased over time in the task of standing guarding. Over a similar timeframe, standing guards disappeared (presumably dying), with observations of day-0 marked standing guards decreasing steadily and halting altogether by day 7 (Figure 1). Together, these results suggest that major bees begin guarding as hovering guards, switching to the task of standing guarding with age.

Wing wear

Most bees had visibly intact wings regardless of task, suggesting that transitions from hovering to standing were not driven primarily by wing wear. Only 19% of 120 measured bees showed any sign of wing wear, with only 6% of bees exhibiting more damage than a single wing tear on a single wing. When comparing across standing guards, hovering guards, and nonguard bees (foragers), there was no significant difference in magnitude of wing wear as measured by wing wear index (GLMM: $X^2 = 1.196$, $df = 2$, $P = 0.550$). There was also no difference in incidence of wing wear (of any magnitude) across task groups (chi-squared test of independence: $X^2 = 0.430$, $df = 2$, $P = 0.806$).

Rapid replacement of missing guards by small bees—naturally occurring colonies

There was a significant difference in live mass among foragers, guards, and guard replacements 5 h after guard removal from a naturally occurring nest in Panama (Anova, $F_{2,171} = 19.27$, $P < 0.001$; Figure 2A). Replacement guards were significantly smaller than the normal guard force (Tukey HSD, $t = 3.41$, $P = 0.002$) and were also significantly larger than foragers (Tukey HSD, $t = 3.14$, $P = 0.006$). Due to equipment resolution, we could neither detect nor rule out bimodality in replacement guard mass distributions. However, Grüter et al. (2012) have previously established a bimodal size distribution among worker subcastes (soldiers and nonsoldiers) when sampling entire colonies of this species. Guard replacements therefore most likely consisted of a mix of both small and large morphological castes, filling the short-term need for guards in crisis situations.

Long-term guard size and number recovery after removal—managed colonies

Replacement guards 24 h after guard removal (day 2) were significantly smaller in live mass than guards prior to removal (day 1) (Figure 2B; LME: $t = -2.84$, $P = 0.005$). Seven of 57 (12.3%) guards weighed 4.0 mg or less, which is the typical size of foragers in this Brazilian population (Grüter et al. 2012). No guard was in this weight category prior to experimental removal of guards. By 9 days after guard removal (day 11), however, guard weight was no longer significantly different from before removal ($t = -0.12$, $P = 0.91$) and was significantly higher than 24 h postremoval (Figure 2B; $t = 3.68$, $P < 0.001$).

Treatment and control colonies did not differ in guard number prior to the removal manipulation (LME: $t = -0.06$, $P = 0.95$). Thus, we used the initial guard count for each colony as a reference value (i.e., 100%) and visualized all later measurements in relation to these values (Figure 2C). One day after removing *T. angustula* guards, treatment colonies had 55.5% fewer guards than control colonies (Figure 2C; LME: $t = -3.64$, $P = 0.01$). The same pattern held for days 3–6 (all $P < 0.05$; Supplementary Table S1). By days 7–11, control colonies still had more guards, but the difference was no longer statistically significant.

Table 1

Task fidelity between hovering guarding, standing guarding, and foraging as represented by frequency of observed bees that were paint marked according to these task groups on day 0. Observations were made at 11:00 each observation day. Numbers depicted are pooled across 3 replicate colonies. Switching between foraging and guarding was rare. Over 24 h hovering and standing guards showed significant preference to continue the same guarding task over switching, but this significance broke down on days 2 and 3. Across all days, hovering guards appeared more likely to switch from hovering guard to standing guard than vice versa

Day 0 task	Hovering	Standing	Foraging
	Day 1		
Hovering	8	5	0
Standing	1	10	0
Foraging	4	0	13
	Day 2		
Hovering	18	9	0
Standing	2	6	0
Foraging	3	0	15
	Day 3		
Hovering	13	5	0
Standing	1	5	0
Foraging	0	0	6

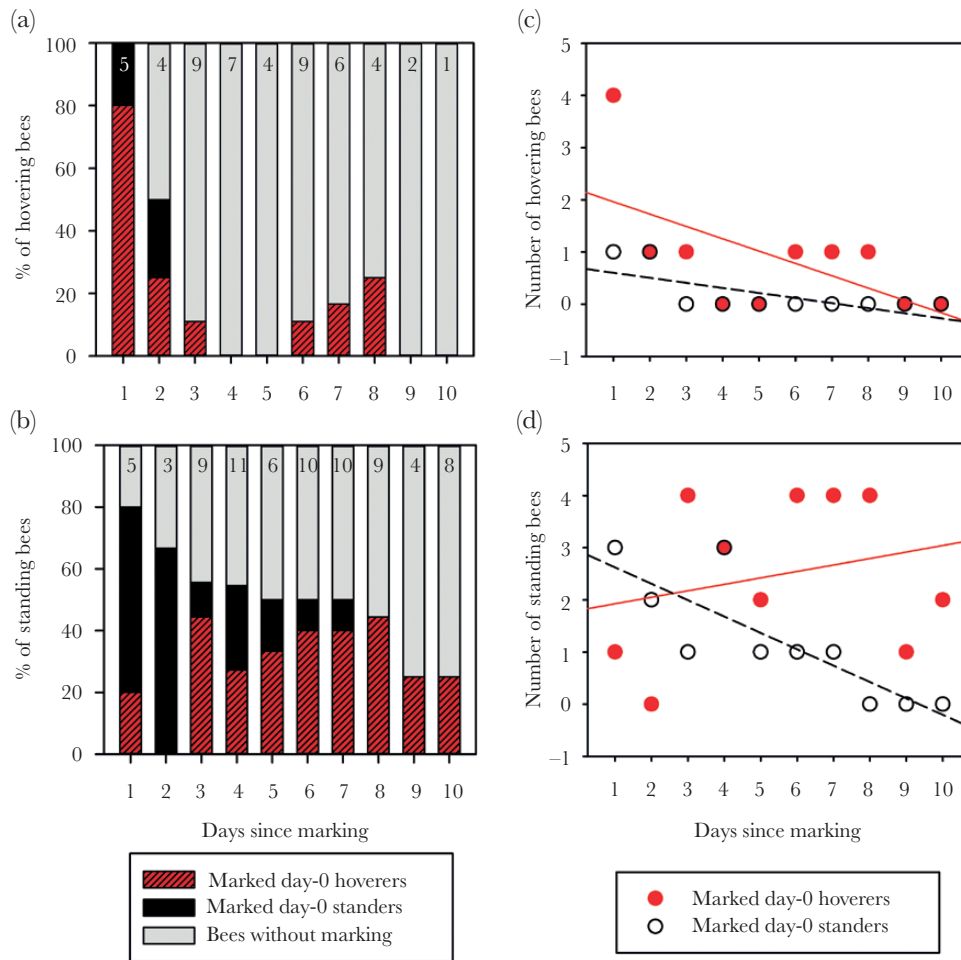


Figure 1

Age-based change in guard task allocation over time as represented by change in tasks performed by marked day-0 task groups over 10 days. (A) and (B) show **daily** percentages of marked and unmarked bees performing each guarding task, **with the total** number of bees performing each task **listed at the top of each column**. (C) and (D) show **daily** counts of marked bees in each day-0 task group performing each task. **All** hovering guards **had** switched to standing guarding after day 8, with all marked standing guards appearing to have died after 7 days **of performing** the task of standing guarding.

DISCUSSION

Colonies of *T. angustula* simultaneously employ multiple defense strategies with different degrees of specialization and flexibility. We present evidence that major bees flexibly specialize on the discrete guarding tasks of hovering guarding and standing guarding, with allocation to each governed by temporal polyethism. Colonies of *T. angustula* are also capable of reallocating minors to these guarding tasks rapidly and for multiple days following the loss of defense-specialized majors. This mixed task-allocation strategy could help solve a conundrum faced by social groups of how to optimally defend under ever-changing environmental challenges. More precisely, colonies achieve the benefits of a highly specialized defensive force while maintaining the potential for rapid reinforcement when exposed to sudden and unexpectedly intense invasion.

Behavioral specialization and age polyethism among majors

Unperturbed guarding majors specialize in 1 of 2 discrete guarding tasks (hovering guarding and standing guarding), and preference for guarding task shifts directionally with age. Younger guarding majors

were more likely to hover; and most hovering guards switched to standing by approximately 1 week. In performing this shift, majors make a transition from flight to standing at the nest entrance, the opposite age polyethism direction from what is typically seen for the onset of foraging as a terminal task in most species that have been studied (Seeley 1982; Jeanne et al. 1992). To our knowledge, this is the first report of discrete guarding behaviors organized by age polyethism in any social hymenopteran. However, due in part to nest defense being thought of as a single task, few studies have explored these dynamics. Jongepier and Foitzik (2016) report discrete defensive behaviors associated with *Temnothorax longispinosus* nest defense, showing high behavioral flexibility between these 2 tasks among guarding ants. However, whether the directionality of this task switching was related to age was not explored.

Majors have a similar lifespan as minors but switch from predominantly within-nest tasks to heavily guarding-biased repertoires toward the last 1–2 weeks of life (Hammel et al. 2016). Consistent with this timeframe, we estimated that guards persist in hovering and standing tasks for 1–2 weeks (Figure 1). We also observed a very low probability of foragers switching to guarding and no instances of guards switching to foraging. Notably, in all cases where a marked forager switched to guarding, they became

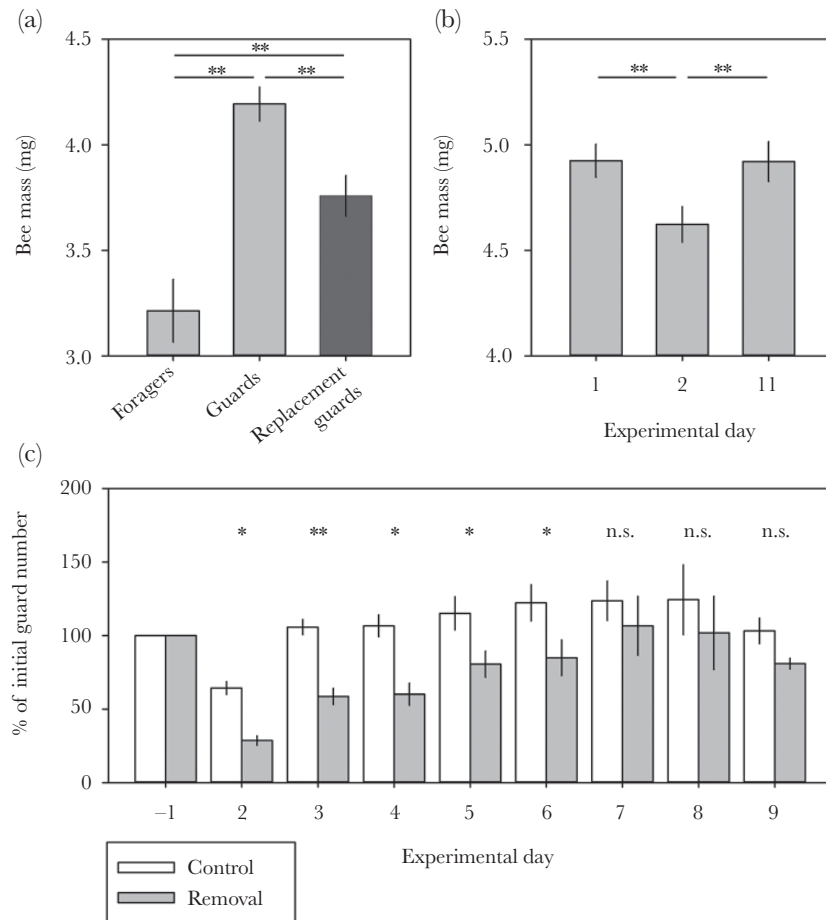


Figure 2

(A) Average mass of foraging and guarding bees prior to guard removal (light gray) and of replacement guards (dark gray) 5 h following removal of guarding majors (naturally occurring Panamanian colony). (B) Weight of *T. angustula* guards from Brazilian colonies before guard removal (day 1), 24 h following removal (day 2), and after guard size had recovered from removal (day 11). (C) Number of bees performing guarding tasks before removal (day -1) and following guard removal (days 2–11), relative to preremoval numbers of guards (% of initial guard number). Gray are treatment (removal) colonies, white are control colonies (no guards removed). Error bars represent standard error in all panels. * $P < 0.05$, ** $P < 0.01$, n.s. = not significant.

hovering guards ($N = 7$). Together, this supports a revised probabilistic major bee age progression from within-nest tasks, to hovering guarding, to standing guarding, with a relatively small percentage of majors foraging between within-nest tasks and hovering guarding.

The age progression of nest guarding in *T. angustula* differs considerably from that of European honeybees, which guard for only a single day after aging out of within-nest tasks and before beginning foraging (Moore et al. 1987). Although age shifts from royal guarding to nest entrance guarding have been described in termites (Yanagihara et al. 2018), the transition from hovering to standing guarding in *T. angustula* involves not only shifts in location but also dramatic shifts in behavior, metabolic demand, and use of sensory modalities.

We observed more switching between hovering and standing tasks than between foraging and guarding in general (Table 1). Most but not all of this switching between discrete guarding tasks could be accounted for by age progression from hovering to standing. However, there were also rare but notable instances of individual bees moving between tasks in an unexpected progression relative to the majority. Similar rare probabilistic events in foraging decisions are beneficial to collective decision-making among ant colonies in dynamic environments (Dussutour et al. 2009). Whether

and to what extent these exceptions benefit stingless bee colony defense remains to be explored.

Potential benefits of age polyethism within guarding task

The clear pattern of transition from hovering to standing guarding raises the question of what adaptive benefit this strategy may provide. In *T. angustula*, older bees performing standing guarding line of behaviors are the last defense before intruders enter the nest and also are primarily responsible for detecting subtler close-range cues to discern nestmates from nonnestmates among groups of conspecifics (Kärcher and Ratnieks 2009; van Zweden et al. 2011). Visual and olfactory cues associated with flowers are learned by foraging bees over time (Cartwright and Collett 1983; Lavery 1994). Accuracy in risk assessment may similarly improve in stingless bee majors with repeated exposure to invaders and returning nestmates over time. Having bees with more guarding experience in the role of standing guard may be beneficial to group defense by offering improved accuracy of detection at the last line of defense before entering the nest.

Further, hovering and standing guards may be constrained in performance by developmental changes in sensory (visual and/or olfactory) acuity or processing. Increases in mushroom body (visual and

olfactory processing regions) volume and synaptic density are associated with adult aging in *Apis mellifera* (Groh et al. 2012). The tendency of hovering guards to intercept predominantly heterospecific invaders and standing guards to intercept both heterospecific and conspecific invaders (van Zweden et al. 2011) may be reflective of a similar age-related neural transition. A nestmate, worker from a close relative colony, or resource-robbing conspecifics each demand appropriate responses. Majors in this species are better able to discern nestmates from nonnestmates (Grüter et al. 2017b). Which guard type is better able to discriminate allies from nonnestmates is a question that might be next studied.

Another possibility is that standing guarding is more dangerous than hovering guarding, motivating the assignment of older bees to this risky task. Nest entrances are not only the site where colonies defend themselves from airborne intruders, but they can also be a target for cursorial threats, such as from predatory ants (Schatz and Wcislo 1999; Ostwald et al. 2018) or jumping spiders (CG, FS, KB, pers. obs.). Standing guards may be more prone to attacks of this nature, because they are often stationary on the exterior surface of the nest entrance tube while facing inward. Age polyethism is predicted to be adaptive when older individuals are assigned to more risky tasks, minimizing the cost of worker loss (Oster and Wilson 1978; Porter and Jorgensen 1981; Tofilski 2002; Gordon et al. 2005; Giraldo and Traniello 2014; Yanagihara et al. 2018). However, whether and to what degree mortality risk differs between hovering and standing guard tasks has not been assessed.

We did not find support for the hypothesis that standing guards are less physiologically capable of flying due to wing damage. Accumulated wing wear was minimal across all bees and was not significantly different between standing guards and hovering guards. However, older majors could be physiologically less capable of flight due to other factors associated with age and wear such as changes in metabolism, fat stores, or flight musculature. These too are avenues for future exploration.

Task flexibility of minors

Even given a highly specialized system of defensive task allocation among majors, colonies maintained the ability to rapidly recruit minors to guarding tasks under conditions of massive loss of majors. Colonies were plastic in their rapid responses to threats, with bees of different morphotypes able to sense the loss of guards and replace missing guards within hours and for up to days at a time (Figure 2A,B). We also observed 2 occasions where morphologically smaller foragers comprised a notable minority of bees attacking a nest robber, even when large bees were present (Supplementary Information). Although minors might be less efficient guards than majors (van Zweden et al. 2011; Grüter et al. 2012; Jones et al. 2012), they would still likely reduce invasions relative to an unattended nest entrance. Minors replace missing guards but they do so with relatively lower numbers of individuals, a condition from which the colony recovers over the course of approximately 5 days (Figure 2C). In this way, these emergency minor guards bridge the span of time it takes *T. angustula* colonies to produce mature majors to replace those lost, as only a small percentage of the reared workers are majors and it takes about 40 days for a bee to progress from an egg to adult (Segers et al. 2015). Even if not specialized colony guards, tropical stingless bee foragers must maintain the ability to contend with often intense competition at food sources (Roubik 1980; Roubik and Villanueva-Gutiérrez 2017). That even small and old foraging minors have some combat ability is therefore not entirely unusual. These findings suggest that colony defense in

T. angustula is aided by both specialist guard response when majors are available as well as flexible nonspecialist reinforcement by minors as needed. This 2-tiered system may help mitigate the costs of having only a small number of specialist soldiers.

Context-dependent task reallocation has long been recognized as an important part of social insect colony function (Calabi 1988; Calabi and Traniello 1989). For instance, honeybees are able to replace missing foragers by reassigning slightly younger nest-worker bees (Huang and Robinson 1996), and in the absence of minors, majors of many ant species expand task repertoires to include brood care (Carlin and Hölldobler 1983; Wilson 1984). However, our observations of forager reassignment to guarding tasks is in contrast to reports of unidirectional behavioral inflexibility in harvester ant (*Pogonomyrmex badius*) foragers (Kwapich and Tschinkel 2016). *P. badius* may exist in less dynamic environments that select for more specialization and less flexibility. When conditions are remarkably consistent, colonies can successfully employ strategies that are more efficient but depend on this consistency to be feasible. Our findings for *T. angustula* further show how social insects can build hierarchies of flexibility to attempt to share the benefits of specialization with the benefits of generalization.

CONCLUSION

Behavioral nest defense of *T. angustula* entails multiple substrategies and timelines of task allocation that mitigate the costs of each in isolation. Although it requires a week for a colony to replace lost soldiers or for soldiers to age from hovering to standing guarding, nonspecialist small bees can switch to guarding tasks rapidly and as needed. This high degree of flexibility among guarding tasks may be beneficial for coping with sudden changes to colony or environmental dynamics. However, rather than investing in a strictly generalist defense strategy at all times, under less dynamic conditions, *T. angustula* colonies return to a very highly specialized defensive strategy. This high degree of specialization includes not only large and combat-effective soldiers but also age-dependent task specialization on particular guarding roles among these soldiers. By employing both flexible and specialized strategies in a context-dependent manner, colonies can minimize losses in a variety of environmental conditions. Considering the adaptiveness of such mixed strategies in the context of group defense is key to making accurate predictions of when and where substrategies such as size polymorphism, age polyethism, or behavioral flexibility are most beneficial (Gordon 1996). We suggest the importance of exploring these patterns across more eusocial taxa and thinking about colony defense tasks more as a series of complementary behaviors as in supply-chain models. Such work can inform our understanding of task allocation dynamics in biological and nonbiological social systems alike.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Baudier et al. (2019).

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