ORIGINAL ARTICLE

Age-based changes in kairomone response mediate task partitioning in stingless bee soldiers (*Tetragonisca angustula*)

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Abstract

Collective defense is one of the most ubiquitous behaviors performed by social groups. Because of its importance, complex societies may engage a set of defensive specialists, with physical and/or neurological attributes tuned for defense against specific invaders. These strategies must be balanced, however, with the need to flexibly respond to different threat levels and sources. Insect societies rely heavily on olfaction for detecting and communicating in the context of defense. We therefore asked whether threat detection via olfaction is specialized towards invader-specific cues and how this may be integrated into defense task specialization. Colonies of the stingless bee, *Tetragonisca angustula*, deploy a morphologically distinct sub-caste of larger-bodied workers (soldiers) for colony defense. These soldiers transition between two different guarding tasks as they age, progressing from guarding in a hovering position near the nest entrance to guarding in a standing position on the nest entrance tube. Hovering and standing guards intercept different types of invaders: primarily heterospecific versus conspecific, respectively. We asked whether hovering and standing guarding behaviors were modulated by differential sensitivity to invader-associated olfactory stimuli; then we compared their responses to these cues to those of smaller workers that perform predominantly non-defense tasks. We exposed bees under both field and lab conditions to citral, a kairomone produced by an obligate heterospecific nest robber, primarily intercepted by hovering guards. Consistent with their roles, hovering guards were more likely to move towards citral than were either standing guards or small-bodied bees within a Y-maze. We also presented guards at field nests with dummies of conspecific versus heterospecific invader types, varying whether they included citral odors. Standing guards were more responsive to conspecific intruder scenarios than hovering guards, but heterospecific response differed by presence of citral. Standing and hovering guards responded in similar proportions when citral was absent, but the addition of citral produced a marginally non-significant reduction in standing guard participation. Our results potentially demonstrate differentiated cue-specific responses that correspond to morphological task specialization and age polyethism in these eusocial societies.

Significance statement

Group defense is a ubiquitous function of any society, but threats are often diverse. Though defense versus non-defense task specialization has been well studied, when and how individuals sub-specialize on particular threats is less understood. We asked how age-differentiated soldier sub-types in a stingless bee differ in their innate responsiveness to olfactory cues associated with nest invader type. Consistent with behavioral roles, younger soldiers were more innately reactive to odors of an obligate

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heterospecific parasite, and older soldiers were more reactive to odors of conspecific intruders. This suggests that either transitions in learning or discrete cue sensitivity mediate defensive sub-specialization. This is the first study to compare behavioral responsiveness to olfactory stimuli across age-specialized soldier sub-types. Results suggest benefits of flexible allocation and prompt further studies exploring the topic of defensive sub-specialization.

Introduction

Group defense is an essential task for social species, because clustering of individuals and resources creates a high-value target for predators and competitors. As such, investigation of how animal societies perform defense has long been—and continues to be—a prominent area of study in behavioral ecology (Wood 1975; Blumstein 1999; Bonanni et al. 2011; Tian and Zhou 2014). Whether an individual within a social group takes part in defense can be related to many factors including age (Bernstein and Ehardt 1985; Cassidy et al. 2017) or fixed morphological differences (Duffy et al. 2002) and can also be mediated by learning (Davies and Welbergen 2009) or genetics (Jaffé et al. 2007). Foundational work in these areas has set the stage for more recent questioning of how these observed behavioral specializations relate to differences in task-specific cognitive capabilities across defensive versus non-defensive functional groups (O'Donnell et al. 2018). However, by virtue of considering group defense as a single task, the behavioral, cognitive, and neurological nuances of defensive sub-specialization in systems where this assumption is not met have remained understudied.

Complex defensive needs may best be met through differentiation in behavioral roles or strategies within the defensive task group. Sub-specialization on different defensive tasks potentially allows for the development of different associated neurosensory abilities that mediate detection and response to different colony threats. Indeed, defensive sub-specialization is the norm in large human military groups and has been reported in morphologically distinct soldiers of at least two eusocial insect species: the termite *Reticulitermes speratus* (Yanagihara et al. 2018) and the stingless bee *Tetragonisca angustula* (Baudier et al. 2019).

The stingless bee *T. angustula* presents an especially tractable model for exploring the sensory processes mediating task roles within defense. In this species, large-bodied soldiers are morphologically specialized guards and additionally switch from the roles of "hovering guarding" in front of the nest entrance to "standing guarding" at the nest entrance as they age. Hovering and standing guards engage predominantly different invader types (Bowden et al. 1994; Kärcher and Ratnieks 2009; van Zweden et al. 2011) that are likely best detected using different sensory modalities (Wittmann et al.

1990; Bowden et al. 1994; Kärcher and Ratnieks 2009). Here, we assess the extent to which these age-differentiated soldier sub-types differ in their innate responsiveness to such task-specific cues associated with these different invaders. It has only come to light recently that standing guarding and hovering guarding are not performed interchangeably but are instead age-partitioned tasks (Baudier et al. 2019). As such, this is the first study to investigate potential specialization in invader cues across these guard types.

Within tropical stingless bee communities, both heterospecific and conspecific nest robbing are common (Breed et al. 2012; Grüter et al. 2016). Such competition from other stingless bees in the environment is hypothesized to have driven the evolution of a morphologically larger soldier sub-caste multiple times among stingless bees in the tribe Meliponini (Grüter et al. 2012; Grüter et al. 2017a). Relative to other sterile workers, soldiers in the stingless bee *T. angustula* are better at detecting nest intruders (Grüter et al. 2017b) and more aptly perform physically demanding defensive maneuvers (Grüter et al. 2012; Shackleton et al. 2018). We are only beginning to understand how these age-differentiated soldier sub-types differ in their ability to perform these tasks.

Morphologically larger soldiers spend the first half of their adult life predominantly performing within-nest tasks before switching to guarding tasks for about the last 2 weeks of life (Hammel et al. 2016). However, discrete guarding tasks are further specialized by age, as soldiers spend the first week of this time hovering guarding and switch to standing guarding for their the final week of life (Baudier et al. 2019). Hovering guards fly in static aerial formations that face inwards towards the nest entrance flyway (Wittmann 1985; Zeil and Wittmann 1989; Kelber and Zeil 1990) and are thought to primarily intercept heterospecific nest invaders based on volatile odors and visual cues (Bowden et al. 1994; van Zweden et al. 2011). Older guards in standing formation on the nest entrance tube are thought to then primarily intercept conspecific nest invaders using close-range olfactory cues at the point of the nest entrance tube (Kärcher and Ratnieks 2009). This raises the question of whether this defensive division of labor arises as a passive result of soldier position at the nest entrance or if these age-based guarding task groups specialize on specific threats via differential cue responses.

Here, we present complementary lab- and field-based explorations of the olfactory responses of these two discrete defense groups, particularly focusing on differences in responses to an important high-threat heterospecific invader cue. At very low concentrations, the volatile chemical citral (3,7-dimethyl-2,6-octadienal) is an important component of foraging recruitment pheromones in many stingless bees (Blum et al. 1970). However, stingless bees in the genus *Lestrimelitta* have evolved an obligately cleptoparasitic life history (Sakagami et al. 1993), using much higher concentrations of citral to instead recruit raiding parties to target nests (Blum et al. 1970; Wittmann et al. 1990). In response, guards of the stingless bee *T. angustula* have evolved the ability to detect high concentrations of citral as an alarm kairomone signaling the threat of nest robbing (Wittmann 1985). Although alarm pheromones in these bees are composed of multiple potentially detectable compounds (Blum et al. 1970), antennae of *T. angustula* soldiers are more sensitive to synthetic citral than those of non-soldiers (Balbuena and Farina 2020), suggesting the importance of citral in colony threat detection. We ask whether soldier responsiveness to citral differs across age types (hovering versus standing guarding) as well as across colony morphotypes in general (soldiers versus non-soldiers). We hypothesized that soldiers are more reactive to citral than non-soldiers and that hovering guards are especially reactive to citral, given that they are age-specialized heterospecific defenders.

Methods

All field and lab manipulations were conducted using subjects from naturally occurring nests in Gamboa, Panama (9.12° N, 79.70° W). Data were gathered in January 2018 and February 2019. Task group assignments for each experiment were made using established behavioral criteria (Baudier et al. 2019). Bees hovering at the entrance for longer than 20 s while facing inwards towards the flyway were considered hovering guards. Bees standing on the wax and resin nest entrance tube for longer than 20 s while facing towards the entrance opening were considered standing guards. Minors (smaller-bodied worker bees that do not typically defend the colony) were collected while in the task of foraging because this task is performed by small workers during 3–4 weeks since adult emergence, making as close as possible an age-matched comparison group to soldiers that perform the task of hovering in approximately the third week following adult emergence and the task of standing guarding in approximately the fourth week following adult emergence (Hammel et al. 2016; Baudier et al. 2019). A bee was considered a forager if it exited the nest and flew immediately away from the nest entrance (unlike hovering guards) but carried no detritus material (unlike dump workers). In a previous study, subject weights confirmed that hovering and standing guards gathered using this method were significantly larger in body size than collected foragers (Baudier et al. 2019).

Y-maze olfactory response bioassays

We conducted two types of Y-maze assays. The first assessed affinity of bees to citral, and the second assessed starvation-motivated bee affinity to a nutrition source (mixed pollen and honey). This allowed for the comparison of defense-specific olfactory response as well as appetitive olfactory acuity across the 3 focal task groups: minor bees, standing guards, and hovering guards. All Y-maze assays were performed in an ambient lab with relative humidity between 81 and 100% and temperature ranging from 24 to 30 °C.

To test the affinity of bees in different task groups for citral, we introduced bees to Y-mazes with citral at the end of one arm. A total of 54 naïve individual bees were used (6 bees × 3 task groups × 3 colonies per assay). Subject bees were first collected from nest entrances by aspirator into 38 × 84 mm vented plastic tubes and placed into a dark environment for 15 min prior to each citral Y-maze assay in order to allow bees to calm from any alarm caused during collection. We used 3D-printed Y-mazes constructed from white PLA plastic (MakerBot Industries, LLC) and, with an interlocking, transparent acrylic lid to enable observations. Y-maze arms were 10 cm long, 1 cm wide, and 1 cm tall (Figure S1). A 2.5 × 1 × 1 cm entrance vestibule adjoined to arms, each offset at 45° (90° angle between the arms). A quantity of 2 µl of 95% citral (Sigma-Aldrich, CAS Number 5392-40-5) was placed on a 4 × 4 mm filter paper, while the other arm was left with an unscented filter paper. This small dose was based on previous studies which approximated citral quantity in natural contexts, given that 1 ml of a 1% citral solution was sufficient to illicit defensive behavioral responses in this species (Jernigan et al. 2018). Air flow was provided in the maze using a pump to inhibit odor diffusion in the non-treatment arm (Figure S1). We set the air flow to 166 ml/s, strong enough to prevent the scent of citral from permeating the opposite arm (according to human olfaction), but mild enough to not inhibit the smallest bees from navigating through the maze. To eliminate directional bias, citral was alternated equally between the left and right arms of the Y-maze. Each replicate of the assay started when the subject bee was placed in the vestibule and ended only when the scented filter paper was contacted by the bee (all bees in the study touched the scented filter paper in under 5 min of observation). The time until each bee contacted the scented filter paper and each bee's first arm choice (towards versus away from odor) were recorded. A subject's first walk > 5 cm down an arm from the vestibule was considered its first arm choice. Y-mazes were scrubbed with soap and water, rinsed with water, then rinsed with ethanol, and allowed to air dry between replicates. Similar Y-maze materials and

cleaning protocols have been used in previous olfactory studies with hymenopteran subjects, without apparent complications associated with scent contamination (Carcaud et al. 2009; Provecho and Josens 2009; Arenas and Farina 2012; Duell 2018).

To test for differences in general olfactory acuity, we similarly compared the 3 groups (minors/foragers, hovering guards, and standing guards; $N = 54$ naïve bees; 6 bees × 3 task groups × 3 colonies per assay) in their ability to locate the smell of food reward in the same Y-maze setup when starved/water deprived. The same collection and Y-maze procedures were used for this, except that bees were deprived of food or water in darkened, vented tubes for 55 min prior to Y-maze introduction (to motivate food search), and a liquid food reward mixture (40% honey and 20% pollen of locally farmed *Apis mellifera*, 40% water) was applied to one end of a Y-maze arm as the focal odor source. This mixture was wiped lightly along the last 0.5 cm of one Y-maze arm. We recorded time until the liquid food mixture was contacted and the bee's first arm choice.

All analyses were conducted in R version 4.0.0 (R Core Team 2020). Variation between task groups in time until solving either food or citral odors in the Y-maze was compared using mixed model survival analyses due to the non-parametric distribution of the data. In each case, we used a Cox proportional hazard analysis that included and colony ID as a random factor and task group (forager/minor, hovering guard, standing guard) as a fixed predictor of time until bees found the odor source within the mazes using the coxme command in the package "coxme" (Therneau 2015). We then followed with a post hoc Tukey HSD test to compare among the three task groups.

To test for task group differences in first Y-maze arm choice, we fit a binomial mixed effects model with arm choice as the response variable, task group (hoverer, stander, forager) as a fixed factor, and colony as a random factor using the glmer function (Bates et al. 2015) and then performed a Type-II analysis on the fitted model to test significance of fixed effects. This was done separately for the citral and starved food choice assays.

At-nest manipulations

To test whether lab-observed patterns in citral reaction were observable in a more naturalistic field setting, we measured guard responses to citral-coated dummies at nest entrances. At each subject colony nest entrance, we introduced two mock invader treatments and two controls. The first treatment was a freshly freeze-killed worker of the stingless bee *Partamona peckolti*, an environmentally abundant competitor in the area that is black in body color (like *Lestrimelitta*) but does not use citral to recruit raids. The second treatment was an obligate nest-robber kairomone dummy consisting of a

black polymer clay figure of roughly equal size, shape, and color (black) as the average *Lestrimelitta danuncia* worker and dipped in 95% citral (approximately 0.02 ml of citral). *Tetragonisca angustula* respond similarly to actual invaders as they do to such color- and odor-matched dummies (Bowden et al. 1994).

Using this design, if the division of labor between hovering and standing guards is (at least in part) driven by differences in responsiveness to olfactory cues, then we predicted that a higher number of hovering guards would respond to the high-citral dummy, compared with the responsiveness of standing guards (Table 1). For reference, we also included a positive control for eliciting standing guard attack behavior: a freeze-killed non-nestmate *T. angustula* worker. Each intruder dummy was harnessed with wire onto the end of a dowel before being presented at nest entrances. An empty wire was also used as a negative control for attack behavior alongside each of the three treatment dummy invaders. A negative result from the empty wire control would confirm that there was no olfactory contamination of instruments and that the effect of non-olfactory disturbance at the nest (human presence, vibrational disturbance, etc.) on the aggressive behaviors of interest was minimal.

Each mock intruder or control was brought directly towards the nest entrance tube (0° angle) and contacted the edge of the tube for 2 s. During each trial, we recorded number of attackers (predominantly soldiers) and each attacker's guard type (hoverer versus stander). This is a standard method for eliciting and measuring defensive guard attack in this species (Bowden et al. 1994). Attacks were defined as contact with a mock invader for greater than 2 s. Each mock intruder and wire were discarded and replaced after use to prevent cross contamination of scents. Treatments and control wire trials were conducted 15 min apart at each subject colony and were presented in a pseudo-random order. A total of five colonies were tested in this manner, and each colony was repeatedly tested on 3 different days.

We used a mixed effect model approach to repeated-measures analysis comparing the effects of guard type and invader type on total responses. Number of bee responses was first z-transformed to improve normalcy of distributions. Next, we fit a linear mixed effect model (lme4 package, lmer function) (Bates et al. 2007; Bates et al. 2015), which included guard type (hovering vs. standing), mock intruder treatment, and the interaction between the two as fixed predictors of number of bee responses (z-transformed number of bees contacting model for at least 2 s) and included colony as a random factor. We used a Type-II Wald chi-square test of this model to test significance of fixed factors. We then followed this analysis with post hoc pairwise comparisons of standing versus hovering guard responses within each intruder treatment (colony kept as a random factor), using multiple Type-II Wald chi-square tests with adjusted p values according to

Table 1   Predictions for and results of field manipulations under the age-based olfactory shift hypothesis for guard responses to three treatment mock-intruders

Intruder treatment	Model	Predictions: citral affinity		Observed: citral affinity	
Conspecific	*Tetragonisca angustula* (no citral)	⬍	stander	⬍	stander
		⬇	hoverer	⬇	hoverer
Heterospecific	*Partamona peckolti* (no citral)	stander = hoverer		stander = hoverer	
Kairomone dummy	Clay model + Citral	⬇	stander	stander = hoverer	
		⬆	hoverer		
Control	Empty wire harness (no citral)	⃠	stander	⃠	stander
		⃠	hoverer	⃠	hoverer

Symbols indicate type of response: (⃠) no response, (⬆) higher response, (⬇) lower response, and (=) equal responses of guard types

these multiple comparisons using the Benjamini and Hochberg method (Benjamini and Hochberg 1995).

Results

Differences in odor affinity using Y-maze assays

We tested whether detection and response to citral vary between morphological sub-castes (small foragers versus large guards) or age-based guarding task groups (younger hovering guards versus older standing guards). Citral affinity, assessed by length of time until bees contacted citral sources in the mazes, differed significantly among task groups ($\chi^2 = 16.69$, $df = 3$, $p < 0.001$; Fig. 1A). Hovering guards made contact with citral odor sources significantly sooner than either standing guards ($z = -2.701$, $p = 0.019$) or minors/foragers ($z = 4.053$, $p < 0.001$). Minors (measured while in the task of foraging) and standing guards were similarly slow in navigating towards citral odor sources ($z = 1.819$, $p = 0.1624$). Task group was a marginally non-significant predictor of first arm choice in the citral baited Y-mazes (bimodal mixed model: $\chi^2 = 5.13$, $df = 2$, $p = 0.077$; Table 2), with hovering guards tending towards the citral arm and foragers tending away from the Y-maze arm that contained citral. Standing guards showed no apparent preference in first arm choice based on citral presence.

Task groups differed inversely in their expediency to find the source of food odors ($\chi^2 = 14.36$, $df = 3$, $p = 0.002$; Fig. 1B). Hovering guards took significantly longer to arrive at the honey/pollen odor source in Y-mazes than did standing guards ($z = 3.516$, $p = 0.001$) or foragers ($z = -2.720$, $p = 0.018$). Standing guards and foragers found the source of food odors in Y-mazes similarly quickly ($z = 1.156$, $p = 0.478$). First arm choice (away or towards food odors) did not differ among task groups (bimodal mixed model: $\chi^2 = 3.22$, $df = 2$, $p = 0.200$; Table 2).

Differences in at-nest intruder responses

Aggressive responses to mock intruder treatments differed according to guard type (interaction between guard type and intruder treatment: $\chi^2 = 11.899$, $df = 3$, $p = 0.008$; Fig. 2). As predicted, significantly more standing than hovering guards responded to conspecific nest intruders ($\chi^2 = 6.83$, $df = 1$, $p = 0.009$, adjusted $p = 0.036$), while neither standing nor hovering guards responded to the negative control (Type-II Wald chi-square $\chi^2 = 2.15$, $df = 1$, $p = 0.142$, adjusted $p = 0.190$). However, the difference in hovering and standing guard responses to the citral (*Lestrimelitta*-like) dummy treatment approached but did not reach significance ($\chi^2 = 3.29$, $df = 1$, $p = 0.070$, adjusted $p = 0.140$). Approximately equal numbers of hovering and standing guards responded to *P. peckolti*, the non-obligate heterospecific robber that does not use citral ($\chi^2 = 0.32$, $df = 1$, $p = 0.570$, adjusted $p = 0.570$).

Discussion

We tested whether soldier bees showed differences in defense-specific cue sensitivity compared with other workers and also whether soldier sensitivities can change with age-associated shifts in defensive task roles. Sensitivity to the kairomone citral, a cue of high-threat cleptoparasites (*Lestrimelitta* spp.), differed both between morphologically distinct worker sub-castes (soldiers vs. minors) and soldier age-associated task groups, as predicted by likelihood of each sub-caste to intercept such threats at

Table 2   Frequencies of first choices of odor versus odorless Y-maze arms across task groups and types of odors

Task	Odor	No odor
Citral		
Forager	5	13
Hoverer	12	6
Stander	9	9
Food		
Forager	13	5
Hoverer	8	10
Stander	12	6

Task group was a marginally non-significant predictor of first arm choice in citral-baited Y-mazes ($p = 0.077$) and a non-significant predictor of first arm choice in food-baited Y-mazes ($p = 0.200$)

the nest entrance. Smaller-bodied workers that were not defense specialists (foragers) showed no such affinity for citral. This result was in contrast to the citral affinity of the hovering guard soldier age group in particular. Hovering guards showed higher responsiveness than standing guards to citral in general while standing guards appeared more reactive to conspecific invader cues in the field. In this way, intruder cue sensitivity appears to vary between defense and non-defensive specialists and is flexible across time. This fits into the general idea that combining temporary specificity in defense and flexibility is beneficial for mitigating complex environmental challenges.

These findings were consistent with the hypothesis that different behavioral sensitivities contribute to a functional benefit of age polyethism and morphological specialization within defensive tasks. Hovering and standing guards have recently been shown to have similar antennal sensitivity to citral, with soldiers

in general showing higher antennal sensitivity than foragers (Balbuena and Farina 2020). Together with our results, this suggests that foragers may be less inclined than soldiers to respond aggressively to citral due to (at least in-part) lowered ability to sense it, but that heightened hovering compared with standing guard citral response is not due to sensory ability, but rather some other age-associated change in behavioral response threshold (Beshers et al. 1999). In other words, although older standing guards are equally able to sense citral, we report that they were less excited into action by citral compared with younger hovering guards. Adaptive age-dependent behavioral sensitivity to odors has been shown in a handful of other insect species, for example, moths (Jarriault et al. 2009; Abrieux et al. 2014), locusts (Ignell et al. 2001) and honey bees (Pham-Delegue et al. 1993; Vergoz et al. 2009). Age-based differences in citral sensitivity may similarly benefit nest defense efficacy in *T. angustula*.

Our Y-maze studies indicated that hovering guards have a heightened affinity for the kairomone citral relative to standing guards or foraging minors when other attacker cues were absent (Fig. 1A). This finding is directionally consistent with the marginally non-significant differences in numbers of responding standing and hovering guards to dummy intruders with the cue presented at nest entrances (Fig. 2). It is also consistent with previous studies showing a higher response of hovering guards versus standing guards to similar citral-scented dummies (Bowden et al. 1994; Kärcher and Ratnieks 2009; Grüter et al. 2011). Because it has only come to light recently that standing guarding and hovering guarding are age-partitioned tasks
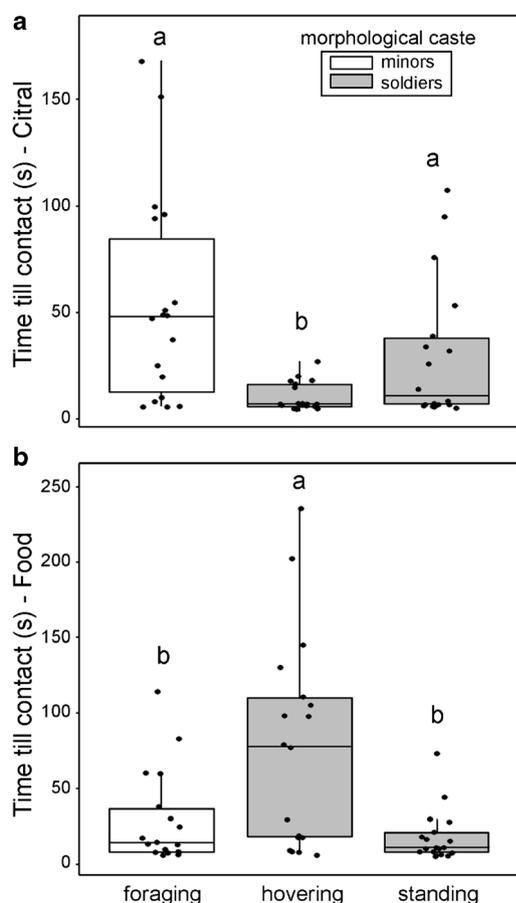

Fig. 1 Results of Y-maze olfactory response bioassays, showing time until bees found either citral (A) or food (B) odor sources within Y-mazes across focal bee task groups. Grey box shading denotes morphologically larger soldier bees, while white denotes morphologically smaller worker bees: "minors." All bees contacted odor sources within 5 min. Lowercase letters represent results of mixed model Cox proportional hazards analyses followed by post hoc Tukey HSD tests. Hovering guards found citral odor sources more rapidly and food odor sources more slowly than either older standing guards or smaller-bodied foragers. $N = 18$ naïve bees were used per task group and stimulus type, evenly sampled across 3 colonies
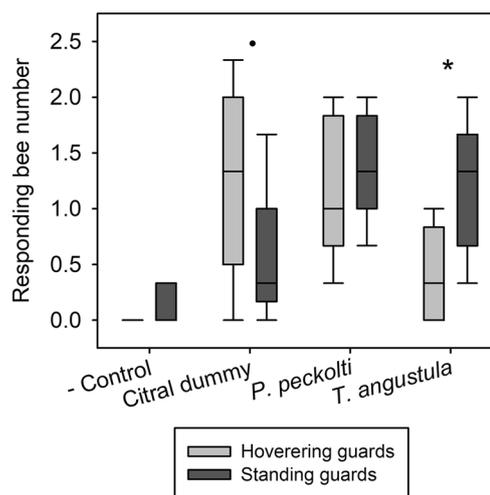

Fig. 2 Results of at-nest manipulations, showing intruder model differences in hovering versus standing guard defensive response. Non-citral-using heterospecific and conspecific intruder models were freeze-killed bees (a *Partamona peckolti* worker and a non-nestmate *Tetragonisca angustula* worker, respectively). The citral dummy was a bee-shaped clay dummy dipped in citral, providing an olfactory signal resembling that of obligate nest robber bees in genus *Lestrimelitta*. Responding guard counts across replicates and per scenario are presented. An un-transformed y-axis is shown here, but mixed effect analyses were performed on z-transformed response numbers. "•" denotes $0.05 > p \leq 0.1$, and "*" denotes $p \leq 0.05$ in this analysis

(Baudier et al. 2019), this is the first evidence of differential sensitivities being innately attributable to younger versus older soldiers across these task groups rather than arising as a passive result of position in defense formations at the nest entrance. As such, this is perhaps the first report of invader cue specialization among soldier sub-types in social insects at large.

One explanation for why differences between standing and hovering guard responses to citral were seen between lab and field studies may have to do with the relatively higher quantity of citral used in the field scenarios. Because this quantity of citral is more akin to the amount of citral present when multiple *Lestrimelitta* attack at once, quantities over a certain amount may be more likely to illicit both standing and hovering guard response. Intriguingly, the marginally non-significant trend of higher hovering than standing responders to citral at nest entrances appears to be driven by a decrease in standing guard response relative to a non-citral-bearing heterospecific invader rather than a higher hovering guard response to invaders with citral (Fig. 2). Although Y-maze results suggest that citral is a stimulator of hovering guard aggression (Fig. 1A), at-nest manipulations suggested that citral may also act as an inhibitor of standing guard action. If this is indeed the case, then this represents a unique form of olfactory-based invader-type task partitioning of colony defense. This possibility therefore merits further investigation.

A possible mechanistic explanation of the differences in citral responsiveness among guard types is that neurologically based olfactory acuity or processing underlies age shifts in task role; in this case, changes manifest in the sensitivity to citral, a major and distinctive cue of an important nest invader. The potential for neurosensory shifts with such rapid forms of age polyethism have not been well studied. However, heightened sensitivity to citral in hovering guards does not appear to be paired with overall heightened olfactory acuity, given that starved hovering guards were significantly slower to locate food in Y-mazes than either standing guards or foraging minors (Fig. 1B). Recent work reports no difference in antennal sensitivity to citral between soldier types (Balbuena and Farina 2020). However, whether neurological differences in sensory processing drive these contrasts in citral reactivity merits further study. We are currently investigating how neural investment between sensory-modality-specific brain regions differs across these task groups.

In addition to potential fixed age-based differences in olfactory sensing, learning may also differ across these age-based task groups. Learning in general has been shown to increase with age in older foraging honey bees (Pham-Delegue et al. 1990), perhaps due to experience-dependent plasticity of the mushroom bodies, the sensory integration center of the brain (Cabirol et al. 2018). Ability to learn olfactory cues in particular also differs with age in young honey bees (Arenas and Farina 2008). It is possible that general

olfactory learning may be higher in older standing guards or in smaller workers engaged in the terminal task of foraging. It might also be the case that minors which spend half of their adult life foraging have a higher learning acquisition to food odors and guards may have a higher propensity for learning temporally relevant invader cues. If this is the case, similar processes can be seen in advanced age honey bees that acquire flower search images while in the terminal task of foraging (Chittka et al. 1999; Ishii and Masuda 2014). Whether hovering guards are similarly forming an intruder search image versus having an innate propensity for responding to citral at this age stage remains an interesting question. However, to our knowledge, there has been no previous study of olfactory learning across different soldier sub-types in this or other species of eusocial insects. Present and future work studying the mechanisms of advanced age task-switching in social insect soldiers opens new avenues for exploring the ontogeny of signal processing as it relates to group defense, but potentially also senescence.

Although here we focused on one important kairomone, many cues across multiple modalities are simultaneously processed by guards on patrol at nest entrances to detect and respond appropriately to a wide variety of nest intruders. *Tetragonisca angustula* guards use visual, olfactory, and potentially also vibrational information to identify different types of colony threats at nest entrances (Wittmann 1985; Zeil and Wittmann 1989; Kelber and Zeil 1990; Bowden et al. 1994; van Zweden et al. 2011; Ostwald et al. 2018). The multimodality of these complex behaviors may account for the difference in citral response contrasts between lab and field components of this study. More work is needed to parse the intricacies of this multimodal integration in nest defense.

Conclusion

The stingless bee *T. angustula* uses a strategy of age-based task partitioning of soldiers to accomplish nest defense in an environment with a diversity of competitors, cleptoparasites, and predators (Baudier et al. 2019). Here we report that these age-differentiated soldier types are further (albeit, temporarily) specialized in threat cue detection. Specialization on specific tasks has been shown to reduce time costs of task-switching in other eusocial insects (Leighton et al. 2016). By age-partitioning guarding tasks with cue-specialized soldier types, *T. angustula* soldiers may improve both efficiency and accuracy of defense. This strategy resembles the highly sub-specialized defensive roles of humans in military units, in which, at the large scale, individuals perform different defensive duties according to individual differences in experience and aptitude. The need for future, taxonomically diverse investigations into defensive sub-specialization is high. Such work has the potential to expose understudied commonalities

of group defense shared by an array of cooperative societies. Possible impacts of such discoveries are diverse and, alongside the findings we present here, provide useful empirical insight to the growing field of social-insect-inspired strategies for problem-solving in scenarios of human social defense, multi-robot systems, and beyond (Korczynski et al. 2016; Strickland et al. 2018).

Data availability Data used in this study are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.h18931zj3)

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