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Journal
Behavioral Ecology and Sociobiology, 73(9)

ISSN
0340-5443

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Publication Date
2019-09-01

DOI
10.1007/s00265-019-2731-7

Behavioral Ecology and Sociobiology (2019) 73: 123
https://doi.org/10.1007/s00265-019-2731-7

ORIGINAL ARTICLE

Task repertoires of hygienic workers reveal a link between specialized necrophoric behaviors in honey bees

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Received: 19 March 2019 / Revised: 7 July 2019 / Accepted: 22 July 2019 / Published online: 9 August 2019

Abstract

The ecological success of social insects is often attributed to their complex social organization and division of labor. Much previous work has investigated the extent to which individual workers within colonies specialize on certain tasks, which presumably increases colony efficiency. Fewer studies have investigated the extent to which individual biases for multiple related tasks adaptively shape division of labor. Here, we focus on honey bee (*Apis mellifera*) workers that perform hygienic behavior, the detection and removal of diseased larvae in order to reduce pathogen transmission within the hive. We individually marked workers observed performing this rare task and tracked their behavior over three days to fully characterize their task repertoires. We then compared the task repertoires of workers who specialized on hygienic behavior to generalist workers who occasionally perform hygienic behavior to identify additional task biases in hygienic specialists. We show that workers specialized in hygienic behavior are much more likely to also specialize in removing dead adult bodies from the hive, a task that is also associated with colony defense against disease. Our results demonstrate that individual specialization in one task can predict specialization in another related task. We argue these associations represent a type of specialization in multiple tasks that may be an important aspect of how insect colonies adaptively allocate their workers.

Significance statement

Any animal group that divides up work among individuals faces the challenge of figuring out the best way to allocate workers towards certain tasks. One mechanism by which large groups can increase their efficiency is by utilizing specialized workers dedicated to a single task. However, it has also been suggested that if multiple tasks require similar skills or are spatially associated with one another, then group efficiency can be further increased by using the same set of workers to perform those tasks as well. Here, we show that honey bee workers specialized in removing dead larvae from the brood zone of the hive are much more likely than bees of the same age to also specialize in removing dead adults from the bottom of the hive. We suggest that this task association has implications for colony fitness and exemplifies an understudied component of how social insect colonies allocate their workers.

Keywords Honey bees · Division of labor · Hygienic behavior · Specialization · Social insects · Social immunity

Introduction

The ecological success of social insects is often attributed to their complex systems of division of labor (Oster and Wilson 1978; Hölldobler and Wilson 1990). Variation in age and morphology often underlie the basic distribution of individuals towards particular tasks (Kerr and Hebling 1964; Oster and Wilson 1978; Seeley 1982; Seeley and Kolmes 1991; Johnson 2010b). Much research has further focused on inter-individual variation in workers within these broadly defined castes of workers (Jeanne 1988). Special attention in these studies is often paid to workers that focus on specific tasks, often labeled as "specialists" (Visscher 1983; Moore et al. 1987; Kolmes 1989; O'Donnell and Jeanne 1992; Johnson 2002; Gardner et al. 2007; Jandt et al. 2009).

Specialist workers have been defined broadly as individuals with a bias for a particular task (Johnson 2010a) or more

Communicated by O. Rueppell

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narrowly as individuals that perform a single task to the exclusion or limitation of other behaviors (Robson and Traniello 2002). Specialized workers, based on either criterion, have been characterized for a number of tasks in social wasps, ants, and bees (O'Donnell and Jeanne 1992, Hart and Ratnieks 2002, Gardner et al. 2007). This work has been particularly pronounced in the study of the honey bee (*Apis mellifera*), where specialists have been identified for many tasks such as allogrooming, guarding the nest entrance, and wax work (Visscher 1983; Moore et al. 1987; Kolmes 1989; Robinson and Page 1989; Page and Robinson 1991; Trumbo et al. 1997; Breed et al. 1990; Johnson 2002). Honey bee workers specialize on tasks that are both common and rare, and individuals vary in the degree to which they persistently perform any particular behavior.

Hygienic behavior in the honey bee is a rare task that has received much attention due to its role as a mechanism of social immunity against brood diseases (Rothenbuhler 1964a, 1964b; Spivak and Gilliam 1993; Spivak 1996; Masterman et al. 2001; Arathi et al. 2006; Paleolog 2009; Wagoner et al. 2018; McAfee et al. 2018). Hygienic behavior is composed of two sequential acts: the uncapping of diseased brood in a cell and the removal of the diseased brood from the hive (Rothenbuhler 1964a, 1964b). Timely removal of diseased pupae has been demonstrated to effectively limit the transmission of various pathogens within a hive, which keeps the colony healthy (reviewed in Wilson-Rich et al. 2009 and Evans and Spivak 2010).

Past work on hygienic behavior has identified variation in the trait at both the colony (Spivak and Gilliam 1993; Pérez-Sato et al. 2009; Bigio et al. 2014) and individual levels (Arathi et al. 2000; Arathi et al. 2006; Scannapieco et al. 2016). Consistent with the response threshold model of division of labor (Robinson 1987; Theraulaz et al. 1998; Beshers et al. 1999; Pankiw and Page 2000), the probability with which workers perform hygienic tasks is the result of variation in sensitivity to the olfactory stimuli associated with recognizing diseased brood (Masterman et al. 2000; Masterman et al. 2001; Spivak et al. 2003; Chakroborty et al. 2015). Studies focused on the volatile chemicals eliciting the behavior have identified a number of odors associated with brood infected with specific pathogens and with dead brood in general (Nazzi et al. 2004; Swanson et al. 2009; McAfee et al. 2018).

A majority of what is known about hygienic behavior comes from experiments using colonies that have been artificially selected for rapid or slow performance of the behavior. Studies using workers from both stocks have revealed that different mixtures of phenotypes within a colony affect the extent to which workers partition hygienic behaviors. That is to say, although some workers perform the full sequence of tasks, other workers focus on either uncapping or removing sick brood (Scannapieco et al. 2016). The number of hygienic workers in a colony and the extent to which they partition these subtasks ultimately affects how efficiently hygienic behavior is used as a defense against disease (Arathi et al. 2006).

Although considerable work has examined the proximate and ultimate causes of hygienic behavior, no studies have attempted to characterize the long-term task repertoires of hygienic workers beyond the context of uncapping and removal behavior. Theory on division of labor predicts that workers focused on one task may also perform tasks that are located in the same area or require similar skills to maximize group efficiency (Oster and Wilson 1978; Seeley 1982). Moreover, regardless of high task fidelity, it is likely that demand for hygienic behavior waxes and wanes based on pathogen load, and hygienic workers are thus required to shift to other tasks or become inactive depending on demand. The middle-aged caste of workers collectively displays a large task repertoire (Johnson 2002, 2008, 2009, 2010b), which includes hygienic behavior and other behaviors that contribute to defense against infection (Wilson-Rich et al. 2009). Researchers have occasionally speculated on whether or not hygienic behavior may be associated with these other social immunity tasks such as allogrooming (the grooming of other workers) or undertaking (the removal of dead adult bodies from the hive) (Spivak 1996; Barron and Robinson 2005). Altogether, there are many reasons to suspect that workers specializing in hygienic behavior may show biases for tasks beyond uncapping or removing diseased brood, and these task associations may have implications for colony ergonomics.

The present work addresses several questions. We sought to quantify the degree of specialization of hygienic workers, defined here as any worker observed engaging in hygienic behavior, and to determine how important specialists are to both hygienic work and other tasks performed by middle-aged bees. We further asked if any other tasks were associated with hygienic behavior based on a comparison of the task repertoires of generalists, who rarely perform hygienic behavior, and hygienic specialists who focus on this task.

Materials and methods

Study site and observation hives

All experiments were performed at the Harry Laidlaw Honey Bee Research Facility at the University of California, Davis. All trials were performed during the summer of 2018 between late-May and mid-August. Four-frame observation hives were set up in early April, giving each colony ample time to acclimate to its location and setting. Frames in each observation hive were arranged in a single vertical column. Frames were arranged such that the bottom two frames contained brood and pollen while the upper two frames contained nectar and honey in order to maintain the normal organization of a honey bee nest (Seeley 1978).

Experiment 1 design

The goal of this experiment was to classify the task repertoires of middle age bees, including hygienic specialists. Although previous work has done this for middle age bees, hygienic behavior is normally a rare task meaning few, if any, observations of it were recorded in past work (Seeley and Kolmes 1991; Trumbo et al. 1997; Johnson 2002; Johnson 2008). Hence, the basic design of the experiment was to introduce a large cohort of individually marked bees to an observation hive, increase colony demand for hygienic behavior and other key tasks, and compare the task repertoires of hygienic specialists to other middle age bees. We replicated this experiment in three different colonies.

Focal bees

We marked newly emerged workers taken from unrelated colonies using a colored number tag attached to the thorax and a paint mark on the lower abdomen as per previous studies (Seeley and Kolmes 1991). For each trial, we individually marked a cohort of 350 bees. Cohorts were introduced to an observation hive when less than 1 day old. Marked bees were observed for three days, ages 15–17 days, when the cohort had on average transitioned into the middle-aged caste (Johnson and Frost 2012).

Stimulating hygienic behavior and other tasks

Fourteen days post introduction, we replaced the upper brood frame of the hive with a frame containing six sections of frozen killed brood, a common technique for eliciting hygienic behavior in which capped pupae sacrificed using liquid nitrogen simulates diseased brood for removal (Spivak and Downey 1998; Arathi and Spivak 2001). We also damaged the top frame of honey using a hive tool to increase demand for more common middle-aged bee behaviors, such as wax work. Lastly, we increased demand for undertaking by introducing 50 dead bodies to the ramp of the focal hive every morning before observation. Scan sampling began the morning after these manipulations were performed.

Experiment 2 design

The goal of this experiment was essentially the same as experiment 1; however, we modified our marking procedure in order to gain more information on the task behavior of hygienic specialists in particular. Previous studies have shown that most tasks, both common and rare, are predominately performed by generalists that show no persistency in their performance of any given task (Visscher 1983; Moore et al. 1987; Johnson 2002). Thus, out of the small percentage of workers that perform hygienic behavior, it is likely that an even smaller proportion of those workers performs the task to a disproportionate extent. The design for this experiment was therefore to mark large groups of workers that were observed performing hygienic behavior and compare the task repertoires of workers that did or did not show a significant subsequent bias for hygienic behavior (i.e., specialists versus generalists).

Essentially, based on past work, we predicted that most of the bees we marked performing hygienic behavior would not be observed to perform this task again because they are generalists, while a small fraction of our marked workers would be hygienic specialists. We performed this experiment in three different colonies. Two of the colonies were used in experiment 1, while one of the colonies was new to this study. We performed this experiment after all the bees in the previous study died, meaning the past use of our colonies for experiment 1 had no effect on this experiment.

Focal bees

We began by replacing the upper brood frame of an observation hive with a frame containing six sections of frozen killed brood. We then also replaced the glass cover of one side of an observation hive with mesh fabric. Over the next 4 h, 90 workers were individually marked using a combination of paint marks to the thorax and abdomen through the mesh fabric (von Frisch 1967). We only marked workers that were observed removing dead brood from their cells. Hence, all workers in these trials had been observed performing hygienic behavior. These workers were then observed over three days, starting the day after marking.

Stimulating hygienic behavior and other tasks

At the end of the marking day, we damaged the comb as in experiment 1, replaced the frozen killed brood frame with a fresh frame of frozen killed brood, and replaced the mesh fabric with the original glass cover. We added 50 dead bodies to the focal hive at the beginning of each observation day as in experiment 1.

Scan sampling

We scan sampled each colony ten times per day at the top of each hour from 0800 to 1700, when workers are most active. Scan sampling was performed as per previous studies (Seeley 1982; Johnson 2002, 2003). Briefly, the entire nest was scanned starting from the top left corner of the colony and then working across to the right and then down incrementally for both sides. For any observed bee, their individual ID, task behavior, and location (using a 2″ × 2″ grid drawn on the glass) were recorded. All scan observations were conducted by the same observer to reduce variance in task discrimination.

Classification of tasks and task repertoires

Definitions for identifying each behavior matched those described in earlier studies (Seeley 1982, Johnson 2002). To focus on task repertoires in particular, we removed all non-task behaviors from the data (e.g., walking, standing) prior to analysis. To keep our comparisons of task repertoires solely focused on bees in the middle-aged caste, we removed behaviors from individuals that were identified as either nurses or foragers based on caste-indicative tasks (e.g., feeding brood, carrying pollen) (Johnson 2008). Prior to analyzing individual task repertoires, we removed all workers that were observed performing less than three tasks to reduce the influence of rarely observed individuals.

Statistics

Experiment 1 did not require formal statistical analysis. Data from all three trials of experiment 2 were pooled together for analysis. We compared the group-level task repertoires of hygienic specialists and generalists using a chi-square test of independence on the task set of both groups. We then used standardized residuals to identify which tasks significantly contributed to the non-independence of group identity and task behavior. To analyze associations among tasks based on individual task repertories, we performed a correspondence analysis using the R packages "FactoMineR" (Lê et al. 2008) and "factoextra" (Kassambara and Mundt 2016). Additionally, we used the "cluster" package (Maechler 2018) to conduct a hierarchical cluster analysis of individuals based on their task repertoires using the "Euclidean" distance method and "complete" clustering method. Data were scaled and centered before calculating distances (Romesburg 2004). Using the "pvclust" package (Suzuki and Shimodaira 2006), we used multiscale bootstrap sampling to estimate p values for task-association clusters based on individual repertoires. All statistical analyses were performed in RStudio version 3.5.0 (RStudio Team 2015).

Results

Experiment 1

Table 1 shows the task performance frequencies of tagged middle-aged bees in the presence of elevated stimulus for hygienic behavior, undertaking, and wax work ($N = 437$ workers). Workers performed a wide variety of tasks in the hive but spent most of their time wax working (34% of observations) and inspecting (29%). Only a single instance of undertaking was observed, and workers were never observed performing hygienic behavior.

Table 1 Task performance frequencies of generalist middle-aged workers in experiment 1 ($N = 437$ workers)

Task	Frequency	Relative frequency
Allogroom	52	2.2%
Cement	49	2.0%
Clean cell	85	3.5%
Fan	65	2.7%
Hygienic behavior	0	0%
Inspect	697	29.0%
Pack pollen	25	1.0%
Process nectar	67	2.8%
Trim brood capping	185	7.7%
Trim honey capping	5	0.2%
Trophallax	237	9.9%
Undertake	1	0.04%
Washboard	115	4.8%
Wax work	819	34.0%

Experiment 2

Identifying hygienic behavior specialists

To test for specialization, the number of observations of hygienic behavior per worker during scan samples was compared with a randomly generated Poisson distribution with the same mean (Kolmes 1989). Figure 1 shows these two distributions. Frequencies from these distributions are significantly different from one another based on Fisher's exact test ($p = 0.002$). The randomly generated distribution predicted that no workers would perform the behavior more than once. We therefore identified 5 specialist workers that performed hygienic behavior more often than what would be expected by chance.

Task performance of hygienic specialists compared with generalists

Figure 2 shows the differentiated task repertoires of hygienic specialists in comparison with generalists. A chi-square test of independence on the task performance frequencies of both groups indicated that there is a significant association between hygienic specialists and particular tasks ($\chi^2 = 81.138$, df = 11, $p < 0.0001$). Based on standardized residuals from the test, the only task that significantly contributes to the χ^2 value is undertaking, a task for which hygienic specialists show a highly disproportionate bias (standardized residual = 8.70). Hygienic specialists were most frequently observed performing hygienic behavior and undertaking, and there were a number of tasks that hygienic specialists did not perform at all. Generalists were most often observed working wax, inspecting, and exchanging food.

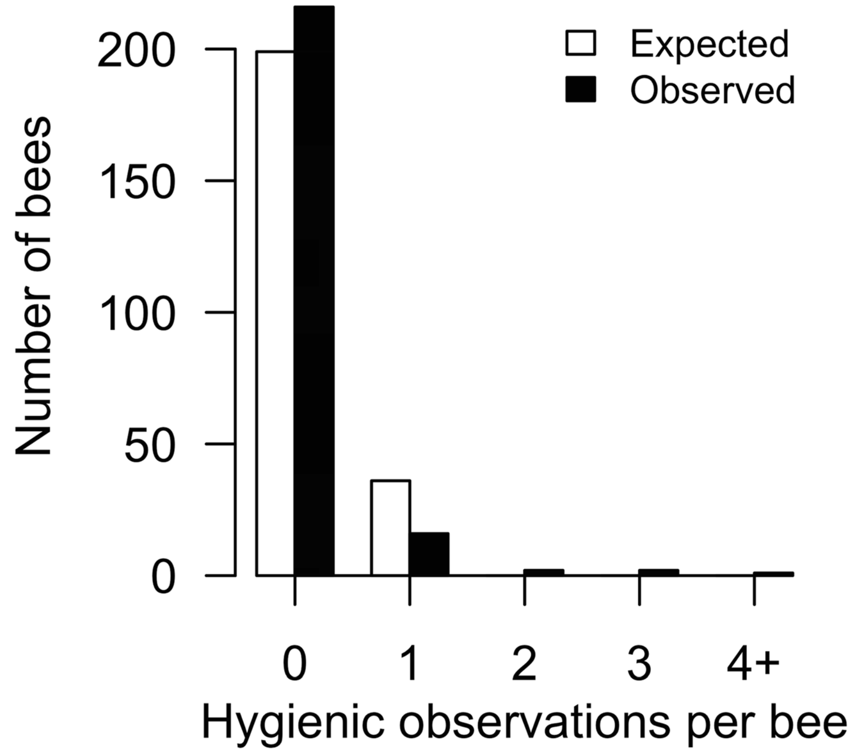


Fig. 1 Test for specialized hygienic workers. A Poisson distribution generated using the average number of hygienic task observations per bee as observed in our data was compared with the actual distribution of hygienic observations per bee

Contribution of hygienic specialists to group-level task performance

Figure 3 shows the contribution of hygienic specialists to group-level performance of the tasks they were observed to also engage in. The black portion of each column shows the proportion of task performance for each task that is attributable to hygienic specialists. Hygienic specialists made up 3% of workers marked during experiment 2 but performed 48% of hygienic behavior and 25% of undertaking observed in this group of workers. The other tasks hygienic specialists performed were done to the relative proportion expected assuming equal probability of performance (indicated by the red line).

Correlated task choices

To explore correlated task choices at the individual level, we examined the task repertoires of workers in experiment 2 without explicitly grouping workers into specialists and generalists. This is useful and important because our previous analysis based on a statistical classification is probably overly conservative in that some of the bees observed performing hygienic behavior only once during scans may have also been specialists. Testing for a correlation between hygienic behavior and undertaking using all collected data is thus warranted.

Results from a correspondence analysis of individual worker repertoires are shown in Fig. 4. Based on the scree plot diagnostic (figure not shown), we kept two dimensions for interpretation (Hayton et al. 2004). Together, these two dimensions explain a considerable amount of variation in the large set of data (36%). Dimension 1 predominately represents allogrooming behavior (91% dimension contribution), and dimension 2 predominately represents undertaking and hygienic behavior (60% and 26% dimension contributions, respectively). The plot shows which tasks are highly discriminating (distance from the origin) and which tasks are highly associated with one another (degree of angle connecting two tasks to the origin). The results show that hygienic behavior, undertaking, and allogrooming are the tasks that most differentiate workers from one another, and that hygienic behavior and undertaking are highly associated with each other (based on the highly acute angle of the two rays connecting the behaviors from the origin).

In contrast to the correspondence analysis, which uses a dimension reduction approach, we also performed a cluster analysis, which quantifies associations among all tasks in worker repertoires. Figure 5 shows the results of this analysis, which also identifies a significant connection between undertaking and hygienic behavior. The results also indicate that inspecting and wax work are strongly associated tasks within individual worker repertoires (based on approximately unbiased p value ≥ 95; Suzuki and Shimodaira 2013).

Discussion

This work provides insights into the behavior of hygienic workers, an important group of bees for social immunity.

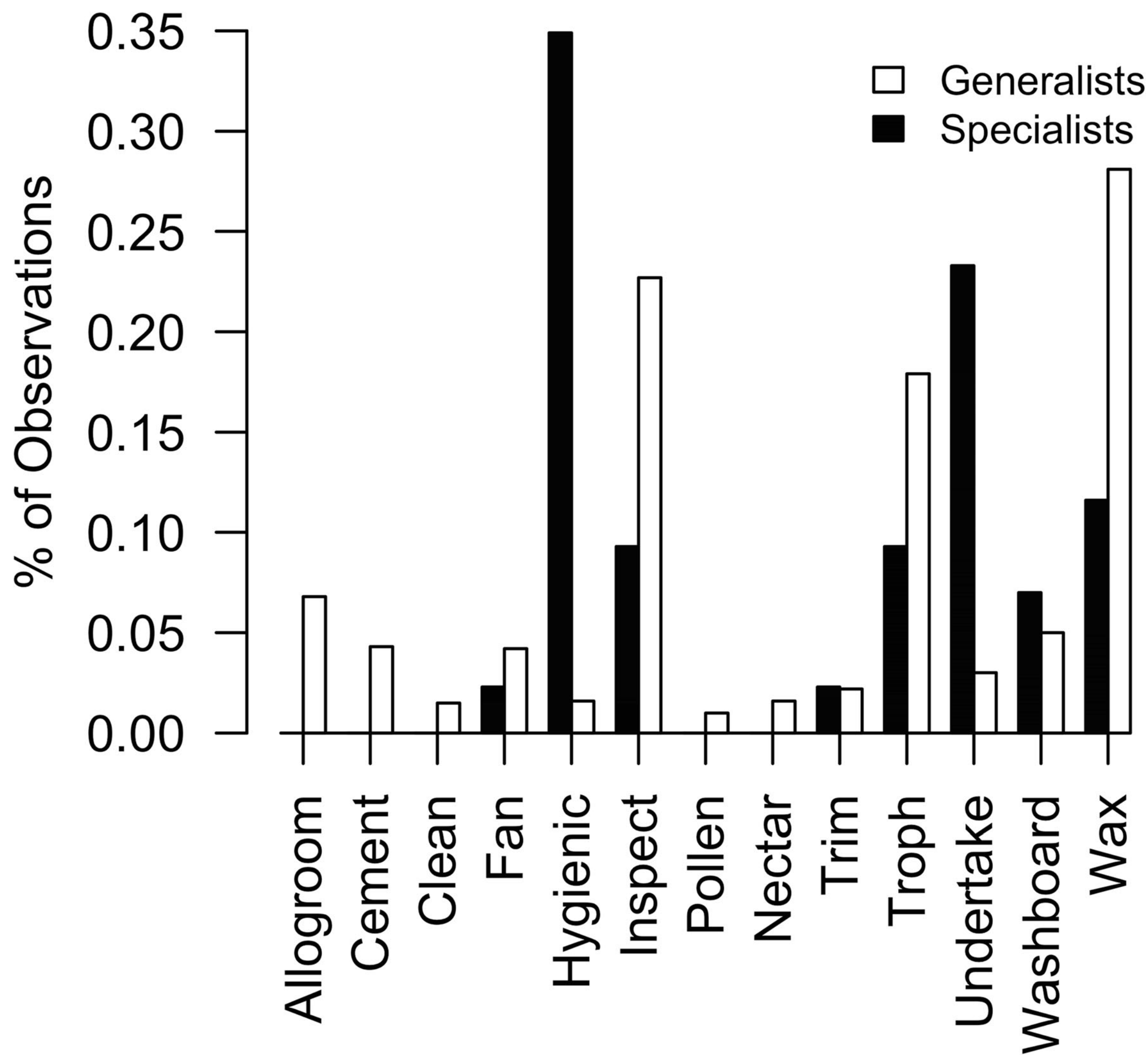
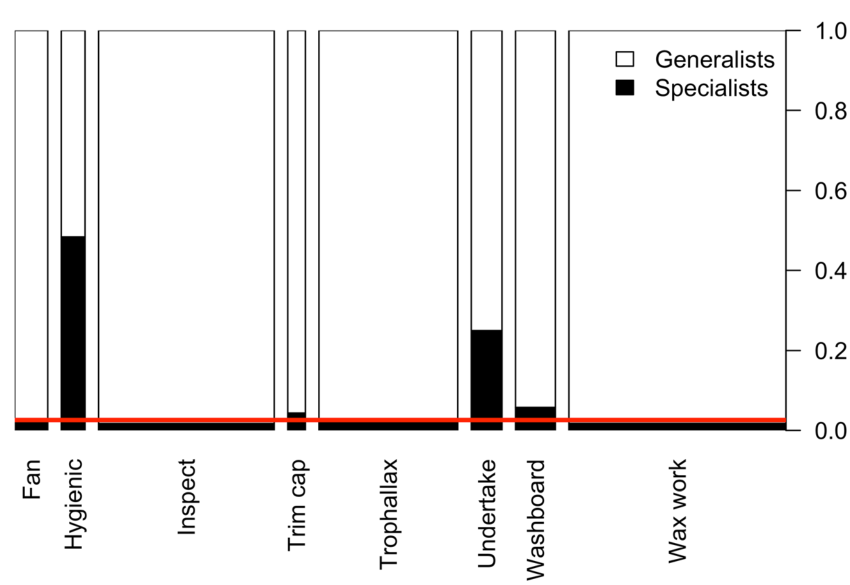


Fig. 2 Task repertoires of hygienic specialists and generalist workers in experiment 2

Fig. 3 Contribution of hygienic specialists to group-level task performance of specialists and generalists ($N = 43$ and 990 task observations for hygienic specialists and generalists, respectively). Tasks never performed by hygienic specialists are not shown to improve readability



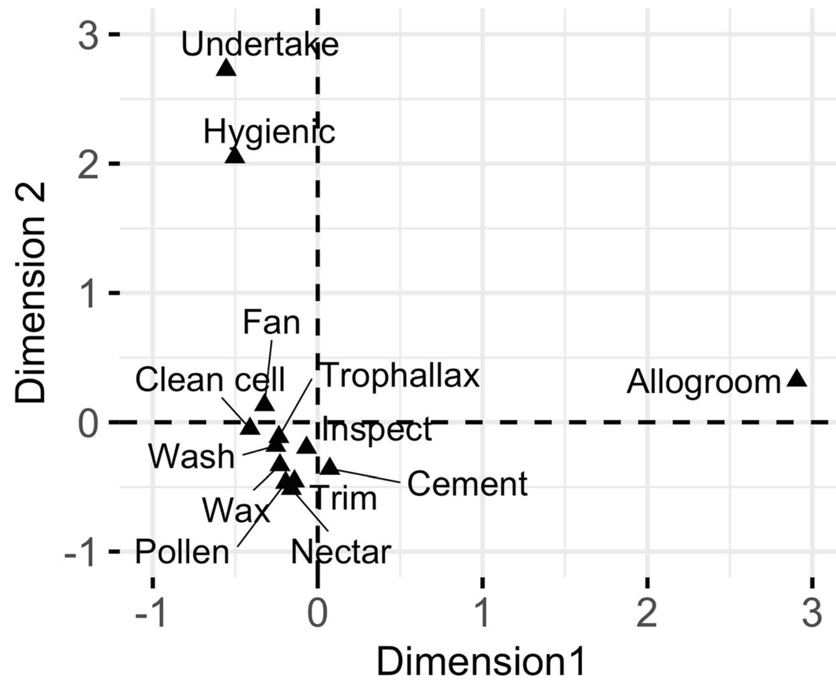


Fig. 4 Correspondence analysis results identifying discriminating tasks and associations among task behaviors

Although previous studies have demonstrated individual variation in the performance of hygienic behaviors (Arathi and Spivak 2001; Arathi et al. 2006), our study is the first to focus on the additional task biases demonstrated by specialists for the behavior. We found that hygienic behavior and undertaking are strongly associated such that individuals with a bias for removing dead brood from their cell are also highly likely to remove dead adults from the hive. We discuss the performance and partitioning of hygienic behavior in our colonies, the differentiated behavior of hygienic specialists, and the proximate and ultimate causes of the correlated task choices observed in specialists.

Performance and partitioning of hygienic behavior

Our initial experiment showed that even when stimuli for hygienic behavior and undertaking are abnormally high, most middle-aged workers do not perform these tasks (Table 1). This result aligns with what others have previously observed (Visscher 1983; Arathi et al. 2000) and suggests that most workers do not engage in these tasks at all. Likewise, the relative frequency with which other tasks were performed agrees with previous studies indicating that wax work and inspecting make up most of the task repertoire of generalist middle-aged bees (Lindauer 1967; Kolmes 1986; Seeley 1995; Johnson 2008).

Experiment 2 showed that the majority of workers that do engage in hygienic behavior show little to no persistency for the task while a few individuals perform the task to a significantly disproportionate extent (Fig. 1). The percentage of hygienic specialists identified in our study (3% of workers) resembles what has been seen in previous work on undertaking, guarding, allogrooming, and wax work (Visscher 1983; Moore et al. 1987; Kolmes 1989; Johnson 2002). A common conclusion from these earlier studies is that task specialists still only contribute a relatively small amount towards group-level performance of their task. Our results, however, indicate that the contribution of hygienic specialists is noticeably important for group-level performance as specialists performed 48% of hygienic behavior observed across all three trials (Fig. 3). Yet, this result is only suggestive as the number of times we observed hygienic behavior is small in comparison with the number of dead pupae that were introduced to the colony. Colony-level performance of the task was thus mostly attributable to works that went unmarked and unclassified as either generalists or specialists.

The extent to which hygienic behaviors have been observed to be partitioned among workers has been variable in previous work (Arathi et al. 2000; Arathi and Spivak 2001; Arathi et al. 2006; Paleolog 2009; Scannapieco et al. 2016). However, even early studies concluding that all hygienic workers perform both subtasks note that some workers show preference for uncapping dead brood but not removing them from their cell, and some workers remove dead brood from their cell but then drop the brood to the bottom of the hive for other workers to then remove from the hive (Arathi et al. 2000; Arathi and Spivak 2001). Our results indicate a strong division between workers that uncap and those that remove as we marked workers that specifically removed dead brood from their cell and only saw removal behavior during our scans, either from a cell or from the bottom of the colony. Although Arathi et al. (2000) attributed the removal of dropped brood to undertakers with no relation to hygienic behavior, our study shows that while these workers do

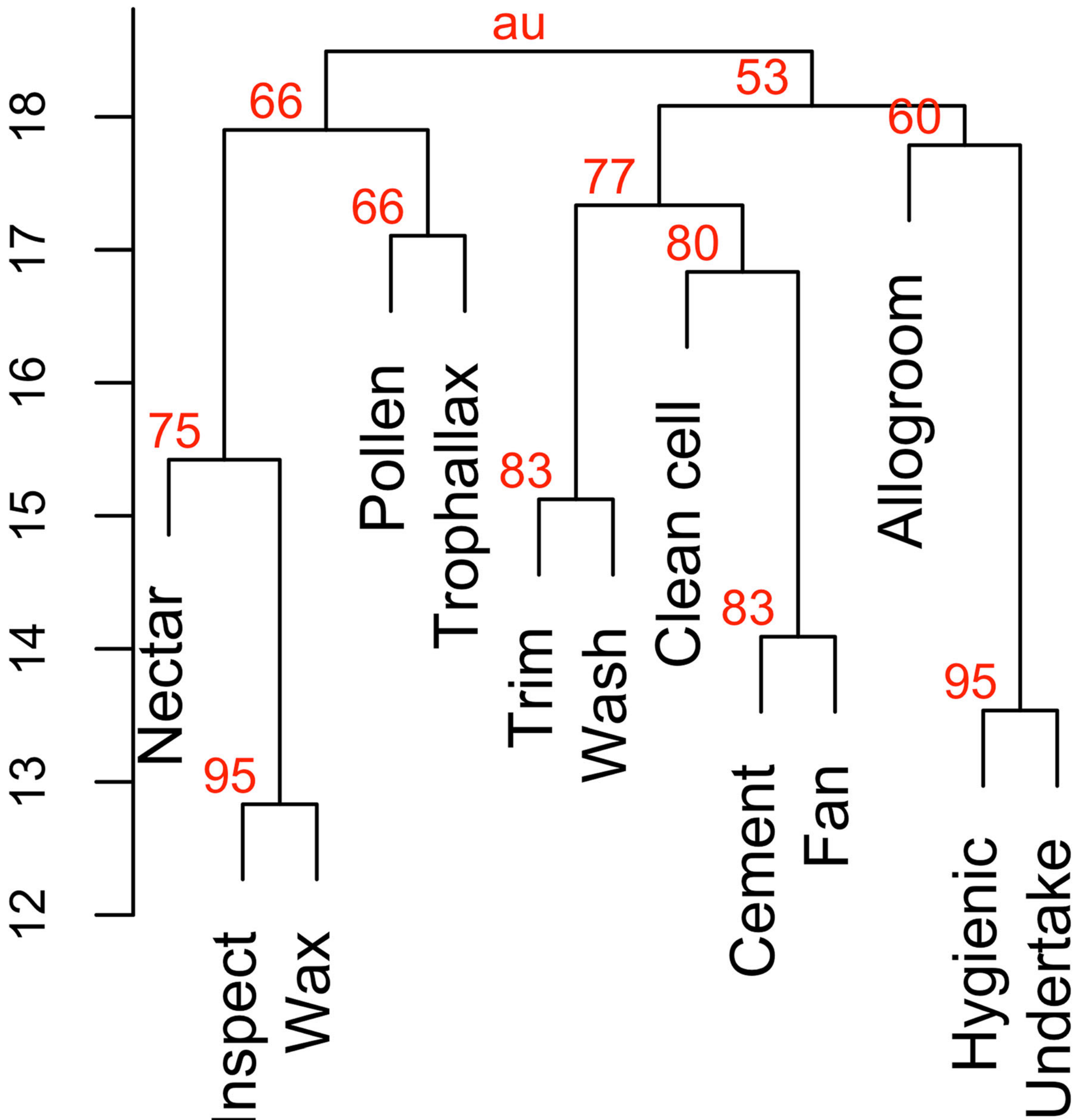


Fig. 5 Task association dendrogram based on the results of a cluster analysis. Approximately unbiased (au) *p* values based on 7500 bootstrap samples are shown. Tasks that cluster together are highly associated with one another based on individual worker repertoires

remove dead adults, they are also workers that were, or are still, involved in removing dead brood from their cells. The lack of uncapping behavior observed by our marked workers provides strong evidence for high levels of task partitioning in our colonies, and a study similar to ours focused on workers that specialize in uncapping behavior could deliver different results.

The differentiated behavior of hygienic specialists

Previous researchers have presented slightly different concepts of what constitutes a specialized worker. Hygienic specialists identified here exhibit a task repertoire in which they spent most of their time performing hygienic behavior and undertaking, and there are a number of tasks that they never engaged in (Fig. 2). Hygienic specialists could therefore be categorized as "idiosyncratic" (Trumbo et al. 1997) in that they perform rare tasks in favor of more common ones although they still occasionally perform the most common middle-aged bee behaviors. They do not, however, show the highly limited task set expected in maximally efficient specialists as their task repertoires do include these additional non-hygienic tasks (Oster and Wilson 1978; Kolmes 1986; Robson and Traniello 2002). There is also no evidence to conclude that hygienic specialists are highly active elites or reserve workers, as they showed no discernable difference in activity level in comparison with generalists (Plowright and Plowright 1988; Dornhaus 2008; Dornhaus et al. 2008).

Models of middle-aged bee task behavior have emphasized that middle-aged bees frequently quit their current task regardless of continued demand in favor of patrolling the nest to potentially gain information on which tasks are currently most imperative (Johnson 2008, 2009, 2010a). The present results indicate that hygienic specialists will consistently focus on hygienic behavior and undertaking if demand for the tasks are high, and they show a trend of inspecting cells much less frequently than generalist middle-aged bees in experiments 1 and 2 (9% of observations compared with 29% in experiment 1 workers and 23% in experiment 2 generalists). Hygienic specialists may therefore exhibit a behavioral algorithm intermediate to the frequent "quit-task-then-patrol" procedure of generalist workers and "single task tenacity" of strictly specialized workers. Hence, hygienic specialists display a type of specialization in which they focus on two rare tasks and will continue to perform these tasks if demand is present.

Proximate and ultimate causes of correlated task choices in hygienic specialists

The results from our correspondence and cluster analyses showed that performance of hygienic behavior, both in specialists and in generalists, is highly associated with the performance of undertaking by the same individuals (Figs. 4 and 5). We further identified that hygienic specialists in particular show a highly disproportionate bias towards undertaking (Fig. 2). That the removal of both dead brood and dead adult bodies from the hive is performed by an overlapping subset of middle-aged workers is probably meaningful for colony fitness and could be explained by an overlap in the mechanisms determining worker responsiveness to both tasks.

At the proximate level, the association between these tasks could be explained in two fundamentally different ways. Perhaps the most parsimonious explanation is that both behaviors require sensitivity to the same cues that elicit removal behavior. Essentially, this hypothesis posits that both behaviors are triggered by the same cue and workers that show sensitivity to removal cues in the brood area remain sensitive to those cues in other parts of the hive. Thus, hygienic workers show a consistently low response threshold to death cues whether they indicate decaying brood or decaying adults.

Alternatively, there could be distinct cues for each behavior that are both perceived via olfactory sensation. In this case, the superior olfactory sensitivity of hygienic bees (Masterman et al. 2000; Spivak et al. 2003) for two related volatile cues could drive the association between these tasks. Although the specific odors associated with eliciting hygienic behavior are becoming increasingly well described (Nazzi et al. 2004; Swanson et al. 2009; McAfee et al. 2018), the signals associated with undertaking are largely still unresolved (Visscher 1983; Sun and Zhou 2013). The most recent work on odors associated with frozen brood specifically shows that one of the main pheromones associated with dead but not diseased brood is oleic acid, which is a conserved "death pheromone" in social insects (McAfee et al. 2018). However, the role of oleic acid in dead adults as the main trigger for undertaking is still contentious, and the quick removal of dead adults from the hive before oleic acid builds up highlights that the signals triggering undertaking behavior are more complex than what can be explained by a single odor (Sun and Zhou 2013). Consequently, further work is required to distinguish between these two explanations, and testing if this task association varies when workers are presented with brood infected with different pathogens, rather than just dead brood, is an intriguing line of future research.

From the perspective of colony fitness, there are a few conceivable reasons why hygienic specialists should also perform undertaking. Because the behaviors are similar in the motor activities involved, it could be that common skills linking the two behaviors allow for a more efficient removal of individuals that pose a risk for disease transmission. Both behaviors are inherently time sensitive, so quicker recognition and removal likely confer greater social immunity for the colony if performed by the same individuals that are most physically capable to perform these tasks. Prior work on undertaking specialists identified a similar link between undertaking and the removal of chalkbrood mummies, another remnant of dead individuals that can spread disease within a hive (Trumbo et al. 1997).

There is also a partial spatial link between undertaking and hygienic removal behavior that could allow for an efficiency benefit in the reduction of travel time between tasks. Both tasks are often initiated at the bottom of the nest, either after dead adults have fallen to the base of the hive or after other hygienic workers have expelled dead brood out of their cell but not completely removed them from the hive. Hygienic specialists in our study were observed removing dead pupae from their cell and removing dead pupae dropped by other hygienic workers. There is therefore a spatial link in the behavior of hygienic specialists that removed both dead adults and dropped pupae from the bottom of the hive. The extent to which hygienic specialists moved back and forth from the brood zone and the bottom of the hive or performed work in the brood zone and then transitioned to exhibiting spatial fidelity for the bottom of the hive would need to be more explicitly tested in future work.

In addition to the possibility that hygienic removal behavior and undertaking are adaptively linked based on colony ergonomics, it is also conceivable that these behaviors are linked due to colony-level benefits conferred through a reduction in disease transmission. The organizational immunity hypothesis of disease transmission suggests that colonies ought to limit the number of workers performing risky tasks involving pathogens and that these workers should in turn interact less with other workers, brood, and the queen (Schmid-Hempel 1998; Naug and Camazine 2002; Evans and Spivak 2010; Stroeymeyt et al. 2014). Previous work in some ant species supports the notion that colonies may partially organize themselves based on these principles. Leaf cutter ants (*Atta cephalotes*) show high amounts of task partitioning between workers cultivating fungus and those involved in waste heap work, and waste workers are kept in the heap area through aggressive interactions (Hart and Ratnieks 2002). More recent work on *Lasius niger* ants showed that the social contact network of a colony adaptively shifts when specific individuals are infected with a pathogen, even before symptoms associated with the pathogen are present (Stroeymeyt et al. 2018). Hence, there is evidence from these studies that potentially contaminated workers are recognizable and show patterns of specialization in task behavior, space use, and social isolation that keeps other nestmates healthy. It is therefore possible that individual workers focused on both hygienic behavior and undertaking increase colony fitness, not by any particular increase in efficiency, but simply by virtue of reducing the number of workers involved in disease-related tasks. Thus, this task association may be an example of how task partitioning increases colony fitness without any necessary increase in individual task proficiency (Seeley 1982; Jeanne 2016). However, much work remains to be done to more fully address any adaptive implications this particular task association has for colony-level survival and efficiency.

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Acknowledgments We thank Charley Nye for support throughout the project. We thank Darian Buckman, Danielle Woody, Emily Zepeda, Isabelle Neylan, Spencer Eberhard, Sarina Bao, and Sharon Pneh for help with tagging and freeze-killing bees. We thank Damien Caillaud for guidance on statistical analyses. We also thank two anonymous reviewers for comments that improved the manuscript.

Data availability Data and R code for the analyses presented here are available from the corresponding author on reasonable request.

Compliance with ethical standards

The work presented here complies with the laws of the USA.

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