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Inefficient task partitioning among nonhygienic honeybees, *Apis mellifera* L., and implications for disease transmission

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Hygienic behaviour in honeybee colonies involves the recognition and removal of diseased brood by worker bees. This task is further partitioned into subtasks of uncapping cells with diseased brood and removing the cell contents. Worker bees that express hygienic behaviour remove diseased brood from the colony before the pathogen reaches the infectious stage. Although all honeybees are capable of uncapping and removing diseased brood, only those colonies with hygienic bees, the genetic specialists, do so rapidly and hence efficiently limit disease transmission. Colonies with nonhygienic bees, however, uncap and remove diseased brood only after it becomes infectious, resulting in the handling and transmission of pathogens. To understand the behavioural repertoire of individual nonhygienic bees and how the performance of the behaviour is influenced by the presence of hygienic bees as nestmates, we observed nonhygienic bees that were maintained in colonies with varying proportions of hygienic and nonhygienic bees. Our results indicated that nonhygienic bees in mixed colonies were not stimulated to perform hygienic behaviour to a higher extent in the presence of hygienic bees. The rate, duration and probability of uncapping and removing dead brood by nonhygienic bees was significantly reduced in the presence of hygienic bees. In mixed colonies, as compared to a colony of hygienic bees, a higher proportion of uncapped cells were subsequently recapped, resulting in delayed removal of dead brood. This inefficient task partitioning would allow the pathogen to reach the infectious stage and increase the probability of disease transmission.

Individuals in social insect colonies distribute their efforts among colony tasks, performing tasks repeatedly and nonrandomly, resulting in a division of labour (Wilson 1971; Beshers & Fewell 2001). The allocation of individuals to particular tasks is dynamic and changes in response to resource availability in the environment and the needs of the colony. As individual worker honeybees age, they generally progress from tasks performed inside the colony (intranidal tasks) that include nest cleaning, feeding larvae and disease control, to tasks performed outside the colony (extranidal tasks) that include foraging, undertaking and guarding. In addition to the age of the workers, worker genotype and task demand regulate the task performance by a worker (Winston 1987). Division of labour results in a flexible and efficient process by which groups work in tandem to maintain homeostasis in the colony, responding and adjusting to changes in the need for various tasks. The performance of a particular intra- or extranidal task may be divided among many individuals, resulting in task partitioning (Ratnieks & Anderson 1999), which has been proposed to be a general mechanism to increase efficiency (Oster & Wilson 1978; Ratnieks & Anderson 1999). With regard to hygienic behaviour, however, this efficiency has specifically been shown to be high only in colonies with large proportions of hygienic bees (Arathi & Spivak 2001). In this study, we present a case of how task partitioning among workers in a honeybee colony could actually lead to an overall inefficiency in the performance of hygienic task, which ultimately impacts disease transmission within the colony.

'Hygienic behaviour' (Rothenbuhler 1964a; reviewed in Spivak & Gilliam 1998) is a complex but discrete behaviour. It is expressed only by those colonies that contain a sufficient number of individual bees with low response thresholds to the stimuli that elicit the behaviour. Hygienic behaviour is a heritable trait (Rothenbuhler 1964a; Lapidge et al. 2002). Individual bees that perform

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this task are genetic task specialists (Calderone & Page 1991), but they perform the behaviour facultatively, because the stimulus to perform the behaviour is not always present in every colony, and they perform normal colony tasks in the absence of the stimulus. Our emphasis in understanding hygienic behaviour has been to study task partitioning and explain how the performance of a discrete task is divided among a group of individuals in the presence of a stimulus that elicits the behaviour. Earlier studies (Arathi et al. 2000; Arathi & Spivak 2001) were aimed at explaining task partitioning among hygienic bees with low response thresholds for performing hygienic behaviour (Masterman et al. 2001) under varying colony-level demands for the task. In the current study, we focus on the task performance of nonhygienic bees, those bees with high response thresholds to perform the behaviour, and how the collective behaviours of bees within mixed colonies influence the colony-level expression of the trait.

Colonies that express hygienic behaviour quickly detect and uncap cells with diseased brood and remove the brood from the nest, thus eliminating the disease-causing pathogen from the colony, while colonies of nonhygienic bees uncap and remove diseased brood very slowly or not at all. If workers from nonhygienic colonies do remove diseased brood, it is done at a stage when the pathogen is already infectious, thus increasing the risk of disease transmission through the colony (Woodrow & Holst 1942; Rothenbuhler 1964a). Hygienic behaviour is a very effective mechanism of disease resistance only if bees are able to remove brood from the nest before the pathogen becomes infectious (Brødsgaard et al. 2000). Hygienic colonies are resistant to American foulbrood (Rothenbuhler 1964a, b; Spivak & Reuter 2001a) and chalkbrood (Gilliam et al. 1983) and are able to reduce the level of infestation of the parasitic mite, *Varroa destructor* Anderson and Truman (Peng et al. 1987; Boecking & Drescher 1991; Spivak 1996; Spivak & Reuter 1998, 2001b). All these advantages make hygienic behaviour economically important for honeybee colonies.

Early studies on the genetic basis of hygienic behaviour postulated that two recessive genes controlled the uncapping and removal motor patterns, respectively (Rothenbuhler 1964a). More recent studies indicate that the trait is quantitative and up to seven loci may influence the expression of the behaviour (Moritz 1988; Gramacho 1999; Lapidge et al. 2002). All bees are capable of performing these motor patterns, but there is great variability at the colony level in the rate of initiation and completion of the uncapping and removal components of the behaviour. Hygienic bees show increased olfactory sensitivity and responsiveness to the odours of diseased brood compared to nonhygienic bees (Masterman et al. 2000, 2001; Spivak et al. 2003). Therefore, it has been hypothesized that the genes influencing hygienic behaviour could also be involved in the olfactory pathway. Variation in olfactory sensitivity is seen even among presumably homozygous hygienic bees selectively bred to perform the behaviour for many generations (Gramacho & Spivak 2003).

In the sequence of temporal division of labour shown in honeybee colonies (Winston 1987), hygienic behaviour is performed largely by the middle-aged bees, between 15 and 20 days old (Arathi et al. 2000). A detailed study of hygienic behaviour at the individual level indicated that in a colony with hygienic bees, only about 20% of the worker bees (which also corresponds to the percentage of middle-aged workers in a colony with an even age distribution) actually uncap and remove dead brood (Arathi & Spivak 2001). However, the efficiency of hygienic behaviour, as defined by the ability of the colony as a whole to remove dead pupae from the cells within 48 h (Spivak & Downey 1998) is higher in hygienic colonies than in nonhygienic colonies. It has also been shown that hygienic bees can modulate the extent of performance of the behaviour depending on the genetic composition of the colony. In colonies composed of 25% hygienic bees and 75% nonhygienic bees, hygienic bees responded to the stimulus of dead brood by increasing the rate of performance of hygienic tasks, by becoming more persistent and by extending the period of intranidal task performance of middle-aged nurse bees. Despite the increase in the rate, persistence and extended performance of hygienic behaviour in individual bees, there was no evidence for a corresponding increase in colony efficiency (Arathi & Spivak 2001). Genetically mixed colonies did not reach efficiency levels of a colony with 100% hygienic bees, suggesting that the proportion of bees in the colony that are able to perform the task at any one time must be large for successful colony-level expression of the trait. Increased activity of individual hygienic bees in mixed colonies is not sufficient to meet the increased demand for task performance.

Our previous studies cited above did not describe the behaviour of individual nonhygienic bees in these mixed colonies, although this is essential to explain the reduced efficiencies of mixed colonies. An analysis of the behaviour of nonhygienic bees in the presence of hygienic bees is necessary to have a clear understanding of how the combined and interactive effects of individual bees with diverse behavioural propensities affect the colony-level expression of this complex behaviour. Preliminary observations suggested that these bees could be recapping previously uncapped cells (Spivak & Gilliam 1993; Boecking 1994; Gramacho 1999). The aim of our current study was to analyse the behavioural profile of nonhygienic bees in colonies with different proportions of hygienic bees (Arathi & Spivak 2001). Additional experiments were performed to test whether colonies containing nonhygienic bees recapped dead brood that previously was uncapped.

METHODS

The details of the breeding programme for isolating and maintaining the hygienic and nonhygienic genetic lines of bees are described in detail in Arathi & Spivak (2001). For the behavioural observations in 2001, one parental hygienic queen and one nonhygienic queen, both selected from among the sixth generation of colonies in the breeding programme, were the source of worker bees that made up four observation (glass-walled) colonies. Frames with eggs from each of the parental colonies were moved into a common 'nursery' colony headed by a naturally mated queen. Cofostering the bees during their

development in the nursery colony has been suggested to eliminate potential behavioural differences that could result from nutritional differences in colony brood rearing (Calderone & Page 1988). Combs containing pupae within 1–2 days of eclosion were removed from the nursery colony and placed in individual cages in an incubator held at 34°C and 50% relative humidity. The eclosing bees were individually marked with a number tag on the thorax. We introduced 100 newly eclosed worker bees that had been individually identified with coloured, number tags into glass-sided observation hives every 3 days for 60 days to ensure an even age distribution in the observation colony.

We established four observation hives in 2000, each differing in the proportions of bees derived from one parental colony of the hygienic line and one of the nonhygienic line, respectively. The 25H:75NH colony had 25% hygienic bees (400 bees) and 75% nonhygienic bees (1200 bees). The 50H:50NH colony had equal proportions of hygienic (800 bees) and nonhygienic bees (800 bees). The 100H colony had all hygienic bees (1600 bees). The 100NH colony had all nonhygienic bees (1600 bees). For details on marking the bees, and creating and maintaining these observation hives, refer to Arathi & Spivak (2001).

Behavioural Observations of Nonhygienic Bees

To observe the onset of hygienic behaviour in the observation hives, we inserted a section of comb containing freeze-killed worker brood in the centre of the bottom (brood) frame of the observation hive. The comb section was 6×4 cm and contained a mean $\pm$ SD of 215.35 ± 5.32 cells. We counted the number of empty cells and the number of cells with intact pupal caps or unsealed larvae in each comb section before inserting the freeze-killed brood into the brood frame and also again at the end of 12 h. The presence of dead brood elicits hygienic behaviour, and is a standard field assay for the behaviour (Spivak & Downey 1998).

Instantaneous Scans

We made video recordings of the region of the observation hive containing the freeze-killed brood between 0900 and 2100 hours, up to 6 h each day. These recordings were made on 3 days for each colony, resulting in 18 h of recording per colony. Each of the 3 days of recording on the same colony was separated by at least 1 week. We performed instantaneous scans of all the number-tagged bees present on the section and noted their behaviour from the videos at 15-min intervals, resulting in 26 instantaneous scans each day and a total of 78 scans for each colony.

To determine whether nonhygienic bees performed hygienic behaviour nonrandomly depending on collective make-up of the colony they cohabited, we used a Kolmogorov–Smirnov test to compare the distribution of the number of performances of hygienic behaviour per bee to a theoretical Poisson distribution. About 1–11 bees in the experimental colonies performed hygienic behaviour more than four times in the day and were pooled into one class for analysis. These bees were accordingly said to be persistent in the task.

Focal Animal Sampling

We randomly picked a number-tagged nonhygienic bee that was uncapping or removing dead brood from the freeze-killed brood section and we recorded all the behaviours that she performed during the next 20-min using a hand-held computer (PSION Workabout PLC 1995). The behaviours recorded during the observation period were: autogrooming (AG), walking (WA), inspecting brood cells (IC: the bee thrust its head into the cell or entered it) and the two subtasks of hygienic behaviour, uncapping cells with dead brood (UC: removing the wax cap of the pupae) and removing dead pupae from these uncapped cells (RE: the bee dragged the dead pupa out of the cell with her mandibles). We observed 30 nonhygienic bees for 20 min each, in each of the colonies. From these data, we calculated the duration of each behaviour (time spent uncapping and/or removing dead bees in 20 min) and the mean rates of performance of each behaviour in terms of frequency per bee per hour.

Do Nonhygienic Bees Recap Previously Uncapped Dead Brood?

In the summer of 2002, we established four new observation hives with the same proportions of marked hygienic and nonhygienic bees as described above. A section of freeze-killed brood was inserted into the frames of the observation hives exactly as described for the previous experiment. In this case, however, all the cells of the freeze-killed brood section were traced onto a clear overhead transparency sheet. The cells that were empty or already uncapped were marked. The process of tracing empty and uncapped cells was repeated on the same transparency sheet at the end of 1, 5 and 7 h. If a previously uncapped cell was sealed in the following tracing, it was said to have been recapped. The procedure was repeated on 11 different days for each colony using different freeze-killed brood inserts and transparency sheets each day.

We calculated the proportion of cells that were recapped at the end of 7 h separately for the 11 days. We performed a Student's t test on the arcsine-transformed data, but we used untransformed data for means and standard deviations in Fig. 5.

RESULTS

Scan Sampling

The data from instantaneous scans showed that the nonhygienic bees performed hygienic behaviour (uncapping and/or removing dead brood) randomly in all colonies, without showing any evidence of persistence in the behaviour (Kolmogorov–Smirnov test: 100NH colony: $D_{\max} = 0.134$, $D_{0.01} = 0.145$, $N = 252$, $P = 0.011$; 25H:75NH colony: $D_{\max} = 0.143$, $D_{0.01} = 0.237$, $N = 94$,

$P = 0.146$; 50H:50NH colony: $D_{max} = 0.142$, $D_{0.01} = 0.211$, $N = 119$, $P = 0.091$; Fig. 1). Varying proportions of nonhygienic bees were observed to perform some component of hygienic behaviour at least once (100NH colony: 190/1600 nonhygienic bees, 12%; 25H:75NH colony: 75/1200 nonhygienic bees, 6%; 50H:50NH colony: 81/800 nonhygienic bees, 10%). An analysis of the proportion of hygienic and nonhygienic bees performing hygienic behaviour in these colonies indicated that a significantly higher percentage of nonhygienic bees in the two mixed colonies did not perform hygienic behaviour compared to the hygienic bees in the same colonies (25H:75NH: 80.3% of hygienic bees versus 95.3% of nonhygienic bees: $\chi^2_1 = 855.44$, $P < 0.01$; 50H:50NH: 80.2% of hygienic bees versus 94.9% of nonhygienic bees: $\chi^2_1 = 186.28$, $P < 0.01$; 100H and 100NH: 81.5% of hygienic bees versus 88.1% of nonhygienic bees: $\chi^2_1 = 27.25$, $P < 0.01$). The total number of instances of performing hygienic behaviour and the degree of persistence were much higher in the 100H colony than in the 100NH colony. As many as 28 bees performed the behaviour four times or more in the 100H colony, representing the increased persistence in the task (see Arathi & Spivak 2001). The persistence on the task was much lower in the 100N colony, with only nine bees performing hygienic behaviour four times or more.

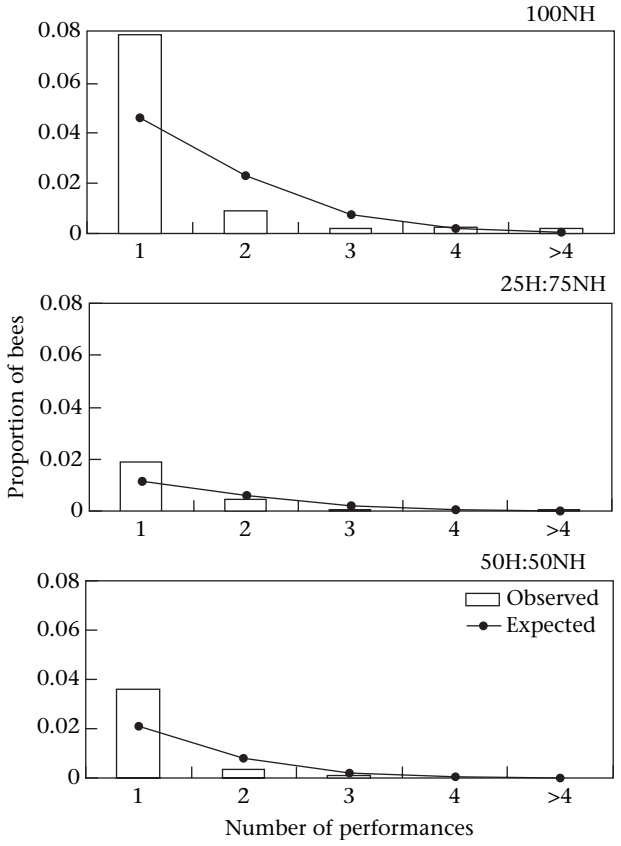

Figure 1. The distribution of the number of performances of hygienic behaviour per bee as a function of the proportion of the total number of nonhygienic bees in the colony (bars). The line indicates the numbers expected from a Poisson distribution.

Focal Sampling

Mean performance of all five behaviours (uncapping cells, removing dead brood, inspecting cell contents, autogrooming and walking) by the nonhygienic bees in the 20-min sampling period was similar in the two mixed colonies and the 100NH colony (Fig. 2). The data on focal nonhygienic bees also indicated that these bees spent significantly less time engaged in uncapping and/or removing dead brood than did hygienic bees in the same colony (Table 1). Hygienic bees in the different colonies spent, on average, 622–757 s of the 20-min observation period performing hygienic behaviour, whereas nonhygienic bees spent 53–74 s of the 20-min observation period performing hygienic behaviour. Consequently, the average proportion of time allocated to the task components of hygienic behaviour differed significantly between hygienic and nonhygienic bees (Table 1). Nonhygienic bees that began to uncap a cell remained on this task for much less time than did hygienic bees, then they switched to other behaviours, such as autogrooming or walking away from the location of the dead brood.

Focal nonhygienic bees performed significantly fewer hygienic-related subtasks (uncapping or removing dead brood) than did hygienic bees in the same colony (Student's t test: 25H:75NH: $t_{28} = 12.76$, $P < 0.01$; 50H:50NH: $t_{27} = 9.65$, $P < 0.01$; Fig. 3). Nonhygienic bees in the 100NH colony also performed significantly fewer hygienic tasks than did hygienic bees in the 100H colony ($t_{58} = 6.54$, $P < 0.01$; Fig. 3). A closer examination of the two hygienic subtasks indicated that cell uncapping was performed at significantly higher frequencies in all the colonies than was the removal of dead brood (100NH: $t_{60} = 14.29$, $P < 0.01$; 25H:75NH: $t_{58} = 5.86$, $P < 0.01$; 50H:50NH: $t_{56} = 6.78$, $P < 0.01$; 100H: $t_{56} = 6.16$, $P < 0.01$; Fig. 4). The two mixed colonies with nonhygienic bees did not differ significantly in the number of cells uncapped per hour (25H:75NH versus 50H:50NH: $t_{57} = 0.82$, $P = 0.41$), but both showed significantly lower frequencies of cell uncapping than did the colony with only hygienic bees (25H:75NH versus

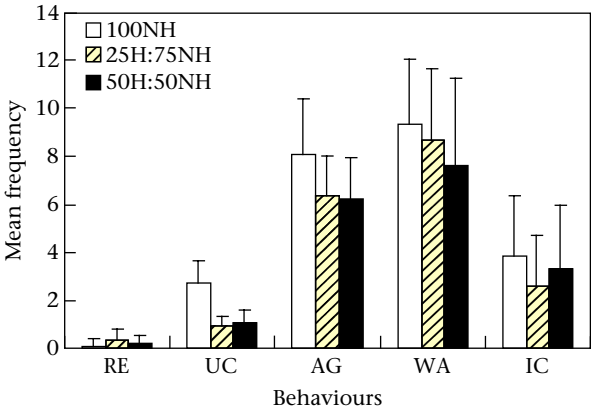

Figure 2. Mean ± SD frequencies of behaviours shown by nonhygienic bees in a 20-min period on the freeze-killed brood section. RE = removing dead brood; UC = uncapping the wax cap of the dead pupa; AG = autogrooming; WA = walking; IC = inspecting cells.

Table 1. Mean ± SD time (in seconds) that hygienic (H) and nonhygienic (NH) bees from different colonies spent in hygienic behaviour during 20-min focal bee observations. The corresponding proportions of time that bees spent in hygienic behaviour are given in parentheses

	Duration of hygienic behaviour (s)					
	Hygienic bees		Nonhygienic bees		Statistics*	
	$\overline{X} \pm$ SD (proportion of time)	*N*	$\overline{X} \pm$ SD (proportion of time)	*N*	*t*	*P*
Colony						
100NH	—		53.93±25.56 (0.04±0.02)	29	12.79	<0.01
100H	635.65±243.57 (0.51±0.2)	29	—			
25H:75NH	757.36±276.22 (0.59±0.2)	30	67.15±28.18 (0.05±0.02)	30	13.62	<0.01
50H:50NH	622.30±258.45 (0.49±0.2)	29	74.41±38.81 (0.06±0.03)	30	11.29	<0.01

*Statistical comparisons were performed for the mean duration of hygienic behaviour. Mean durations for colony 100NH were compared with those for colony 100H. The results were similar when the proportions were compared.

100H: $t_{57} = 9.86$, $P < 0.01$; 50H:50NH versus 100H: $t_{56} = 8.95$, $P < 0.01$) and the colony with only nonhygienic bees (25H:75NH versus 100NH: $t_{59} = 9.38$, $P < 0.01$; 50H:50NH versus 100NH: $t_{58} = 8.08$, $P < 0.01$). The two mixed colonies also showed significantly lower frequencies of removing dead brood than did the 100H colony (25H:75NH versus 100H: $t_{57} = 4.02$, $P < 0.01$; 50H:50NH versus 100H: $t_{56} = 4.52$, $P < 0.01$), but these frequencies did not differ significantly from that of the 100NH colony (25H:75NH versus 100NH: $t_{59} = 2.03$, $P = 0.05$; 50H:50NH versus 100NH: $t_{58} = 0.85$, $P = 0.39$; Fig. 4).

Recapping Dead Brood

The proportion of previously uncapped cells that were recapped at the end of a 7-h period was significantly higher in mixed colonies (18–31%) than in the 100H colony (5%) (25H:75NH versus 100H: $t_{20} = 2.64$, $P < 0.01$; 50H:50NH versus 100H: $t_{20} = 2.49$, $P < 0.01$), but did not differ significantly from the proportion of recapped cells in the 100NH colony (25H:75NH versus 100NH: $t_{20} = 0.58$, $P = 0.58$; 50H:50NH versus 100NH: $t_{20} = 1.11$, $P = 0.28$; Fig. 5). The proportion of cells recapped in the 100NH colony was not significantly different from that in the 100H colony ($t_{20} = 1.21$, $P = 0.23$). However, the bees in the 100NH colony had very few cells that were initially uncapped, and hence there were very few cells available to be recapped, whereas the bees in the 100H colony uncapped cells and removed dead brood in rapid succession, resulting in very few uncapped cells remaining to be recapped. The variation in behaviour in these two colonies resulted in many days where there were no instances of recapping observed. We therefore recalculated the proportion of cells recapped after considering only days when both colonies had uncapped cells available to be recapped. We found that the average proportion of recapped cells

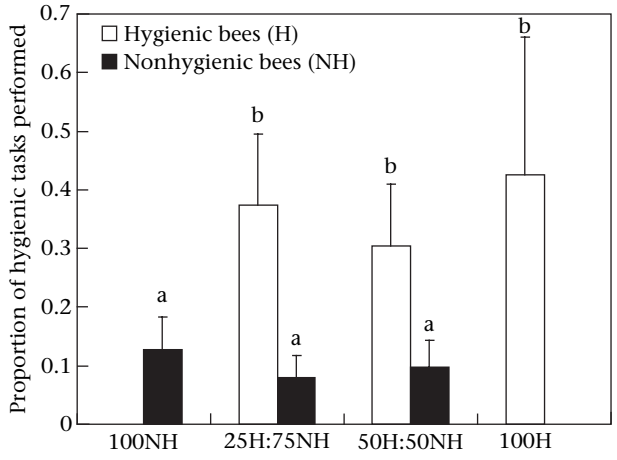

Figure 3. Mean ± SD proportion of hygienic-related tasks performed by hygienic (H) and nonhygienic (NH) bees relative to other intranidal tasks. Bars with different letters indicate significantly different frequencies (Student's *t* test).

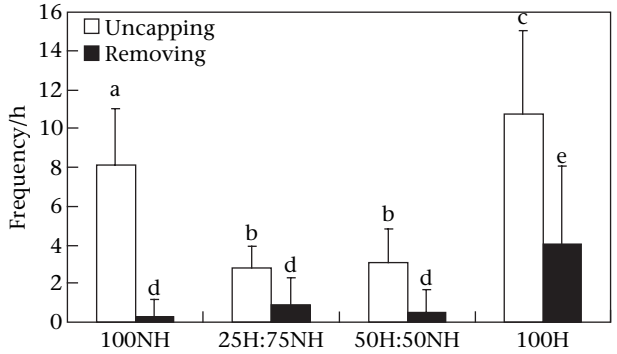

Figure 4. Mean ± SD frequency (per hour per bee) of uncapping cells and removing dead brood in the different colonies. Statistical comparisons were performed between the two behaviours within each colony and across different colonies for the same behaviour. Bars with different letters indicate significantly different frequencies (Student's *t* test).

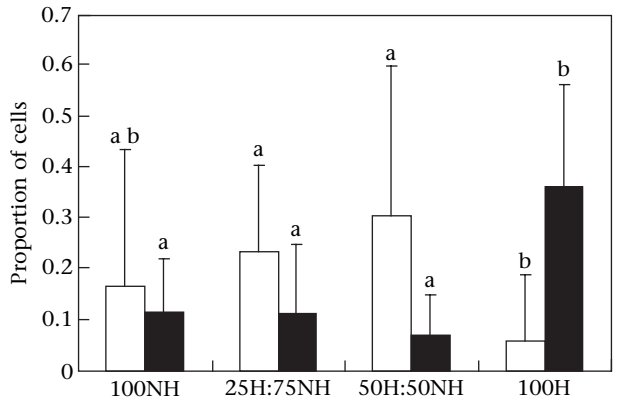

Figure 5. Mean ± SD proportion of cells with contents recapped (□) or removed (■) at the end of 7 h. Bars with different letters denote significant differences between colonies in the proportion of cell contents that were recapped or removed (Student's *t* test).

was higher in the 100NH colony (0.45 ± 0.26, $N = 4$ days) than in the 100H colony (0.15 ± 0.19, $N = 4$ days). Statistical comparisons of these numbers were not performed because of the small sample size.

Efficiency at the Colony Level

A further examination of the removal of dead brood by bees from these colonies indicated that colony-level efficiency did not differ between colonies that had different percentages of nonhygienic bees (25H:75NH versus 50H:50NH: $t_{20} = 0.93$, $P = 0.36$). However, colonies with different percentages of nonhygienic bees removed significantly fewer dead brood by the end of 7 h than did the colony with only hygienic bees (25H:75NH versus 100H: $t_{20} = 3.34$, $P < 0.01$; 50H:50NH versus 100H: $t_{20} = 4.32$, $P < 0.01$; Fig. 5). About 35% of freeze-killed pupae were removed from the cells in the 100H colony by the end of 7 h, whereas less than 20% of the freeze-killed pupae were removed in the colonies that contained nonhygienic bees. This result further supports the premise that the 100H colony would have had fewer cells that were available to be recapped.

DISCUSSION

Our study on the behavioural profiles of nonhygienic bees sheds light on how, in the presence of a stimulus that elicits a discrete behaviour, task partitioning among individual workers can affect colony-level efficiency in the performance of a behaviour that has a direct impact on disease transmission. Our results provide a mechanism to explain why colonies composed of a majority of nonhygienic bees are inefficient in uncapping and removing diseased brood from the nest. The lack of efficiency emerges because mixed colonies with nonhygienic bees tend to recap dead brood that has been uncapped by other bees, resulting in the recurrent performance of uncapping and recapping cells instead of the linear progression from uncapping cells to removing diseased brood. The delay in removing diseased brood allows the pathogen time to reach the infectious stage (Woodrow & Holst 1942), resulting in disease transmission.

The results of the scan and focal sampling showed that individual nonhygienic bees in colonies composed of 75% and 50% nonhygienic bees were significantly less predisposed to perform hygienic behaviour compared to their sisters in 100NH colonies. These findings show how colony composition influences the performance of important behaviours by individuals and confirm observations from earlier studies (Trump et al. 1967) in which nonhygienic bees did not appear to either learn from or be stimulated by the activity of hygienic bees. The reduced performance of a task by worker bees in the presence of other task specialist bees has also been shown in colonies with bees bred for pollen hoarding. Bees selected for low pollen hoarding reduce pollen foraging when the complementary high pollen-hoarding genotype is present in the colony (Calderone & Page 1992).

Analysis of the task components of hygienic behaviour from focal sampling indicated that cell uncapping occurred at a significantly higher frequency in all colonies than did the removal of cell contents. Furthermore, nonhygienic bees in the different colonies uncapped cells at similar frequencies but removed brood from these cells at much lower frequencies. Earlier studies suggested that nonhygienic bees could have a higher predisposition to recap cells that were previously uncapped either by themselves or by other bees in the colony (Spivak & Gilliam 1993). Such recapping by nonhygienic bees instead of removing cell contents was suggested as a possible reason for lowered efficiencies in colonies with nonhygienic bees (Arathi & Spivak 2001). Our current experiments using an indirect assay of monitoring the same set of cells over time to detect and measure rates of recapping provided clear evidence that the two colonies with nonhygienic bees recapped dead brood cells that had been previously uncapped. This recapping helps to explain the reduced efficiency observed by Arathi & Spivak (2001), in which reduced efficiencies were observed despite the presence of hygienic bees in these colonies that worked beyond their nursing age to clean the colony of dead brood (Arathi & Spivak 2001).

There are at least two explanations for why a bee might recap brood that has been previously uncapped. One hypothesis is that bees that recap diseased brood are actually attempting to entomb the pathogen or dead brood within the nest. For example, alkali bees, *Nomia melanderia*, and sweat bees, *Lasioglossum versatum*, use dirt to wall off brood cells infected with fungi (Batra 1968; Batra & Bohart 1969). Similarly, termites avoid nestmates infected with fungal infections or entomb them in the dirt (Culliney & Grace 2000), ensuring that the diseased individuals are not handled. Entombing is an effective strategy when the insects do not reuse cells in which brood is reared. Honeybees are somewhat unique among other closely related solitary bees and bumblebees in their reuse of brood cells, but they do tend to entomb foreign objects and intruders such as hive beetles (Neumann et al. 2001) with propolis.

An alternative hypothesis is that nonhygienic bees recap cells containing diseased brood because they have

reduced sensitivity to olfactory cues associated with diseased brood. Some evidence for this hypothesis comes from the fact that the genes that influence hygienic behaviour are also involved in the olfactory pathway. This may be modulated by increased expression of octopamine in localized regions of the brain, or by a difference in the distribution and responsiveness of the octopamine receptors in hygienic bees and nonhygienic bees (Masterman et al. 2001; Spivak et al. 2003; Goode et al. 2006). Thus, nonhygienic bees may perceive a hole in the pupal cap, but may not necessarily detect that the brood within the cell is dead or diseased, and respond by resealing the hole with wax instead of continuing the process of uncapping. This would result in the cell remaining sealed for a longer period. Colonies composed of a majority of hygienic bees may show lower proportions of recapping than colonies with a majority of nonhygienic bees because they would have a larger proportion of bees with relatively high olfactory sensitivity for diseased and dead brood (Gramacho & Spivak 2003; Spivak et al. 2003). Colonies with hygienic bees, therefore, would uncap and remove the contents more quickly. A colony with a high proportion of hygienic bees will tend to detect infected brood, uncap the cells that contain the brood, and remove the diseased brood from these cells with relatively higher speed and efficiency. In contrast, a colony with a high proportion of nonhygienic bees will tend to take longer to detect infected brood, and may then proceed to uncap, recap and uncap these cells multiple times, and remove the diseased brood much later, if at all. This repetitive performance of the initial subtask of uncapping cells increases the probability that these bees will make repeated contact with the pathogen, resulting in an increased probability that the pathogen is transmitted through the colony.

Our results indicate that the initial step of detecting diseased brood is necessary but not sufficient for the completion of the hygienic behaviour motor patterns of uncapping and removing diseased brood. Even when a cell containing dead brood has been detected, nonhygienic bees have a lower probability of performing hygienic behaviour compared to hygienic bees. In addition to the influence of colony composition, the expression of the behaviour is also influenced by environmental factors external to the colony, such as abundance of nectar (Trump et al. 1967; Momot & Rothenbuhler 1971) and seasonal factors that remain obscure (e.g. Mondragon et al. 2005).

Although the colonies used in this study were not replicated, our results are supported by data collected by Gramacho (1999), which document the frequency with which the cells containing dead brood are uncapped and recapped repeatedly before the brood is removed by bees. While it is possible that our results could have been influenced by factors other than colony composition, our previous studies on the behaviours of hygienic bees have been robust in all replications across different years (Arathi et al. 2000; Arathi & Spivak 2001; Gramacho & Spivak 2003). A detailed comparative study of how an individual larva naturally infected with disease is handled by hygienic and nonhygienic bees in the colony (e.g. Palacio 2005) could help to identify more directly the differences between these bees. Further explorations on the neural mechanisms of reduced olfactory sensitivity of nonhygienic bees could explain why these bees may recap diseased brood instead of completing the task and removing the cell contents as a hygienic worker bee does.

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