Ethology **106,** 365—379 (2000)

ISSN 0179–1613

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Ethology of Hygienic Behaviour in the Honey Bee *Apis mellifera* L. (Hymenoptera: Apidae): Behavioural Repertoire of Hygienic Bees

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Arathi, H. S., Burns, I. & Spivak, M. 2000: Ethology of hygienic behaviour in the honey bee *Apis mellifera* L. (Hymenoptera: Apidae): behavioural repertoire of hygienic bees. Ethology **106,** 365—379.

Abstract

Hygienic behaviour performed by middle-aged worker bees is an important intranidal task in colonies of the honey bee *Apis mellifera* (L.). It comprises detecting diseased brood in the larval and pupal stages and removing all such infected brood, thereby decreasing the incidence of infection. Hygienic behaviour consists of two task-components: uncapping cells and removing the cell contents. The aim of this study was to observe bees performing hygienic behaviour to determine their age at performance of the behaviour and to describe their behavioural repertoire. The bees performing hygienic behaviour were middle-aged bees, younger than foragers. In the colonies where the behaviours of individual bees were observed, all bees performing the hygienic behaviour were seen to exhibit both the components, though at different frequencies. One behavioural class performed the task of uncapping cells at higher frequencies than the task of removing cell contents, while another class performed both tasks to the same extent. While these two classes had higher frequencies of the tasks comprising the hygienic behaviour but lower frequencies of other common behaviours in their repertoire, a third class of bees included those that performed all behaviours in their repertoire at similar frequencies. There was no difference in the ages of the bees in these three behavioural classes. These results suggest that there is no evidence of task partitioning among bees performing the hygienic behaviour. The segregation observed could, however, be based on their response thresholds to the stimulus and/or on their ability to discriminate the various cues emanating from the dead brood.

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Introduction

Hygienic behaviour involves the detection of diseased and infected brood and their selective removal from the cells by worker honey bees. Among social insects,

hygienic behaviour is apparently unique to honey bees and could be an adaptation for cell reuse because, unlike other closely related social insects such as bumble bees and stingless bees, honey bees reuse cells after brood emerges rather than building new cells (Michener 1974). Some other social insects are known usually to leave diseased brood under a capped cell (entombing) (Spivak & Gilliam, 1993). Hygienic behaviour has become increasingly important in honey bees because, in addition to being a behavioural mechanism of disease resistance (Rothenbuhler 1964a; Gilliam et al. 1983), it is one mechanism of behavioural defence against the ectoparasitic mite *Varroa jacobsoni* Oud., a pest which damages bee colonies (Boecking & Spivak 1999; reviewed in Spivak & Gilliam 1998a,b).

Worker honey bees perform a gamut of tasks in their adult lives, beginning with tasks inside the hive (intranidal) and then moving to the riskier task of foraging for the colony's needs (extranidal). A comparison of the number and complexity of the intranidal tasks that need to be carried out by a worker bee with the number and complexity of extranidal tasks indicates that there are more intranidal tasks and that the cues involved with these tasks are more subtle. Highly flexible division of labour in honey bee colonies is an important factor that has contributed to their ecological success (Seeley 1995). Individual workers undergo various ontogenetic changes in behaviour, progressing from one intranidal task to another and then onto extranidal tasks (Lindauer 1953). There is considerable interindividual variability at every stage (Winston 1987; Seeley 1995) and this variability leads to task specialization among worker bees. Even among workers of similar ages, task specialization can be seen for undertaking (Visscher 1983), grooming (Kolmes 1989), guarding (Moore et al. 1987), foraging for pollen, nectar or water (Robinson & Page 1989; Page & Robinson 1991) and performing hygienic behaviour (Rothenbuhler 1964a,b) and some of these specializations could have a genetic basis.

Hygienic behaviour consists of two tasks, uncapping a cell containing dead brood and removing the contents of such an uncapped cell. Rothenbuhler (1964b), from his genetic experiments, proposed a two-locus model for the inheritance of hygienic behaviour with each locus controlling the expression of one component. Based on this model, there would be 'hygienic bees', homozygous for at least one of the two loci that would result in their expressing different aspects of the behaviour, and 'non-hygienic bees', heterozygous at both loci and not expressing the behaviour. Thus, according to this two-locus model, there would be a class of hygienic bees homozygous at one locus that would uncap cells with dead brood and another class of hygienic bees homozygous at another locus that would remove the cell contents. Bees that are homozygous at both loci would therefore be predicted to perform both uncapping and removing. Further genetic analysis suggested that a two-locus model for a complex social behaviour could be an oversimplification. Moritz (1988) proposed a three-locus model suggesting that hygienic behaviour could, in fact, be the result of more complex genetic mechanisms than simple Mendelian segregation.

Studies so far have tried to determine the genetic control of hygienic behaviour and its expression at the colony level. However, there have been few attempts to study the behavioural repertoire of individual hygienic bees to determine if there

is variability in the expression of the behaviour. The present study aims to document the age range and behavioural repertoire of individual hygienic bees and to determine if a specific age class and set of workers are involved in performing the task-components of hygienic behaviour.

Methods

Breeding for Hygienic Bee Colonies

The breeding programme for hygienic behaviour was initiated in 1993 by selecting two colonies of Italian-derived *A. mellifera* bees using a freeze-killed brood (FKB) assay described in Spivak & Downey (1998). Colonies that remove FKB within 48 h are considered hygienic, and those that take over 6 d to remove FKB are considered non-hygienic. The breeding methodology has been described in Spivak & Gilliam (1998b). Daughters of the most hygienic queens are inseminated with sperm of drones from the most hygienic colonies of the previous generation, resulting in queens and their worker progeny being homozygous for the alleles that govern hygienic behaviour.

Setting up Observation Hives

A comb containing stored honey and pollen, a laying queen, an empty comb, and approximately 1000 unmarked bees of various ages were used to establish two-frame observation hives. The queens in the observation hives were allowed to lay eggs, but the brood combs were replaced with empty combs every 18 d to ensure that no brood eclosed in the observation hive and that the bees in the observation hives were marked and derived from the parental colony. Combs containing pupae within 1–2 d of eclosion were removed from the parental colonies and placed in individual cages in an incubator held at 34°C and 50% relative humidity (RH). One-day-old bees eclosing from this comb were either marked with coloured paint on the thorax or individually number-tagged and introduced periodically into the observation hive. Behavioural observations were begun when the youngest of the unmarked bees were over 28 d old, and were therefore either predominantly foragers or dead.

Estimating the Age Range of Bees Performing Hygienic Behaviour

In 1996, two third-generation parental hygienic colonies formed the source of bees for two observation hives. Every 3 d, for 30 d, cohorts of 1-d-old bees were added to each observation hive after marking the bees with a unique colour of paint on the thorax using Testor's enamel paint (Testor Corp., USA). In the first cohort, 500 1-d-old bees were marked and in each subsequent cohort, 50 fewer bees were marked, until the fifth cohort had 300 marked bees. Thereafter, 300 bees were marked each time. This marking scheme ensured the maintenance of a

relatively equal age distribution of bees in the observation hive (Table 1). Approximately 3500 marked bees (10 colour-coded age cohorts) were added to each observation hive. Two sections of FKB, one in the bottom comb containing brood and the other in the top comb containing honey, were inserted into each observation hive through circular, removable plexiglass portals to provide the stimulus to elicit hygienic behaviour. One to two hours after introducing the FKB section, the colour marks on the bees observed uncapping and/or removing dead brood from each insert were recorded every 2 h from 07:30 to 21:30 h daily until all of the freeze-killed brood was removed from the section. To compare the age of bees performing hygienic behaviour with the age of foragers from the same colonies, the colour mark of the returning foragers was recorded at the hive entrance. In one of the colonies, all the remaining marked bees in each age cohort were counted at the end of the experiment to estimate the number of bees lost during the experimental period.

Behavioural Observations of Bees Performing Hygienic Behaviour

In 1998, one parental hygienic colony, selected from among the fifth generation of colonies in the breeding programme, was the source of all individually number-tagged bees observed while performing the hygienic behaviour. The eclosing bees were individually marked with a number-tag on the thorax. Fifty number-tagged 1-d-old hygienic bees were introduced to the observation hive every other day for a period of 30 d to ensure an even age distribution in the observation colony. Two such observation hives were used for the study, from Jun. to Sep. 1998. One of the colonies was observed from mid-Jun. until mid-Jul., and the other (established 1 mo later) was observed from mid-Jul. through Aug. The first observation hive

Table 1: The number of marked bees remaining in one of the observation hives (colony 1) at the end of the experiment

Date marked	Colour	Age of bees on Jul. 9, 1996 (d)	No. of bees introduced	No. of bees on Jul. 16, 1996	% bees lost
11 Jun.	Silver	28	505	180	64.4
14 Jun.	White	25	455	244	46.4
17 Jun.	Red	22	405	226	44.2
20 Jun.	Orange	19	346	246	28.9
23 Jun.	Yellow	16	304	253	16.8
26 Jun.	Green	13	300	278	7.3
29 Jun.	Blue	10	302	231	23.5
2 Jul.	Purple	7	304	229	24.7
5 Jul.	Pink	4	301	258	14.3
8 Jul.	Teal	1	311	267	14.1
Total			3533	2412	32%

had approximately 850 tagged hygienic bees added to it, and the second had approximately 750 bees added. To observe the onset of hygienic behaviour in the observation hives, a section of FKB was inserted in the centre of the bottom (brood) frame of the observation hive. Behavioural observations were begun 1 h after inserting the FKB.

Sampling the Behaviour of Hygienic Bees

Two techniques of behavioural sampling were employed: (1) instantaneous scans from video recordings of the comb section with bees performing the hygienic behaviour were taken, and (2) focal animal sampling of individual number-tagged bees was carried out for two 20-min periods, one in the presence and another in the absence of the FKB section.

Instantaneous scans

Video recordings of up to 12 h each were made of the region of the observation hive containing the FKB section from 09:00 to 21:00 h. From the videos, instantaneous scans were taken at 15-min intervals, and all the number-tagged bees present on the section and their behaviour were recorded. The proportion of number-tagged hygienic bees actually observed on the FKB section and the proportion of time spent by these bees in various behaviours were then calculated.

Focal animal sampling

A number-tagged bee present on the FKB section was picked and all the behaviours she exhibited in the next 20-min period were recorded on a hand-held computer (PSION Workabout PLC 1995 PSION, London). The time at which a behavioural act was commenced by the bee was also recorded to calculate the duration of each behavioural event. The FKB section was removed after a series of 20-min observations were completed. About 2 h later, the same bees were located and followed for another 20 min to record their behaviour in the absence of the stimulus. A total of 57 bees (31 bees from one colony and 26 bees from the other) were thus observed from the two observation colonies over a period of 3 mo.

A total of seven behaviours were recorded during the observation period. The most common behaviours exhibited both in the presence and in the absence of the FKB section were autogrooming (AG), walking (WA), and inspecting brood cells (IC; the bee thrust its head into the cell or entered it). In presence of the FKB section, two task-components of hygienic behaviour, uncapping cells with dead brood (UC; the bee removed the wax cap of the pupa) and removing dead pupae from these uncapped cells (RE; the bee dragged the dead pupa out of the cell with her mandibles), were observed. The other relatively rare behaviours recorded include standing (ST) and interaction (IN; antennating another bee or mouth-to-mouth contact).

The mean rates of performance of various behaviours (except ST and IN, which were rare) in terms of frequency per bee and the mean duration for which these behaviours lasted were calculated from the data. The last behaviour was

avoided when determining the duration since the behaviour was still ongoing when the observations were terminated. The mean rates of performance of these behaviours were subjected to principal components analysis followed by a cluster analysis of the points in the principal component space to determine whether there was any evidence of clustering of the bees based on the task-components of hygienic behaviour. In addition, the relative frequency of task performance for each task for every bee was calculated as the probability that a bee would perform a task relative to the other tasks it performs:

$$P_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$$

where P_i is the relative frequency of a task 'i', n_i is the number of times task 'i' is performed and k is the total number of tasks.

Results

Age of Bees Performing Hygienic Behaviour

The bees performing the hygienic behaviour were found to be 15–17 d of age. In the first colony, the bees observed uncapping and those observed removing were significantly younger than the foragers, while, in the second colony, only the bees observed uncapping were significantly younger than the foragers (Table 2). An estimate of the mean ages of bees uncapping and removing FKB and those of foragers indicates that the bees performing the hygienic behaviour were middle-aged bees.

Repertoire of Bees Performing Hygienic Behaviour

Analysis of behaviours on FKB section based on instantaneous scans

From the time activity budgets, it was evident that the time spent in the two components of hygienic behaviour was as follows: uncapping cells, about 40%,

Table 2: Mean age ($\bar{x} \pm$ SD) of bees observed uncapping and removing freeze-killed brood, and that of foragers (n given in parentheses)

Colony	Uncappers	Removers	Foragers
1	15.4 ± 7.4 a (165)	15.1 ± 6.1 a (77)	22.1 ± 5.1 b (79)
2	15.1 ± 7.2 a (199)	17.5 ± 6.8 b (100)	19.6 ± 5.2 b (66)

Different letters within each colony (across a row) indicate significant differences between the means, from single classification ANOVA followed by multiple comparisons of means by Tukey's HSD test. 'n' refers to the number of bees observed uncapping, removing, or foraging, respectively.

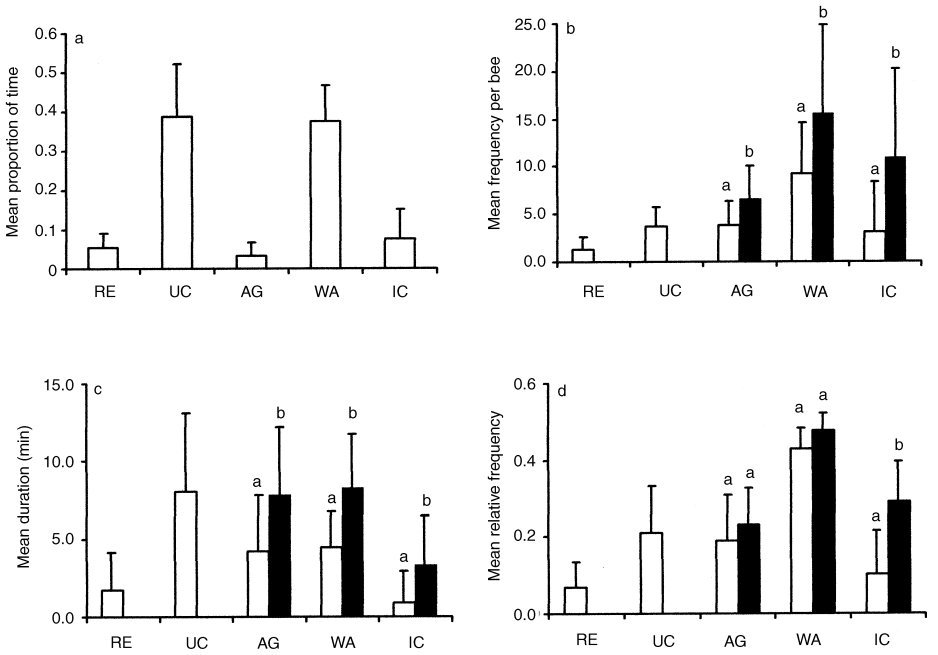

Fig. 1: a. Proportion of time spent in the various behaviours ($\bar{x} \pm SD$) calculated from instantaneous scans of bees present on an FKB section. The proportions are averaged across two colonies for a period of 12 d (the bees spent 7% of their time standing, which is not shown in the figure). b. Frequencies of behaviours ($\bar{x} \pm SD$). c. Durations of behaviours ($\bar{x} \pm SD$). d. Relative frequencies of behaviours ($\bar{x} \pm SD$). Open bars represent behaviours performed by the bees on the FKB section and solid bars represent behaviours performed by the same bees in the absence of the FKB section calculated from focal animal samples. Statistical comparisons are based on a paired t-test. Bars with different letters are significantly different ($p < 0.001$, $n_1 = n_2 = 57$ bees from two colonies; colony 1 = 31 bees and colony 2 = 26 bees). RE = removing dead brood, UC = uncapping the wax cap of the dead pupa, AG = autogrooming, WA = walking, and IC = inspecting cells

and removing dead brood, 2% (Fig. 1a). The bees, however, spent 55% of their time in the common behaviours like autogrooming, walking and inspecting cells, even in the presence of the FKB section in the colony. The number of different number-tagged bees seen working on the FKB section ranged from 22 to 120^{d-1}, over a span of 14 video-days. On average, 48.2 ± 28.0 bees were seen per day on the FKB section at least once. Assuming that a similar number of bees were working on the FKB section on the other side of the frame, the average provides an estimate of $12.8 \pm 3.7\%$ of the number-tagged bees in the colony actually seen performing the hygienic behaviour. From the 1996 study, it was evident that 32% of the bees were lost during the experimental period (Table 1) and, by correcting the above estimate for this loss, the percentage of bees performing hygienic behaviour is calculated to be $17.7 \pm 5.4\%$.

Analysis of behaviours on FKB section based on focal animal sampling

The mean frequency of behaviours per bee in a 20-min focal observation period in the presence and in the absence of the FKB section is shown in Fig. 1(b). In the presence of the FKB section in the colony, there was a significant decrease in the frequency of walking (paired t-test, $t_s = 6.19$, $p < 0.001$; $n = 57$) and in the frequency of inspecting cell contents (paired t-test, $t_s = 7.59$, $p < 0.001$; $n = 57$). A comparison of the mean duration that these behaviours lasted indicated that there was also a significant decrease in the duration of common behaviours (paired t-test, walking: $t_s = 11.24$, $p < 0.001$; $n = 57$; inspecting cells: $t_s = 7.12$, $p < 0.001$; $n = 57$) in the presence of the freeze-killed brood (Fig. 1c). The relative frequency evaluates the performance of each task relative to the other tasks. A comparison of the means indicates that the relative frequency of inspecting cell contents decreased significantly in the presence of dead brood (Fig. 1d). In other words, in the absence of dead brood stimulus, the observed hygienic bees had a higher probability of inspecting the cell contents of the colony.

There was a significant negative correlation (Fig. 2) between the rates of performance of the two task-components of the hygienic behaviour. Bees that uncapped cells at higher frequencies removed dead pupae at lower frequencies. A principal component analysis of the frequencies of the five tasks was carried out. The first two principal components explained about 93.7% (colony 1) and 85.6% (colony 2) of the variance in the data. The points in the principal component space fall into three clusters by visual delineation without prior assumptions about the number of clusters desired. The discreteness of these clusters was confirmed by iteration using the nearest centroid method (the distance between any individual point and the centroid of the cluster to which it belongs was less than its distance to the other two centroids) as described by Gadagkar & Joshi (1983). The centroid of a visually defined cluster was calculated iteratively including each outlier in the scatter plot in every iteration. Each iteration would have a different centroid depending on the points included in the cluster being tested. The cluster was said to be discrete when all the points in the cluster were at their minimum distance to the centroid of that cluster. Figs 3a and b depict the scatter of points in principal

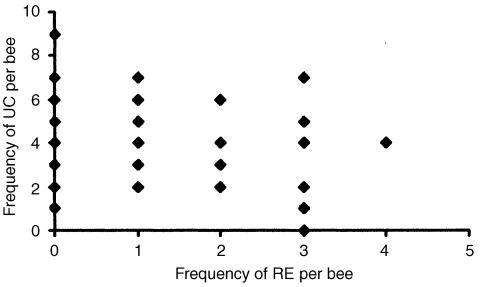

Fig. 2: Frequency of removing cell contents with dead brood is significantly negatively correlated with the frequency of uncapping cells (Kendall's rank correlation, $\tau = -0.29$, $p < 0.01$; $n = 57$)

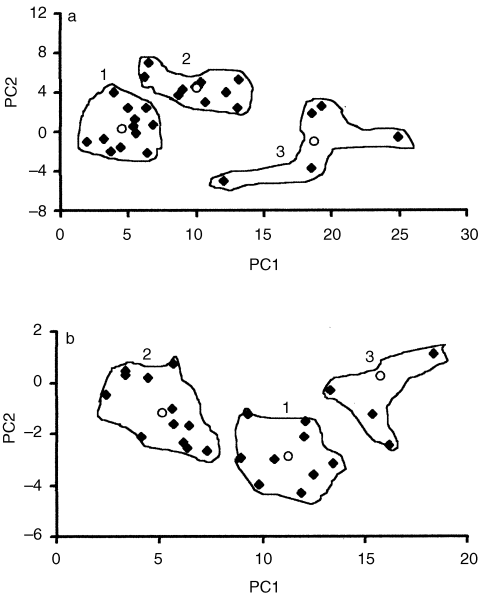

Fig. 3: Cluster diagrams of points distributed in the principal component space for colony 1 (a) and colony 2 (b). Closed diamonds represent the projections of each bee on PC1 and PC2 and the open circle is the centroid of each cluster. '1' refers to the cluster of bees that predominantly uncapped cells with dead brood, '2' refers to the cluster of bees that uncapped cells and removed dead brood to similar extents and '3' refers to the cluster that performed all behaviours (hygienic and others) to similar extents

component space and the different clusters resulting from the scatter. The first cluster (13 bees) in Fig. 3a is low on PC1 but intermediate on PC2. These bees exhibited a significantly higher frequency of UC than RE. The second cluster (11 bees) was high on PC2 but intermediate on PC1, and these bees exhibited similar frequencies of UC and RE. The remaining six bees fell in a third cluster, performing all the five tasks at similar frequencies and not conforming to any of the above classes. Similarly, when the scatter of points in the PC space for colony 2 was analysed, three such behavioural classes were obtained with 13, nine and four bees in the three classes, respectively. Similar results were obtained when the principal component analysis was repeated with relative frequencies of task performance. The first two principal components explained about 88% (colony 1) and 82.1% (colony 2) of the variance in the data. Though the classes obtained by both the measures were discrete in principal component space, the bees in each of the classes performed both the task-components of hygienic behaviour.

In both the colonies observed, the frequency of UC was significantly higher (Figs 4A,B) than the frequency of RE for bees falling into class 1 (Wilcoxon's matched pairs signed ranks test, $p < 0.05$). There was no significant difference in the frequencies of UC and RE for the bees that fell into class 2 (Wilcoxon's matched pairs signed ranks test, $p > 0.05$). In addition, the bees in class 1 in both the

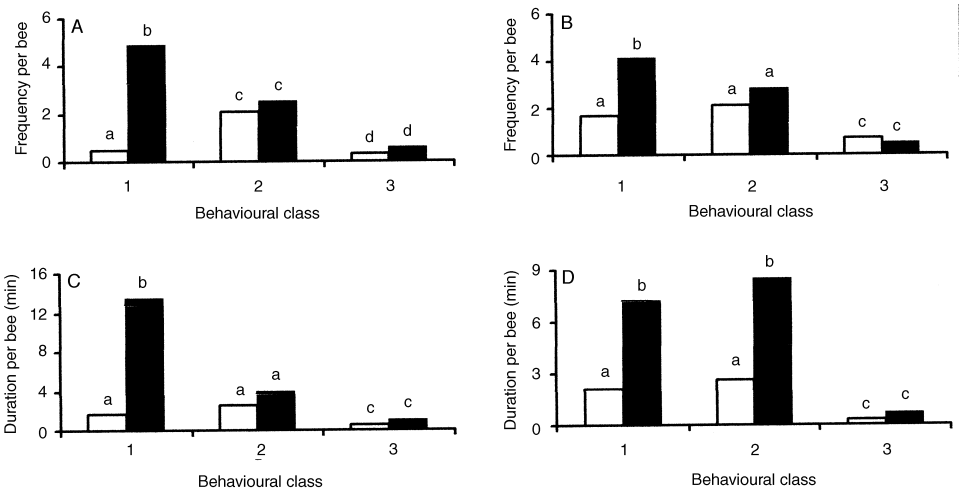

Fig. 4: Mean frequency of uncapping cells with dead brood (solid bars) and removing cell contents (open bars) by bees in the three classes in colony 1 (A) and colony 2 (B), and mean duration of uncapping cells with dead brood (solid bars) and removing cell contents (open bars) by bees in the three classes in colony 1 (C) and colony 2 (D). Paired bars were compared by Wilcoxon's matched pairs signed ranks tests [class 1: (A, C) $T_s = 0$; $n = 13$; $p < 0.05$; (B, D) $T_s = 3.5$ and 2, respectively; $n = 9$; $p < 0.05$; class 2: (A, C) $T_s = 22.5$ and 20, respectively; $n = 11$; $p > 0.05$; (B) $T_s = 21$; $n = 13$; $p > 0.05$; (D) $T_s = 7$; $n = 13$; $p < 0.05$]. Bars across each behavioural class were compared by Mann–Whitney U-test [uncapping: (A, B, C) $U_s = 165.5$, 119.5 and 150, respectively; $p < 0.05$; (D) $U_s = 75$, $p > 0.05$; removing: (A, C, D) $U_s = 67.5$, 97.5, 78.5; $p < 0.05$; (B) $U_s = 71.5$, $p > 0.05$). Bars with different letters represent values that are significantly different

colonies exhibited significantly higher frequencies of UC than the bees in class 2 (Mann–Whitney U-test, $p < 0.05$). However, the colonies differed in the frequency of RE exhibited by the bees in class 1 and class 2. In colony 1, bees in class 2 exhibited significantly higher frequencies of RE than bees in class 1, while in colony 2 the two classes of bees exhibited similar frequencies of RE. Bees in both categories exhibited significantly higher frequencies of IC in the absence of dead brood (Figs 5A,B; Wilcoxon's matched pairs signed ranks test, $p < 0.05$).

In both the colonies, bees coming under class 1 exhibited a longer duration of UC than RE (Figs 4C,D; Wilcoxon's matched pairs signed ranks test, $p < 0.05$). While the bees in class 2 in colony 1 performed UC and RE for similar durations (Wilcoxon's matched pairs signed ranks test, $p > 0.05$), the bees in class 2 in colony 2 showed a significantly longer duration of UC than RE (Wilcoxon's matched pairs signed ranks test, $p < 0.05$). In addition, the durations of UC and RE were not significantly different for the bees in the two classes in colony 2 (Mann–Whitney U-test, $p > 0.05$) while in colony 1 the bees in class 1 showed a significantly longer duration of UC but a similar duration of RE.

There was no significant difference in the frequencies of UC and RE for bees in class 3 (Figs 4A,B). However, these frequencies were significantly lower than the respective frequencies for bees in classes 1 and 2. The bees belonging to class 3 also

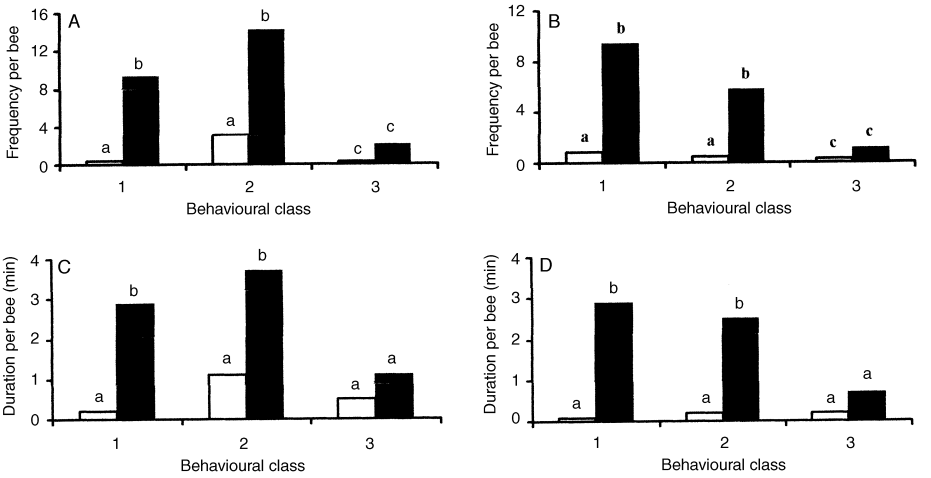

Fig. 5: Mean frequency of inspecting cell contents by bees in the three classes in the presence (open bars) and absence (solid bars) of freeze-killed brood in colony 1 (A) and colony 2 (B), and mean duration of inspecting cell contents by bees in the three classes in the presence (open bars) and absence (solid bars) of freeze-killed brood in colony 1 (C) and colony 2 (D). Paired bars were compared by Wilcoxon's matched pairs signed ranks test [class 1: (A, C) $T_s = 0$; $n = 13$; $p < 0.05$; (B, D) $T_s = 0$; $n = 9$; $p < 0.05$; class 2: (A, C) $T_s = 3$ and 5, respectively; $n = 11$; $p < 0.05$; (B, D) $T_s = 0$; $n = 13$; $p < 0.05$]. Bars with different letters represent values that are significantly different

had similar frequencies of IC (Figs 5A,B) both in the presence and in the absence of the FKB section, and these frequencies were significantly lower than those for bees in classes 1 and 2.

Interestingly, the mean duration of IC for bees in classes 1 and 2 was significantly longer in both the colonies in the absence of the FKB section (Figs 5C,D; Wilcoxon's matched pairs signed ranks test, $p < 0.05$), lending support to the possibility that the bees predisposed to perform hygienic behaviour inspect cell contents at higher frequencies and for longer durations.

There was no detectable difference in the ages of bees in the different classes. The mean age of bees in class 1 was 16.6 ± 10.2 d and 15.8 ± 5.5 d in the two colonies, respectively. This was not significantly different from the mean age of bees in class 2 (16.1 ± 5.5 d and 22.1 ± 8.3 d for the two colonies, respectively; Mann–Whitney U-test, $U_s = 81.5$ and $U_s = 27$ for the two colonies, respectively; $p > 0.05$). These ages were also not significantly different from that of the bees observed uncapping and removing FKB in the 1996 study.

Discussion

Hygienic behaviour is an important intranidal task in a honey bee colony and is performed by middle-aged bees. The above estimates of the ages of bees performing hygienic behaviour confirm that these bees have brood-rearing experience but have

not yet begun foraging. It is evident from the time activity budgets of the bees performing hygienic behaviour that 42% of their time is spent in the two components of hygienic behaviour, while in the remaining time the bees are engaged in common behaviours such as walking, autogrooming and inspecting cell contents. The common behaviours described above are known to be the main components of the behavioural repertoire of middle-aged bees (Kolmes 1985; Seeley 1995). Hygienic behaviour is exhibited by a small percentage (18%) of the bees in the colony. A similarly small percentage of bees that are task specialists has also been reported for bees performing undertaking, the removal of dead adult bees from the colony. Bees performing the hygienic behaviour did not perform undertaking but were seen to drop the dead brood they pulled out to the base of the colony which would then be cleaned out by other worker bees (Arathi, pers. obs.). Undertakers are members of the forager age class, unlike the bees performing the hygienic behaviour, and were also found to constitute a small subset of colony workers: about 1–2% at any time (Sakagami 1953; Visscher 1983). It is interesting, however, to note here that the small percentage of bees found performing the hygienic behaviour in this study is despite the fact that these colonies were selected for hygienic behaviour. The bees in these colonies should be homozygous for the character and all of them are therefore equally likely to perform the behaviour. The small percentage of bees that performed the hygienic behaviour, however, performed both components of it and did not show any evidence of clear task partitioning between uncapping cells and removing cell contents.

The analysis of behaviours performed in the presence and in the absence of dead brood indicated that there was a decreased frequency of task performance of common behaviours in the presence of dead brood. Such a decrease in frequency could be due to an increased need to uncap cells with dead brood and remove the cell contents, and also to the prolonged nature of these tasks. Walking, an intermediary step between any two behavioural events, could have reduced in frequency in the presence of freeze-killed brood, due to the possibility that, once the bees started uncapping cells, they remained at it for a length of time and did not switch behaviours.

The increased frequency of inspecting cell contents in the absence of dead brood in the colony could have an important implication for the behavioural profile of these bees selected for hygienic behaviour. Inspecting cells could provide the worker bees with information about the general well-being of the larvae via pheromones that the larvae secrete (Le Conte et al. 1990). Huang & Otis (1991) have proposed that honey bee workers inspect larval cells frequently, not just to assess the hunger status of larvae but also to obtain other information on colony status. Bees that perform hygienic behaviour could be more likely to inspect cell contents in a colony, once they have been exposed to the stimulus of dead brood. The handling of dead brood and emptying of the cell contents could stimulate inspection of cells for signs of disease or any abnormalities. Alternatively, bees selected for hygienic behaviour could be more predisposed to inspect cells irrespective of any abnormalities in the colony.

Ergonomic analysis of task partitioning predicts that, if two task-related

behaviours are to be performed in the same location and require similar skills, then it should be more efficient for the same set of individuals to perform both tasks (Oster & Wilson 1978). The two task-related behaviours of uncapping cells and removing cell contents require to be performed at the same location but do not necessarily require similar skills. The requirement of differential skills could lead to task partitioning, but the need for tasks to be performed at the same location may still result in non-discrete behavioural classes. The two-locus genetic model may explain to some extent the behavioural classes in accordance with Rothenbuhler (1964b). The bees in class 1 could correspond to the class of 'Uncappers' of Rothenbuhler (1964b) and could be homozygous for at least one locus. The bees in class 2 perform the two behaviours of uncapping and removing to similar extents, and could be homozygous for both the loci. However, the behavioural classes obtained in this study were not behaviourally discrete and all the bees performed both components of hygienic behaviour, though at significantly different frequencies and for different durations, which suggests that the two-locus model may be an oversimplification of the actual genetics of the behaviour. The results of this study seem to support the predictions of Moritz (1988) that hygienic behaviour may be governed by genetic mechanisms more complex than a simple two-locus model of inheritance. In addition, expression of hygienic behaviour may also depend on the resources available (Momot & Rothenbuhler 1971) or the colony composition.

The partitioning of tasks among workers in a social insect colony could also result from differential response thresholds of individual workers to the associated stimulus (Theraulaz et al. 1998). The expression of hygienic behaviour thus may depend on the response thresholds of hygienic bees in addition to their ability to perceive the necessary cues to detect and remove dead brood. These response thresholds could be genetically based, and workers that have a low response threshold to abnormal cues associated with diseased brood could begin uncapping cells.

Learning has long been known to influence the efficiency of task performance, especially in foragers (Dukas & Visscher 1994), but there is very little known about the effects of learning on in-hive tasks except in nest repair in the paper wasp *Polistes fuscatus* (Downing 1992). It has been suggested that, in honey bees, learning may not be an important component of specialization in most undertakers and guards, given the brief tenure of these tasks (Breed et al. 1992; Trumbo et al. 1997). Though the performance of hygienic behaviour is also brief in tenure, it is very unlikely that learning is not an important component. On the contrary, given that the behaviour is performed for a brief time in a worker bee's life and is associated with handling diseased brood, it is likely that its performance becomes more efficient through learning. Learning could lead to better detection of the stimulus with continued exposure (Masterman et al. 2000). Earlier experiments by Trump et al. (1967) proposed that bees do not learn from their association with other hygienic bees, but just being in the same hive as other hygienic bees may not have the same effect as actively performing the behaviour. Uncapping cells with dead or diseased brood could result in bees performing these tasks more efficiently. Further

studies are necessary to determine the factors that lead to the expression and categorization of the components of hygienic behaviour.

Acknowledgements

This research was supported by a National Science Foundation grant (IBN-9722416) to MS. The authors thank John Hickey for his enthusiastic assistance with behavioural observations, and Rebecca Masterman, Elaine Evans, Laura Heuser Kimball and Jeremiah Baumann for their help at various stages of the experiments. AHS wishes to thank Niranjan Joshi for valuable discussions and suggestions and Gary Reuter, Jenny Warner and David Tronrud for assisting with the maintenance of the honey bee colonies. This is contribution #99117–0013 from the Minnesota Agricultural Experiment Station.

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Received: June 11, 1999

Initial acceptance: September 15, 1999

Final acceptance: October 30, 1999 (J. Brockmann)