



Influence of colony genotypic composition on the performance of hygienic behaviour in the honeybee, *Apis mellifera* L.

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Hygienic behaviour, an intranidal task performed by middle-aged worker bees is an important behavioural mechanism of resistance to disease and to attack by *Varroa destructor*, an ectoparasitic mite. We studied the effect of a colony's genotypic composition on the expression of this behaviour among worker bees by creating normal age-structured colonies with different proportions of bees belonging to hygienic and nonhygienic lines. We established four colonies with 0, 25, 50 or 100% of worker bees belonging to the hygienic line. Analyses of the behaviour of hygienic bees in these colonies indicated that the performance of hygienic behaviour depended on the proportion of hygienic bees in the colony. Hygienic bees in the 25% hygienic colony performed the behaviour well beyond middle age and were more persistent at the task compared with bees from the same genetic line in the other colonies. However, the colony with all worker bees from the hygienic line was more efficient in achieving the task despite a lack of persistence. We also observed that in the colony with 50 and 100% hygienic bees, the behaviour was partitioned into subtasks, and some bees performed the subtask of uncapping cells at higher frequencies than the subtask of removing cell contents. These results suggest that a colony's genotypic composition influences the performance and partitioning of hygienic behaviour. We propose that the performance of hygienic behaviour and its partitioning into subtasks could be determined by response thresholds of individual worker bees and that the rate of behavioural ontogeny may be controlled by the demand for specific tasks.

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The dominant feature of insect societies is that of individual workers performing behavioural acts repeatedly and nonrandomly. As a result, colony organization and worker allocation, are important themes of behavioural studies of social insects (Wilson 1971; Oster & Wilson 1978; Gordon 1996). Insect societies show organization at two levels: the individual level, with a worker performing an array of activities; and the colony level, characterized by complex interactions among the individual colony members (Calderone & Page 1991). The behavioural profile of workers in a colony is determined by their collective activity, both with respect to the relative frequencies with which they perform various tasks and the social organization within which those tasks are performed.

Studies of division of labour in the honeybee *Apis mellifera* have demonstrated that workers switch tasks as they age, a phenomenon known as age polyethism

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(Lindauer 1953a, b; Wilson 1971; Seeley 1982, 1995; Winston 1987; Page & Robinson 1991; Robinson 1992; Calderone 1998). Age polyethism in social insect colonies is a highly flexible system, reflecting the ability of workers to respond constantly to changing needs determined by factors within and outside the colony (Robinson 1992). Factors influencing this flexibility include changing hormonal levels (Robinson et al. 1994), genotypic variability for task performance (Rothenbuhler 1964; Calderone & Page 1988, 1991, 1992; Frumhoff & Baker 1988; Robinson & Page 1988, 1989; Rothenbuhler & Page 1989; Breed et al. 1990), response thresholds of workers to task stimulus (Page 1997; Page et al. 1998; Theraulaz et al. 1998; Pankiw & Page 2000) and the demand for task performance (Tofts & Franks 1992; Franks & Tofts 1994).

A classic study of animal behaviour involves the behavioural genetics of hygienic behaviour by workers of the honeybee *A. mellifera* (Rothenbuhler 1964; reviewed in Spivak & Gilliam 1998a). Rothenbuhler (1964) reported that worker bees from a line (Brown line) resistant to American foulbrood (a bacterial disease of honeybee larvae), uncapped cells containing diseased brood and removed the brood from the nest, eliminating the

pathogen before it sporulated, while workers from a susceptible Van Scoy line did not uncapped or remove the diseased brood. Rothenbuhler (1964) postulated that the behaviour is controlled by two, independently assorting recessive loci; one for uncapping diseased brood, and the other for removing it from the nest. Bees could thus be uncappers, removers, or both depending on homozygosity of the alleles at one or both loci. Hygienic behaviour is apparently unique to the honeybees (Michener 1974; Spivak & Gilliam 1993). It is becoming increasingly important as a behavioural mechanism of disease resistance (Rothenbuhler 1964; Gilliam et al. 1983) and as a behavioural defence against the ectoparasitic mite *Varroa destructor* (formerly *Varroa jacobsoni* Oudemans, Anderson & Trueman 2000), a serious pest damaging bee colonies (Spivak & Reuter 1998; Boecking & Spivak 1999). We have selected new resistant and susceptible lines of hygienic and nonhygienic bees, respectively, using a freeze-killed brood assay (Spivak & Downey 1998). The hygienic bees were bred through artificial insemination to be homozygous for the loci that govern the trait. We have found that hygienic behaviour is predominantly performed by the middle-aged worker bees that have not yet begun foraging and that about 18% of the bees in the colony are actually involved in the task at any given time (Arathi et al. 2000).

An important feature of the organization of work in insect societies is 'task partitioning', a mechanism by which two or more individuals contribute sequentially towards the performance of a task (Jeanne 1991; Ratnieks & Anderson 1999). Hygienic behaviour is a task partitioned into subtasks of uncapping cells and removing cell contents. In our previous work (Arathi et al. 2000), the hygienic bees in two different colonies allocated themselves into one class that performed a higher frequency of uncapping than removing, and another class of bees that performed both uncapping and removing at similar frequencies. Because worker honeybees show considerable flexibility in their responses to colony environments (Ribbands 1952; Lindauer 1953a, b), it is possible that while the potential to perform hygienic behaviour is determined genetically, the actual partitioning of hygienic behaviour into subtasks could be influenced by the colony environment.

Previous studies on the effects of worker genotype on behavioural flexibility have concentrated on extranidal tasks such as foraging for nectar, pollen and water. Here, we studied the effect of colony genotypic composition on the expression of an important intranidal task, by creating colonies with normal age structures but with different proportions of bees belonging to the hygienic and nonhygienic lines. Worker bees were observed while performing hygienic behaviour, and we report evidence showing that a colony's genotypic composition influences: (1) the persistence in the performance of hygienic behaviour, (2) the partitioning of hygienic behaviour, (3) the age at which bees performed hygienic behaviour, and (4) the efficiency of task performance. In addition to making a direct test of the behavioural responses of hygienic bees to varying colony genotypic composition, we also evaluated the effect of task demand on age polyethism.

METHODS

Maintaining Field Colonies

The breeding programme for hygienic behaviour was initiated in 1993 by selecting colonies of Italian-derived *A. mellifera* bees using a freeze-killed brood (FKB) assay described in Spivak & Downey (1998). Colonies that removed FKB within 48 h were considered hygienic, those that took over 6 days for the same were considered nonhygienic. The breeding methodology is described in Spivak & Gilliam (1998b). For each generation, we instrumentally inseminated daughters of the most hygienic queens with 6–8 μ l of sperm from drones of the most hygienic colonies of the previous generation, resulting in the queens and their workers being homozygous for the alleles that govern the behaviour. Similarly, we inseminated daughters from the most nonhygienic queens with sperm from drones of the most nonhygienic colonies, resulting in workers being heterozygous for the alleles. One parental hygienic and one nonhygienic queen, both selected randomly from among the sixth generation of colonies in the breeding programme, were the source of bees that made up the four observation colonies in the present study. The colonies were maintained in standard Langstroth beekeeping equipment. Frames with eggs from each of the parental colonies were moved into a common 'nursery' colony headed by a naturally mated queen (Calderone & Page 1988). Cofostering the bees during their development in the nursery colony eliminated potential effects that could result from nutritional differences in colony brood rearing. 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% RH. The eclosing bees were individually marked with a number-tag on the thorax. A total of 100 newly eclosed worker bees individually identified with coloured, number-tags were introduced into glass-sided observation hives every 3 days for a period of 60 days in order to ensure an even age distribution in the observation colony. Each of the observation hives contained different proportions of hygienic and nonhygienic bees derived from one parental colony of the hygienic line and one of the nonhygienic line, respectively.

Setting Up Observation Hives

We used a comb containing stored honey and pollen, a laying queen, an empty comb and approximately 1000 unmarked bees of various ages to establish two-frame observation hives. We allowed the queens in the observation hives to lay eggs, but we replaced the brood combs with empty combs every 18 days to ensure that no brood eclosed in the observation hive; thus all bees in the observation hives were marked and derived from the parental hygienic and nonhygienic colonies. We began behavioural observations when the marked bees were 3–21 days old.

We thus established four observation hives each differing in the proportions of bees from the hygienic line and

the nonhygienic line. The 25H colony had 25% hygienic bees and 75% nonhygienic bees. To create this colony, we introduced 25 newly eclosed, number-tagged hygienic bees and 75 newly eclosed, number-tagged nonhygienic bees every 3 days over a period of 60 days. We established the 50H colony by adding 50 hygienic and 50 nonhygienic bees every 3 days, and similarly established the 100H and 100N colonies by adding 100 hygienic or 100 nonhygienic bees, respectively.

Behavioural Observations

Instantaneous scans

To observe the onset of hygienic behaviour in the observation hives, we inserted a section of FKB in the centre of the bottom (brood) frame of the observation hive. The section was 6 × 4 cm in dimension and contained a mean of 215.3 cells (SD=5.3). We counted the number of cells with intact pupal wax caps and the number of empty cells in each comb section before inserting the FKB into the brood frame and again at the end of 12 h. We made video recordings up to 6 h each day of the region of the observation hive containing the FKB section between 0900 and 2100 hours. We made video recordings on each colony on 3 days resulting in 18 h of recording for each colony. Each day of recording on the same colony was separated by at least a week. We performed instantaneous scans of all the number-tagged bees present on the section and noted their behaviour from the videotapes at 15-min intervals resulting in 26 instantaneous scans each day and a total of 78 scans for each colony.

Focal animal sampling

We picked a number-tagged hygienic bee performing hygienic behaviour on the FKB section and recorded all the behaviours she displayed in the next 20-min period using a hand-held computer (PSION[®] Workabout PLC 1995). The behaviours recorded during the observation period were autogrooming, walking, inspecting brood cells (the bee thrust its head into the cell or entered it) and the two subtasks of hygienic behaviour: uncapping cells with dead brood (uncapping the wax cap of the pupae) and removing dead pupae from these uncapped cells (dragging the dead pupa out of the cell with her mandibles).

Data Analysis

Instantaneous scan data

The observation colonies with different proportions of hygienic and nonhygienic bees accordingly had different proportions of bees with the genetic potential to perform hygienic behaviour. To determine whether hygienic bees performed the behaviour nonrandomly depending on the kind of 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 Poisson distribution. There were a few bees (1–11 bees in the three different colonies) that performed the

Table 1. Colony composition and performance of hygienic behaviour in the four different observation colonies

Colony	Number of hygienic bees (nonhygienic)	Number of bees performing hygienic behaviour (%)	
		Hygienic	Nonhygienic
25H	400 (1200)	315 (78.75)	75 (6.25)
50H	800 (800)	317 (39.62)	81 (10.13)
100H	1600 (0)	307 (19.18)	—
100N	0 (1600)	—	190 (11.87)

behaviour more than four times in the day and therefore, we pooled these into one class for analysis. Accordingly, the bees that performed the behaviour more than four times were said to be persistent in the task. Although we used the absolute frequency of bees for the statistical test, we present proportions in Fig. 1 for ease of comparison across colonies. In addition, we compared the age of bees performing the hygienic behaviour and the percentage of cleaned cells in the FKB section among the four colonies to determine the effect of colony composition on the behavioural development of the worker bees and their efficiency in the different colonies, respectively.

Data from the focal samples

We observed 30 hygienic bees 20 min each in each of the colonies. We calculated the mean rates of performance of various behaviours in terms of frequency per bee from the data. We analysed the mean rates of performance of these behaviours using 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.

RESULTS

Analysis of Behavioural Data from Instantaneous Scans

The data from the instantaneous scans indicated that the overall percentage of hygienic bees performing hygienic behaviour ranged from 19.2% in the 100H colony to 78.8% in the 25H colony. The percentage of nonhygienic bees performing the task ranged from 6.3% in the 25H colony to 11.9% in the 100N colony (Table 1).

Persistence of Hygienic Bees Performing the Behaviour

The number of times an individual bee performed hygienic behaviour differed depending on the genotypic composition of the colony. Figure 1 shows the distribution of the number of performances of hygienic behaviour per worker bee for all the bees that encountered the FKB section in the three different colonies pooled over all the video-days.

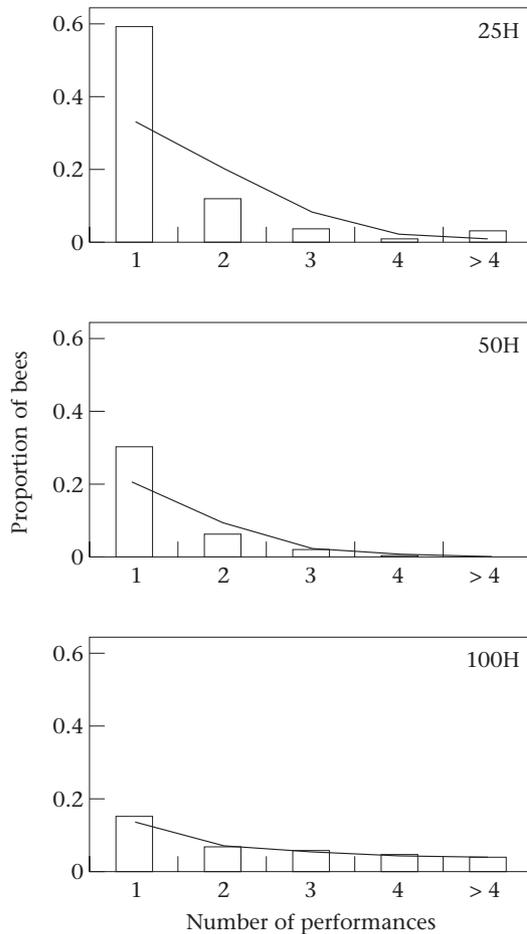


Figure 1. The distribution of the number of performances of hygienic behaviour per bee versus the proportion of the total number of hygienic bees in the colony (bars). The line indicates the numbers expected from a Poisson distribution. Kolmogorov–Smirnov test: 25H colony: $D_{\max}=0.138$, $D_{0.001}=0.119$, $N=374$, $P<0.001$; 50H colony: $D_{\max}=0.128$, $D_{0.001}=0.110$, $N=437$, $P<0.001$; 100H colony: $D_{\max}=0.097$, $D_{0.001}=0.128$, $N=307$, NS.

In the 25H colony, the observed distribution of the number of performances differed significantly from that expected by a Poisson distribution (Fig. 1a; Kolmogorov–Smirnov test: $P<0.001$). The coefficient of dispersion was 1.4, indicating that the distribution was clumped. More than the expected number of bees performed hygienic behaviour once, and the number of bees performing the behaviour more than four times was also higher than expected. These latter data provide evidence of increased persistence in the task, with a few bees performing the behaviour repeatedly.

In the 50H colony, the observed distribution of the number of performances differed significantly from that expected by a Poisson distribution (Fig. 1b; Kolmogorov–Smirnov test: $P<0.001$). The coefficient of dispersion was 0.77, indicating that the distribution was repulsed. More than the expected number of bees performed hygienic behaviour once but the number of bees performing the behaviour more than four times did not differ from expected. This suggests that there was no evidence of persistence in the task and the performance of

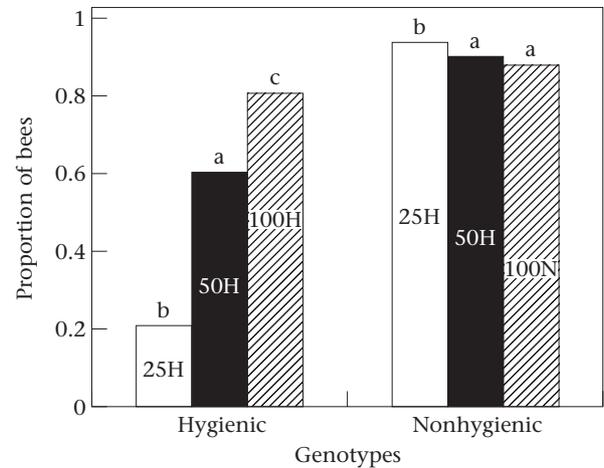


Figure 2. Proportion of bees of the respective genotypes that never performed hygienic behaviour in the colonies with different compositions of hygienic and nonhygienic bees. The comparison was for bees of a similar genotype across colonies using G test of proportions. Bars with different letters were significantly different from each other. Hygienic bees: 25H versus 50H: $G_1=171.99$, $P<0.01$; 25H versus 100H: $G_1=501.22$, $P<0.01$; 50H versus 100H: $G_1=117.76$, $P<0.01$. Nonhygienic bees: 25H versus 50H: $G_1=9.82$, $P<0.01$; 25H versus 100N: $G_1=26.37$, $P<0.01$; 50H versus 100N: $G_1=1.66$, NS.

the behaviour among the bees was more uniformly distributed.

In the 100H colony, the observed distribution of the number of performances was not significantly different from a Poisson distribution (Fig. 1c; Kolmogorov–Smirnov test: NS). Under conditions where every worker in the colony was capable of performing the task, only very few individuals actually performed the task at any given point.

The behaviour of nonhygienic bees did not show any evidence of being affected by the genotypic composition of the colony. The nonhygienic bees performed the task randomly in all colonies with the distribution of the number of performances of hygienic behaviour not being significantly different from that expected by a Poisson distribution (Kolmogorov–Smirnov test: NS).

A comparison of the proportion of bees that never performed hygienic behaviour and the ones that performed the task at least once indicated that the 25H colony had a very low proportion of hygienic bees that never performed the task (0.213; Fig. 2). This proportion was significantly lower than those in the 50H and 100H colonies (G test of proportions: $P<0.01$). In addition, the proportion of hygienic bees in the 50H colony that never performed the task (0.603) was significantly lower than the proportion in 100H colony (0.808; G test of proportions: $P<0.01$; Fig. 2). Among the nonhygienic bees, the proportion of bees in the 25H colony that never performed the task (0.938) was significantly higher than that in the 50H and 100N colonies (G test of proportions: $P<0.01$). However, the proportion of bees in the 50H colony that never performed hygienic behaviour (0.899) was not significantly different from that in the 100N colony (0.881; G test of proportions: $P>0.01$; Fig. 2).

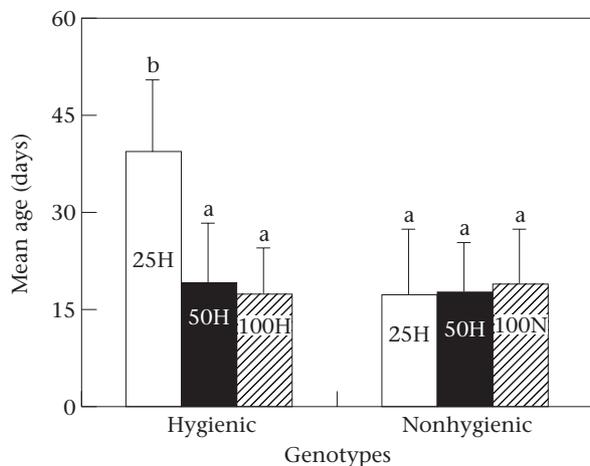


Figure 3. Age (mean \pm SD) of the bees performing hygienic behaviour. The comparison was for bees of a similar genotype across colonies, and among bees of different genotypes within a colony using Student's *t* test. Bars with different letters were significantly different from each other. Hygienic bees: 25H versus 50H: $t_{630}=1.98$, $P<0.05$; 25H versus 100H: $t_{620}=2.32$, $P<0.05$; 50H versus 100H: $t_{622}=0.23$, NS. Nonhygienic bees: 25H versus 50H: $t_{154}=0.02$, NS; 25H versus 100N: $t_{263}=-0.19$, NS; 50H versus 100N: $t_{269}=-0.19$, NS. Hygienic bees versus nonhygienic bees: 25H colony: $t_{388}=2.01$, $P<0.05$; 50H colony: $t_{396}=0.22$, NS; 100H versus 100N: $t_{495}=-0.21$, NS.

Although the proportion of bees never seen performing the task was similar in 100H and 100N colonies, bees in the 100H colony cleaned the FKB section, whereas bees in the 100N colony did not (discussed in detail below).

Age of Bees Performing Hygienic Behaviour

A comparison of the ages of the bees performing hygienic behaviour in the different colonies indicated that the age at task performance varied significantly depending on the composition of the colony the bees cohabited and their genotype. Figure 3 depicts the mean ages of the bees performing hygienic behaviour in the different colonies. The hygienic bees in the 25H colony performed hygienic behaviour well beyond middle age and the oldest bee seen performing the task was 56 days old. Hygienic bees in the 50H and 100H colonies were within the middle-aged category (17–19 days). The mean age of hygienic bees in the 25H colony performing hygienic behaviour was therefore significantly higher than that in the 50H and 100H colonies. There was no significant difference between the mean ages of hygienic bees performing the task in 50H and 100H colonies. The nonhygienic bees also performed the task within the middle-aged category irrespective of the composition of the colony they cohabited (Fig. 3). There was no significant difference in the mean age at task performance among nonhygienic bees in the 25H, 50H and 100N colonies. However, in the 25H colony, hygienic bees that performed the behaviour were significantly older than nonhygienic bees that performed the task, but there was no significant difference in the mean ages of hygienic and

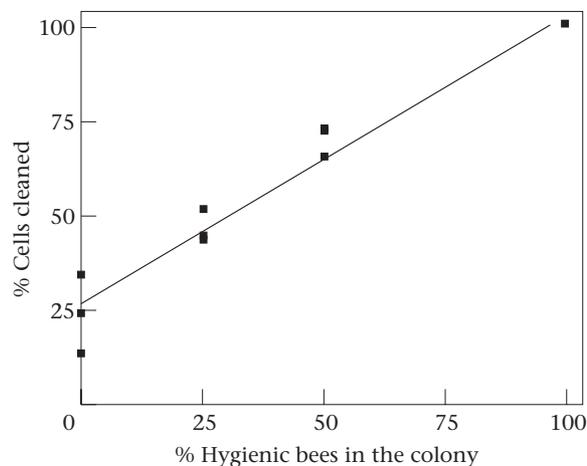


Figure 4. Efficiency of task performance in terms of the percentage of cells in the FKB section cleaned increased significantly with the increase in the percentage of hygienic bees in the colony (linear regression analysis; $r^2=0.957$, $Y=0.755X+27.223$, SE of slope=0.051, $t_{11}=14.89$, $P<0.001$).

nonhygienic bees performing hygienic behaviour in the 50H colony.

Efficiency of Bees in the Different Colonies

We measured the efficiency of hygienic behaviour based on the average percentage of FKB cells that were cleaned after a given period of time (12 h). The 100H colony was the most efficient, consistently cleaning up all cells in the FKB section in a span of 12 h. The 100N colony cleaned an average of 26.1% of the cells and 25H and 50H colonies cleaned 46.2 and 69.6% of the cells, respectively, in the same amount of time. A linear regression analysis indicated that there was a significant increase in the percentage of cells cleaned with an increase in the percentage of hygienic bees in the colony ($r^2=0.957$, $P<0.001$; Fig. 4).

Analysis of Behavioural Data from Focal Bee Sampling

Mean absolute frequencies of performance of the activities performed by the hygienic bees in the four colonies are presented in Fig. 5. Data from the focal bee sampling indicated that hygienic bees performed all five behaviours (autogrooming; walking; inspecting brood cells; uncapping cells; removing dead brood) and the mean frequency of performance of these behaviours per bee in the 20-min sampling period was similar irrespective of the colony's genotypic composition.

Principal components analysis of the frequencies of the five behaviours revealed that the grouping of hygienic bees based on the performance of the two subtasks, uncapping cells and removing cell contents, differed depending on the genotypic composition of the colony. The points in principal component space were visually delineated into clusters without any prior assumptions of the number of clusters desired, and the discreteness of

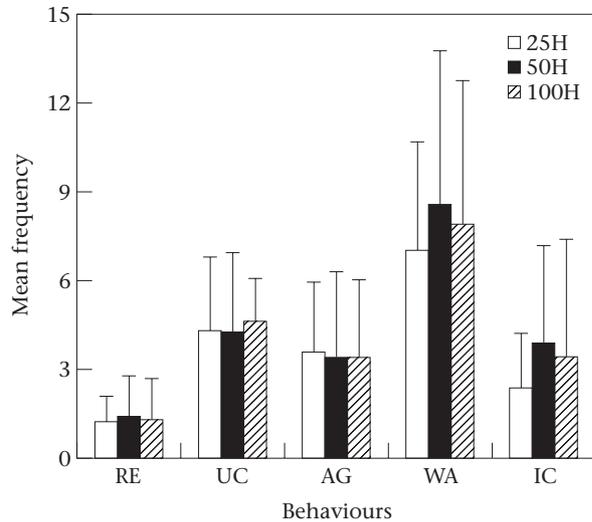


Figure 5. Frequencies of behaviours (mean \pm SD) shown by hygienic bees in a 20-min period on the FKB section. RE=removing dead brood, UC=uncapping the wax cap of the dead pupa, AG=autogrooming, WA=walking and IC=inspecting cells.

these clusters was confirmed by iteration using the nearest centroid method (Arathi et al. 2000). The first two principal components (PC1, PC2) explained about 77% of the observed variance in the data in the 25H colony, about 88% in the 50H colony and about 87% in the 100H colony. The hygienic bees in the 25H colony did not show any evidence of task partitioning (Fig. 6) and were distributed all across the principal component space. The hygienic bees from the 50H colony allocated into two classes (Fig. 6). One class that was intermediate on PC1 and PC2 showed similar frequencies of uncapping and removal and another with seven bees high on PC1 and distributed all along the PC2 showed significantly higher frequencies of uncapping than removal (Fig. 7). Hygienic bees in the 100H colony allocated into three classes (Fig. 6). The cluster high on PC2 and low on PC1 showed significantly higher frequencies of uncapping than removal. Another cluster intermediate on PC1 and PC2 showed similar frequencies of uncapping and removal, and the third cluster with three bees had equal frequencies of all five behaviours observed (Fig. 7).

DISCUSSION

These results support earlier studies of genotypic interactions influencing task performance among foraging honeybees where it was shown that an individual's pattern of task performance was affected not only by her own genotype but also by the genotypes of her nestmates. Studies on task specialization in honeybees have shown that genotypic variability has a major effect on the rate of behavioural development (Calderone & Page 1988, 1992; Rothenbuhler & Page 1989; Page & Robinson 1991; Page et al. 1992). Our experiments were aimed at performance of hygienic behaviour, an important intranidal task in a honeybee colony. The results show that the genotypic composition of a colony affects the performance of

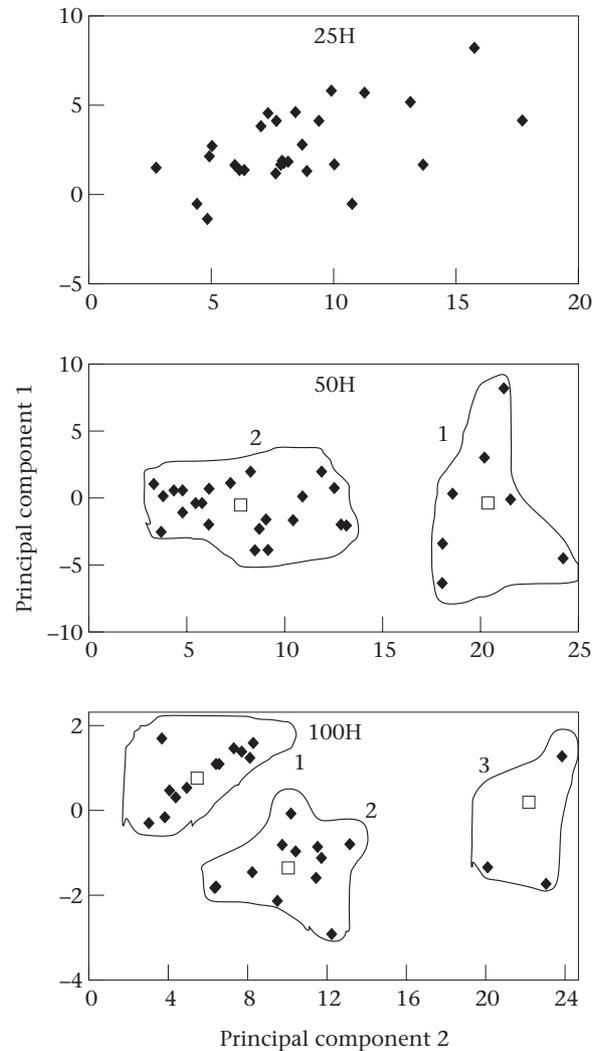


Figure 6. Points distributed in the principal component (PC) space for each colony (25H, 50H and 100H) and the observed clusters in the space. ◆: The projections of each bee on PC1 and PC2; □: the centroid of the relevant cluster. Numbered clusters: '1' refers to the cluster of bees that predominantly uncapped cells with dead brood; '2' refers to the cluster of bees that uncapped cells and removed cell contents to similar extents; '3' refers to the cluster of bees that performed all behaviours (hygienic and others) to similar extents.

hygienic behaviour by influencing the persistence of bees in performing the task, the age of the bees performing the task, the efficiency of task performance and also the partitioning of the task among the hygienic bees.

Task Performance by Hygienic Bees and Their Efficiency

Hygienic bees in colonies composed of 50% or fewer hygienic bees showed a nonrandom performance of hygienic behaviour with a large number of bees performing the task once (25H and 50H colonies), and a higher number of bees persisting in the task and performing it more than four times (25H colony). About 78% of the hygienic bees in the 25H colony performed the task at

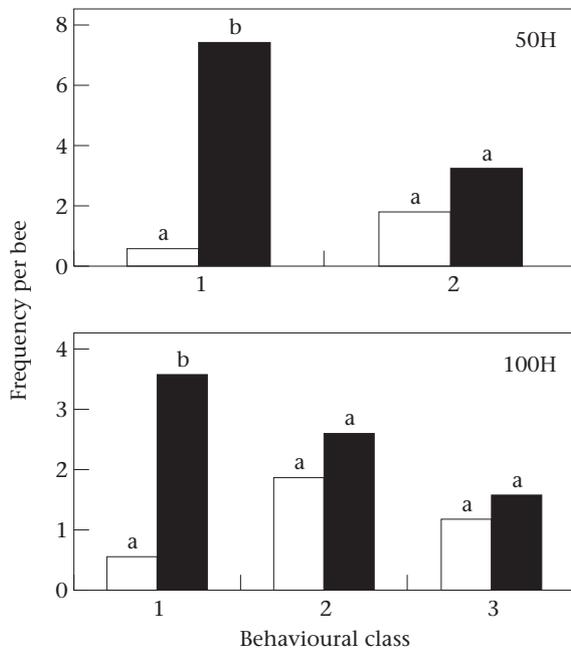


Figure 7. Frequency (mean±SD) of uncapping cells with dead brood (■) and removing cell contents (□) by bees in the various classes for the 50H colony and 100H colony. Paired bars within a class were compared by Wilcoxon matched-pairs signed-ranks tests. Bars with different letters were significantly different from each other. 50H class 1: $T=0$, $N=7$, $P<0.01$; class 2: $T=50.5$, $N=22$, NS; 100H class 1: $T=0$, $N=14$, $P<0.01$; class 2: $T=5.5$, $N=11$, NS; class 3: $T=5$, $N=4$, NS.

least once in contrast to 19% in the 100H colony. Because resources did not allow us to replicate each type of colony, it is possible that the observed differences between bee types contain sources of variability other than our treatment component. However, these findings confirm our earlier study of two 100H colonies in which all individuals were theoretically capable of performing the task but only a small percentage actually did so at any given time (Arathi et al. 2000). Our present findings are not counterintuitive given the number of hygienic bees available to perform the task in the three different colonies. A Poisson approximation of the probability that a bee never performed the task yielded a value of 0.82 in 100H colony versus 0.46 in 25H colony, values which are close to the observed proportions of 0.81 and 0.21, respectively. Although the amounts of FKB provided to each of the colonies were of the same dimensions, the demand on the colonies differed because the number of bees of the hygienic line varied. The hygienic bees in the 25H colony had to work harder to complete the task, which resulted in a larger proportion of bees performing the task at least once and also a larger number of individuals being persistent.

However, the hygienic bees in the 25H and 50H colonies were still not able to perform the task to its completion as evidenced by 25–50% of the cells of the FKB still remaining capped at the end of 12 h, whereas the bees in the 100H colony had completely cleaned the FKB section. A genotypically diverse group of workers may perform tasks less efficiently than a group of workers of

similar genotypes (Calderone & Page 1992). The inefficiency of the hygienic bees in the 25H and 50H colonies could have been because the number of hygienic bees available to perform the task was not enough to complete the task in the 12-h period. Alternatively, the inefficiency of the 25H and 50H colonies could have been because the nonhygienic bees recapped cells uncapped by hygienic bees. A previous study showed that bees of the nonhygienic line recapped partially uncapped FKB cells (Spivak & Gilliam 1993). Nonhygienic bees have a higher response threshold to the task stimulus, as indicated in our neuroethological studies and electroantennogram recordings of individual hygienic and nonhygienic bees exposed to varying concentrations of extracts of brood infected with chalk brood, a fungal disease (Masterman 2000; Masterman et al. 2000). If the nonhygienic bees were actually recapping the FKB in our present experiment, then the stimulus for hygienic behaviour in mixed genotype colonies would remain low, and also would cause the hygienic bees with a low response threshold to persist in the task. In the 100H colony however, there was little chance that an uncapped cell would be recapped. With every instance of hygienic behaviour, the stimulus levels would be reduced, resulting in fewer bees remaining on the task. Workers with higher response thresholds would be less likely to perform the behaviour as the FKB was being progressively cleaned, and bees would also be less likely to return to the task. This agrees with the suggestions of the response-threshold mediated-task performance model (Page 1997; Theraulaz et al. 1998) according to which the performance of the behaviour by the hygienic bees should vary depending on the extent of the stimulus in the colony.

Task Partitioning among Hygienic Bees

Another way to increase efficiency within a set of individuals of similar genotypes is by partitioning a task into its component subtasks (Oster & Wilson 1978; Ratnieks & Anderson 1999). In our experiment, task partitioning was observed in the 50H and 100H colonies where the bees partitioned hygienic behaviour and clustered into behavioural classes, while bees in the 25H colony did not display such task partitioning, resulting in a lack of any behavioural classes. However, unlike the 50H colony, the hygienic bees in the 100H colony both partitioned the task and were efficient as evidenced by the cleaned FKB section. Individuals in the 100H colonies that showed higher frequencies of uncapping than removing could perform the subtask of uncapping leaving the other bees to complete the remaining subtask of removal. Individuals in the 25H colony probably attempted to uncap cells and remove dead brood without partitioning the behaviour into subtasks because there were fewer individuals with the required competence. This could have resulted in the bees trying to work in tandem, which would have reduced their efficiency. Because the parental queens of the hygienic bees used in our study were inseminated with sperm from drones of the sixth generation of selection and all the worker offspring were presumably homozygous for the alleles

governing hygienic behaviour, it was not surprising that all observed bees in the 100H colony performed both uncapping and removing as postulated by [Rothenbuhler \(1964\)](#). However, of interest is that the 100H colony had a more discrete class of uncappers and was more efficient than the bees in the other two colonies, which suggests that the expression of the trait is modulated by colony environment.

The response-threshold model also can explain the observed behavioural classification and task partitioning among hygienic bees in the 100H colony. The response threshold for the stimulus that elicits hygienic behaviour could be expressed as a continuum among bees in a colony. Our preliminary electroantennogram (EAG) data confirm that hygienic bees collected from a colony while uncapping FKB have a lower threshold response to diseased brood odour than hygienic bees collected while removing FKB (R. Ross, unpublished data). Bees with the lowest threshold begin uncapping cells, which leads to a few cells being uncapped and exposing the dead brood within, resulting in increased stimulus levels in the colony. Bees with a high response threshold would then begin uncapping cells and once a few cells are completely uncapped, these individuals would then also remove the cell contents. Once a few cells with dead brood have been cleaned the overall stimulus levels would be reduced, and the bees with the lowest response threshold would discontinue performing the behaviour, while other bees with medium–high response thresholds would remain on the task until the whole section has been cleaned. Such a pattern could result in the observed behavioural classification of bees into ones that performed a high frequency of uncapping and ones that performed both uncapping and removing to similar extents. By such response-threshold mediated-task partitioning, the bees in the 100H colony could have achieved higher efficiency.

Another mechanism to explain the observed classification among hygienic bees in the 100H colony is the possibility of individuals following simple rules of increasing and decreasing propensities for a subtask as proposed by [Spencer et al. \(1998\)](#). According to this model, the performance of a particular subtask by an individual increases its propensity to perform that task and vice versa. The second subtask would initially have no individuals in the group that had previously encountered the subtask. But once they encounter and perform the second subtask their propensity to perform it would gradually increase until there are individuals performing both subtasks at equal rates. Accordingly, the performance of uncapping by a worker bee could increase the propensity to remain in that subtask, and after a short period, the sequential second subtask of removing cell contents would be initiated. Initially some individuals would perform only uncapping, and later on individuals would begin removing cell contents in addition to uncapping cells resulting in the second class of bees that perform both tasks at equal frequencies. Such a classification results in maintaining specialization as long as tasks are available and task switching when circumstances change leads to individuals specializing according to the needs of the colony ([Bourke & Franks 1995](#)).

Behavioural Development among Hygienic Bees

The age at which bees performed hygienic behaviour indicated that bees in the 25H colony overworked and performed the task well beyond middle age. The mean age of hygienic bees performing hygienic behaviour in the 25H colony was 39.43 days compared with 17.56 days in the 100H colony ([Fig. 3](#)). The nonhygienic bees in the 25H colony performing the behaviour were within the middle-aged category, being 17.41 days old, and possibly went on to become foragers at their normal foraging ages. This result suggests that hygienic bees in the 25H colony had a reduced rate of behavioural ontogeny and continued to perform intranidal tasks even when they reached the normal foraging age. The age and identity of foragers from hygienic and nonhygienic lines was not recorded in these experiments, as that was not the main focus of the study. However, it is reasonable to assume that nonhygienic bees progressed in their behavioural ontogeny to become foragers at their normal foraging age since the age range of the nonhygienic bees seen performing hygienic behaviour was between 6 and 25 days and older nonhygienic bees were never seen on the FKB section.

This result of overaged bees of the hygienic line performing hygienic behaviour cannot be explained on the basis of lack of individuals of a particular age class because the age distribution in the colonies was maintained close to that of a normal colony. In the other reports of overaged nurses ([Robinson & Page 1989](#); [Robinson et al. 1992](#)), the age structure of the colony was altered to consist only of older bees, and it was accordingly observed that older bees revert to nursing due to the lack of bees of younger ages. In our experiment, the bees in the 25H colony remained in intranidal tasks, suggesting retarded development rather than behavioural reversal seen in the earlier studies ([Robinson & Page 1989](#); [Robinson et al. 1992](#)), while the bees of the nonhygienic line probably progressed normally through their behavioural ontogeny to become foragers. Genotypic variability leading to differential rates of behavioural development has been reported ([Robinson et al. 1989](#); [Rothenbuhler & Page 1989](#); [Page et al. 1992](#)), but all of these studies used colonies with an atypical age structure, with the bees belonging to single or limited age cohorts.

Our results lend support to the possibility that demand for a task within the colony can regulate the rate of behavioural ontogeny ([Tofts & Franks 1992](#); [Franks & Tofts 1994](#); [Bourke & Franks 1995](#); [Calderone 1998](#)) even in a colony that has a normal age structure. In the colony with very few hygienic bees, the demand for cleaning the FKB cells remained high with very few bees actually capable of performing the task, resulting in the hygienic bees retarding their behavioural ontogeny, while the nonhygienic bees displayed normal rates of behavioural development. [Huang & Robinson \(1996\)](#) demonstrated that age demography of a honeybee colony can influence temporal division of labour. They suggested that the schedules of division of labour in a honeybee colony can be maintained independent of task needs but claimed that further experiments need to be performed keeping age demography constant and altering demand. Our

study does precisely this by keeping the area of the comb providing the stimulus for hygienic behaviour constant and varying the number of individuals competent to perform the task, thus indirectly altering demand for the task in the different colonies. We observed that under such conditions of varying demand, individuals that were capable of performing a task remained at the task as long as the demand persisted while others moved on to other tasks, thus ensuring that the colony needs were accomplished. This suggests that the schedule of division of labour in a honeybee colony is determined more by task needs than entirely by the age demography. This form of flexible behavioural development determined by demand on the colony is more beneficial than an age-based behavioural ontogeny since individual workers can respond to the needs of the colony irrespective of their age-scheduled task performances. This result also provides evidence for the suggestion that skilful workers (the hygienic bees) could be taking the task of performing the hygienic behaviour away from the less specialized workers (nonhygienic bees), which then tend to find other tasks like receiving nectar or foraging (Franks & Tofts 1994).

A differential schedule of age polyethism among bees within a colony is more advantageous than all bees following a fixed rate of behavioural development with age. If individual worker bees can respond to the needs of the colony by remaining behaviourally competent to perform the required tasks, it could cause changes in rates of behavioural development among workers even in a normal age-structured colony. Such a regulation of behavioural development in response to colony needs provides more flexibility and maintains social homeostasis by providing a way to regulate the required levels of response to changing environments.

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