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Differential responses of honeybee (*Apis mellifera*) patrilines to changes in stimuli for the generalist tasks of nursing and foraging

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Abstract Which task a social insect worker engages in is influenced by the worker's age, genotype and the colony's needs. In the honeybee, *Apis mellifera*, genotype influences both the age a worker switches tasks and its propensity of engaging in specialist tasks, such as water collecting, which only some workers will perform. In this study, we used colonies with natural levels of genetic diversity and manipulated colony age demography to drastically increase the stimuli for the generalist tasks of foraging and nursing, which all workers are thought to engage in at some point in their lives. We examined the representation of worker patrilines engaged in nursing and foraging before and after the perturbation. The representation of patrilines among foragers and nurses differed from that of their overall colony's population. In the case of foraging, over- and underrepresentation of some patrilines was not simply due to differences in rates of development among patrilines. We show that replacement foragers tend to be drawn from patrilines that were overrepresented among foragers before the perturbation, suggesting that there is a genetic component to the tendency to engage in foraging. In contrast, the representation of patrilines in replacement nurses differed from that in the unperturbed nursing population. Our results show that there is a genetic influence on even the generalist tasks of foraging and nursing, and that the way patrilines in genetically diverse colonies respond to increases in task stimuli depends upon the task. The possible significance of this genetic influence on task allocation is discussed.

Keywords Task allocation · Task plasticity · Stimulus response threshold · Polyandry · Age demography

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Introduction

In an insect society, workers allocate themselves to the tasks required by their colony in a manner that maximises colony productivity. Task allocation must be flexible so that the colony can respond appropriately to changing circumstances, such as an unexpected increase in food resources or a loss of foragers due to inclement weather. In honeybee (*Apis mellifera*) colonies, the primary division of labour among workers is based on age polyethism (Lindauer 1971; Seeley 1985; Winston 1987; Seeley and Kolmes 1991). Younger workers perform tasks within the nest, such as nursing brood. As they mature, workers perform tasks at the nest periphery, and finally become foragers at 20–24 days of age (Lindauer 1971; Seeley 1985; Winston 1987).

Although this is the typical behavioural ontogeny, it is flexible and can be modified according to colony needs. This is an important ability because catastrophes can eliminate large proportions of the forager population, while the natural process of reproduction by fission results in a daughter colony composed mainly of older workers (Winston 1987; Robinson et al. 1989; Robinson 1992). If

there are insufficient foragers, some younger workers will accelerate their behavioural development and become foragers (Huang and Robinson 1996). Alternatively, if there are insufficient nurses, then some older workers and even foragers will reverse their development to become nurses again (Page et al. 1992; Robinson et al. 1992; Huang and Robinson 1996).

A second important factor influencing the division of labour in honeybee colonies is genotype. *A. mellifera* queens are polyandrous, mating with 14 males on average (Palmer and Oldroyd 2000). Colonies consequently consist of workers of multiple patrilines that all share the same mother but which have different fathers. Because of different alleles carried by the fathers of each patriline, a worker's patriline often affects the probability that it will engage in a range of specialist tasks that are only performed by a small proportion of the available workers. These specialist tasks include collecting water (Robinson et al. 1984; Kryger et al. 2000), defending the nest (Breed et al. 1990; Guzman-Novoa and Page 1994), removing corpses (Robinson and Page 1988, 1995) or dead brood (Rothenbuhler 1964) and ventilating the nest (Oldroyd et al. 1994; Jones et al. 2004).

Patriline also influences the age at which a worker makes the major transition from 'middle age tasks' at the nest periphery, such as guarding, corpse removal or nectar receiving, to foraging (Lindauer 1971; Seeley 1985; Winston 1987). All workers are believed to engage in nursing and foraging tasks at some time in their lives; these tasks can then be referred to as 'generalist' tasks as opposed to 'specialist' tasks that are only performed by some workers (Winston 1987; Calderone and Page 1988; Robinson et al. 1989; Kolmes et al. 1989; Page et al. 1992; Robinson and Huang 1998). These patrilineal differences in the probability and age that a worker will perform a particular task are thought to arise from patrilines requiring different levels of stimuli before they will engage in a particular task (e.g. Crozier and Page 1985; Calderone and Page 1988; Robinson 2002; Myerscough and Oldroyd 2004). Increased stimulus levels result in the response threshold being met for an increased number of patrilines, and in more workers engaging in the task (Robinson 1992; Fewell and Page 1993; Jones et al. 2004).

It has been suggested that this genetic polyethism results in a more flexible and appropriate allocation of workers to tasks and thus to be one potential benefit of polyandry (Page et al. 1995; Palmer and Oldroyd 2000; Fuchs and Moritz 1998; Crozier and Fjerdingstad 2001). Computer simulations suggest that the response of genetically diverse colonies to changing needs is smoother and more optimal than that of more genetically homogenous colonies (Bertram et al. 2003; Cox and Myerscough 2003; Myerscough and Oldroyd 2004; Graham et al. 2006). Empirical evidence supports this hypothesis: genetically diverse colonies maintain a more homeostatic within-colony environment than do genetically uniform colonies (Jones et al. 2004).

Despite good evidence for genetic diversity in stimulus thresholds and some support for this contributing to a more optimal division of labour, there are few data showing how genetic polytheism works in practice when stimuli change. An increase in a stimulus, for example, will result in more workers engaging in the relevant task(s). However, these additional workers could come from patrilines that were already predisposed to engage in that task, or from other patrilines whose response threshold has now been reached, or from all patrilines in a colony to a similar extent. Several studies have found that increasing stimuli results in workers of more patrilines engaging in the specialist tasks of pollen collecting and nest defence (Fewell and Page 1993, 2000; Fewell and Bertram 1999; Hunt et al. 2003), while Jones et al. (2004) found that workers of different patrilines fanned at different temperatures. Analogous studies on the allocation of workers to the generalist tasks of foraging and nursing are less clear-cut, being based on colonies with very few patrilines and/or consisting of workers with different mothers, but it appears that worker patrilines that tend to forage precociously are also those that are likely to accelerate their development when there is a shortage of foragers (Robinson et al. 1989; Page et al. 1992; Giray and Robinson 1994).

In contrast, there appears to be no genetic influence on the likelihood an existing forager will revert to nursing behaviour. Rather, reversion to nursing tasks is more likely in individuals that have more recently switched to foraging tasks (Page et al. 1992; Robinson et al. 1992).

Here we investigate how patriline influences the probability that a worker will engage in the generalist tasks of foraging and nursing. We do this both before and after experimentally altering colony demography and thus the colony-level need for foragers or nurses. We show that in naturally mated colonies some patrilines are disproportionately represented in foraging and nursing task groups compared to the colony as a whole, and that patriline also influences which workers switch task after a demographic perturbation. The results show how patrilineal proportions among foragers and nurses arise from a combination of both colony need (task stimulus) and genotype.

Materials and methods

Fieldwork was conducted at the University of Western Sydney, Richmond Campus. The study colonies were headed by naturally mated queens of commercial Italian type. The colonies comprised six brood combs and 12 combs covered in adult bees.

Experiment I. Replacement of foragers

Experiment I was conducted in March 2004 using three replicate colonies (1, 2, 3). To estimate the frequency of each patriline in the colony we collected a sample of approximately 200 adult workers for each colony by vacuuming them haphazardly throughout the nest into a container. Although genetic polyethism means that patrilines will to some extent be distributed according to task rather than in a homogenous manner, these samples consisted of workers taken from all areas of the nest and were thus likely to be representative of the colony as a whole. We then removed 1–2 combs of emerging brood and placed them in separate cages in an incubator at 35°C and 80% relative humidity. We checked the combs daily for emerging workers and marked newly emerged workers (< 24 hours old) with non-toxic paint (Posca Paint Pens, Mitsubishi Pencil, Japan.) on the thorax and/or abdomen for 13 days. The colour and position of the marks allowed us to determine the age of each worker throughout the experiment. After marking the bees we returned them to their parent colony.

When the oldest marked workers were 24 days old, we moved the experimental colonies 20 m away from their original locations at 1100 hours. We immediately placed a hive box containing a single comb of honey at the original site of each relocated colony to act as a 'trap' for the foragers. All experienced foragers, including both those foraging at the time of the manipulation and any flying afterwards from the relocated colonies, would have orientated to the original locations and thus returned from their foraging trips to the trap hives (Page et al. 1992; Robinson et al. 1992; Huang and Robinson 1996). All workers that became foragers after the manipulation undertook orientation flights directed at the hives' new locations and therefore returned to the relocated hives.

The next morning, before foraging commenced, we closed the trap hives and put them in a freezer at −20°C, thus obtaining a sample of each colony's original foragers (hereafter O-foragers). The trap hives contained many hundreds of marked and unmarked workers, supporting the assumption that experienced foragers returned to the trap hives. Two days after the relocation, we closed the entrance to the relocated hives and sampled approximately 200 returning foragers via aspiration at the hive entrance. These replacement foragers (hereafter R-foragers) were workers that must have initiated foraging after the relocation of the colony. The samples therefore allowed us to compare the relative ages and genotypes of the O- and R-foragers, and to compare these to the colony.

Experiment II. Replacement of nurses and foragers

Experiment II was carried out in April 2004 using three colonies (A, B, C), different from those used in Experiment I. For each colony, we collected a random sample of approximately 200 workers using the same method as in Experiment I to determine the frequency of patrilines in the overall colony. To establish the patriline frequencies in the original nurse (hereafter O-nurse) task group, we also sampled approximately 200 workers from open brood cells containing larvae, as most workers on brood combs can be assumed to be engaged in nursing (Winston 1987; Page et al. 1992; Huang and Robinson 1996). At 1100 hours we relocated the three colonies approximately 500 m (a greater distance than in Experiment I because robbing was prevalent at the original apiary) from their original sites and placed new hive boxes containing a comb of honey/pollen, a comb of young brood and three empty combs at the original sites. As in Experiment I, experienced foragers returned to the trap hives; the relocated colonies consisted of younger bees. The combs of young brood were placed in the trap hives to stimulate some O-foragers to revert to nursing, which occurs within about 2 days (Huang and Robinson 1996).

We collected approximately 200 returning O-foragers from the entrance of the trap hives via aspiration immediately after the split. Five days after the split, we sampled approximately 200 workers from open brood cells of the trap hives. These replacement nurses (hereafter R-nurses) comprised workers that were originally O-foragers that are assumed to have reverted to nursing after splitting the colonies. We also sampled R-foragers from the entrances of the relocated hives. Unlike Experiment I, no age data were collected in Experiment II because too few marked workers were recovered due to a change in marking technique.

DNA extraction and microsatellite analysis

DNA was extracted with the Chelex® method (Estoup et al. 1994). Microsatellite loci (A7, A8, A14, A29, A35, Ap53, A79, A88, A107, B124; Estoup et al. 1994; Franck et al. 2000) were amplified using a standard PCR program of 94°C for 3 min, followed by 35 cycles of 94, 55 and 72°C for 30 s each, and finally 72°C for 9 min. PCR products were electrophoresed on a GS2000 (Corbett Research, Sydney) or analysed on a 3130*xl* Genetic Analyzer (Applied BioSystems, CA, USA). Maternal alleles were assigned at each locus based on all workers having the allele or having one of two alleles (indicating that the queen was homozygous or heterozygous at the locus, respectively). Paternal alleles for each locus were then inferred by subtraction and individuals assigned to patrilines based on their deduced genotypes. After testing workers from each colony with all ten microsatellite loci, we chose 3–5 loci that efficiently differentiated among the patrilines of each colony.

Statistical analyses of patriline proportions

We compared the frequencies of patrilines in the O-forager, R-forager and O-nurse task groups with their frequencies in the colony (hereafter overall colony) samples using a sampling version of Fisher's exact test using Monte Carlo RxC Contingency Table Test V2.1 (W. Engel, University of Wisconsin, Madison, WI, USA; Fowler et al. 1998; Zar 1999). We compared the frequencies of patrilines in the O-forager and R-forager task groups to determine if the same patrilines were over- or underrepresented in these forager task groups. The R-nurse task group was compared to the O-forager task group, as the population from which the R-nurses were allocated consisted only of O-foragers. Figures summarising the number of patrilines and sample sizes for each colony can be found in the online supplementary material (S4–S9). To control for the possibility of false discoveries resulting from the multiple tests performed we used sequential Bonferroni corrections (Sokal and Rohlf 1995).

The power of the tests was determined using the programme G*Power 2.1.2 (Erdfelder et al. 1996). We calculated the effect size w (the degree of deviation from the null hypothesis in our experiments) following Cohen (1988) and present it as the mean ± SE of the three colonies.

We examined the relationships between the representation of patrilines in the task-group samples (O-foragers, O-nurses, etc) relative to the overall colony. The patrilineal frequencies were adjusted to equalise their sample sizes. This was done using the ratio of the overall colony sample to the task-group sample. We then calculated, for each patriline, the empirical logit of its frequency in each task group (O-foragers, R-foragers, etc) divided by its frequency in the overall colony (Sokal and Rohlf 1995). This provided a measure of a patriline's over- or underrepresentation, or bias, in a task group relative to its representation in the overall colony.

We then weighted the empirical logits by the reciprocals of the binomial variances ($n/p[1\text{-}p]$), where n is the total number of workers sampled and p is the adjusted proportion in the overall colony; Zar 1999), so that more abundant patrilines had greater weight than those in which fewer workers were sampled. After these initial calculations, we used bivariate correlations of the weighted empirical logits of patrilines within colonies to examine the relationships between patriline proportions in task groups. This allowed us to determine, for example, whether a patriline's bias toward or against being an O-forager is correlated with its bias towards or against being an R-forager. We pooled the three experimental colonies for each test; individual colony results are presented in electronic supplementary material (S1). Results are presented as a Pearson's correlation (r) of the null hypothesis that, for example, the O-forager bias is not significantly correlated with the R-forager bias.

Statistical analysis of worker age

In Experiment I we used univariate analyses of variance to examine, for each colony, whether the age of workers differed among patrilines or between the O-forager and R-forager task groups, and whether there was any interaction between patriline and task group. We also examined the relationships between the average age of each patriline and their representation in the O-forager and R-forager task groups using correlations to determine if, for example, patrilines overrepresented in the O-forager or R-forager task groups were precocious foragers. We pooled the results for each test; individual colony results are presented in the online supplementary material (S2–S3).

Results

Experiment I. Replacement of foragers

There were significant differences between the representation of patrilines in the O-foragers and the overall colony (Table 1; w=0.468±0.058; see S4 for figure). There were also significant differences between patriline representation in the samples of R-foragers and the overall colony (Table 1; w=0.362±0.006; see S5 for figure). There was no difference in patriline representation when O- and R-foragers were compared directly to two of the three colonies (Table 1). There was a significant tendency for patrilines that were over (or under) represented in the O-forager task group, given their representation in the overall colony, to also be over (or under) represented in the R-forager task group (r=0.580, P<0.001, df=53; Fig. 1; S1).

There was no interaction between the effects of patriline and task group (O- or R-foragers) on the average ages of foragers (ANOVA: $F_{20,633}$=0.973, P=0.494; S3). R-foragers were significantly younger (18.3 days±0.296) than the O-foragers (19.7 days±0.206; $F_{1,57}$=12.8, P=0.001; S3). There was no variation among patrilines in the age of their foragers (ANOVA: $F_{36,633}$=1.33, P=0.100; S3). Neither O-forager bias or R-forager bias of a patriline correlated with its average age (O-forager bias: r=0.087, P=0.545, df=51; R-forager bias: r=0.110, P=0.444, df=51; S2). There was a significant correlation between the mean age of a patriline's O-foragers and R-foragers (r=0.326, P=0.020, df=51; Fig. 2; S2).

Experiment II. Replacement of nurses and foragers

As in Experiment I, there were significant differences between the representation of patrilines in the O-forager task group and the overall colony (Table 1; w=0.423±0.02; see S6 for figure). There were no significant differences in

Table 1 *P* values and power for Fisher's exact tests comparing the representation of patrilines in the different task groups for experiments I and II

	P	Power
Experiment I		
Overall colony vs O-forager		
Colony 1	0.007*	0.83
Colony 2	<0.001*	0.82
Colony 3	0.144	0.82
Overall colony vs R-forager		
Colony 1	0.046*	0.81
Colony 2	0.020*	0.84
Colony 3	0.011*	0.86
O-forager vs R-forager		
Colony 1	0.002*	0.82
Colony 2	0.311	0.85
Colony 3	0.120	0.86
Experiment II		
Overall colony vs O-forager		
Colony A	<0.001*	0.75
Colony B	0.002*	0.71
Colony C	0.039*	0.77
Overall colony vs R-forager		
Colony A	0.873	0.54
Colony B	0.433	0.47
Colony C	0.001*	0.71
O-forager vs R-forager		
Colony A	0.289	0.44
Colony B	0.443	0.35
Colony C	0.524	0.62
Overall colony vs O-nurse		
Colony A	0.027*	0.80
Colony B	0.049*	0.75
Colony C	0.001*	0.75
O-forager vs R-nurse		
Colony A	0.014*	0.72
Colony B	0.022*	0.70
Colony C	0.408	0.72

* indicates significance at the α=0.05 level after sequential Bonferroni corrections were performed.

patriline proportions between the R-forager task group and the overall colony in two of the three colonies (Table 1; w=0.356±0.066; see S7 for figure). However, the sample sizes of R-foragers were very low due to experiment II being conducted late in the season, so statistical power was very limited (Table 1). When O and R-forager task groups were directly compared there was no significant difference in patrilineal distribution (Table 1). There was no correlation between patriline bias in O and R-forager tasks groups (r=0.201, P=0.196, df=37; Fig. 3; S1). However, this again is likely to be due to the low power of the test, and thus should not be taken as evidence against the findings of Experiment I.

The representation of patrilines in the O-nurse task group differed from the overall colony (Table 1; w=0.401±0.029; see S8 for figure). Patriline proportions differed between the R-nurses and the O-forager task group from which they were drawn in two of the three colonies (Table 1; w=0.377± 0.026; see S9 for figure). There was no relationship between a patriline's relative bias in the O-nurse and R-nurse task groups (r=0.145, P=0.347, df=44; S1). There was a significant positive relationship between a patriline's O-forager and O-nurse bias (r=0.670, P<0.001, df=45; Fig. 4; S1). R-nurse bias was significantly negatively correlated with O-forager bias (r=−0.354, P=0.016, df= 46; Fig. 5; S1). There was a similar relationship between O and R-forager task groups as in Experiment I, but due to the low power of the test this was not statistically significant (r=−0.033, P=0.850, df=35; S1).

Discussion

Our results show that even in an unperturbed colony of *A. mellifera*, some patrilines are over- or underrepresented in the O-foraging task group (Fig. 1). After the increases in stimuli that resulted from the removal of O-foragers, many workers switched to become R-foragers, and patriline also influenced which workers were most likely to switch. Here, patrilines that were over- or underrepresented in the O-forager population were similarly over- or underrepresented in the R-forager population. All workers are thought to become foragers in later life (e.g. Lindauer 1971; Seeley 1985; Winston 1987; Seeley and Kolmes 1991), and so the representation of patrilines among foragers would be expected to mirror that of the overall colony. This is clearly not the case. What mechanisms, therefore, determine why workers of some patrilines are more likely to become foragers than others?

The only established genetic influence on foraging tendency is the age at which workers begin foraging (Calderone and Page 1988; Robinson et al. 1989; Kolmes et al. 1989; Page et al. 1992; Robinson and Huang 1998). However, this could not have generated the non-random representation of patrilines in the O-foragers that we observed. This could only happen if there is patrilineal variation in the length of time workers survive as foragers. Otherwise, patrilines that begin foraging at a younger age would be expected to die younger, and their representation in the O-foragers should not be different from that in the colony. There is no convincing evidence for genetic variation in survival time, as studies have not distinguished between genetic, maternal and developmental cause (Guzman-Novoa and Gary 1993; Guzman-Novoa et al. 1994).

We did not find a correlation between a patriline's average O- or R-forager age and its O- or R-forager bias. If the difference in a patriline's likelihood of foraging was caused by differential longevity, with younger age of first

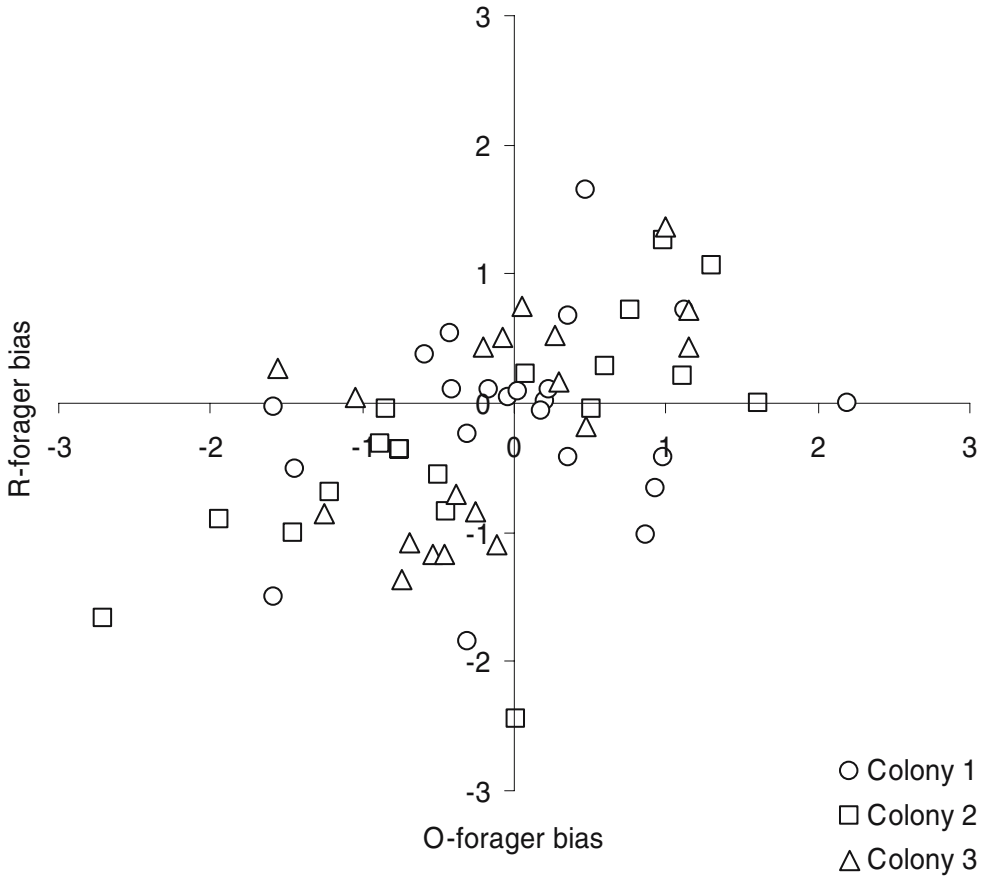

Fig. 1 Relationship between O-forager bias and R-forager bias for patrilines in the three experimental colonies used in Experiment I, with each point being the bias of a patriline. Biases are the empirical logits of the frequency of a patriline in the O- or R-forager task group relative to the colony. Positive and negative logits indicate patrilines that were over- or underrepresented in the task-groups respectively

foraging resulting in a longer foraging life, then we could expect that average age would be correlated with bias. Furthermore, genetic variation in survival time cannot explain the very similar representation of patrilines in the O- and R-forager task groups because the R-foragers were sampled just 2–5 days after the demographic manipulations. This is insufficient time for differential longevity among patrilines to have an effect on patrilineal proportions.

Unequal sperm usage by queens is another potential mechanism by which patrilineal proportions could differ between the forager task group and the general worker population (Oldroyd et al. 1992, 1994). This effect could arise if the queen over-sampled the sperm of some drones when fertilising the eggs that developed into O- and/or R-foragers. Such biased utilisation of sperm from particular drones has been found to occur shortly after mating and sperm use can also change over long periods (12 months; Haberl and Tautz 1998; Frank et al. 1999, 2002; Schlüns et al. 2004). However, as all our queens were at least 1 year old and as each experiment lasted less than a month, unequal sperm usage is also an unlikely explanation for our results.

Given that changes of sperm usage and differential survival of foragers among patrilines are unlikely causes of the observed patrilineal biases between forager and overall colony populations, the only alternative explanation is that patrilines differ in the likelihood that they engage in foraging. It is well established that genotype influences the probability of a worker engaging in many specialist tasks (Rothenbuhler 1964; Robinson et al. 1984; Robinson and Page 1988, 1995; Breed et al. 1990; Guzman-Novoa and Page 1994; Jones et al. 2004). It may be that there is a similar influence on the probability of a worker foraging.

Leoncini et al. (2004) showed that the number of foragers in a colony is regulated by a pheromone, ethyl oleate, produced by existing foragers and spread to pre-foragers via trophallaxis. If there are sufficient foragers in a colony, the production of ethyl oleate is high, and the transition to foraging in younger workers is delayed. If there are insufficient foragers the production of ethyl oleate in the colony is reduced, and this increases the rate at which younger workers make the transition to foraging (Leoncini et al. 2004). This regulatory mechanism provides a plausible means by which patrilines could differ in their probability of becoming a forager: differential sensitivity to ethyl oleate among patrilines would have the effect of changing the average age at which workers of different patrilines mature to foraging tasks.

Our study also shows that as with foraging, there is significant variation among patrilines in their relative representation in the O-nurse samples (Experiment II). This

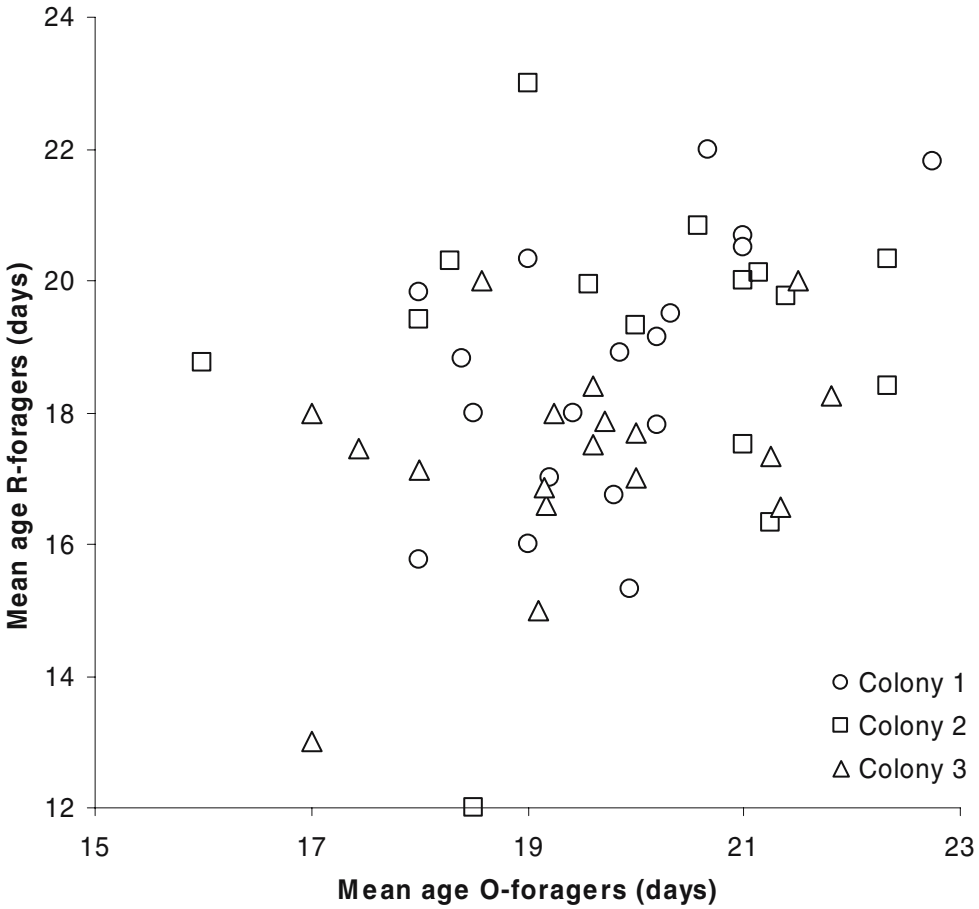

Fig. 2 The relationship between the mean ages of O- and R-foragers for patrilines of the three colonies used in Experiment I, with each point being the mean age of a patriline

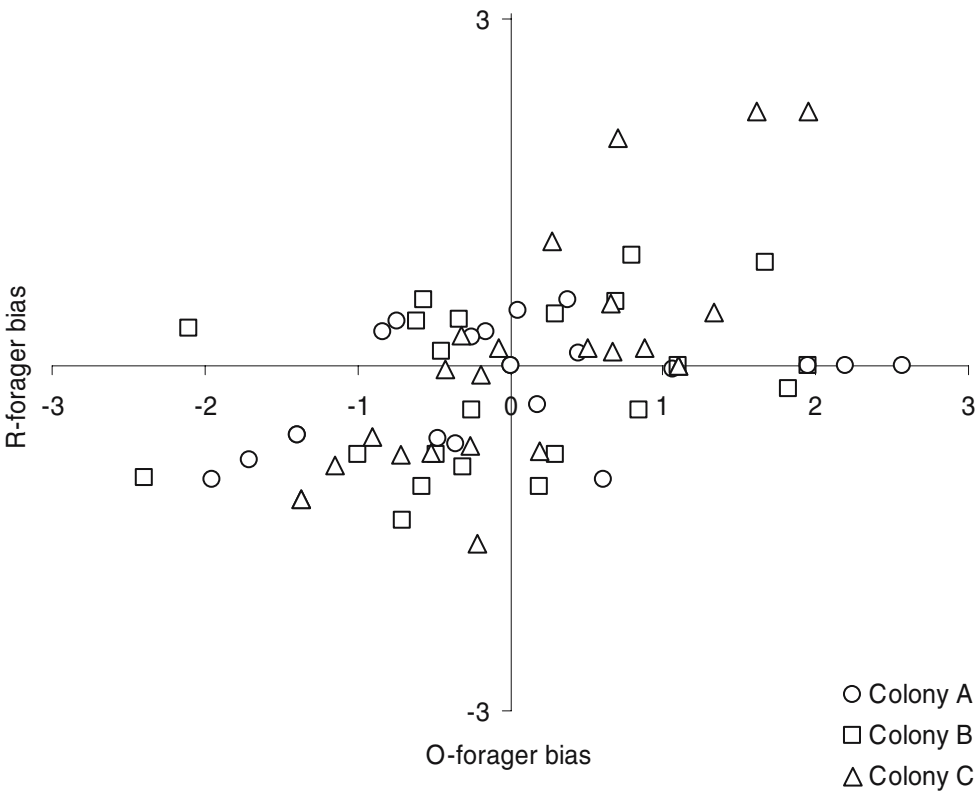

Fig. 3 Relationship between O-forager bias and R-forager bias for patrilines in the three experimental colonies used in Experiment II, with each point being the bias of a patriline. Biases are the empirical logits of the frequencies of patrilines in the O- or R-forager task group relative to the colony. Positive and negative logits indicate patrilines that were over- or underrepresented in the task-groups, respectively

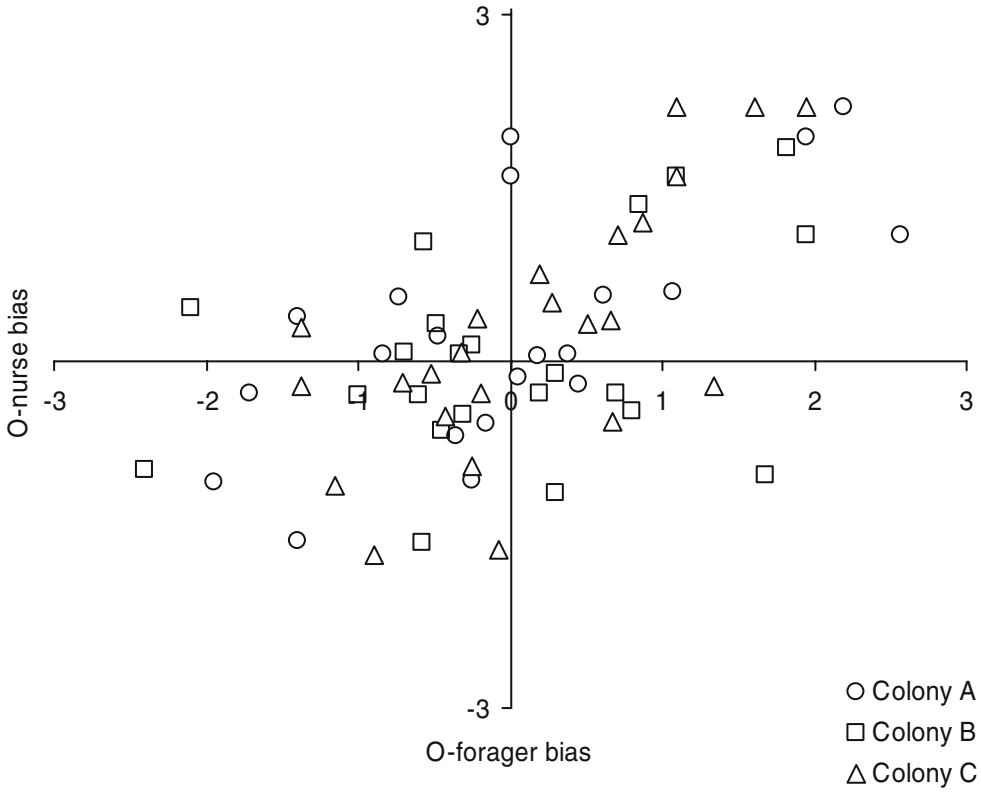

Fig. 4 Relationship between O-nurse bias and R-forager bias for patrilines in the three experimental colonies used in Experiment II, with each point being the bias of a patriline. Biases are the empirical logits of the frequencies of patrilines in the O-nurse or R-forager task group relative to the colony. Positive and negative logits indicate patrilines that were over- or underrepresented in the task groups, respectively

is in line with Robinson et al. (1992) who found a bias toward nursing in a colony composed of two patrilines. All workers are thought to begin life as nurses (Seeley 1985; Winston 1987), and patrilines are known to switch from nursing to other tasks at different ages (Robinson et al. 1989). Any patriline that switches at a later age will be overrepresented in the nursing population by virtue of remaining there longer. This seems the most likely

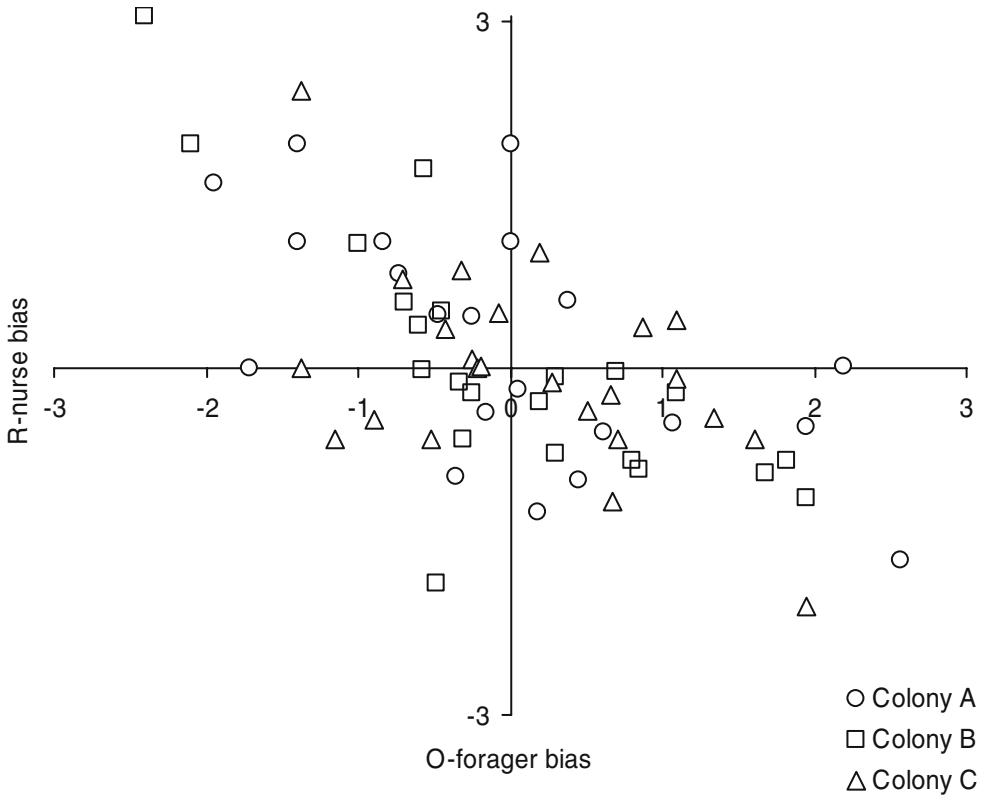

Fig. 5 Relationship between O-forager bias and R-nurse bias for patrilines in the three experimental colonies used in Experiment II, with each point being the bias of a patriline. Biases are the empirical logits of the frequencies of patrilines in the O-forager or R-nurse task groups relative to the group from which they came (the colony and the O-forager task group, respectively). Positive and negative logits indicate patrilines that were over- or underrepresented in the task groups, respectively

explanation for the difference in genotype representation that we observed in the O-nurses. Surprisingly, O-forager and O-nurse bias were positively correlated, so patrilines over- or underrepresented in the nurses were also over- or underrepresented in the foragers.

Possibly there are some patrilines that simply work at a higher rate than others. More likely, however, the genes influencing the response thresholds for nursing and foraging are related, as the cues for nursing and foraging are interrelated. Increased brood numbers increase the likelihood of nursing (Huang et al. 1989), while Huang and Otis (1989) observed that broodless colonies foraged less than brood-right colonies. Pollen is consumed by nurse workers and therefore increased brood will increase the colony's requirement for pollen and pollen foragers (Dreller and Tarpy 2000; Free 1967; Pankiw et al. 1998).

There was also a genetic influence on the representation of workers in the R-nurses, but different patrilines were involved than those in the O-nurses. The only factor known to affect the likelihood of a worker reverting to nursing is the length of time she has been a forager (Page et al. 1992; Robinson et al. 1992; Giray and Robinson 1994). If the original genetic influence on nursing was due to patrilines differing in the age at which they switched from nursing to other tasks, then patrilines that were youngest when switching might be least able to revert. However, this would result in a positive relationship between O-nurse bias and R-nurse bias, and no such relationship was evident. There was also a similar negative relationship between R-nurse bias and R-forager bias, but the test had low statistical power and was not significant. This suggests that patrilines that are most predisposed to engage in foraging also require a greater stimulus to revert to nursing than do other less foraging-biased patrilines. There is therefore at least, to some extent, a genuine genetic influence on the probability of a worker reverting to nursing, but it is distinct from that normally involved in nursing.

The genetic influences on the tasks of foraging and nursing contrast strikingly. Workers switched to engage in both tasks after increases in stimuli. Whereas the genetic influence on switching to become an R-forager is the same as that on the probability of being an O-forager, the genetic influence on becoming an R-nurse is different from that on O-nurses under unperturbed conditions. The lack of relationship between switching to the two tasks suggests that no genotypes are in general more sensitive to changing conditions, as also concluded by Giray and Robinson (1994). Rather, any genetic influences on sensitivity to changing conditions are specific to the particular stimuli. It seems likely that the difference between the two tasks may relate to their relative importance.

The loss of nurses probably presents a much greater problem for a colony than the loss of foragers, both because of the potential cost of brood death due to lack of nursing and the need for R-nurses to redevelop their hypopharyngeal glands for production of brood food (Huang and Robinson 1996). Consequently, the stimulus to nurse will be greater and the response threshold will be reached for more patrilines, resulting in more patrilines being represented in the R-nurse population. The results demonstrate that genotype influences not only which workers engage in the generalist tasks of nursing and foraging, but also which workers switch after an increase in task need. The results provide support for the response threshold model as a basis for genetic polyethism and place the results of earlier studies using smaller, less genetically diverse colonies into a more natural context. Further studies using colonies with natural levels of genetic diversity are needed to clarify how genetic polyethism works in practice and whether or not it does indeed provide a benefit of polyandry.

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