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Promiscuous honeybee queens generate colonies with a critical minority of waggle-dancing foragers

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Abstract Honeybees present a paradox that is unusual among the social Hymenoptera: extremely promiscuous queens generate colonies of nonreproducing workers who cooperate to rear reproductives with whom they share limited kinship. Extreme polyandry, which lowers relatedness but creates within-colony genetic diversity, produces substantial fitness benefits for honeybee queens and their colonies because of increased disease resistance and workforce productivity. However, the way that these increases are generated by individuals in genetically diverse colonies remains a mystery. We assayed the foraging and dancing performances of workers in multiple-patriline and single-patriline colonies to discover how within-colony genetic diversity, conferred to colonies by polyandrous queens, gives rise to a more productive foraging effort. We also determined whether the initiation by foragers of waggle-dance signaling in response to an increasing sucrose stimulus (their dance response thresholds) was linked to patriline membership. Per capita, foragers in multiple-patriline colonies visited a food source more often and advertised it with more waggle-dance signals than foragers from single-patriline colonies, although there was variability among multiple-patriline colonies in the strength of this difference. High-participation patrilines emerged within multiple-patriline colonies, but their more numerous foragers and dancers were neither more active per capita nor lower-threshold dancers than their counterparts from low-participation patrilines. Our results demonstrate that extreme polyandry does not enhance recruitment effort through the introduction of low-dance-threshold, high-activity workers into a colony's population. Rather, genetic diversity is critical for injecting into a colony's workforce social facilitators who are more likely to become engaged in foraging-related activities, so boosting the production of dance signals and a colony's responsiveness to profitable food sources.

Keywords Intracolonial genetic diversity · Polyandry · Recruitment signaling · Response thresholds · Task allocation · Waggle-dance communication

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Introduction

Many species of bees, ants, and wasps in the Order Hymenoptera form spectacular societies that are characterized by extreme cooperation and striking reproductive division of labor (Wilson 1971). Typically, a colony is staffed by worker sisters who cooperate to rear the offspring of a single reproductive individual, their queen mother. Haplodiploidy, the hymenopteran system of sex determination, fosters altruism within these colonies because it generates an unusually high level of relatedness among a queen's sterile daughters, increasing their inclusive fitness through kin selection (Hamilton 1964). Most hymenopteran queens maintain maximal within-colony relatedness through monandry or single-male mating (reviewed by Strassmann 2001; Hughes et al. 2008). However, a tendency to mate with multiple males, or polyandry, has evolved in several lineages of social insects [honeybees

(reviewed by Tarpy and Nielsen 2002; Tarpy et al. 2004), yellow jacket wasps (Ross 1986), army ants (Denny et al. 2004; Kronauer et al. 2004, 2007), leafcutter ants (Boomsma et al. 1999; Fjerdingstad and Boomsma 2000; Fjerdingstad et al. 1998), seed-harvester ants (Gadau et al. 2003; Pol et al. 2008; Rheindt et al. 2004; Wiernasz et al. 2004), and desert ants (Timmermans et al. 2008)]. Polyandry in the genus *Apis* (the honeybees) is particularly noteworthy because honeybee queens of every species are extremely promiscuous. Depending on the species, each queen mates with an average of 8–44 males (Tarpy et al. 2004), a behavior that substantially inflates the number of genetically distinct patrilines within a colony but simultaneously lowers within-colony relatedness to a level that makes the maintenance of altruism surprising. Thus, the extreme promiscuity of honeybee queens is paradoxical because obligate sociality has evolved universally in *Apis* despite limited kinship among essentially sterile work forces.

In light of Hamilton's (1964) rule, it is predicted that polyandry must confer to an entire colony substantial increases in reproductive output that offset low relatedness between workers and the sibling reproductives that they rear. There is strong evidence that colonies of some species of social Hymenoptera gain significant fitness benefits when queens mate multiply, including improved resistance to disease [honeybees (Seeley and Tarpy 2007), leafcutter ants (Hughes and Boomsma 2004)], higher long-term population growth and reproductive output [honeybees (Mattila and Seeley 2007), harvester ants (Cole and Wiernasz 1999), and yellow jackets (Goodisman et al. 2007)], greater nest microclimate stability [honeybees (Jones et al. 2004)], and reduced likelihood of producing diploid males [Hymenoptera (reviewed by van Wilgenburg et al. 2006), honeybees (Tarpy and Page 2002)]. The increased productivity of genetically diverse, multiple-patriline colonies is attributable in part to enhanced acquisition of food resources through a more expansive foraging effort (Mattila and Seeley 2007; Mattila et al. 2008; Wiernasz et al. 2008). In honeybees, colonies with multiple patrilines generate higher foraging rates and larger food reserves than similarly sized colonies that consist of only a single patriline (Mattila and Seeley 2007). Among the collective, foragers in genetically diverse colonies exchange more foraging-related recruitment signals (e.g., waggle dances and shaking signals) to direct nest mates to the location of profitable food resources, and they report discoveries that are farther afield from their home than do foragers in genetically uniform colonies (Mattila et al. 2008).

These studies highlight the significant benefits of polyandry for improving the productivity of honeybee colonies, but it remains unclear how the actions of individuals generate these differences. While we do not yet understand the individual-level mechanisms that enhance the activity of multiple-patriline colonies, the increases in productivity and communication that accompany it indirectly support the response threshold model of division of labor, which proposes that a colony's workforce is allocated among tasks based on each individual's genetically determined threshold for responding to task-specific stimuli (Robinson and Page 1989). This elegant model is often invoked to explain the emergence of division of labor and increased efficiency in social insect colonies. Explored heavily from a theoretical perspective (e.g., Bonabeau et al. 1998; Jeanson et al. 2007; Waibel et al. 2006), most of the model's empirical support comes from indirect evidence of "specialist" patrilines in colonies [reviewed by Oldroyd and Fewell (2007) in Table 1]. For instance, the observation that waggle-dancing workers are disproportionally represented by a subset of a colony's patrilines (Arnold et al. 2002) is consistent with, but not proof of, the idea that some workers have a genetic bias toward responding to less profitable food rewards with dancing. The response threshold model was based on observations of this nature (Robinson and Page 1989); however, indirect evidence cannot sustain it without risk of circular reasoning. Actual response thresholds of individuals are seldom measured (Beshers and Fewell 2001) and, where they have been assayed, patriline-based differences in thresholds were not assessed (Mailleux et al. 2000; O'Donnell and Foster 2001), or were not relevant for monandrous species (Weidenmüller 2003), or thresholds were an out-of-context correlate of task preference (Page et al. 1998), or response was potentially confounded as worker activity altered stimulus levels [e.g., fanning response to increasing nest temperatures (Jones et al. 2004)]. A stringent test of the response threshold model of division of labor requires connections to be made empirically between an individual's response threshold, activity level, genetic differences among colony members, *and* the colony-level response that is generated as many individuals respond concurrently to the same stimulus.

Here, we present research that examines for the first time how intracolonial patriline diversity—and thus polyandry—shapes the organization of a colony's workforce through effects on the task execution and response thresholds of its workers. We created singly and multiply "mated" queens through instrumental insemination and then assayed the foraging effort and recruitment signaling response of workers marked for individual identification in single-patriline and multiple-patriline colonies as each queen's workforce utilized a food resource in a greenhouse. The patriline membership of each worker was subsequently identified by genotyping. This assay permitted us to determine the following: (1) the effect of patriline diversity

on performance at the level of the colony; (2) whether a subset of workers, defined by shared paternity, was responsible for enhanced foraging effort and recruitment signaling in multiple-patriline colonies; and (3) whether foragers from patrilines that participated heavily in dancing had lower dance response thresholds than foragers from patrilines whose members were less commonly engaged as dancers.

Materials and methods

Manipulating patriline diversity in colonies

Two groups of honeybee colonies, with each colony composed of either multiple patrilines or a single patriline, were created by instrumentally inseminating the queens that headed them with semen from either 15 drones or from one drone, respectively. Each queen received semen from a unique drone or set of drones, and thus, a drone's semen was used for only a single insemination (i.e., an individual drone's semen never inseminated more than one queen). The queens were reared and inseminated by a queen breeder (Glenn Apiaries, Fallbrook, CA, USA) who did not reveal the insemination status of the queen groups until collection of all behavioral data was completed. All of the queens were highly related sisters—daughters of a single Carniolan queen and one Carniolan drone—who shared 75% of their genes in common. For the inseminations, a pool of 1,000 drones was amassed from five drone "bank" colonies. Each bank held drones from one of five honeybee strains: Minnesota Hygienic Italian (n=8 source colonies), Carniolan (n=3 source colonies), Russian (n=4 source colonies), Cordovan Italian (n=2 source colonies), and Varroa sensitive hygienic (n=6 source colonies). From each bank, 200 sexually mature drones were collected into a container (i.e., one container per bank; five containers in total), and then, using a random-numbers table to dictate the container from which each was to be drawn, drones were selected and their semen was harvested, either to inseminate one queen or to contribute to a pool of semen that was assembled across strains/banks for the insemination of a "polyandrous" queen. All queens were inseminated with 1 μl of semen, but multiple-drone semen mixtures were stirred with a glass rod for 2 min before a 1-μl aliquot was withdrawn for insemination. Analysis of the genetic structure of each multiple-patriline colony's population indicated that workers fathered by ≥14 of the inseminating drones were present in each colony (see "Results").

The queens were shipped to Ithaca, NY, USA on 1 May 2007, where they were introduced into host colonies at Cornell University's Liddell Field Station. Behavioral assays were not initiated until 7 weeks later, after the host colonies had been repopulated by the offspring of the inseminated queens. Over this period, colonies were medicated and checked weekly for signs of disease or oviposition failure by the inseminated queens, as described by Mattila and Seeley (2007). Only healthy colonies with solid brood patterns were used in the study.

Assaying foraging and dancing performance of individuals

Three multiple-patriline and three single-patriline colonies were used in the assay; colonies were studied one at a time, alternating between treatment groups. For each colony, we transferred ~4,000 bees, the inseminated queen, and a frame of brood (all stages and with minimal stored food) from the parental colony into a two-frame observation hive. An empty frame of comb was installed in the upper position of the observation hive; it was separated from the brood frame below by a queen excluder, which prevented the queen from accessing the top frame to fill it with eggs and provided empty cells to stimulate workers to forage. This configuration resulted in a large hungry colony that had little stored food and whose foragers (presumably) had great incentive to visit and recruit nest mates to new resources. Once an observation hive was assembled, it was moved into a greenhouse (5×6.5 m^2), where we could maintain a consistent foraging environment over the duration of the study (19 June to 20 August 2007). Workers could fly freely in the greenhouse and had unlimited access to water but had no access to food unless we stocked our experimental feeder (during the assay only). Once installed, we left the hive covered and undisturbed for one full day so that foragers could orient to their new entrance and surroundings.

After the adjustment period was over, a 5-day behavioral assay commenced, during which time the foraging and dance activity of a different set of foragers was assessed each day. Every morning, a group of workers was trained to visit a feeder that was 7 m from the hive and was stocked with 1.5 M anise-scented sucrose solution, according to Seeley (1995). Training began at 7 a.m. each day, and by 9 a.m., 15–30 uniquely marked workers (designated as the focal foragers) had made multiple trips to the feeder. After the marked foragers had become accustomed to visiting the feeder, it was removed for 1 h, during which time all of the trained foragers and any recruits who were searching the greenhouse generally returned to the hive and the colony became quiescent.

At this point, the assay began. The feeder was repeatedly stocked over the course of the day with a series of five anise-scented sucrose solutions that increased incrementally in reward (0.5, 1.0, 1.5, 2.0, and 2.5 M). Each solution was in the feeder for 0.5 h at a time (a "trial"); then, the feeder was emptied for a l-h intertrial interval before it was refilled with the next sucrose solution. At the start of each trial, a droplet of

the scented sucrose solution was placed 5 cm inside the entrance of the hive to reactivate the focal foragers to the feeder. Once a trial commenced, an observer who was positioned at the feeder recorded the number of visits made to it by each focal forager. A second observer who was stationed at the observation hive monitored the area of comb immediately adjacent to the hive's entrance (the "dance floor"), where the returning foragers unloaded their sucrose solution payload to food-receiving bees and danced to advertise the food reward at the feeder, if they were so inclined. The dance floor was videotaped during the trial with a digital video camera (Sony, DCR-HC90), and the observer identified dancing workers for the video record. Unmarked recruits that landed on the feeder during each trial were captured (before they sampled the food reward), held in a screened cage, and then released after the feeder was emptied. This action stifled recruitment by unmarked bees and prevented overcrowding at the feeder with unmarked recruits, which could have affected our focal foragers' perception of the profitability of the sucrose reward. It also limited the prior experience of unmarked bees with the food reward, should they become focal foragers on a subsequent day.

At the end of a day-long assay, each focal forager was collected so that her patriline membership could be determined at a later date. This sequence—training a group of workers to the feeder, assaying their foraging and dancing performance, and then removing them from the colony at the end of the day—was repeated daily over 5 days with each colony (a total of 79–153 workers were assayed per colony) before it was removed from the greenhouse and replaced with another colony. A sample of workers was collected from each colony before it was returned to its parental hive. The patrilines' membership of workers from this sample (n=150) was determined (see below) to compare the patriline profile of the colony's population to that of its focal foragers.

Estimating dance activity of focal foragers

Videotapes of the dance floor, which recorded for every colony's assay all of the dance activity of the focal foragers as they returned to the hive from the feeder, were analyzed to glean the following: (1) the number of foraging trips per focal forager that ended in dancing upon return to the hive; (2) the amount of time that each forager danced (per trip, per trial, and in total over the assay), if she danced; and (3) the number of circuits she completed as she danced (per trip, per trial, and in total). Because dancing foragers were reporting a resource that was 7 m from their nest, they all danced "round" dances, where workers move in tight circuits on the dance floor, each circuit containing only a very brief waggle phase (Gardner et al. 2008). Thus, it was easy to identify the moment at which a moving forager swung into or out of the characteristic circular movements of the dance to start or to stop her signaling. The number of circuits completed by a dancer was determined by counting the times that she rounded the top of her circular dance path as she danced. The videotapes were analyzed using a digital video editor (Sony, DSR-30), which resolved the duration of each dance to 1/30 s.

Determining patriline membership of workers

For each multiple-patriline colony, worker paternity was determined by comparing the genotype of each worker, after subtracting alleles inherited from the queen, to those of the drones that were used to inseminate the colony's queen (based on preserved material). DNA was extracted from the hind legs of drone and worker samples using a DNeasy blood and tissue extraction kit (Qiagen Inc., Valencia, CA, USA) and then amplified using fluorescent-dye-labeled primers (Life Technologies Corp., Carlsbad, CA, USA) for eight highly variable microsatellite loci [A7, A79, A113, Ap1, Ap33, Ap36, Ap256, AB124; summarized by Solignac et al. (2003)] that were separated in a single reaction by size and fluorescence. Reactions were conducted in 10 μl of the following solution: 5 μl of master mix from a polymerase chain reaction (PCR) multiplexing kit (Qiagen Inc., Valencia, CA, USA), 1 μl of DNA extract, 0.8 μl of water, and 0.2 μl of each of the 16 primers. PCR amplifications were carried out in a thermal cycler (Thermo Electron Corp., Milford, MA, USA) that was programmed for 15 min at 95°C (for activation of multiplex *Taq* polymerase) followed by 35 cycles consisting of 50 s at 94°C, 45 s at 50–57°C (annealing temperature dropped 1°C per cycle during the first seven cycles and was held at 50°C for the remaining cycles), and 90 s at 72°C. Size of the dye-labeled DNA fragments was determined with an automated gene sequencer and associated software (3730×l DNA Analyzer and GeneMapper version 3.0, Life Technologies Corp., Carlsbad, CA, USA). If definitive paternity was not yielded by comparing a worker's genetic profile for these eight markers to those of her potential drone fathers' genotypes, then a second set of eight loci [A28, A43, Ap43, Ap68, Ap85, Ap226, Ap289, At3; summarized by Solignac et al. (2003)] was employed for further discrimination, which resulted in the conclusive identification of every worker's drone father.

Statistical analyses

Differences in forager activity (e.g., feeder visitation, duration of dancing) between multiple- and single-patriline colonies were examined with repeated-measures, two-way analyses of variance (ANOVAs; colony type and sucrose solution concentration effects); all tests were conducted with colony means that were based on worker measures. Percentage or proportional data were arcsine-

square-root-transformed prior to analysis. For each multiple-patriline colony, patriline profiles for the focal forager sample (observed frequencies) and the sample of workers collecting from inside the hive (expected frequencies) were compared with a chi-squared test. A dance response threshold score (1–5) was calculated for each worker based on the lowest concentration of sucrose solution for which she danced upon return to the hive (e.g., 1 if she danced for the lowest concentration of food reward offered, 5 if she only danced for the richest); low scores represented low thresholds of response. Foragers who never danced were excluded from this analysis. The probability that a forager would dance after returning from the feeder was also determined for each focal individual by estimating the proportion of trips to the feeder that ended in dancing once a forager's response threshold for dancing was met or exceeded. Foragers were excluded from this analysis if they never danced or if they made fewer than ten trips to the feeder during the trials when it was stocked with a reward that met or exceeded their threshold. The probability of foraging and foraging response thresholds were not calculable for focal workers because they were already participating as foragers in the assay. Mean activity (number of trips to the feeder and total dance time), dance response threshold score, and dance response probability per worker were compared across patrilines with a one-way ANOVA for each multiple-patriline colony; where a significant effect of patriline was found, means were separated using Tukey's studentized range test. Mean worker activity and dance response threshold scores, separated according to patriline, were contrasted between all patrilines in each multiple-patriline colony and the whole-colony patrilines that made up the three single-patriline colonies. Relative frequency distributions of per capita response were compared between colony types with a Kolmogorov–Smirnov test. Linear regression lines were determined for both colony types based on each individual's total number of trips to the feeder and her total time spent dancing over the course of the assay; the slope of each regression line was compared using a dummy variable model that tested the equality of the regression coefficients. All statistical tests were conducted with SAS version 9.1 (SAS Institute, Inc, Cary, NC, USA) and a significance level of α=0.5. Means are reported with standard errors.

Results

Performance at the colony level

The level of genetic diversity of a colony's workforce affected the performance of its workers considerably, both in how strongly foragers exploited the feeder and how they behaved in the hive. Although similar percentages of focal workers were reactivated to the feeder each time it was stocked with a new sucrose solution (mean 80–84% of marked workers per colony, over all concentrations and 5 days of assay; repeated-measures ANOVA patriline diversity effect: $F_{1,\ 4}$=0.8, p=0.42), the average number of times that a forager visited the feeder was 37% higher if she was from a colony that was comprised of multiple patrilines rather than only a single patriline (Fig. 1a; repeated-measures ANOVA patriline diversity effect: $F_{1,\ 4}$= 7.7, p=0.049). On the hive's dance floor, differences in signaling performance were unambiguous: over the assay, compared to a forager from single-patriline colony, a forager from a multiple-patriline colony made an average of 30% more trips to the feeder that ended in waggle dancing, she completed an average of 34% more dance circuits per trip to the feeder and 60% more circuits over each 0.5-h trial (Fig. 1b; repeated-measures ANOVAs patriline diversity

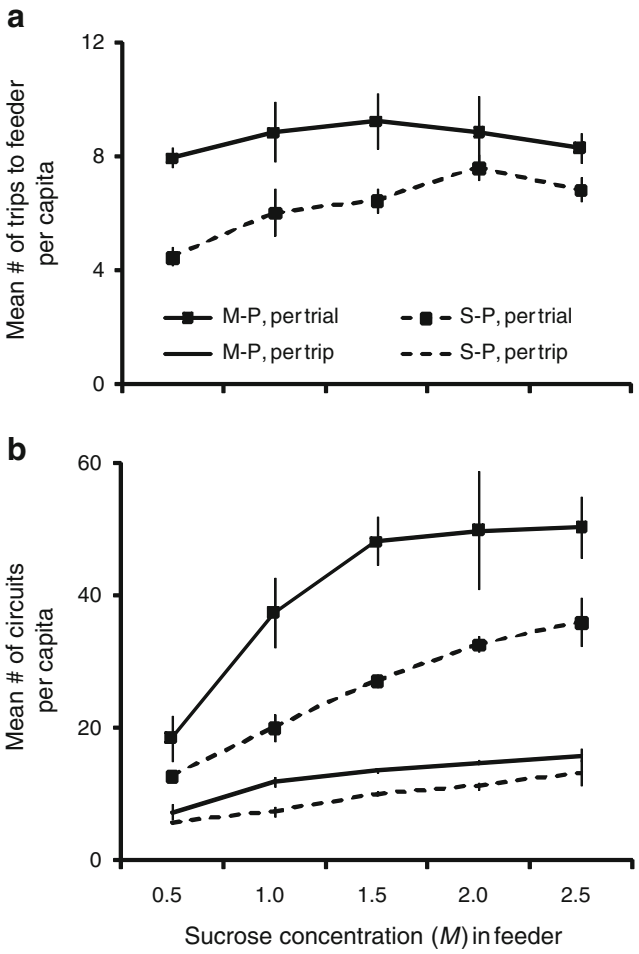

Fig. 1 If reactivated to the feeder over each 0.5-h trial, per capita **a** feeder visitation (mean number of trips) and **b** dance activity (mean number of circuits completed; per trip and per trial) in multiple-patriline (*M-P*) and single-patriline (*S-P*) colonies. Calculations are based on colony means (±SEM; n=3 colonies per mean)

effects, respectively: $F_{1,\ 4}$=10.3, p=0.03; $F_{1,\ 4}$=23.8, p=0.01; $F_{1,\ 4}$=12.3, p=0.02;).

Figure 2 provides for each of the six colonies that were studied relative frequency distributions of the activity of the focal foragers as they visited the feeder and danced in the hive (the response of each individual was summed over the day-long assay). Conspicuous differences in foraging and recruitment signaling between colony types were matched by the high percentage of foragers that never danced *at any point* during the assay in single-patriline colonies (20–57%) compared to multiple-patriline colonies (1–35%; Fig. 2; t_4=3.3, p=0.03). Within each colony, there was considerable variability in the extent to which individuals visited the feeder and danced to advertise it over the course of the

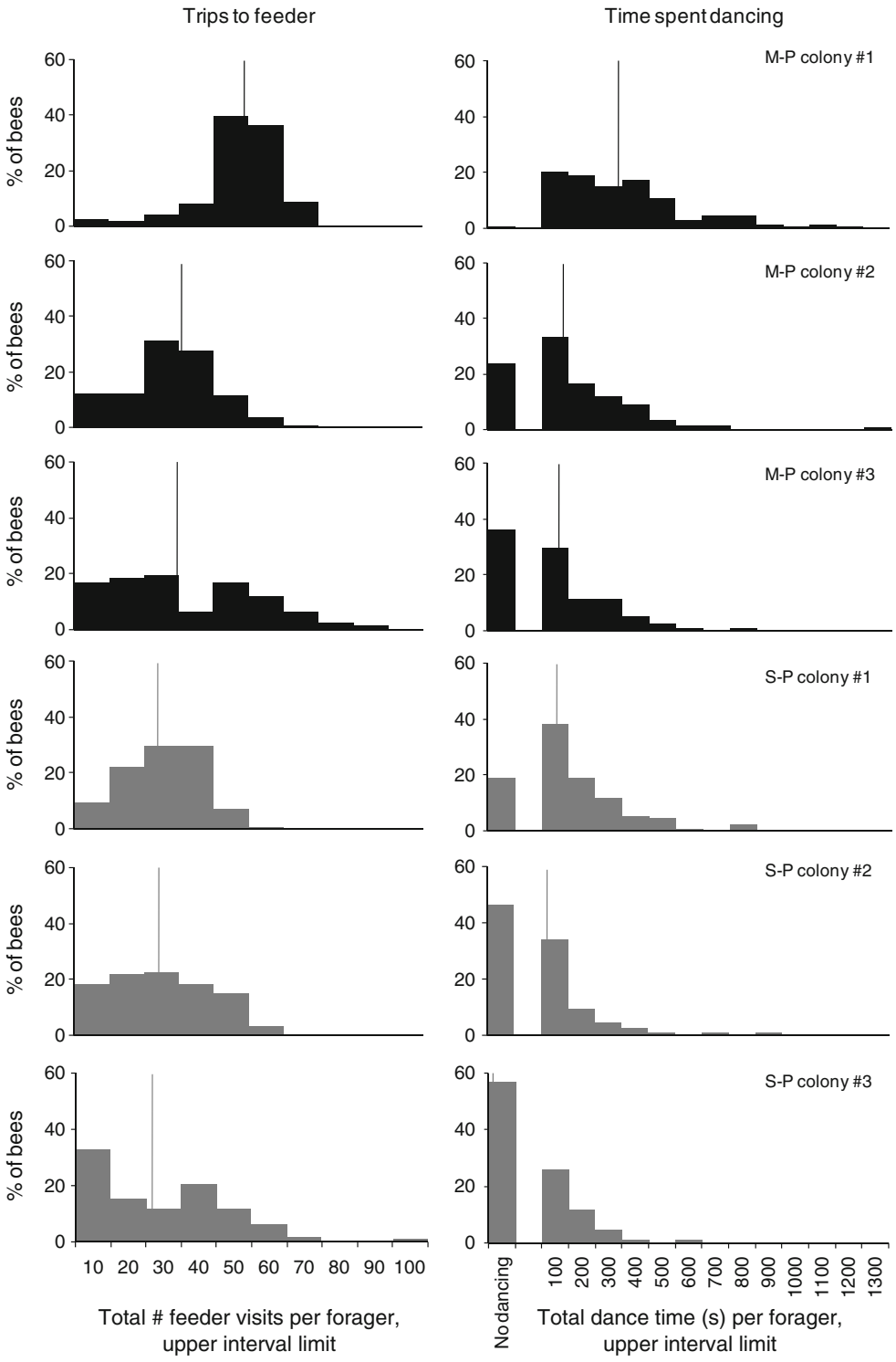

Fig. 2 Percentage of focal foragers for each multiple-patriline (*M-P*) colony (*black*) and each single-patriline (*S-P*) colony (*gray*) that foraged and danced to various degrees over the course of the assay. The total number of trips to the feeder (*left*) and total time spent dancing (*right*) were summed over the entire assay (all five sucrose concentrations) for each forager; the extent of her activity was classified into one of several categories of performance, and the number of foragers who were similarly classified was determined to create for each colony a relative frequency distribution. *Vertical lines* indicate median values

assay. Furthermore, there was a significant difference between multiple- and single-patriline colonies in how individuals were distributed across the range of observed activity levels (Fig. 2; comparison of distributions after pooling by colony type; for both per capita foraging and dancing: $D=0.3$, $p<0.0001$). Using the median value for each colony's per capita activity as a benchmark (the vertical lines in Fig. 2), colonies with a shift toward greater activity were multiple-patriline colonies and those with, in general, less active individuals were single-patriline colonies. However, the two colony types met in the middle: the distribution of performance of the population of focal individuals in the least-active multiple-patriline colony (#3) was similar to the best-performing single-patriline colony (#1).

Apportioning performance among patrilines

It is apparent that having multiple patrilines was linked in some colonies to an increase in per capita rates of feeder visitation and subsequent production of waggle dances, but the question remains: was elevated activity generated by a widespread increase in worker performance or was it produced by only a narrow subset of especially lively foragers and dancers and, if so, were these workers from specific patrilines?

We found that the majority of the workers who visited the feeder and advertised it with waggle dances in multiple-patriline colonies were from a minority of subfamilies (Figs. 3, 4, and 5). Across all of the multiple-patriline colonies, three out of the 14–15 patrilines that made up each colony produced workers who were responsible for 52–64% of the total time spent by all focal foragers waggle dancing to signal the presence of the stocked feeder (Figs. 3a, 4a, and 5a). Moreover, foragers from these top three patrilines also made 54–61% of all trips to the feeder over the 5 days that each colony was assayed (Figs. 3b, 4b, and 5b). Notably, the most active patrilines also contributed relatively large numbers of individuals to their colony's pool of focal foragers. In each multiple-patriline colony, workers from the three patrilines that ranked highest for contribution to total dance time greatly overrepresented the individuals who responded to initial training and subsequently participated in the assay compared to that expected based on the genetic structure of each colony (Fig. 3: $\chi_{(13)}^2=171.3$; Fig. 4: $\chi_{(13)}^2=260.1$; Fig. 5: $\chi_{(14)}^2=44.9$; all $p<0.0001$). Across colonies, 38–53% of all foragers came from these three patrilines, whereas only 12–25% of foragers were expected had the profiles of the forager populations matched those of the colonies' greater populations (see tables at the bottom of Figs. 3, 4, and 5; multiple-patriline colonies #1–3, respectively). For these reasons, we have designated these patrilines as high-participation patrilines because their workers became engaged as recruiting foragers in unexpectedly high proportions. Overrepresentation of a minority of patrilines in the forager force was accompanied by underrepresentation of other patrilines; in two of three colonies, some subfamilies were never detected in the forager fleet, even though they were present in the colony's population (Figs. 3 and 5).

However, the top three patrilines' share of colony activity did not typically inflate because a forager from one of these patrilines foraged more vigorously or danced longer on average than a forager from one of the colony's remaining patrilines. This observation is evidenced by mean per capita activity levels, separated according to patriline, that are indicated in Figs. 3a, b, 4a, b, and 5a, b (black bars). Within each multiple-patriline colony, mean per capita foraging rates and dance activities were similar between the top-ranked and lower-ranked patrilines, either because there was no statistical difference among patrilines in performance (Figs. 3b and 5a, b; one-way ANOVA patriline effects, respectively: $F_{11,\ 117}=0.7$, $p=0.78$; $F_{13,\ 63}=1.1$, $p=0.38$; $F_{13,\ 63}=1.1$, $p=0.35$) or because patrilines with significantly greater per capita activity (compared to other patrilines in their colony) were not the ones that contributed the most workers to the pool of recruiting foragers (Figs. 3a and 4a, b; one-way ANOVA patriline effects with means comparisons, respectively: $F_{11,\ 117}=3.5$, $p=0.003$; $F_{13,\ 139}=2.3$, $p=0.01$; $F_{13,\ 138}=3.3$, $p=0.0002$). The only exception among all of these comparisons was in multiple-patriline colony #2, where foragers in patriline 11 were, on average, less active per capita than foragers in patriline 1 (only for time spent dancing, Fig. 4a) or patriline 2 (feeder visitation only, Fig. 4b). Furthermore, there was no difference among patrilines within each of the three multiple-patriline colonies in the probability that a forager would dance upon return from the feeder, from the trial when they first danced onward (Figs. 3d, 4d, and 5d; one-way ANOVA patriline effects, respectively: $F_{11,\ 113}=1.8$, $p=0.06$; $F_{13,\ 89}=1.6$, $p=0.11$; $F_{9,\ 32}=2.\ 0$, $p=0.08$). Taken together, these trends reveal an overarching result that the total foraging and dancing effort in multiple-patriline colonies was dominated by minority of patrilines that contributed more workers to the pool of foragers and dancers, but once these workers were engaged in these tasks, their performance did not differ significantly from that of workers from other less well-represented patrilines who were executing the same tasks.

Although we found no suggestion that particular patrilines supplied especially active foragers and dancers to each colony's forager workforce, there is evidence that foragers in multiple-patriline colonies were generally more active than foragers from single-patriline colonies. Firstly, the per capita duration of dance signaling rose almost two times

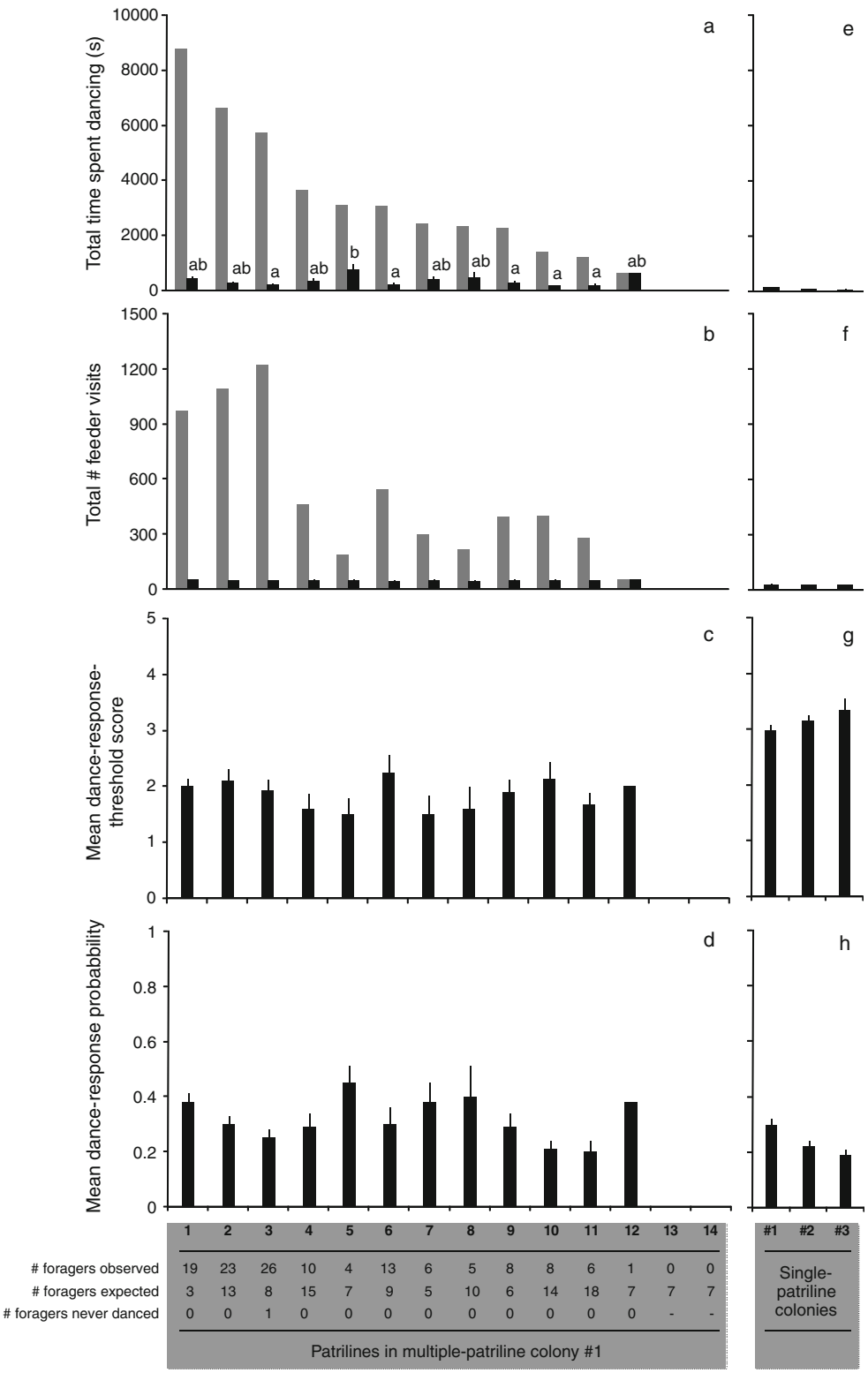

Fig. 3 Comparison over the entire assay of total dance time, total feeder visits, dance response thresholds, and response probabilities of foragers from multiple-patriline colony #1 (separated by patrilines; **a**–**d**) and the single-patriline colonies (separated by colony; **e**–**f**). The *gray bars* represent total activity per patriline (dance time and feeder visitation only; summed for all active foragers in that patriline), and the *black bars* represent per capita means per patriline (all measures). *Letters* indicate significant differences among patriline means. Forager counts are listed below each patriline

faster as feeder visitation increased for foragers in multiple-patriline colonies compared to their counterparts in single-patriline colonies (Fig. 6; $\text{slope}_{\text{M-P}}=6.0$ versus $\text{slope}_{\text{S-P}}=3.5$; comparison of regression line slopes; $F_{1,\ 741}=10.4$, $p=0.001$). This difference indicates that a forager in a multiple-patriline colony had a tendency to dance longer upon return to the hive than a forager from a single-patriline colony, given the same amount of feeder visitation. Secondly, mean per capita activity levels for individual patrilines in colonies with polyandrous queens were often

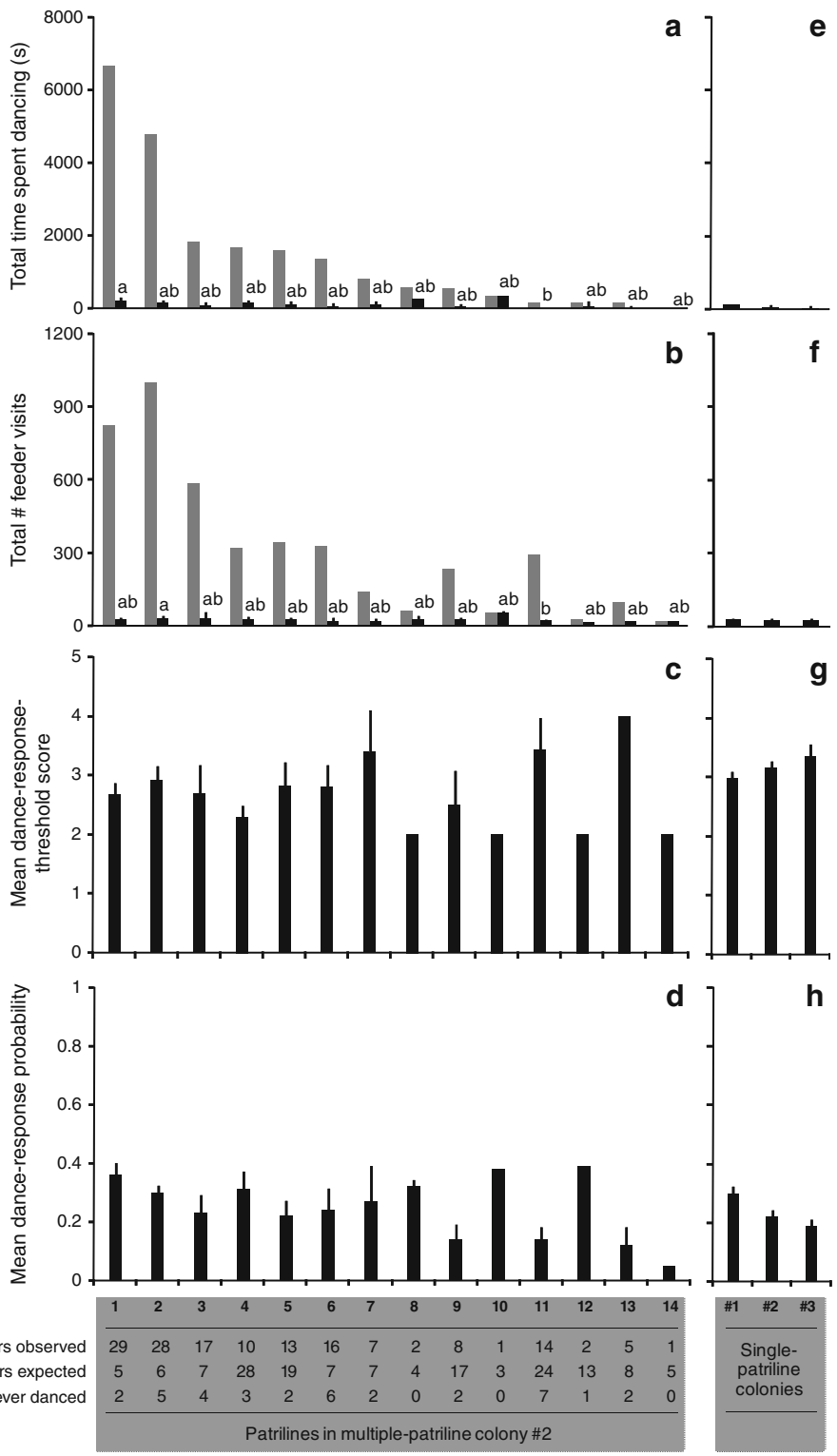

Fig. 4 Comparison over the entire assay of total dance time, total feeder visits, dance response thresholds, and response probabilities of foragers from multiple-patriline colony #2 (separated by patrilines; **a**–**d**) and the single-patriline colonies (separated by colony; **e**–**f**). The *gray bars* represent total activity per patriline (dance time and feeder visitation only; summed for all active foragers in that patriline), and the *black bars* represent per capita means per patriline (all measures). *Letters* indicate significant differences among patriline means. Forager counts are listed below each patriline

higher than the per capita activity of the patrilines that made up the entire colonies where queens were singly mated. For both foraging and dancing, per capita performance for the patrilines in multiple-patriline colony #1, as a group, was significantly greater than the patrilines in single-patriline colonies #1–3 (means contrasts; Fig. 3a vs. e: $F_{1,\ 475}$= 103.9, p<0.0001; Fig. 3b vs. f: $F_{1,\ 475}$=176.9, p<0.0001). A similar comparison with multiple-patriline colony #2 showed that patrilines in that colony had higher per capita dance activity than the whole-colony single patrilines (Fig. 4a vs. e; means contrast; $F_{1,\ 497}$=6.6, p=0.01), but not higher foraging activity (Fig. 4b vs. f; means contrast;

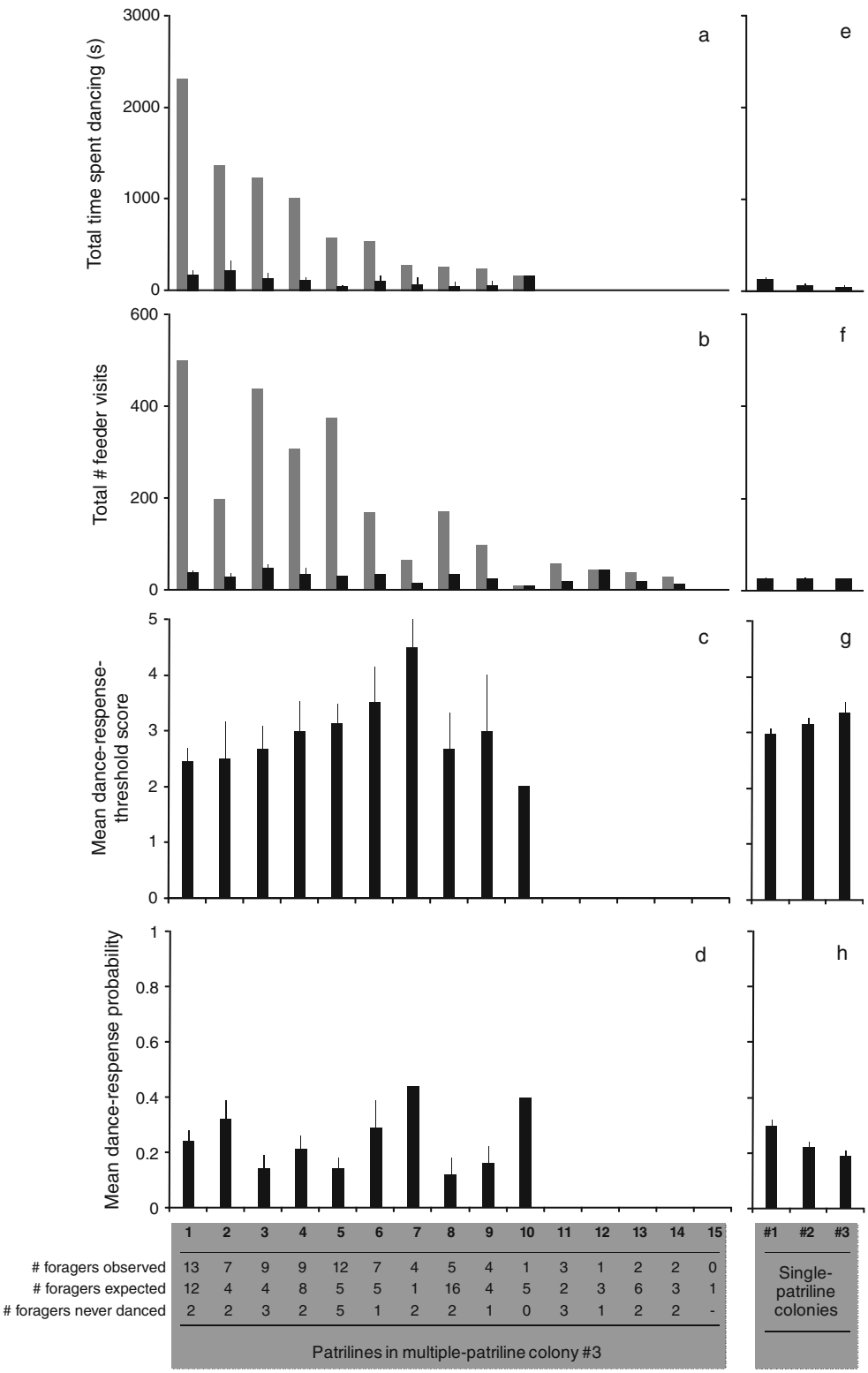

Fig. 5 Comparison over the entire assay of total dance time, total feeder visits, dance response thresholds, and response probabilities of foragers from multiple-patriline colony #3 (separated by patrilines; **a**–**d**) and the single-patriline colonies (separated by colony; **e**–**f**). *Letters* indicate significant differences among patriline means. The *gray bars* represent total activity per patriline (dance time and feeder visitation only; summed for all active foragers in that patriline), and the *black bars* represent per capita means per patriline (all measures). Forager counts are listed below each patriline

$F_{1,\ 497}=1.4$, $p=0.24$). Mean per capita performance was similar between the patrilines of multiple-patriline colony #3 when contrasted to those of the single-patriline colonies (means contrasts; Fig. 5a vs. e: $F_{1,\ 417}=2.3$, $p=0.13$; Fig. 5b vs. f: $F_{1,\ 421}=1.2$, $p=0.28$).

No differences in dance response thresholds among patrilines

Our results thus far suggest that there are patrilines in multiple-patriline colonies whose workers are more likely

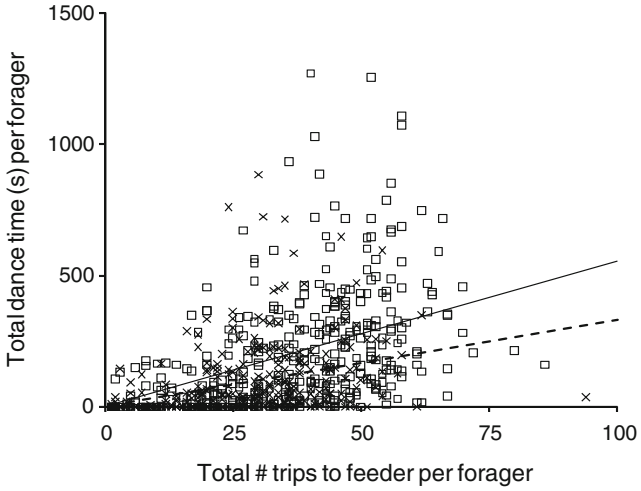

Fig. 6 Relationship between per capita total feeder visits and total dance activity for foragers (n=745 individuals) from multiple-patriline (*squares*) and single-patriline (*x marks*) colonies. Regression lines are provided for each type of colony (*solid trend line*: multiple-patriline colonies; *dashed trend line*: single-patriline colonies)

to become engaged in foraging and dancing tasks. The response–threshold model predicts that these differences in task execution occur because patrilines whose workers are more likely to become employed as foragers and dancers have lower response thresholds than workers in other patrilines that participate less in the same task (Page and Robinson 1989). To test this reasoning, we looked for differences among patrilines within each multiple-patriline colony wherein foragers initiated dancing (reflected by their dance response threshold scores) as the food reward increased over the course of the day. Within each multiple-patriline colony, there was never a difference in mean dance response threshold score among patrilines (Figs. 3c, 4c, and 5c; one-way ANOVA patriline effects, respectively: $F_{11,\ 116}$=0.7, p=0.72; $F_{13,\ 103}$=0.9, p=0.60; $F_{9,39}$=0.9, p=0.50). However, on average, foragers in two of the three multiple-patriline colonies started dancing for lower concentrations of sucrose than foragers in single-patriline colonies (means contrasts; Fig. 3c vs. g: $F_{1,\ 332}$= 78.2, p<0.0001; Fig. 4c vs. g: $F_{1,\ 319}$=6.3, p=0.01; Fig. 5c vs. g: $F_{1,\ 225}$=0.0, p=0.98). These results indicate that a tendency to dance for a low-stimulus feeder did not characterize certain patrilines, particularly those whose workers were more likely to become engaged as foragers but that, overall, foragers in a genetically diverse colony were often more likely to signal the presence of a lower-reward resource by waggle dancing (i.e., had lower dance response thresholds in two of three comparisons) than foragers in a colony with little genetic diversity.

To untangle in multiple-patriline colonies the relationship between dance response thresholds and the relative extent to which foragers from each patriline participated in dancing, we compared across sucrose concentrations the dance response of patrilines as the number of workers per patriline who responded to the feeder with dancing relative to the number that were expected to respond per patriline based on the genetic structure of the colony (Fig. 7). Compared to the way in which foragers from patrilines were predicted, according to the response threshold model, to participate in the dance assay as the sucrose solution in the feeder became increasingly rewarding (Fig. 7a), we found that, in two of three multiple-patriline colonies, several patrilines greatly overresponded to the feeder with dancing (relative to expected numbers) across a broad range of stimuli (Fig. 7b, c). Furthermore, in all multiple-patriline colonies, a substantial number of patrilines showed a limited dance response to the feeder or showed no response at all across the range of stimuli with which foragers were presented (Fig. 7b–d).

Discussion

Superior forager workforces were created in most multiple-patriline colonies in two ways: first, by introducing through multiple mating a subset of patrilines whose individuals were highly likely to become engaged as foragers, and second, by a tendency of foragers in a multipatriline milieu to make trips to and perform dances for a food source with greater frequency (regardless of paternal descent) than foragers from colonies with only one patriline. Because of these differences, when all six colonies were compared across a continuum of performance, the colonies that best utilized the available food source were those that had multiple patrilines and, in general, the colonies with relatively poorer performance because of the lackluster activity of their workers were those that had no patriline diversity among their ranks (Fig. 2). These observations begin to reveal the reasons why foraging success and fitness are enhanced when social insect queens mate multiply (Cole and Wiernasz 1999; Goodisman et al. 2007; Mattila and Seeley 2007; Mattila et al. 2008; Wiernasz et al. 2008). Notably, there were considerable differences among the colonies (within and across treatment groups) in the amount of activity that was generated by each workforce in response to the feeder and the colonies at either extreme of the performance spectrum were revealing. Foragers from one multiple-patriline colony (#1) vastly outstripped all other colonies in the activity of its foragers at the feeder and on the dance floor, and out of 129 trained foragers, every individual in that colony but one danced during the assay (Fig. 2). At the other end of the spectrum, focal workers from the least-active single-patriline colony (#3) were shockingly miserable dancers and mediocre foragers at best (Fig. 2). On one test day, not one focal

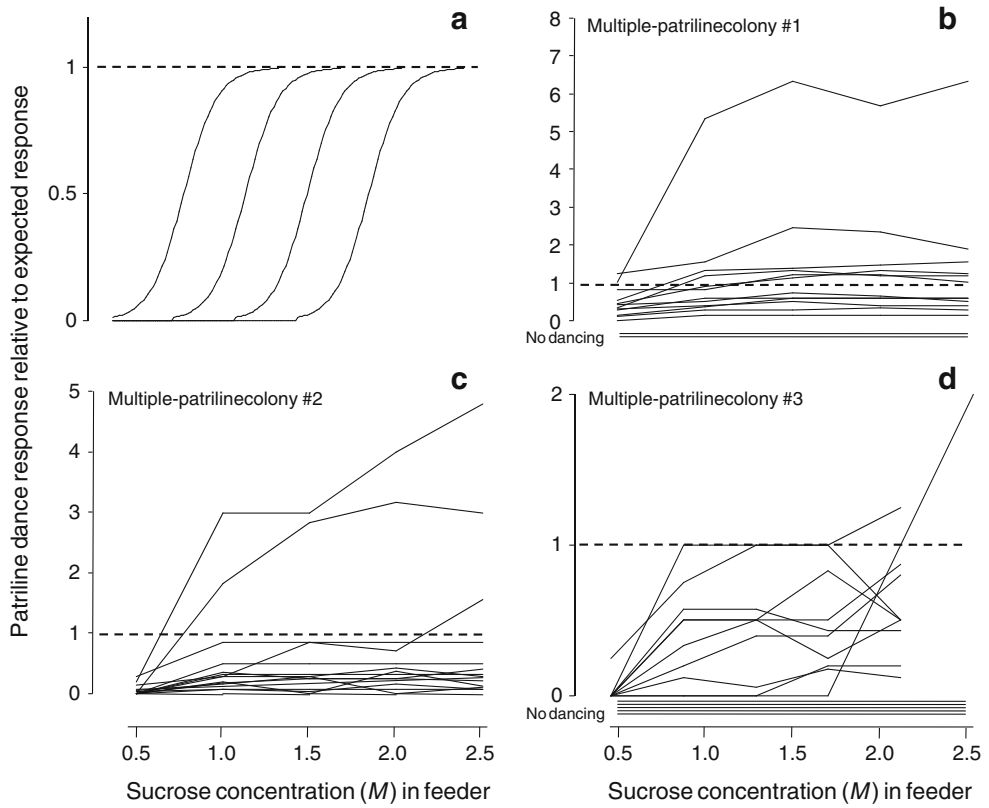

Fig. 7 The relative recruitment signaling response of patrilines to increasing sucrose stimulus as predicted by the response threshold model (**a**) compared to the response of patrilines in the three multiple-patriline colonies studied here (**b**–**d**). The *horizontal dashed line* indicates the upper limit of response if foragers in patrilines participated in dancing according to expected frequencies (provided at the bottom of Figs. 3, 4, and 5); relative response to each sucrose stimulus was determined for patrilines depicted in **b**–**d** by dividing the number of workers from each patriline that responded with dancing to a given sucrose stimulus by the number of workers from that patriline that were expected to join the forager work force, based on the genetic structure of each colony. Thus, values <1 indicate that relatively fewer of a patriline's workers participated in recruitment signaling than was expected; values >1 indicate that relatively more workers than were expected participated in recruitment signaling. Patrilines whose foragers never responded to the sucrose resource with dancing are indicated at the *bottom* of each figure part (i.e., response = 0 over the course of the assay)

forager from this colony danced to recruit nest mates to the feeder, a dysfunctional state for a colony that was in need of food. The extent of these differences suggest that, given only limited genetic background from a single mate, a queen increases the probability of her colony suffering from exceptionally poor performance of a task by its workers or that, by increasing patriline number through polyandry, a queen improves her odds of assembling a workforce that responds better or even unusually well to task stimuli. The need to strike the right mix of a workforce that executes well a wide range of tasks is clearly an important selective force driving greater sampling by queens of the drone population.

The nature of the assay, a single feeder that was available within a closed foraging environment, made it possible to pinpoint the parts of the foraging and recruitment effort that each colony might have excelled at or failed to execute in a proficient manner. For the slower colonies, exploitation of the feeder by the collective was hindered at almost every point in the recruitment process. One of the biggest stumbling blocks to reactivating the focal forager workforce was the length of time that it took for the first forager to discover that food was available once the feeder was restocked (despite the hint that we provided of a droplet of the feeder's solution in the entrance of the observation hive). Very often, especially in the least-active single-patriline colony (#3), even after the first foragers discovered a rich food reward, they would not dance to advertise it upon return to the hive, or they would initiate hesitant or listless dances that attracted little attention from other workers on the dance floor. At times, in some of the less-responsive colonies (usually a single-patriline colony but also multiple-patriline colony #3 at points), the mobilization by focal foragers of their nest mates to the feeder was painfully sluggish to watch. On the other hand, the eagerness with which foragers in the more-responsive colonies greeted the feeder was remarkably different—especially multiple-patriline colony #1, the most active of

the three diverse colonies. When we reentered the greenhouse to start a new trial, it was typical to find several marked foragers inspecting the empty feeder. The inspecting foragers would hover around the person who was preparing the feeder, land on it as soon as it was in place, drink heartily, and fly swiftly back to the hive, where most of them initiated vigorous dances that attracted significantly more dance followers than the dances in single-patriline colonies (M.B. Girard, H.R. Mattila, and T.D. Seeley, unpublished data). These early dance signals promptly reactivated most of the other marked workers to the feeder and, in short order, both the feeder and the dance floor became scenes of bustling activity as the focal foragers shuttled back and forth between the food and their hive. Working so closely with each colony and over many days, we felt that it was not a singular element of the foraging effort that made a responsive colony successful or an unresponsive colony fail. Foragers in the more-responsive multiple-patriline colonies appeared to execute more productively (as a group) almost every aspect of the foraging and recruitment effort compared to similar foragers in the less-responsive single-patriline colonies. We are currently working on studies that will rigorously explore these points.

Although dance response thresholds of foragers were generally lower in multiple-patriline colonies relative to single-patriline colonies (for two of three multiple-patriline colonies that were compared to the latter), within multiple-patriline colonies, the mechanisms by which workers from different subfamilies were allocated to tasks were not clarified by this study. Contrary to the prediction of the response threshold model of division of labor, we found no evidence that mean dance response thresholds were lower for workers in high-dancing patrilines compared to patrilines whose members were less frequently observed dancing, a pattern that was consistent across all three multiple-patriline colonies. The response threshold model is based heavily on an assumption that polyandrous queens introduce into colonies patrilineal differences in thresholds (Robinson and Page 1989), and since its proposal, this model has been embraced as an underlying mechanism for division of labor and to explain empirical observations of "specialist" patrilines [reviewed by Oldroyd and Fewell (2007)]. Yet, we are not aware of any data that link response thresholds of individuals, determined by assaying their response to changing stimuli, with patriline membership *and* colony-level response. Our dance assay is uniquely positioned to overcome this challenge. It demonstrated that lower dance response thresholds were indeed associated with enhanced colony-level response but that the link between lower dance thresholds and paternal descent of workers could not be made. Importantly, we repeatedly documented the presence of high-participation patrilines whose workers became engaged in signaling tasks in overwhelming numbers. The occurrence of patrilines that engage strongly in tasks is often cited as evidence in support of the response threshold model, but we show here that patrilines that participate strongly in the task of recruitment signaling can emerge in colonies without being invoked by differences in dance response thresholds among participators. Thus, we avoid describing workers from high-participation patrilines as specialists because their per capita level of task execution and response thresholds for dancing did not differ on average from the performance of similarly engaged workers from other patrilines.

What then is the role of foragers from high-participation patrilines? They were more likely to begin visiting the feeder (i.e., to participate in the assay) than their nest mates, but once engaged in foraging, they were neither more productive foragers nor more inclined to recruit through dancing. For a task such as foraging, the success of which relies heavily on building momentum among the collective through discovery and recruitment (Seeley 1995), it may be important to have workers who will readily engage in foraging and dancing. These bees will function as the catalysts who get a critical mass of individuals mobilized for exploiting newly available resources. Their rabble-rousing activity generates more foraging-related stimuli in the colony, thereby initiating the positive feedback required to mobilize rapidly workers to the feeder. This response would elevate the per capita activity of all foragers over time if they were similarly responsive to the feeder (as we observed here). Colonies with little or no patriline diversity may miss the opportunity to staff their ranks with these individuals and, thus, may be slow to get their food collection operation going.

It is not clear why workers from high-participation patrilines were more likely to become engaged as dancing foragers than their half-sisters from other patrilines, given that they did not appear to have lower thresholds for responding to task-related stimuli, for dancing at least (foraging thresholds were not determined here). We note, however, that animals do not always respond reflexively and often integrate various lines of information when deciding to respond to a stimulus with action, once it is perceived. This point was evidenced by the variability among patrilines in the probability that workers would dance after a feeder visit once response thresholds were exceeded (Figs. 3d, 4d, and 5d). The element of decision making is often neglected in models that generate division of labor, probably because of the potential complexity of the decision-making process and its evaluation.

In a colony with a polyandrous queen, total foraging and signaling effort was greatly boosted by the presence of a minority of patrilines whose members were *more likely to become engaged* as foragers and dancers, but who, once engaged, were *not more active* than their counterparts from

other patrilines in the colony. Greater differences in foraging performance and dance response thresholds may have been found among patrilines in genetically diverse colonies had we been able to evaluate the performance of workers who did not participate in the assay, proportionally more of which came from low-participation patrilines (a difficult scenario to overcome). However, our self-selected focal foragers responded during training to a sucrose stimulus (1.5 M) that was as rich or richer than almost two thirds of food resources that are collected naturally by foragers in the local environment [Seeley (1995), p. 41] and our resource should have been perceived as highly desirable because it was the only food available to colonies in the greenhouse. If such a reward did not entice more workers from underrepresented patrilines to forage in the assay, then it is difficult to know if any level of stimulus would elicit such a response. A worker may never take up a task if she has an extraordinarily high threshold of response to stimuli that signal the need for the task to be completed, or if she is incapable of, for physiological reasons, executing a task, or if she simply chooses not to execute the task even though an internal threshold for response is exceeded. With any of these scenarios, the outcome is the same: a subpopulation of unresponsive individuals would emerge in colonies, as we found here. Our study shows that the greatest difference among patrilines within a colony was not the strength of stimulus required to initiate a response from a patriline's workers (as predicted by the response threshold model and depicted in Fig. 7a) but rather in the proportion of a patriline that responded across the range of stimuli (Fig. 7b–e; note that, in most patrilines, some proportion of foragers responded with dancing to the lowest sucrose stimulus). A more expansive model of division of labor must acknowledge that, for a given task, not all patrilines may participate and that the proportion of a patriline that responds differs widely among subfamilies.

The presence of high-participation patrilines and broadly elevated levels of per capita activity illustrate important means by which a genetically diverse workforce galvanizes foraging effort in honeybee colonies. Such diversity may be key to ensuring that most patrilines participate well in foraging—colony-level response was clearly weaker without foragers from high-participation patrilines to act as social facilitators for the entire forager workforce, reactivating experienced foragers and recruiting inexperienced workers speedily to a food source. In insect societies, where an expansive foraging effort is required to meet the enormous energy demands of a large population, the need to discover and expeditiously recruit nest mates to profitable resources is crucial for survival. Many species of social Hymenoptera are characterized by colonies that forage successfully without polyandrous queens and, thus, intracolonial genetic diversity (e.g., bumble bees, most stingless bees), but the trend toward extreme polyandry in social taxa with populous and energy-hungry colonies, especially those that require high levels of productivity to support colony fission (e.g., army ants, honeybees), connotes the competitive edge that genetic diversity confers for the organization of labor in these social insect colonies.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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