Behav Ecol Sociobiol (2011) 65:799–811
DOI 10.1007/s00265-010-1083-0

ORIGINAL PAPER

Does a polyandrous honeybee queen improve through patriline diversity the activity of her colony's scouting foragers?

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Received: 1 July 2010 / Revised: 13 September 2010 / Accepted: 16 September 2010 / Published online: 2 November 2010

Abstract Recent studies indicate that the foraging success of a honeybee colony is enhanced when it has numerous genetically diverse patrilines because of queen polyandry. We determined whether foraging is improved in part because patriline diversity generates more responsive populations of scouting foragers. Scouts search for new food sources and advertise them with waggle dances to inform other foragers about unexploited discoveries. We moved multiple-patriline and single-patriline colonies to unfamiliar locations so that colonies relied heavily on successful scouts to initiate recruitment and then compared the development of foraging effort between the two types of colonies. More waggle dance signals were produced during the incipient stages of foraging in multiple-patriline colonies compared to single-patriline colonies because scouts reported food discoveries with longer dances. Scouts also returned to multiple-patriline colonies at rates that were two thirds higher than those of single-patriline colonies, although return rates for general forager populations were not significantly different between colony types. The distance of reported food sources from hives increased with time for all colonies, but by the end of their first day in an unfamiliar environment, maximal foraging reach was greater if colonies had multiple patrilines. Most scouts in multiple-patriline colonies came from a minority of scout-rich patrilines that were generally not those from which general forager populations were derived; the presence of such scout-rich patrilines was correlated with the extent of recruitment signaling in colonies. We show how a honeybee colony's scouting effort is (and is not) enhanced when extremely polyandrous queens produce genetically diverse colonies.

Keywords *Apis mellifera* · Extreme polyandry · Genetic diversity · Scouts · Recruitment communication · Waggle dancing

Communicated by M. Beekman

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Introduction

Across a diversity of taxa, animals that live in social groups often forage as a collective for resources that they share commonly (e.g., hawks: Bednarz 1988; ravens: Marzluff and Heinrich 1991; Wright et al. 2003; killer whales: Hoelzel 1991; Smith et al. 1981; dolphins: Gazda et al. 2005; lions: Scheel and Packer 1991; Stander 1992; hyenas: Holekamp et al. 1997; Kruuk 1972; wild dogs: Creel and Creel 1995; bats: Dechmann et al. 2009; and social insects: Hölldobler and Wilson 1990; Wilson 1971). The exchange of information with family or group members about patchy and ephemeral food resources is probably a key selective force that has fostered the evolution of sociality in these animal systems (Buckley 1997; Campobello and Hare 2007; Marzluff et al. 1996; Safi and Kerth 2007).

Coordination of a collective's foraging effort becomes particularly impressive when large, energy-hungry groups are faced with the challenge of organizing an efficient food search using individuals who have limited cognitive capacity, as occurs in colonies of social Hymenoptera (bees, ants, and wasps). For insect species that forage socially, each day, without a leader or a centrally formed

plan, colony members comb their surroundings to discover new food sources or to check the availability of sources that were visited previously. In some species, workers follow signals that direct them to profitable food items that their nest mates have found. Integrating the activities of all of these workers in a way that generates a productive foraging effort is a formidable task because each workforce must respond sensitively to constant changes in foraging opportunities and needs (Seeley 1995). Thus, many social Hymenoptera, particularly those species characterized by populous colonies, have evolved astonishingly complex recruitment systems that direct the search and recovery effort of foraging colony members to high-quality resources, such as waggle dancing in honeybees (von Frisch 1967; Seeley 1995), pheromone trail marking in ants (Hölldobler and Wilson 1990), odor trail guides and acoustic signaling in stingless bees (Nieh 2004), and tandem following in ants (Hölldobler and Wilson 1990).

Recent studies suggest that one way that a colony can enhance the productivity of its foraging effort is by increasing the genetic diversity of its workforce through polyandry, or multiple mating by a queen (e.g., harvester ants: Wiernasz et al. 2008; honeybees: Mattila and Seeley 2007, 2010; Mattila et al. 2008). Extreme polyandry (six or more mates/queen) is uncommon in the social Hymenoptera (Hughes et al. 2008; Strassmann 2001), but it has evolved repeatedly in species that have large group sizes and foraging strategies that permit well-coordinated colonies to dominate available resources [e.g., army ants (Kronauer et al. 2007), leaf-cutter ants (Boomsma et al. 1999), harvester ants (Rheindt et al. 2004), vespine wasps (Ross 1986), and honeybees (Tarpy et al. 2004)]. That a colony's foraging effort is enhanced when it is composed of a diversity of patrilines is compellingly supported in the honeybees. A honeybee colony that has a polyandrous queen and, consequently, a workforce that comprised multiple patrilines has higher rates of foraging, stores more food, and, ultimately, enjoys greater long-term growth and reproduction than a colony that has limited genetic diversity because its queen has not mated with multiple drones (Mattila and Seeley 2007). Greater colony-level foraging activity is fueled in part by boosts in the frequency of foraging-related signals, including the well-known waggle dance, the signal that foragers produce to inform their nest mates about the location of rich resources (Mattila and Seeley 2010; Mattila et al. 2008). It is challenging to overlay the influence of genetic diversity on the already complex problem of understanding how colonies coordinate their foraging effort, but it is clear that as the forager pool gets organized, the genetic constitution of a worker affects her probability of task execution (reviewed by Oldroyd and Fewell 2007). There is mounting evidence that patriline membership is linked to the extent of participation by workers in foraging and dancing (Arnold et al. 2002; Mattila and Seeley 2010; Robinson and Page 1989; Oldroyd et al. 1991, 1992; Waddington et al. 1998), as well as to their foraging preferences (Oldroyd et al. 1992, 1993). The fact that obligatory and extreme polyandry has evolved in every species of honeybee (reviewed by Palmer and Oldroyd 2000; Tarpy and Nielsen 2002; Tarpy et al. 2004) suggests the significance of the benefits that high levels of genetic diversity confer to the organization of workforces in honeybee colonies (Mattila and Seeley 2007, 2010; Mattila et al. 2008) and their health (Seeley and Tarpy 2007; Tarpy 2003; Tarpy and Seeley 2006).

One important subpopulation of a colony's forager workforce is its scouts—the foragers who are responsible for independently searching for novel food sources without using information contained in their nest mates' recruitment dances (Biesmeijer and de Vries 2001). The proportion of foragers that find new food sources by scouting ranges from 5% to 35% in honeybee colonies depending on how foraging conditions change from day to day (Biesmeijer and Seeley 2005; Lindauer 1952; Seeley 1983). Bees who scout are crucial to the success of a focused foraging effort because their activities allow the majority of foragers to exploit rich forage patches while the scouting minority invests their energy in searching for new, ephemeral resources that are patchily distributed over a relatively widespread foraging range (Dornhaus et al. 2006; Seeley 1983). There is conflicting evidence about whether workers from different patrilines vary in the probability of becoming engaged in scouting (evidence of genetic bias for scouting: Dreller 1998; no evidence of genetic bias: Beekman et al. 2007). If patrilines in a colony vary in the propensity of their members to go scouting, then a queen who mates multiply should have a higher likelihood relative to a singly mated queen of inserting into her colony's population workers who will take up the task of searching for new forage patches. Presently, it is not understood how scouting activity may differ as a function of patriline diversity or whether monandrous colonies suffer a competitive disadvantage in the race amongst colonies to capitalize on lucrative forage sources.

This study evaluates comprehensively the effect of patriline diversity on the development of a colony's foraging effort when its mobilization depends initially on the activity of scouts. As both multiple-patriline and single-patriline colonies foraged for the first time in a common but unfamiliar environment, we inferred the efficacy of each colony's search for and discovery of novel resources by decoding dances that advertised their location and by evaluating the relative richness of their scouting workers' food finds. We also examined the first dances performed by scouts for new forage patches to compare between colony types the amount of time that scouts spent reporting their

finds and the rapidity with which they discovered them. By plotting on maps the food sources that were advertised by foragers, we compared the extent to which the foraging reach of multiple-patriline and single-patriline colonies grew over the course of the day as foragers explored their new environment. We also compared between the colony types the rate at which scouting bees and foragers of all types returned to the hive over their day of foraging. Finally, we compared the patriline profile of each multiple-patriline colony's scout bee population to those of the colony's general forager and general worker populations to determine whether scouts were drawn evenly from the workforce or from a minority of patrilines that were rich in scouting foragers. Ultimately, measures of dance activity in multiple-patriline colonies were linked to the presence in colonies of scout-rich patrilines.

Materials and methods

Creating multiple-patriline and single-patriline colonies

To assemble colonies that were composed of either one patriline or multiple patrilines, we used instrumental insemination to control the number of drones with which queens were "mated." Our methods to create monandrous and polyandrous queens were exactly those described by Mattila and Seeley (2010); we will review them briefly here. A monandrous queen was created by inseminating her with 1 μL of semen from a single drone who was chosen at random from a pool of 1,000 drones drawn from 23 drone source colonies. A total of six monandrous queens were used for this study, and each one produced a single-patriline colony that was fathered by a different drone from the drone pool. The six monandrous queens were paired with six polyandrous queens, each of which was inseminated with a 1 μL mixture of semen from 14–16 drones that were drawn randomly from the drone pool. The groups of drones that were selected for inseminations were unique to each queen. The queens were daughters of a single-drone-inseminated Carniolan queen and, thus, were highly related supersisters (r=0.75). Queen rearing and inseminations were conducted by a queen breeder (Glenn Apiaries, Fallbrook, CA, USA) who kept secret the insemination status of the queens by labeling queen groups only as group A or B; the number of drones used to inseminate queens in either group was not revealed to us until all behavioral data had been collected.

Once the queens were installed in host colonies in an apiary in Ithaca, NY, behavioral observations were deferred for a period of 8 weeks so that the colonies' populations could be replaced by the offspring of the inseminated queens. During that time, colonies were inspected weekly to ensure that inseminated queens were ovipositing well and to examine the general health of colonies. To reduce the incidence of diseases, the impacts of which can be more severe in colonies that lack genetic diversity (Seeley and Tarpy 2007; Tarpy 2003; Tarpy and Seeley 2006), colonies were medicated against ectoparasitic mites (*Varroa destructor*) and the brood disease American foulbrood (causative agent: *Paenibacillus larvae*), according to Mattila and Seeley (2010). All 12 colonies and their queens were visibly healthy for the duration of the study.

General approach for evaluating scouting performance

To compare the foraging and recruitment activity of scouting foragers between multiple-patriline colonies and single-patriline colonies, we randomly paired colonies of each type and then simultaneously moved each pair to a novel environment so that both colonies' forager populations would be forced to rely heavily on scouting to discover food resources. Foraging rates and recruitment signaling of colonies were evaluated during each pair's first day in this new locale. Relocating colonies to an unfamiliar environment has been shown previously to permit the identification of true scouts to an extent comparable to other sampling techniques where the foraging environment remains unchanged (Seeley 1983). Each pair of colonies was subsequently and simultaneously moved to a second unfamiliar environment where we prevented foragers from reentering their hive so that we could collect samples of successful scouts. Six pairs of colonies were studied in total and all data were collected from a single pair of colonies before moving on to the next pair. Behavioral observations were completed between 25 June 2007 and 8 September 2007, and during that time, each pair of colonies was manipulated in the manner described herein.

A pair of colonies was prepared for their move to the first of two unfamiliar environments by transferring each colony's queen, a portion of the worker population, and frames of brood and food into a two-frame observation hive. We were careful to match with no more than a 10% margin of difference between colonies the amount of brood for which workers had to care and the size of their food reserves. For each colony, approximately ~3,500 workers (0.45 kg of bees; Mitchell 1970) were shaken into a container and weighed before they were transferred to their observation hive. Colonies were installed in observation hives in the early morning so that population sizes were not inflated by the return of foraging workers. Each observation hive, once assembled, was placed in the location where its colony's hive once was. The now queenless parental hive, which still held many frames of bees, was screened up and moved to the apiary at Cornell University's Liddell Field Station, which was >5 km away from the apiary where the

observation hives remained. This distance prevented any of the queenless hive's workers from returning to the queen-right observation hive in the original apiary and inadvertently increasing the latter's carefully controlled population size. Either a blue or yellow card (30×20 cm) was placed at the entrance of each observation hive to facilitate orientation of foragers to their new entrance and to reduce drifting of foragers between observation hives. Once one of the colored cards was assigned to a hive, the card was used constantly at its entrance to aid forager orientation as the colonies were moved between locations on subsequent days. When the installation of a pair of colonies was complete, they were left undisturbed for 2 days of foraging so that workers could become accustomed to their new observation hive and its color-coded entrance.

After the period of adjustment was over, we returned to the pair of observation hives at night, screened their entrances, and moved them to the Liddell Field Station, which was a new and distant foraging environment for the workers within the hives. Each pair of observation hives was installed in the same building at the Liddell Field Station; foragers in each colony had access to the outdoors through a Tygon tube (2.5 cm inner diameter) that ran through the building's wall. The entrances for both observation hives were north facing, 6.5 m apart, and were marked with appropriately color-coded cards and landing platforms. We moved the hives to Liddell only if over the subsequent 2–3 days a period of rain-free weather was forecast because we did not want foraging activity to be stalled by periods when bees were unable to fly. In only one instance was the move of a pair of hives delayed for this reason (by 2 days).

Evaluating the effect of scouting activity on foraging performance

When a pair of observation hives was screened up and moved overnight to the Liddell Field Station, the first of two unfamiliar foraging environments, the colony entrances were unscreened at 6 A.M. the next morning and we began to record over the next 8 h all dances that were performed by foragers in both hives. Dance activity was captured by videotaping each observation hive's "dance floor," the area on one side of the hive's combs immediately adjacent to its entrance where returning foragers advertise food resources with waggle dances (see Fig. 4.2, Seeley 1995). The locations of food discoveries that were reported by foragers over the course of the day, as encoded in the dances that they performed, were estimated as described by Mattila et al. (2008). We decoded all waggle dances that were performed by foragers in the first minute of every 5-min interval of videotape. From these data, we constructed maps that showed how the advertised food sources were distributed around the pair of colonies and how the distances to the sources increased over time. Besides sampling one in every 5 min of videotape, we also watched the tapes continuously from the start of the foraging day until the first ten food site discoveries were reported in each colony (we assumed that these were discovered by successful scouts) to determine the timing of their discovery and the number of waggle runs produced by scouts as they tried to recruit nest mates to these sites. Finally, we compared relative foraging rates between multiple-patriline and single-patriline colonies by counting the number of workers that entered each hive over a 5-min period at the end of every hour of the 8-h period of observation.

Collecting scouts to determine patriline membership and nectar loads

After evaluating the effect of scouting activity on foraging effort in the first unfamiliar environment, each pair of colonies was closed with screens and moved at night from the Liddell Field Station to a second unfamiliar location in downtown Ithaca, NY (>6 km west of Liddell). At this second location, we documented the return rate of scouts to colonies and collected them to assess the volume and sugar content of the food that they were trying to bring into the hive. For the colonies that were later revealed to comprise multiple patrilines, successful scouts (defined below) were genotyped to determine their patriline memberships. In downtown Ithaca, both observation hives were installed in the same building, with east-facing, color-coded hive entrances that were 7.5 m apart. For each hive, the outdoor end of the Tygon entrance tube through which foragers exited was modified with a device that permitted bees to exit the hives freely, but it prevented them from reentering the hive upon their return from a foraging flight. We did this by fitting the end of the entrance tube with a cylinder (1.5 cm in diameter, 10 cm in length) made of aluminum wire window screening that was attached through its side wall to the entrance tube, tilted upward at 30° from the horizontal, and left unplugged at the higher distal end. This configuration allowed environmental conditions (light and air flow) to be readily perceived at the hive's entrance through the screen cylinder, and any worker who wanted to leave her observation hive could do so with minimal delay by exiting the Tygon tube and then walking into and then out of the screen cylinder that was attached to her hive's entrance. However, a worker who wanted to reenter the hive was prevented from doing so because she would typically land on the screen cylinder at the point where it covered the Tygon entrance tube and then would walk around, trying to determine how to get into the hive. These individuals were easily caught before they discovered the

opening at the cylinder's distal end. Thus, on the day that workers foraged in this second unfamiliar environment, the design of the hives' entrances permitted workers to receive cues at the entrances that signaled outside conditions and foragers could leave the hives freely, but all foragers were prevented from reentering the hives and recruiting their nest mates to food sources that they had discovered.

At 6 A.M. on this second day of observation, the entrances of both colonies were opened by unplugging the distal end of the screen cylinder. Once a hive's screened entrance was unplugged, an observer monitored the entrance to catch any forager who, upon return from a foraging flight, tried to reenter the hive. Each returning forager was captured with an insect aspirator, placed immediately into a glass vial, and plunged into an ice bath. The ice bath immobilized the forager in <60 s, preventing her from digesting any of the food that she was about to bring into the hive before she was caught. This process was repeated until 200 foragers were collected per colony (3–12 h, depending on the colony). At the end of the day, each forager was classified as either a successful scout or unsuccessful forager by evaluating the food that she carried back to the colony. We labeled a returning forager as a successful scout if she had pollen pellets on her hind legs or if she had >1 μL of nectar in her honey stomach. If a returning forager had ≤1 μL of nectar in her honey stomach, we considered her to be an unsuccessful forager who did not independently discover food (either a non-scout or an unsuccessful scout). This threshold was chosen because a worker carries on average ≤1 μL of nectar as she leaves her colony to forage (Gary and Lorenzen 1976), so if a worker returned to the hive with less than this volume of food, we assumed that she did not discover a new resource while she was outside. Thus, our method of identifying foragers who had scouted excluded those foragers who may have independently searched for food, but failed to find any. Nectar loads were recovered from returning foragers by detaching each worker's abdomen from her body when it was frozen, letting it thaw, and then squeezing it laterally so that the load was expressed out of the honey stomach through the pedicel (modified from the description of Gary and Lorenzen 1976). This method revealed that 50–95% of the returning foragers that we collected were successful scouts. Their bodies were stored immediately in 95% ethanol for genotyping (see below). A scout return rate was calculated for each colony based on the number of successful scouts that was collected from it and the time that it took to get them. We evaluated the stomach contents of 60 successful scouts from each colony so that the quality of their food discoveries could be compared between multiple-patriline and single-patriline colonies. Each worker's nectar load was collected into 10-μL capillary tubes and its volume was determined by estimating the proportion of the tubes' length that was filled by the load. The sugar content of each load was determined subsequently by transferring the nectar from the capillary tubes into a refractometer to estimate % Brix.

After 200 returning workers were collected from each colony, the colonies were sealed and returned to the Liddell Field Station so that the bees in the observation hives could be reunited with their queenless parental colony, which had been foraging undisturbed at Liddell. As soon as each observation hive was unsealed, a sample of ~400 workers was collected from the comb (from the sides, top, and bottom of both frames) into 95% ethanol so that the patriline structure of the entire colony could be determined at a later date. About 10 min after the observation hives were opened at Liddell, a cluster of bees began to form at the (now abandoned) observation hive entrances that each colony had learned to use the previous day. These bees were familiar enough with the entrance to reorient to it once they were flying about the apiary; a sample of ~400 of these workers was also collected into 95% ethanol so that the patriline structure of the general forager population could be determined. Once the insemination status of the colonies' queens was revealed, the paternities of workers from each multiple-patriline colony's scouting, foraging, and whole colony populations (75 workers per population group) were determined using 8–16 microsatellite markers (Solignac et al. 2003) and DNA extraction, PCR, and genotyping techniques that are described by Mattila and Seeley (2010).

Statistical analyses

Paired *t* tests were used to compare single-value measures (e.g., colony means) between multiple-patriline and single-patriline colonies because tests for normality indicated that parametric tests were appropriate. Where workers were subsampled from colonies, one-way ANOVAs with subsampling were used to determine differences between colony types (e.g., evaluation of nectar loads). Repeated measures ANOVAs compared treatments whenever data were collected from colonies at multiple time intervals (e.g., forager return rates, distance of advertised food sources). Goodness-of-fit comparisons of the genetic structure of each multiple-patriline colony's scout population, general forager population (i.e., all types of foragers), and whole colony populations were made with Monte Carlo estimates of exact *p* values (1,000,000 estimates per test). Where significant differences were found between the patriline structure of comparison populations, we identified patrilines that were rich either in scouts or generally in foragers (i.e., there was at least a 50% increase in the number of workers who were scouts or foragers in a patriline relative to that expected based on the colony's genetic structure,

with a minimum of five individuals observed in one of the groups) or poor in scouts (i.e., at least a 50% relative decrease in observed scout numbers, otherwise as above). Spearman correlations were used to determine the relationship between the presence of scout-rich patrilines in colonies and recruitment signaling. For all tests, α=0.05. Statistical tests were conducted with SAS version 9.1.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Who were the scouting foragers?

Three key results were revealed when the patriline profile of workers who were identified as successful scouts was compared in each multiple-patriline colony to the patriline profiles of the general forager workforce and the entire colony population. The first and most important finding was that in all six multiple-patriline colonies, the patriline profiles of the scouting subpopulation and the whole colony populations were significantly different—scouts were not drawn from patrilines in a way that mirrored the genetic structure of each colony's entire population (Table 1). In five of the six multiple-patriline colonies, a few scout-rich patrilines made disproportionately heavy contributions to their colony's pool of scouts relative to the number that was expected if the scout population mirrored the worker population (Fig. 1). For example, patriline 1 in colony 1 yielded 16 successful scouts, but only three were expected based on the sample of the entire worker population (Fig. 1a). Sometimes a scout-rich patriline's contribution to the total pool of scouts in a colony was modest (e.g., 12% of scouts came from scout-rich patriline 4 in colony 2; Fig. 1b), but in other instances, it was hefty (e.g., 33% of multiple-patriline colony 3's scouts came from its only scout-rich subfamily, patriline 1; Fig. 1c). In each of the five colonies with scout-rich patrilines, one to three patrilines were identified that contributed a much higher number of scouts than was expected based on the numerical representation of their workers in the worker population (these are marked with stars in Fig. 1), and generally, these subsets of patrilines produced an average of 44% of their colony's scouts (range 33–53%; Fig. 1). No scout-rich patrilines were identified in only one of the six colonies, multiple-patriline colony 4 (Fig. 1d), even though the distribution of scouts across patrilines differed significantly from that of the whole colony population (Table 1).

Secondly, as a counterpoint to our first observation, there was a minority of patrilines in most colonies whose workers were markedly underrepresented in the scouting workforce despite the patrilines being otherwise well represented in their respective worker populations (marked by a cross in Fig. 1; Table 1). Nine such patrilines were identified in the five multiple-patriline colonies that showed differences in the distribution of scouts relative to the colony population (Fig. 1). Scout-poor patrilines had a 55–83% reduction in the number of scouts that they generated relative to the number that was expected based on the genetic structure of their colony's worker population (Fig. 1). Hence, in five of six multiple-patriline colonies, there was both a small number of patrilines that contributed disproportionately strongly to the scout population in each multiple-patriline colony and a small number of patrilines that contributed disproportionately weakly to the scout workforce.

Thirdly, for five of six multiple-patriline colonies, general forager populations (i.e., not just scouts) differed in their patriline composition compared to the patriline profiles of the whole colony worker samples (Table 1). Similar to scout-rich patrilines, there were forager-rich patrilines in these five multiple-patriline colonies whose workers were more numerous in the forager population than was expected based on their occurrence in the entire worker population (marked with a solid circle in Fig. 1). More often than not, forager-rich patrilines did not overlap with scout-rich patrilines. Twelve scout-rich patrilines and 13 forager-rich patrilines were identified in the six multiple-patriline colonies that were studied, and in only four instances was a patriline a productive source of both scouts and foragers (Fig. 1). Colonies varied in the number of patrilines that were rich sources of foragers; one multiple-patriline colony had no forager-rich patrilines, another had four such patrilines, and the remainder had intermediate numbers of forager-rich patrilines (Fig. 1). The contribution from a single patriline to its colony's forager population

Table 1 Values of p determined by comparing for six multiple-patriline colonies the patriline profiles of the: (a) scout subpopulations to general colony populations, (b) scout subpopulations to entire forager subpopulations, and (c) entire forager subpopulations and general colony populations

Colony	Patriline profile comparison		
	a Scouts vs. colony	b Scouts vs. foragers	c Foragers vs. colony
1	<0.001[a]	<0.001[a]	0.005[a]
2	0.005[a]	<0.001[a]	<0.001[a]
3	<0.001[a]	1.0	0.21
4	0.001[a]	1.0	<0.001[a]
5	0.004[a]	0.039[a]	0.016[a]
6	<0.001[a]	1.0	<0.001[a]

[a] Instances where the distribution of workers across patrilines differed significantly from that expected based on the comparison population

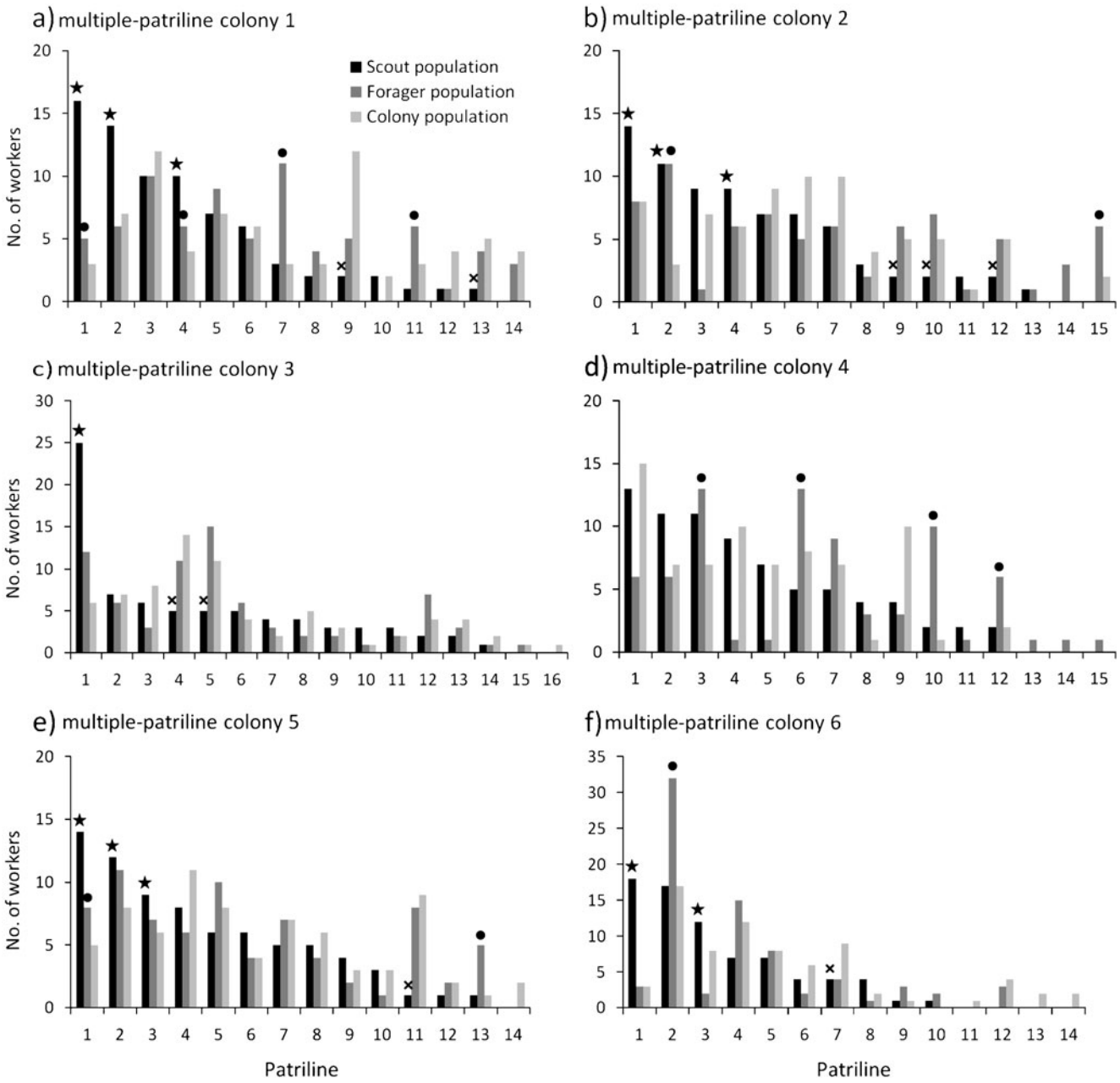

Fig. 1 For each of the six multiple-patriline colonies (colonies 1 to 6 are parts **a**–**f**, respectively), the number of workers in each patriline who were scouts or foragers compared to the number of workers in a similarly sized sample of the entire colony population. *Symbols* highlight patrilines identified as relatively scout-rich (*star*), scout-poor (*cross*), or forager-rich (*circle*) patrilines. A patriline was defined as rich in scouts or foragers if there was at least a 50% increase in the number of scouts or foragers observed for the patriline relative to the number that was expected based on the colony sample (with a minimum of five individuals in either the observed or expected group). A patriline was poor in scouts if there was a 50% decrease in the observed numbers of scouts relative to the expected number (otherwise as above)

was sometimes profound; for example, in multiple-patriline colony 6, 43% of its foragers came from the colony's only forager-rich patriline (Fig. 1f). Where they occurred, forager-rich patrilines yielded an average of 35% of their colony's forager workforce (range 17–56%; Fig. 1).

Comparing the performance of multiple- and single-patriline colonies' scouts and foragers

With clear evidence that most multiple-patriline colonies were stocked with a minority of patrilines that were rich in

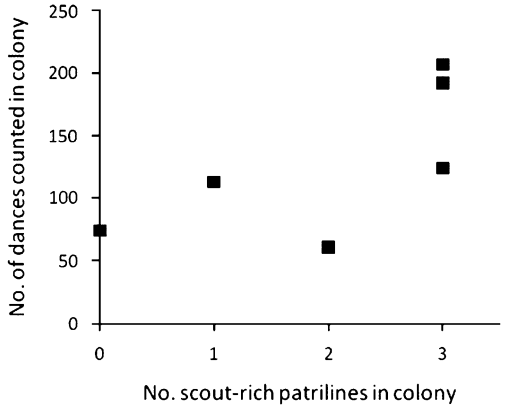

Fig. 2 Relationship between the number of dances counted in each multiple-patriline colony over the course of the first day that colonies foraged in an unfamiliar environment and the number of scout-rich patrilines in each colony

either scouts or foragers, or both, we determined whether having multiple patrilines in a colony, and thus a greater chance of having a scout-rich or forager-rich patriline than a single-patriline colony, resulted in consistent differences in performance between colony types when foraging activity depended initially on the activity of scouts.

As workers in colonies began to explore the first unfamiliar environment to which they had been relocated (Liddell Field Station), the first reported food discovery was advertised by scouts within virtually the same time frame on average in multiple-patriline and single-patriline colonies (mean 78±24.9 and 78±24.8 min after foraging commenced, respectively; t_5=0.02, p=0.98; range 15–191 and 19–168 min). Similarly, there was no difference between multiple-patriline and single-patriline colonies in the length of time that it took for the first ten dances to be performed in each colony (mean 149±35.4 and 156± 14.9 min after foraging commenced for multiple-patriline and single-patriline colonies, respectively; t_5=0.2, p=0.82). However, more information about these sites was available to potential recruits in multiple-patriline colonies because the scouts that danced during this incipient stage of recruitment produced 155% more waggle runs per dance (mean 23±3.0 runs/dance) relative to their counterparts in single-patriline colonies (mean 9±2.5 runs/dance; t_5=3.1, p=0.03).

As foragers surveyed their new surroundings, initial differences between multiple-patriline and single-patriline colonies in per capita signal production did not translate into significant differences in the number of dances that were performed in colonies over the remainder of the day. A mean of 129±27 dances/colony were counted in multiple-patriline colonies and 124±44 dances/colony were counted in single-patriline colonies using our sampling method (t_5=0.1, p=0.92). However, the total number of dances counted in each multiple-patriline colony was positively correlated with the number of scout-rich patrilines that was present in each colony (ρ=0.83, p=0.04; Fig. 2).

Forager return rates were higher, although not significantly so, in multiple-patriline colonies relative to single-patriline colonies over the course of the first day of foraging (Fig. 3; treatment effect: $F_{1,10}$=2.5, p=0.14; time effect: $F_{9,90}$=21.2, p<0.0001; effects interaction: $F_{9,90}$=0.8, p=0.66). During each time interval that colonies were monitored, return rates were consistently higher for multiple-patriline colonies compared to single-patriline colonies, ranging from a 20% to a 155% relative increase (Fig. 3). Small sample size and variability in response across colonies prevented us from clarifying the nature of this pattern (but see scout return rates from the second unfamiliar environment, to follow).

By decoding distance and direction information contained in the waggle dances that foragers produced as they explored the first unfamiliar environment, we were able to determine the location of the food sources that foragers were advertising to their nest mates. Figure 4 provides the foraging maps that were constructed based on the information that was extracted from dances. Dances were performed for food finds that were relatively close to the hives; 45% of dances were for sites that were <1 km away and 93% of dances were for sites <5 km from home (determined across all colonies; Fig. 4). Food source sites were located in relatively similar distributions around the hives of all of the multiple-patrilines colonies, whereas single-patriline colonies varied markedly in the spatial distribution over which food sources were reported (e.g., compare the far-reaching foraging sites plotted for the

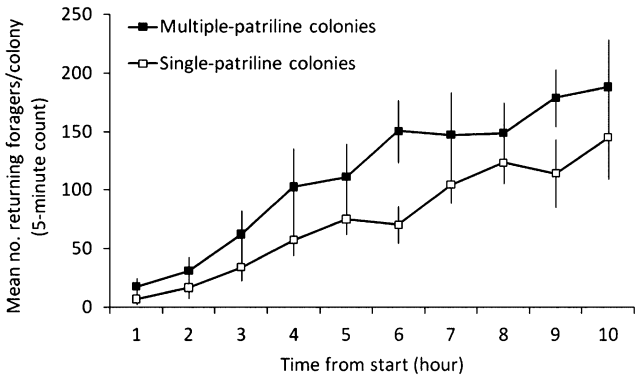

Fig. 3 Mean number of workers returning to the hive (per colony; ±SE) as workers from multiple-patriline and single-patriline colonies foraged over the course of a day in the first of two unfamiliar environments. Colonies from each treatment group were paired and studied simultaneously; six pairs of colonies were studied in total over six different days. Forager counts were made over a 5-min period at the end of every hour of an 8-h observation period that began at 6A.M.

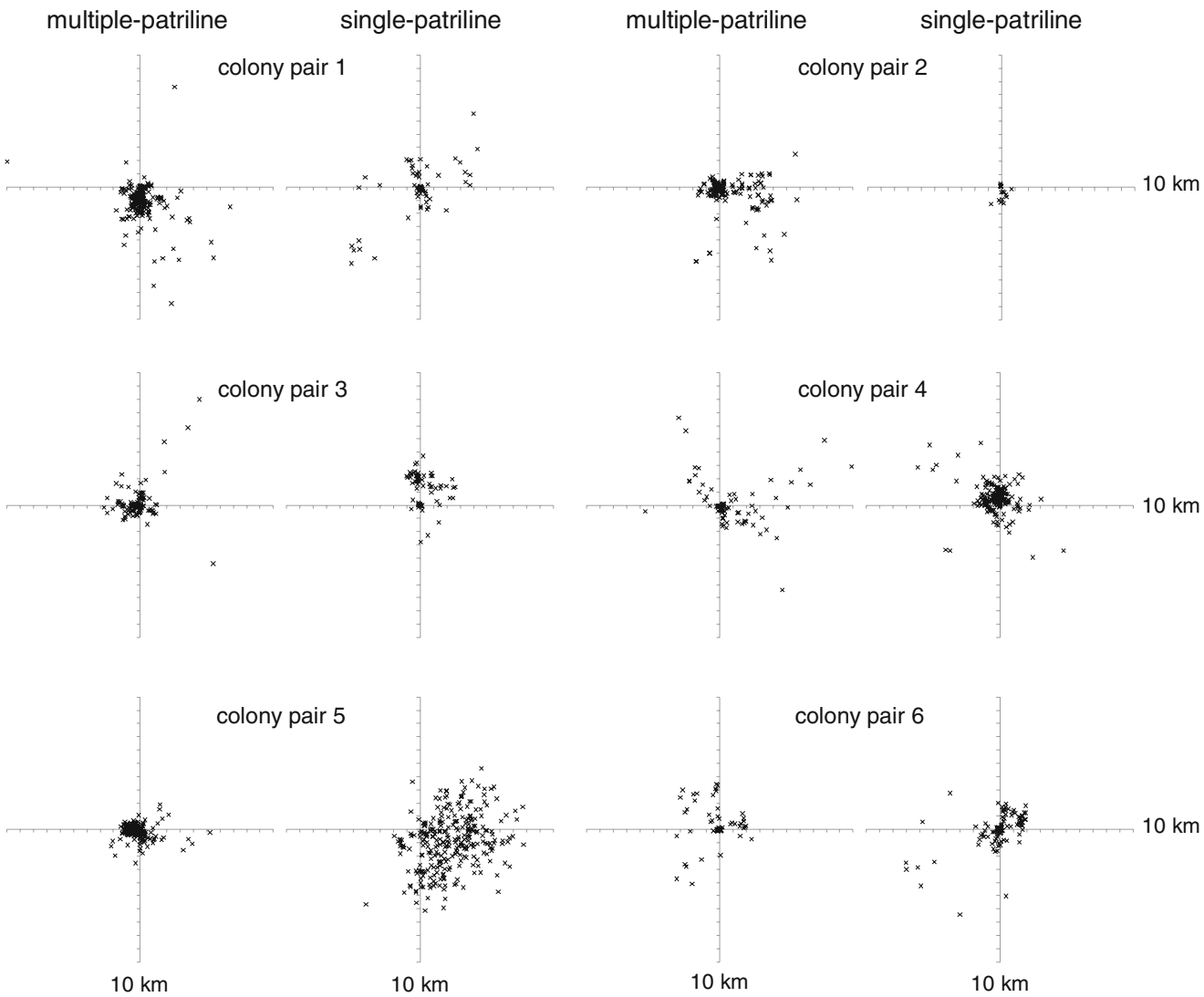

Fig. 4 Maps of the locations of food sources (indicated by a *cross*) encoded by foragers in their waggle dance signals as six pairs of multiple-patriline and single-patriline colonies foraged side by side, over an 8-h period, and in an unfamiliar environment. See Mattila et al. (2008) for methods regarding sampling regimen and the estimation of the location of a food site based on distance and direction information in dance signals. Each pair of colonies was examined on a separate day, from July to September 2007. Plots for each pair of colonies show a 10-km radius around the colonies, which are located at the intersection of the axes (each pair's multiple-patriline colony is on the left and the corresponding single-patriline colony is on the *right*)

single-patriline colony in pair 5 to the close-to-home sites reported by its counterpart in pair 2; Fig. 4). The foraging reach of the colonies, or the mean distance of food sources from the hive as reported by dances, increased hourly over the course of the day (time effect: $F_{7,70}=3.4$, $p=0.001$), but this change was not influenced by the patriline diversity of colonies (treatment effect: $F_{1,10}=0.5$, $p=0.50$; effects interaction: $F_{7,10}=0.4$, $p=0.90$). By the end of the day, however, the most distant food site advertised in a colony by a forager was, on average, 66% farther away from hives with multiple-patriline colonies compared to single-patriline colonies (mean 6.8±1.2 km vs. mean 4.1±0.9 km, respectively; $t_5=2.6$, $p=0.04$).

When foragers were prevented from reentering their hive in the second unfamiliar environment (in downtown Ithaca) so that a sample of successful scouts could be collected, scouts returned to multiple-patriline colonies at rate that was 58% higher on average than the rate at which scouts returned to single-patriline colonies (mean 0.49±0.06 vs. 0.31±0.04 returning scouts/minute, respectively; $t_5=3.4$, $p=0.02$). Nectar loads carried home by successful scouts were similar in quantity and quality between colony types (mean 4.7±0.8 μL and 27±3% Brix for scouts from multiple-patriline colonies and mean 3.9±0.4 μL and 23±2% Brix for scouts from single-patriline colonies; volume: $F_{1,10}=0.7$, $p=0.42$; sugar content: $F_{1,8}=1.0$, $p=0.34$).

Discussion

When a polyandrous queen produces a workforce that is made of multiple patrilines of workers, a disproportionately large number of the workers that scout routinely come from a small number of her colony's patrilines. Thus, workers from only a subset of each colony, determined in part by their genetic constitution, were responsible for much of their colony's effort to discover and report the location of novel food resources to their nest mates. These findings were consistent across most, but not all, of the multiple-patriline colonies, which may reconcile the disparate results of Dreller (1998) and Beekman et al. (2007) who found strong and weak genetic biases, respectively, in a patrilinial propensity to scout in genetically diverse colonies. Interestingly, where they were identified, patrilines that contributed relatively heavily to a colony's scout population consistently were *not* the patrilines that contributed heavily to the colony's general pool of foragers (i.e., scouting and non-scouting foragers). In the six multiple-patriline colonies that we studied, 21 patrilines met the criterion for a scout-rich or a forager-rich patriline, but only four patrilines were identified as both (Fig. 1). This result implies that workers who are highly likely to engage in scouting are not drawn evenly from across the greater population of workers who engage in foraging.

Because scout-rich patrilines were uncommon in each multiple-patriline colony, it is likely that a single-patriline colony will, more often than not, be staffed by a patriline of workers who are not inclined to participate in scouting. Thus, we predicted that on average, the development of a colony's recruitment effort (here represented by waggle dance activity) and foraging effort (represented by the return rate of a colony's foragers and the expansion of a colony's foraging range) would be hindered in single-patriline colonies relative to multiple-patriline colonies by a lack of participation in scouting. We predicted this difference to be especially noticeable when the mobilization of the colony's forager force relied mainly on the activity of scouts, as it did in this study. These predictions were partially realized. Overall, greater numbers of recruitment signals (waggle runs) were produced early in the foraging day by workers in multiple-patriline colonies compared to single-patriline colonies, but this difference was due to an increase in the mean number of waggle runs per dance, not an increase in the rate at which dances were produced by returning foragers (we assume that these dances were produced mostly by bees that had scouted because they were counted so early in the day.) In other words, in multiple-patriline colonies, more information was shared with naïve nest mates about food discoveries because each dancer danced for a longer time when she returned to the hive, not because more individuals were observed dancing. An increase in per dance production of waggle runs has been found previously in colonies with greater patriline diversity (Mattila and Seeley 2010; Mattila et al. 2008), and it is likely that these increases persistently amplified recruitment signaling over the remainder of the day in multiple-patriline colonies.

However, increases in dance duration like those observed in this study have been accompanied previously by increases in total dance number that were not found here (colony level: Mattila et al. 2008; individual level: Mattila and Seeley 2010). There may have been differences in the dance activity of scouts, differences that were hidden within daily dance totals (which did not differ between colony types), but because workers were not individually marked, we could not separate out dancing scouts from the larger pool of dancing foragers. Even without an increase in multiple-patriline colonies in the total number of dances that were performed, early morning recruitment efforts likely benefited from a substantial boost (i.e., a 155% increase) in the production of recruitment signals that generated interest in newly discovered food sources. Indeed, as the day progressed, there was a consistent (although non-significant) pattern of higher foraging rates in multiple-patriline colonies compared to single-patriline colonies. The legitimacy of this pattern, which we believe would have been significant had the laborious task of decoding dances permitted an increase in the sample size of colonies, is supported by our finding that in the second unfamiliar environment, scout return rates were 58% higher in multiple-patriline than in single-patriline colonies. These results, combined with increased signal production, suggest that a marginal edge in the race to discover and exploit new food sources was conferred to forager workforces in colonies that comprised a diversity of patrilines, in part because of a greater effectiveness in scouting.

There is an accumulating body of evidence that suggests that a colony generates a more active and responsive foraging effort when it is headed by a polyandrous queen who has infused her workforce with genetic diversity derived from her many mates (e.g., honeybees: Mattila and Seeley 2010; Mattila et al. 2008; harvester ants: Wiernasz et al. 2008). For this reason, we were surprised that there was not a more decisive difference between the performances of colonies headed by singly and multiply mated queens as their foragers operated in unfamiliar environments. Dance duration and the return rate of successful scouts were enhanced if colonies had multiple patrilines, but day-long foraging rates were boosted only weakly and the speed with which scouts reported new food sources was essentially equal for single-patriline and multiple-patriline colonies. With respect to this latter result, it is possible that by moving colonies into an unfamiliar environment and dampening the early morning production

of waggle dance signals while foragers searched for new food, we created a suite of stimuli inside both single-patriline and multiple-patriline colonies that conveyed to foragers a great need to initiate scouting (for instance, a reduction in the availability of dances to follow—the "failed-follower" mechanism; Beekman et al. 2007). Previous work shows that relocating colonies to a new environment does not result in an unnaturally high number of scouting workers (Seeley 1983), but if stimuli were so strong that foragers who would be unlikely to scout when there are dances to follow became motivated to engage in scouting rather than to remain otherwise inactive inside their hive, then it would explain why similar numbers of dances were observed in both single-patriline and multiple-patriline colonies. Despite a relative lack of effect of patriline diversity on the total number of dances that were executed over the course of a day, polyandrous queens benefit by introducing into their colonies small, genetically defined subpopulations of scouts who return home at higher rates and spend more time per dance reporting food finds to their nest mates.

When foraging is initiated at its outset by foragers of all stripes (e.g., scouts, recruits, inspectors, reactivated or employed foragers; Biesmeijer and de Vries 2001), the collective experience of the forager workforce with a given environment cultivates an enormous head start that allows foraging momentum to accrue in a way that may not have been possible over the course of a day for the naïve multiple-patriline and single-patriline colonies in this study, even though most multiple-patriline colonies had a subset of scout-rich patrilines. However, that the most distant food sites reported in multiple-patriline colonies were on average 66% farther afield from the hives than the most distant sites advertised in single-patriline colonies suggests that patriline diversity may promote a superior foraging range in multiple-patriline colonies that could develop—in the long run—into a substantial increase in resource discovery and exploitation relative to single-patriline colonies (Mattila et al. 2008).

By decoding the dances of workers as they reported food sources in an unfamiliar environment, we have provided the first spatial account of the discovery process that unfolds after a honeybee colony goes through a natural relocation event, such as migration, absconding, or colony fission. Throughout their first day in their new locale, scouting foragers slowly expanded the range of their search effort, suggesting that the collective explores novel foraging space by gradually increasing the range at which foragers search rather than a mixed strategy whereby some individuals initiate searches at farther-flung locations from the outset. This approach limited the area over which a colony's foragers exploited food sources. By the end of their first day in a new environment, neither type of colony exceeded the foraging reach recorded the previous year for multiple-patriline and single-patriline colonies whose forager populations were well acquainted with the same locale (farthest site reported with dances in colonies: 1–9 km for this study vs. 3–22 km for colonies that had not been recently relocated; data from Mattila et al. 2008). Furthermore, the rate of production of recruitment signals was noticeably lower when foragers were not able to visit and recruit to previously exploited resources: the mean daily dance totals for colonies that were just relocated to the Liddell Field Station were 89% lower compared to colonies that were familiar with the same location (Mattila et al. 2008), even though the former colonies had almost twice the number of bees in them.

Small improvements in the performance of many of the tasks that workers execute in colonies should be additive because they clearly generate large fitness benefits for colonies when productivity is assessed at the colony level over long periods of time (e.g., honeybees: Mattila and Seeley 2007; ants: Cole and Wiernasz 1999; Wiernasz et al. 2004; social wasps: Goodisman et al. 2007). When food sources are fleeting and patchy, the presence of a scouting minority in colonies provides the advantage of keeping the forager workforce informed about high-quality resources (Dornhaus et al. 2006; Seeley 1983), but the ways that patriline diversity enhances the performance of this subpopulation are not always readily apparent or observed broadly. However, it is important to note that comparisons of scout bee activity in multiple-patriline and single-patriline colonies revealed performance differences that were either neutral or in favor of colonies with a diversity of patrilines—at no point were colonies with polyandrous queens disadvantaged as they searched a new foraging environment for novel resources. We predict that these small and, presumably, cumulative measures of increase enhance the adeptness with which a genetically diverse colony's workforce responds to temporal or spatial shifts in the foraging landscape. Such an ability to shift nimbly among varyingly available resources is likely under intense selective pressure as these large colonies coordinate a massive collective foraging effort to feed their hungry families.

Acknowledgments We are grateful to Madeline Girard for her extensive and capable assistance with field work, video analysis, and genotyping. We thank Mark Jandricic and Steven Bogdanowicz for lending their time and skills to the genotyping effort. We also thank Justin Armstrong for helping to move colonies late at night and for assisting with field work at the downtown locale. This project was funded by a Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship (H.R.M.) and two Cooperative State Research, Education, and Extension Service National Research Initiative grants (T.D.S.: 2003-35302-13387 and 2007-35302-18174). This experiment complies with the current laws of the country in which it was performed (USA).

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

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