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ERRATUM

Bumble bee pollen foraging regulation: role of pollen quality, storage levels, and odor

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Owing to an unfortunate error, an inappropriate addition was made to the article title.

The correct title should read:

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Communicated by: R. Moritz

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ORIGINAL PAPER

Manuscript in preparation for *Behavioral Ecology and Sociobiology* Bumble bee pollen foraging regulation: role of pollen quality, storage levels, and odor

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Abstract The regulation of protein collection through pollen foraging plays an important role in pollination and in the life of bee colonies that adjust their foraging to natural variation in pollen protein quality and temporal availability. Bumble bees occupy a wide range of habitats from the Nearctic to the Tropics in which they play an important role as pollinators. However, little is known about how a bumble bee colony regulates pollen collection. We manipulated protein quality and colony pollen stores in lab-reared colonies of the native North American bumble bee, *Bombus impatiens*. We debut evidence that bumble bee colony foraging levels and pollen storage behavior are tuned to the protein quality (range tested: 17–30% protein by dry mass) of pollen collected by foragers and to the amount of stored pollen inside the colony. Pollen foraging levels (number of bees exiting the nest) significantly increased by 55%, and the frequency with which foragers stored pollen in pots significantly increased by 233% for pollen with higher compared to lower protein quality. The number of foragers exiting the nest significantly decreased (by 28%) when we added one pollen load equivalent each 5 min to already high intranidal pollen stores. In addition, pollen odor pumped into the nest is sufficient to increase the number of exiting foragers by 27%. Foragers directly inspected pollen pots at a constant rate over 24 h, presumably to assess pollen levels. Thus, pollen stores can act as an information center regulating colony-level foraging according to pollen protein quality and colony need.

Keywords Communication · Recruitment · Foraging · Information flow · Collective behavior · Social insect

Communicated by R. Moritz

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Introduction

In social insects, the need to optimize food collection has led to the evolution of a wide range of foraging strategies, including remarkable adaptations such as the honey bee waggle dance, which communicates the distance and direction to food sources to nestmates (von Frisch 1967). In social ants and stingless bees, we also find species that can recruit nestmates to a specific food location (Hölldobler and Wilson 1990; Nieh 2004). However, some social insects activate nestmates to visit food sources without communicating food location. Such foraging activation has received less attention than location-specific communication, but is an important alternative strategy because it is reported in all bumble bee species tested to date (Dornhaus and Chittka 2004) and in some stingless bee species (Lindauer and Kerr 1958; Lindauer and Kerr 1960).

Bumble bees (Hymenoptera, Apidae, Bombini) play a major ecological role as pollinators in palaearctic, nearctic, and tropical ecosystems (Goulson 2003). Unlike honey bees and stingless bees, bumble bee colonies are annual (not perennial), are generally founded by a single queen (not a swarm), and thus pass through stages in which a relatively small number of individuals must meet colony needs (Michener 1974). Bumble bees thus yield insight into how the collective behavior of a relatively small number of individuals can nonetheless result in the well-tuned behav-

ior of a colonial superorganism, a reproductive unit consisting of individuals who must work together to allow the colony to survive and reproduce (Wilson 1990).

Foraging regulation is a prime example of colony cooperative action. In honey bees, colonies regulate nectar foraging according to nectar quality and location (Seeley et al. 1991, 2000) and colony need (Seeley 1989). Honey bees also regulate pollen foraging based on food availability and colony demand (Fewell and Winston 1992; Camazine 1993; Dreller et al. 1999; Dreller and Tarpy 2000; Calderone and Johnson 2002). Similarly, studies of bumble bees have demonstrated that colonies can activate nectar foraging (increase the number of nectar foragers) and adjust the number of nectar foragers according to colony nectar stores. Foraging activity increased in *Bombus terrestris* and *Bombus transversalis* colonies after foragers returned from nectar collection (Dornhaus and Chittka 2001; Dornhaus and Cameron 2003), and the increase in foraging depended upon food sucrose concentration (Dornhaus and Chittka 2005). Moreover, the level of colony nectar stores serves as a general information center that allows nectar foragers to allocate their efforts according to colony need. Foragers spend more time probing nectar storage pots when these pots are full compared to when they are depleted. *B. terrestris* foragers also spent more time running around excitedly at a higher average speed inside the nest and activate more nestmates to forage when colony nectar stores are low compared to when they are high (Dornhaus and Chittka 2005).

These running bouts may help broadcast a pheromone that activates nectar foraging (Dornhaus and Chittka 2001). One component of the activation pheromone is believed to be the terpene eucalyptol which is able to produce a recruitment response comparable to that elicited by successful returning foragers (Granero et al. 2005). Moreover, the response to recruitment pheromones in *B. terrestris* was stronger in colonies with low food reserves (Molet et al. 2008). Additionally, Dornhaus and Chittka (1999) found that bumblebees can communicate the availability and quality of nectar sources to nestmates by using food source scent.

Pollen is the sole source of colony protein, and pollen foraging is important for reproduction in many insect-pollinated plants (Heinrich and Raven 1972; Heinrich 1979). However, no studies have examined the detailed effect of pollen odor, pollen forage quality, or colony pollen stores on bumble bee foraging activation. Pollen quality and protein levels in bee-pollinated plants can vary widely (2.5–61.0% dry mass protein content, Roulston et al. 2000). Thus, bumble bees discriminate between and prefer flowers with viable, cytoplasm-containing pollen that can support bees nutritionally (Robertson et al. 1999). When foragers were allowed to choose between low- and high-quality pollen patches, the foragers visited flowers that had higher quality pollen more often than flowers offering low quality pollen. Moreover, bumble bees prefer flowers with increased pollen availability and will spend more time visiting such rewarding flowers, visit inflorescences within the same plant more often, and increase their rate of revisits (Harder 1990). Free (1955) suggested that bumble bee pollen foraging reflects colony demand. However, whether and how bumble bees regulate the number of pollen foragers is unknown.

The goal of our study was to determine how bumble bee colonies regulate pollen foraging and whether individual foragers can activate colony foraging based on pollen protein quality. We tested the effect of low and high pollen stores on pollen foraging. If bumble bees can assess colony pollen needs, we hypothesized that adding small amounts of pollen to low pollen stores (the result of successful foragers bringing back pollen) would increase foraging activity, even when no pollen was available in the foraging arena. The added pollen could inform the colony of an available pollen source and thus motivate the colony to forage when pollen stores were low. Under high pollen stores, we expected added pollen to have no effect on foraging activation because bees already had sufficient pollen for colony needs.

We determined the effect of protein quality on pollen foraging activation by using different pollen concentrations (50%, 75%, and 100% pollen diluted by mass with inert, indigestible alpha-cellulose). During these experiments, the colony was given 0.5 g of 100% pollen intranidally each day to support brood development but prevent pollen storage. We hypothesized that foragers increase foraging and excitatory intranidal behavior for high quality pollen compared to low-quality pollen. Finally, we examined potential mechanisms for assessment of pollen stores by (1) continuous video recording of the pollen pots and counting the number of pollen pot inspections, and (2) measuring the effect of fresh intranidal pollen odor on forager activation and nestmate behavior.

Materials and methods

Colonies and study site

We studied a relatively common species of North American bumble bee, *Bombus impatiens*, which ranges from Ontario and Maine south to Florida and West to Michigan, Illinois, Kansas, and Mississippi (Heinrich 1979). We conducted three experiments with three successive *Bombus impatiens* colonies containing approximately 100 to 200 worker bees from BioBest (Leamington, Ontario, Canada, lab-reared, size class B colonies) in a temperature-controlled (22°C)

laboratory at the University of California, San Diego. We housed each colony in a wood nest box (45 cm×27 cm× 15 cm) with a transparent plastic cover. Bees foraged in a 78 cm×31 cm×33 cm plastic arena connected to the nest box with a clear vinyl tube (4.5 cm in diameter). We collected data from September 2006 through March 2008 and conducted experiments between 0900 and 1300 hours. We typically began experiments with each colony within 7 days of receiving it and were able to work with the colony for approximately 8 weeks.

General methods

We labeled all foragers with numbered plastic bee tags (The Bee Works, Orillia, Ontario, Canada) attached with cyanoacrylate to cold-anesthetized bees. Pollen foragers must learn to forage for pollen (Raine and Chittka 2007). Therefore, each colony was given a week to adjust to collecting pollen from a dish in the foraging arena. We fed colonies unscented 1.5 M reagent-grade sucrose solution provided ad libitum in the nest. To ensure there was no sugar in the pollen, we filtered honey bee-collected pollen with distilled water (2.5 L per 225 g of pollen). To test for residual sugar, we suspended five samples of 1 g filtered pollen each in 500 μL distilled water, centrifuged this solution for 30 min, and then tested the supernatant with a refractometer (Fisher Scientific Refractometer, Model No. 13-947, 1% accuracy). The filtered pollen contained no residual sugars. We then measured pollen protein content with a Bradford protein concentration assay (Bio-Rad, catalog no. 500-0006, modified protocol from Roulston et al. 2000) using *Typha latifolia* L. pollen as a standard. The foraging arena was illuminated with a halogen lamp on a 12-h cycle (0800–2000 hours).

Experiment 1: Effect of colony pollen stores

To test the hypothesis that colony pollen stores affect pollen foraging, we ran 27 trials February–May 2007 with Colony 1 (13 trials with high pollen stores and 14 trials with low pollen stores). We either emptied pollen pots of their contents or packed them full of pollen 24 h before the beginning of a trial. We conducted five-minute censuses of the foraging response (number of bees exiting the nest) over a 30-min control phase followed by a 60-min treatment phase. We previously determined that an individual forager deposits an average load of 5 mg. Therefore, during the treatment phase, we added 5 mg of pollen to the pots each 5 min to test the effect of fresh pollen being deposited into the pots, as it is by foragers. No foraging stimuli (neither pollen or sugar solution) were present in the foraging arena throughout the 90-min trial.

Experiment 2: Effect of pollen odor on foraging activation

To determine if fresh intranidal pollen odor alone can increase foraging activity, we ran 14 trials during November 2007 with Colony 2 (seven trials with high pollen stores and seven with low stores), manipulating pollen levels and censusing foraging as in Experiment 1. Each trial consisted of a 30-min control phase (air only, no pollen odor) followed by a 60-min treatment phase (pollen odor). To control for air flow as a stimulus, we used an aquarium air pump to constantly pump fresh air at *all times* into the nest through a 1-cm diameter vinyl tube positioned above the pollen pots (airflow of 4.4 L/min passing through the nest, exiting primarily into the foraging arena). The air inside the nest was therefore fully exchanged at least each 4.1 min.

To generate pollen odor, we attached a 5-ml tube, containing 0.5 g of freshly ground pollen to the air pump system. The tube was connected to a syringe filter (Whatman, 0.2 μm pore size) to prevent pollen grains from entering the air flow. No foraging stimuli (neither pollen or sugar solution) were present in the foraging arena throughout the 90 min trial (0830–1000 hours). Sucrose was supplied ad libitum in the nest at random times between 1300 and 1600 hours.

Dornhaus and Chittka (2001) hypothesized that forager fanning could play a role in communicating food availability such as in diffusing forager-activating pheromone. In our experiment, no food was available in the foraging arena, but we observed fanning by stationary workers inside the nest during the pollen odor experiment. We therefore counted the maximum number of bees fanning (stationary bees that had visible wing movement) and the number of pollen pot inspections (bee placing her head inside a pollen pot). To monitor behavior over a 24-h period, we used a Lorex Digital Video Recorder (Strategic Vista, Markham Ontario, Canada) recording at 10 fps. We measured behavior in three 10-min intervals during the 90-min trial period and seven 10-min intervals during the remaining 1,350 min period. We used two-tailed paired *t* tests to compare the effect of pollen odor and air-only on these behaviors, calculating an average for each behavior for each treatment within a 24-h period. We used post hoc Tukey HSD tests to compare behavioral measures among the different time intervals within a 24-h period.

Experiment 3: Effect of pollen quality on colony foraging and forager intranidal behavior

To quantify the behavior of focal pollen foragers, we videotaped (Panasonic PV-GS39) the intranidal behavior of foragers collecting either 50% or 100% pollen in ten separate trials. In order to video record more than one foraging bee per trial, we left the pollen in the foraging

arena throughout these trials. We randomly chose ten intranidal runs (making sure each run was from a different bee) for 50% and 100% pollen and analyzed the entire path of each returning forager with VideoPoint v2.5.0 software, measuring the total path length, the average velocity, the area covered, and clustering. To calculate area covered, we divided the nest area into 1-cm^2 grids and counted the number of *different* grids that the bee traversed. We then calculated "clustering" by dividing the area covered by the total path length. For example, a forager might repeatedly move back and forth between a limited area of the nest (high clustering) or move the same total distance over a large area of the nest (low clustering).

To test the hypothesis that pollen protein quality affects foraging activation, we ran 14 trials during January 2008 through March 2008 with Colony 3. We used 50%, 75%, and 100% pollen by mass, diluted with powdered alpha cellulose by mass. Alpha cellulose (Sigma, EC 232-674-9, St Louis, MO, USA) is an odorless, inert, indigestible compound that has been used to vary the protein nutrient value of pollen for foraging honeybees (Pernal and Currie 2001; Waddington et al. 1998) and caterpillars (Lee et al. 2004). We allowed all bees access to the foraging arena and censused the foraging response for 30 min. We then placed a dish containing 1 g of 50%, 75%, or 100% pollen into the foraging arena for the entire trial. While still conducting a census of the foraging response, we monitored the foraging bees and made note of the time that the first foraging bee returned to the nest. Then, we measured the foraging response for an additional 60 min.

We did not alter pollen store levels in this experiment. The colony was provided inside the nest with 0.5 g of 100% pollen each day, an amount we found sufficient to provide for daily needs without allowing pollen to accumulate in pots. We counted the total number of nondepositing foragers and depositing foragers, measured the time each forager spent before and after depositing pollen, and measured the total time each nondepositing forager spent inside the nest.

Statistical analyses

Our data conformed to parametric assumptions (we tested for normality and conducted residual analyses). We therefore performed analysis of variance and *t* tests using JMP Statistical Software v5.1 (alpha=0.05). To control for baseline differences in colony activity levels, we compared the first 30 min control period to the second 30 min of the treatment period. We used this protocol because the level of foraging tends to build up and then stabilize 30 min after foraging stimuli are presented (Dornhaus et al. 2006). We use two-tailed paired *t* tests for analyses involving repeated measures. We report averages as mean±SD.

Results

Experiment 1: Effect of colony pollen stores

Foraging activation (number of bees exiting the nest) depended upon colony pollen store levels. The number of bees entering the foraging arena significantly decreased (by 27.8%) when 5 mg of pollen was added each 5 min to full pollen pots, slightly overfilling them (t_9=−2.79, P=0.021, Fig. 1a). However, when pollen stores were depleted, adding pollen each 5 min to pollen stores did not significantly increase or decrease the number of bees leaving the nest (t_3=−1.06, P=0.37, Fig. 1a).

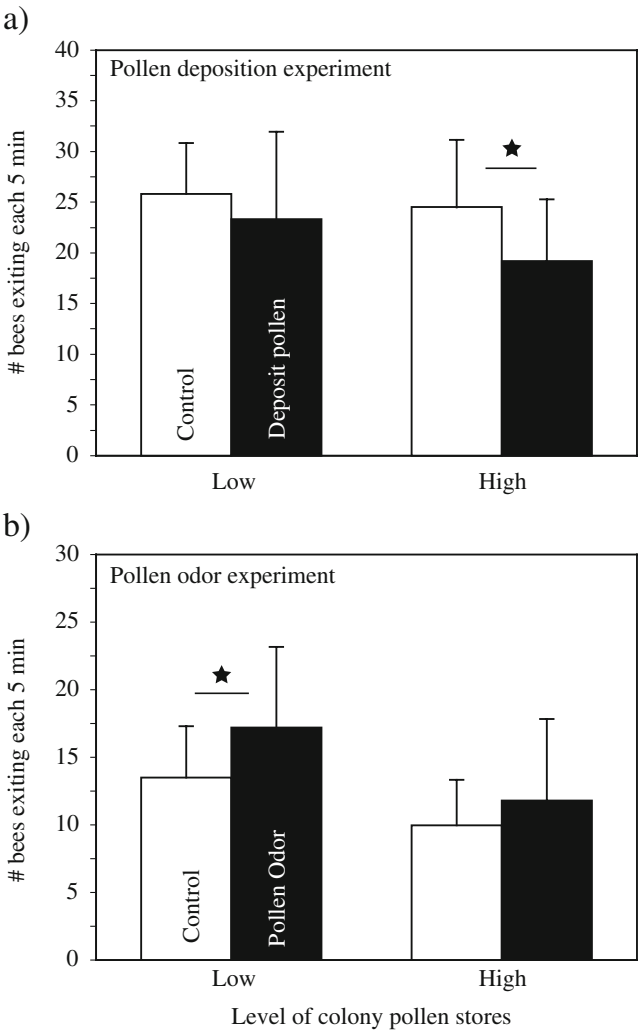

Fig. 1 Colony pollen store levels affect pollen foraging activation. In the *bar graphs*, a *horizontal line* with a star above it denotes a significant difference (P<0.05) and *SD bars* are shown. **a** Pollen deposition experiment. *Open bars* show data from control phases (no pollen added to pots) and *filled bars* show data from treatment phases (pollen added to pots). **b** Pollen odor experiment. *Open bars* show data from control phases (control air) and *filled bars* show data from treatment phases (pollen odor)

Experiment 2: Effect of pollen odor on foraging activation and intranidal behavior

There is a strong effect of pollen odor on foraging activation despite an absence of pollen in the foraging arena. Adding intranidal pollen odor significantly increased the number of bees exiting the nest when pollen stores were low (t_6=3.65, P=0.011), but not when pollen stores were high (t_6=1.35, P=0.23, Fig. 1b). When pollen stores were low, the number of bees exiting the nest increased by 27% when pollen odor was pumped in (Fig. 1b).

Bees inspected pollen pots at a constant rate that was not affected by pollen odor being pumped in, regardless of pollen storage levels (Fig. 2a, low stores t_5=−1.74, P=0.14; high stores t_4=0.89, P=0.42, Fig 2b). In addition, the number of pollen pot inspections did not differ over 24 h under high or low pollen stores (Tukey HSD, P>0.05, Q=3.35).

Pollen odor immediately decreased the number of fanning bees inside the nest under both low (t_5=5.03, P=0.0040) and high (t_4=3.17, P=0.034, Fig. 2c) pollen store conditions. In the absence of pollen odor, there was no change in fanning levels over a 24-h period when pollen stores were low (Tukey HSD, P>0.05, Q=3.29). However, when pollen store levels were high, fanning levels changed and increased at 1300 hours and remained high at 1600 hours after the pollen odor pumping ceased (Tukey HSD, P<0.05, Q=3.35). Moreover, in this time interval, there is a significant effect of colony pollen stores. Fanning significantly increased at 1300 hours and remained equally elevated at 1600 hours at high compared to low pollen store conditions (for these time intervals: full model $F_{3,20}$=4.70, P=0.012, R^2=0.41; pollen stores effect $F_{1,20}$=14.0, P=0.001; time effect $F_{1,20}$=0.06, P=0.81, interaction $F_{1,20}$=0.002, P=0.97, Fig. 2d). Thus, fanning levels did not significantly change between 1300 and 1600 hours under either pollen store condition. However, there is a strong effect of colony pollen stores. When pollen stores were full, fanning significantly increased after pollen odor pumping stopped, increasing on average by 102% over fanning levels that existed before pollen odor pumping began (Fig. 2d).

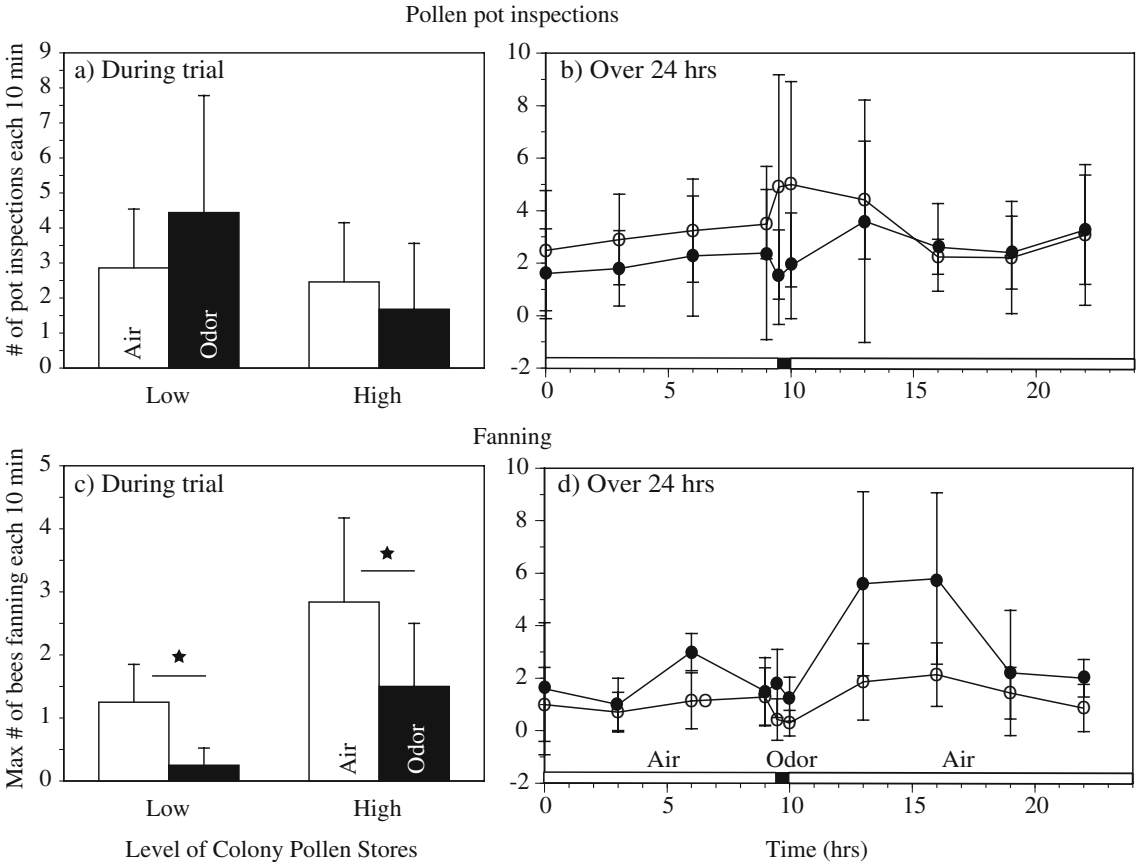

Fig. 2 Intranidal worker behavior (pollen pot inspections and fanning). In *bar graphs*, a *horizontal line with a star* above it denotes a significant difference (P<0.05) and *SD bars* are shown. **a**, **c** *Open bars* show data from control phases (air only) and *filled bars* show data from treatment phases (pollen odor). **b**, **d** *Filled circles* show data from high-pollen store trials, *open circles* show data from low pollen store trials over a 24-h period

Experiment 3: Pollen quality and foraging activation

Our filtered pollen consisted of 32.9% protein by mass as measured by the Bradford assay, and each experiment was run using pollen from the same homogenous filtered pollen reserve. Thus, the 50% and 75% dilutions with alpha cellulose contained, respectively, 16.5% and 24.7% protein by mass (which falls into the natural range, 2.5–61.0%, of bee-pollinated flowers, Roulston et al. 2000). As pollen concentration increased, the number of foragers also increased. Overall, the number of bees exiting the nest was significantly and positively affected by the quality of available pollen ($F_{2,17}$=5.06, P=0.019, Fig 3a) The number of exiting bees was significantly higher (55%) in 100% trials versus 50% trials (Tukey HSD, P<0.05, Q=2.57, Fig. 3a)

The quality of the collected pollen positively influenced the number of foragers depositing pollen inside the nest ($F_{2,13}$=5.1, p=0.023). The number of different individual depositing foragers (all bees were individually marked and each bee was thus counted only once) was significantly higher (233.0%) in 100% pollen trials versus 50% pollen trials (Tukey HSD, P<0.05, Q=2.64, Fig. 3b).

Figure 4 shows the typical path of a forager who deposited pollen inside the nest. Foragers traveled throughout the nest and passed by several different pollen storage pots before choosing one in which to deposit their pollen. Foragers that deposited their pollen loads into pollen pots spent significantly more time in the nest than foragers that did not deposit pollen ($F_{1,65}$=7.0, P=0.01, $\text{mean}_{\text{deposit}}$= 190.4±114.5 s, $\text{mean}_{\text{nodeposit}}$=114.7±120.0 s).

Pollen concentration had no significant effect on the total time a depositing forager spent inside the nest ($F_{2,33}$= 0.015, P=0.98). There was no significant effect of pollen concentration on the time spent within the nest before ($F_{2,33}$=0.35, P=0.70) or after ($F_{2,33}$=0.50, P=0.61) pollen deposition. Pollen concentration also had no significant effect on the total time a nondepositing forager spent inside the nest ($F_{2,28}$=0.69, P=0.51). From the video analysis, pollen concentration did not significantly affect focal

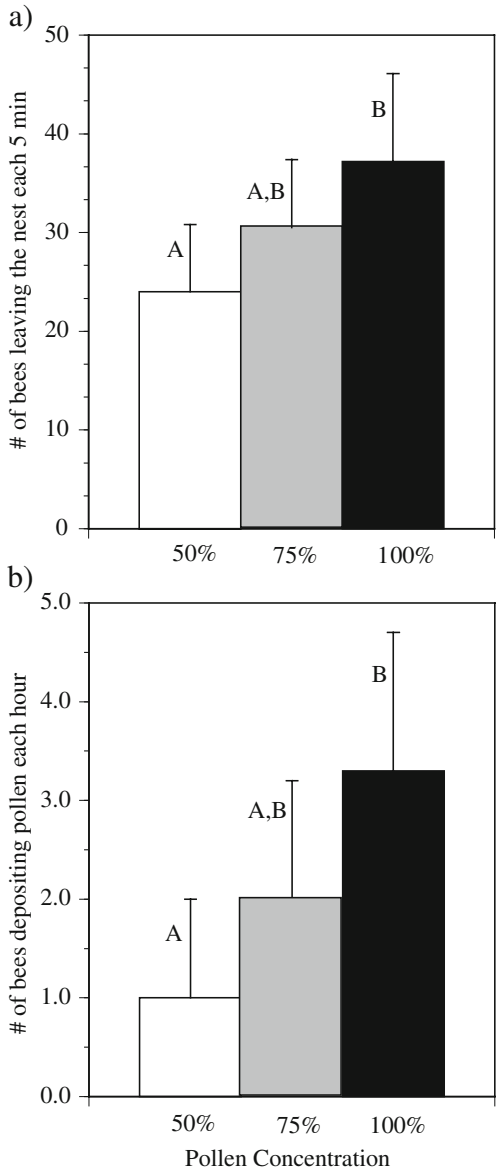

Fig. 3 Pollen protein quality affects pollen foraging activation. Different letters denote significantly *different bars*. SDs shown. This experiment included seven 50% (white) pollen trials, seven 75% (*gray*) pollen trials, and six 100% (*black*) pollen trials. The 50%, 75%, and 100% pollen concentrations correspond to 16.5%, 24.7%, and 32.9% protein by dry mass, respectively

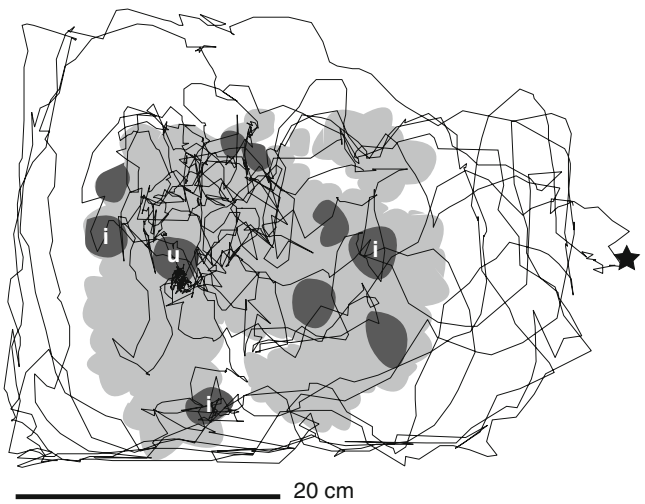

Fig. 4 Typical intranidal path taken by a successful pollen forager collecting 100% pollen. The *light gray areas* show the extent of the nest including the area for nectar pots, brood cells, and empty cells (colony 3). The *dark gray* areas show the location of pollen pots (full in this example). The *star* shows the entrance to the colony, "i" where she inspected pollen pots, and "u" where she unloaded her pollen

forager total path length, average velocity, total time in nest, area covered, or clustering ($F_{1,18} \leq 2.2$, $P \geq 0.15$, Fig. 4, Table 1).

Discussion

Bumble bees, *B. impatiens,* tuned their foraging activation (the number of bees exiting the nest) to the level of colony pollen stores and to the protein quality of available pollen. The degree of foraging activation adjusted to the level of stored pollen, increasing or remaining the same when pollen stores were low and decreasing or remaining the same when pollen stores were high. To simulate the results of successful foraging, we added 5 mg (one pollen load equivalent) of pollen each 5 min. The number of foragers leaving the colony decreased when these small amounts were added to high pollen stores. Pollen odor alone was sufficient to elicit increased foraging when pollen stores were low. In addition, the number of bees exiting the nest was significantly higher when available pollen had a higher protein concentration than when it had a lower protein concentration. Thus, pollen stored in pollen pots, can serve as an information center. Bees consistently inspected pollen pots at a constant rate over 24 h, and their inspection rate was not affected by high or low colony pollen stores. Foragers may thus perceive colony pollen stores through direct inspections and be influenced to check pollen stores through the diffusion of pollen odor inside the colony.

Pollen protein concentration

In addition to determining colony need for pollen, bumble bees can also discriminate high-quality, highly nutritional, viable floral pollen from low-quality, nonviable pollen. For example, *B. terrestris* can use pollen odor to find rewarding flowers (Dobson et al. 1999). *Bombus impatiens* foragers may be able to directly (by taste) or indirectly (by odor) detect protein concentration differences and elevate their thoracic temperature for pollen with higher protein content (Mapalad et al. 2008). In honey bees, pollen foraging is also affected by pollen quality. Choice tests suggest a positive correlation between honey bee preference and pollen with higher concentrations of essential amino acids (Cook et al. 2003). Moreover, honey bee recruitment communication alters according to pollen quality. Recruiters decrease the number of round dance circuits per unit time when fed diluted pollen (50% alpha cellulose by volume) versus pure pollen (Waddington et al. 1998).

We show that bumble bees regulated their foraging activation according to our pollen quality treatments, ranging in our experiment from 17% to 33% protein (by dry mass). This falls well within the range of pollen protein concentration of bee-pollinated plants (Roulston et al. 2000). Inside the nest, nestmates may determine the quality of this newly collected pollen by directly assessing new deposits in pollen pots or by interacting with foragers. For example, Diaz et al. (2007) reported that honey bee dance followers contacted the legs of waggle dancing pollen foragers (which carry pollen) more often than legs of dancing nectar foragers. Contacts between successful bumble bee foragers and nestmates can increase the probability of contacted foragers leaving the nest to search for nectar (Renner and Nieh 2008). In our study, successful pollen foragers also made contact with multiple nestmates while they were inside the nest, providing an opportunity for direct inspection of pollen loads. Alternatively, differences in odor of the diluted pollen may also contribute to the differences in foraging activation.

Intranidal behavior

Foragers inspected pollen pots at a constant rate over 24 h, even at times of no foraging (Fig. 2c). Moreover, this rate of pollen inspection did not depend upon the level of colony pollen stores (Fig. 2a).

Pollen concentration had no significant effect on the average velocity, total path length, area covered, clustering (Table 1), the total time spent in the nest, or the time spent before and after pollen deposition by a returning forager. Thus, a *B. impatiens* forager who has collected only pollen may not provide information about pollen quality through her movements inside the nest. Instead, pollen pot inspections and detection of pollen odor are evidently

Table 1 Intranidal behavior of focal foragers collecting different pollen concentrations

Pollen concentration (by mass; %)	Total path length (cm)	Average velocity (cm/s)	Total time in nest (s)	Area covered (no. of 1 cm^2 grids)	Clustering (no. of grids/cm moved)	*N*
50	181.6±100.7	2.9±1.4	103.4±27.0	104.5±61.4	0.59±0.13	10
100	270.7±260.0	2.1±0.8	139.9±27.0	126.3±85.4	0.53±0.12	10

The path length is the total distance moved by each forager. The area covered is the total number of different 1 cm^2 grids crossed by the forager. Clustering is the total number of different 1 cm^2 grids through which the forager passed divided by the total distance she moved. This is a measure of whether the forager tended to move within the same region of the nest or covered a wide area of the nest

sufficient to account for the observed changes in foraging activation. Integrating the results of multiple sources of information would allow the colony to better allocate its protein foraging efforts not only to colony need but also to the quality of available protein forage.

Dornhaus and Chittka (2005) reported that *B. terrestris* foragers increased their level of excitatory behavior inside the nest for higher-quality nectar (more concentrated sucrose solution). Foragers that collected high-quality nectar spent more time running at a speed higher than 40 mm/s and deposited their nectar load more quickly than foragers that collected low-quality nectar. There may be species-specific differences in intranidal foraging activation behavior or differences in the way that bumble bees activate colony foraging for pollen and nectar.

Dornhaus and Chittka (2001) also observed fanning by successful *B. terrestris* nectar foragers inside the nest and hypothesized that fanning may play a role in recruitment communication, perhaps helping to disperse food-alerting pheromone. In our experiment with *B. impatiens*, we observed stationary nest workers, not foragers, fanning. This could be due, in part, to our experimental design. We pumped in air continuously at all time periods to control for potential disturbances due to airflow.

Pumping in pollen odor immediately reduced the level of fanning inside the colony, regardless of whether colony pollen stores were empty or full. This reduction in fanning was not due to increased airflow because fresh air was pumped in at constant rate over a 24-h period. Changes in temperature or CO_2 concentration are also unlikely to account for the reduction in fanning because the room (and air being pumped in) was maintained at a constant temperature with a full exchange of air inside the colony at least each 4.1 min. In addition, there was no food in the foraging arena 30 min before (control phase) or during the 60-min presentation of pollen odor. Thus, changes in fanning by stationary nest workers were not related to changes in foraging.

It is possible that the influx of pollen odor may have drawn worker attention and thus decreased their level of fanning. When pollen odor pumping ceased, fanning levels increased (between 1300 and 1600 hours) when colony pollen stores were full, but not when they were empty. The reason for this effect is not clear. Under full pollen conditions, the colony does not need more pollen. Thus, we speculate that foragers may have fanned to maintain elevated pollen odor levels *after* odor pumping ceased to circulate the odor from the full pollen pots, and thereby indicate a sufficiency of pollen stores and reinforcing the message that there is no need to collect more pollen. The elevated fanning stopped at 1600 hours, after which the colony normally entered a state of quiescence.

Effect of colony pollen levels

Allocating pollen foraging according to colony needs is beneficial because it can increase pollen collecting efficiency, allowing honey bee foragers to shift between pollen and nectar collecting (Harder 1990; Rasheed and Harder 1997). Thus, like honey bees, bumble bees are able to regulate pollen foraging according to colony need. Mechanisms regulating pollen foraging activity in honeybees include olfactory assessment of pollen quality (Pernal and Currie 2001), assessment of pollen stores' phenolic content (Liu et al. 2006), and brood pheromone (Pankiw 2007). The role of brood pheromone in bumble bee pollen foraging remains to be determined, but pollen odor alone can lead to foraging activation in *B. impatiens* when colony pollen stores are low.

Bumble bee colonies are annual and, as they age, have different levels of brood and thus pollen demand (Heinrich 1979). The pollen odor and pollen supplementation experiments show the same tuning of foraging activation to colony pollen stores, but in slightly different ways that may be related to the age of the colonies and natural fluctuations in the number of pollen foragers during the trials. Adding intranidal pollen odor increased the number of bees exiting the nest, but only when pollen stores were low. However, when we added one pollen load equivalent each 5 min to the pollen stores (simulating the results of successful foraging), the number of exiting bees significantly decreased only under high pollen store conditions. We expect the number of exiting bees to increase upon addition of a pollen cue (deposit or odor) when pollen stores are low. However, this can only occur if the number of foragers is below the maximum (as when we added pollen odor) or stays the same if the number of foragers is already at a maximum (as when we added pollen to the pollen pots). Conversely, if pollen stores are high, there is no need to activate foraging (no response to pollen odor, as found), or there may be a significant decrease in the number of foraging bees (as found in the short-term pollen supplementation experiment).

Honey bees and bumble bees are faced with the challenge of how much time and space to allocate to nectar and pollen resources. However, their needs differ. Bumble bees do not overwinter and therefore require only moderate stores of honey and pollen to provide sustenance during rainy days (Heinrich 1979). Honey bees tend to tightly regulate their pollen foraging activity and pollen stores around a homeostatic point (Fewell and Winston 1992). Honey bees can store an excess of 25 kg of honey in the hive, while only 1 kg of pollen is stored (Seeley 1985). Wild bumble bee colonies, specifically *Bombus vosnesenskii*, average around 6 g of stored pollen and 260 g of stored honey (Heinrich 1979). While both honey bees and bumble

bees tend to have an overabundance of honey in comparison to pollen, it is yet to be determined if nectar and pollen are more tightly regulated in honey bees than bumble bees or vice versa.

There are intriguing differences between how honey bees and bumble bees assess colony food need. Honey bee foragers rely more upon information directly received from nestmates via behavioral interactions such as eliciting food samples from nestmates (Weidenmüller and Tautz 2002) or indirectly from nestmates through the amount of time they must wait before they can unload their collected nectar to a nestmate (Seeley and Tovey 1994). Bumble bees do not exchange food with nestmates and place their collected food directly in pollen and nectar pots (Heinrich 1979). Thus, bumble bees rely upon direct inspections of storage pots containing nectar (Dornhaus and Chittka 2005) or pollen (this study) to assess colony stores. Dornhaus and Chittka (2005) hypothesize that these different approaches to information collection may relate to colony size. Direct inspections could provide more reliable information in annual bumble bee colonies that can vary in size from a single foundress to a few hundred workers compared to honey bee colonies which are perennial, reproduce by fission, and generally contain thousands of workers. Our finding that pollen odor alone can activate bumble bee foraging also supports this idea that direct interactions are not necessary to transfer foraging-related information. In bumble bee colonies, it seems that each individual generally judges food stores for herself.

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