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UNIVERSITY OF CALIFORNIA, SAN DIEGO

# Bumble bee pollen foraging activation: role of colony stores and pollen quality and odor

A Thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

Biology

by

Traci Kimiko Kitaoka

Committee in charge:

Professor James Nieh, Chair
Professor David Holway
Professor David Woodruff

2008

The Thesis of Traci Kimiko Kitaoka is approved and it is acceptable in quality and form for publication on microfilm:

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Chair

University of California, San Diego

2008

# DEDICATION

First and foremost I want to thank my loving boyfriend Morgan. Thank you for your continual support. The many Sundays I dragged you to lab to help me with experiments will never be forgotten; I am in your eternal debt.

To my parents, Jack and Jenny, thank you for always having open phone lines and sympathetic ears (and the occasional, but necessary “deal with it!”).

To my sister, Brynee, thank you for begrudgingly helping me euthanize the bees. You’re the best!

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ABSTRACT OF THE THESIS

Bumble bee pollen foraging activation: role of colony stores and pollen quality and odor

by

Traci Kimiko Kitaoka

Master of Science in Biology

University of California, San Diego, 2008

Professor James Nieh, Chair

Bumble bees, like the closely-related honey bees, can activate nestmates to forage for nectar. However, little is known about how a bumble bee colony regulates pollen collection. Using four colonies of the North American bumble bee, *Bombus impatiens*, we studied pollen foraging activation and colony-level responses to (1) changes in nest pollen stores and (2) changes in the protein quality of extranidal pollen. We provided pollen of different qualities (50%, 75%, and 100% protein diluted with indigestible alpha-cellulose by mass) in a foraging arena and manipulated pollen nest stores. We present the first demonstration that bumble bees can activate nestmates to forage for pollen alone. Colony foraging levels are tuned to the amount of stored pollen inside the colony and to

the protein quality of pollen available outside the colony. Moreover, pollen odor alone is sufficient to increase pollen foraging and pollen pot inspection. We continuously recorded intranidal (within nest) behavior with infrared-illuminated video cameras and found no effect of pollen concentration on the velocity, path length, or nest area traversed by pollen foragers. However, foragers significantly increased pollen pot inspections when pollen odor was pumped into the colony when pollen stores were low. Thus, pollen stores can act as an information center regulating colony-level foraging according to pollen protein quality and colony need.

## Introduction

The ability of bumble bee foragers to activate nestmates to forage for food sources has received renewed attention over the last decade, in part because of this primitively eusocial group's close phylogenetic relationship with the highly social honey bees. Several studies have examined nectar foraging (Dornhaus and Chittka, 1999; Dornhaus and Chittka, 2001; Dornhaus, 2002). However, regulation of pollen foraging remains poorly understood, despite the importance of this source of protein in colony growth. Both the developing larvae and queen require protein consumption (Crailsheim, 1992; Haydak, 1970; Heinrich, 1979; Sagili and Pankiw, 2007), and pollen quality can significantly influence worker lifespan in honey bees (Standifer, 1967). Thus, the goal of our study was to determine how bumble bee colonies regulate pollen foraging and whether individual foragers can activate colony pollen foraging based on pollen availability and protein quality.

Regulating pollen foraging is beneficial because it can increase pollen collecting efficiency, allowing foragers to appropriately allocate their efforts between pollen and nectar collecting (Harder, 1990; Rasheed and Harder, 1997). Honey bees are able to regulate pollen foraging based on food availability as well as colony demand (Calderone and Johnson, 2002; Camazine, 1993; Dreller et al., 1999; Dreller and Tarpy, 2000; Fewell and Winston, 1992). Proposed mechanisms for regulating foraging activity in honeybees include: olfactory assessment of pollen quality (Pernal and Currie, 2001); brood food as an inhibitory cue (Camazine, 1993); and assessment of pollen stores' phenolic content (Liu et al., 2006). Pollen foraging in honeybees is also affected by pollen quality. In choice-tests, honey bees prefer pollen that has a higher composition 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).

Relatively few studies have examined the regulation of bumble bee pollen foraging. However, bumble bees have been shown to prefer flowers with increased pollen availability; foragers visit more rewarding flowers longer, visit inflorescences within the same flower more often, and demonstrate an increased frequency of revisits (Harder, 1990). Bumble bees are also able to discern high quality, viable pollen from low quality, nonviable pollen. In field studies, bumble bees have been shown to discriminate between and prefer flowers that have pollen with viable pollen; i.e. pollen containing cytoplasm that can support bees nutritionally (Robertson et al., 1999). When foragers were allowed to choose between low and high quality pollen patches, the forgers visited the flowers that had higher quality pollen more often flowers offering low quality pollen.

Dornhaus and Chittka (2005) showed that bumble bees can assess the nectar storage contents of their nest and allocate their nectar foraging appropriately. This raises the intriguing possibility that bumble bee foragers may also regulate pollen foraging based upon nest pollen stores. Prior research suggests that bumble bees' pollen foraging reflects colony demand (Free, 1955); however, no studies have demonstrated if bumble bees can monitor pollen pots to assess a colony's pollen demand. Foragers may individually inspect pollen pots and may also be sensitive to the odor of fresh pollen brought into the colony. Bumble bees are known to use pollen odor to assess rewarding

flowers (Dobson et al., 1999). However, it is not known whether forager-collected pollen odor is the cue for bumble bees to check pollen stores and adjust foraging efforts.

We therefore tested the effect of low and high pollen stores on pollen foraging. If bumble bees can assess colony pollen needs, we hypothesized that the addition of pollen to low pollen stores would increase foraging activity. 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 then tested the effect of pollen protein quality on pollen foraging activation by using different pollen concentrations (50%, 75% and 100% pollen diluted by mass with inert, indigestible alpha-cellulose). We expected less foraging activation for pollen with a lower protein quality and expected foragers to exhibit less excitatory intranidal behavior for lower pollen quality or when the colony was full of pollen. Finally, we examined potential mechanisms for pollen stores assessment by (1) continuously video recording the pollen pots and counting the number of pollen pot inspections and (2) measuring the effect of fresh intranidal pollen odor on the foraging activation of and nestmate behavior under conditions of intranidal pollen dearth and plenty.

## Methods and Materials

*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, West to Michigan, Illinois, Kansas and Mississippi (Heinrich, 1979). We conducted four experiments with four successive *Bombus impatiens* colonies containing approximately 100 to 200 worker bees from BioBest Incorporated (Leamington, Ontario, Canada) in a temperature-controlled (22$^{o}$C) laboratory at the University of California, San Diego (N32°52.690, W117°14.464). We housed each colony in a wood nest box (45cm x 27cm x 15cm) with a transparent plastic cover. Bees foraged in a 78cm x 31cm x 33cm plastic arena connected to the nest box with a clear vinyl 4.5 cm diameter tube. We collected data from September 2006 through March 2008.

*General Methods*

We labeled all foragers with individually numbered plastic bees tags (The Bee Works, Orillia, Ontario, Canada) attached to cold-anesthetized bees with cyanoacrylate adhesive. We fed the colonies pollen and unscented 1.5M reagent-grade sucrose solution *ad libitum* in the foraging arena unless otherwise noted. To ensure there was no sugar in the pollen, we filtered honey bee collected pollen with distilled water (2.5 L per 225 grams of pollen) and tested a small sample using a sugar concentration assay. We took five samples of 1 g filtered pollen suspended in 500 µl distilled water, centrifuged this for 30 min and then tested the supernatant for residual sugars (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 #500-0006). The foraging arena was illuminated on a 12-hour cycle (0800-2000). We counted the total number of bees exiting the nest during five- min intervals for 90 min (unless otherwise noted).

*Experiment 1: Pollen foraging activation*

To test the hypothesis that a successful pollen forager can activate colony pollen foraging, we ran 24 trials (11 control and 13 experimental) with Colony 1 September-October 2006, 0800-1100. Two hours before beginning a trial, we placed all foraging bees inside the nest, closed off the entrance to the foraging arena, and cleaned the foraging arena with distilled water and ethanol. In the experimental trials, we placed 0.5 g of pollen in a dish at the end of the foraging arena, 63 cm away from the nest box. Using a double-gated passageway to control egress, we allowed one bee to enter the foraging arena. If she did not forage within three min, we captured her with an aspirator and did not place her back in the colony until the end of trial to avoid disturbing the colony. Upon her return to the nest, we removed the empty or full dish and replaced it with a new, clean dish so that subsequent bees entering the foraging arena were not exposed to pollen. We then removed both gates, allowing all bees access to the arena, and censused the number of bees entering the arena for 90 min. We alternated these experimental trials with control trials in which we placed an empty dish in the foraging arena and ran the same experiment. This measured the baseline recruitment response when there was no pollen available.

*Experiment 2: Effect of colony pollen stores*

To test the hypothesis that colony pollen stores affect pollen foraging, we ran 27 trials February- May 2007, (0800-1200) with Colony 2. We either emptied the pollen pots of their contents (thereby removing the majority of colony pollen stores) or, packed them full of pollen 24 hours before the beginning of a trial. We conducted 13 trials with high pollen stores and 14 trials with low pollen stores.

We conducted five- min censuses of the foraging response over a 30 min control phase followed by a 60 min experimental phase. During the experimental phase, we added 5 mg of pollen to the pots each five min to test the effect of fresh pollen being deposited into the pots, as it would be by foragers. No foraging stimuli (neither pollen or sugar solution) were present in the arena throughout the entire trial.

*Experiment 3: Effect of pollen odor on foraging activation*

To determine if intranidal pollen odor alone can increase foraging activity, we ran 14 trials during November 2007 (0800-1100) with Colony 3. We ran seven trials with high pollen stores and seven with low pollen stores. In the high pollen store trials, we fed the colony *ad libitum* for at least four days before the first trial was run as well *ad libitum* during the days we ran trials with high pollen stores. For low pollen store trials, we removed the pollen from the pots and provided the colony with approximately 0.5 g pollen/day. When provided with this quantity, the colony did not store the pollen. We censused the foraging response over a 30 min control phase followed by a 60 min experimental phase. During the control phase, we used an aquarium air pump to pump fresh air without pollen scent into the nest through a 1 cm diameter vinyl tube positioned

above the pollen pots (airflow of 4.4 liters/min). During the experimental phase, we pumped fresh pollen odor into the nest. 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, 0.2µm pore size NYL w/ GMF) to prevent pollen grains from entering the airflow. No foraging stimuli (neither pollen nor sugar solution) were present in the arena throughout the entire trial.

To monitor the pollen pots over a 24-hour period, we used a Lorex High Definition Digital Video Recorder (Strategic Vista Corp., Markham Ontario, Canada) recording at 10 fps. We observed the behavior of the bees in three ten- min intervals during the 90- min trial period, and seven ten- min intervals during the remaining 1350 min period. We quantified the number of inspections (the act of a foraging bee sticking her head into the pollen pot) and the maximum number of bees fanning (stationary bees that had visible wing movement) at one time.

*Experiment 4: Effect of pollen quality on colony foraging and forager intranidal behavior*

To test the hypothesis that differing pollen quality affects the foraging response and a forager's behavior inside of the nest, we ran 14 trials during January 2008 through March 2008 (0800 and 1600) with colony 4. Using alpha cellulose to decrease pollen quality, we ran trials with 100% pollen, 75% pollen (25% alpha cellulose by mass), or 50% pollen (50% alpha cellulose by mass). The colony was fed sucrose *ad libitum* in the nest box.

We allowed all bees access to the foraging arena and censused the foraging response for 15 min. We then placed a dish containing 50%, 75%, or 100% pollen into

the foraging arena and did not remove the dish during the 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 45 min. For the statistical analysis, we compared the 15 min control phase to the last 15 min of the experimental phase. We did not alter pollen store levels in this experiment; the colony was given 0.5g of 100% pollen each day. When fed this amount, the colony did not store the pollen. We counted the total number of non-depositing foragers and depositing foragers during each trial for the different pollen concentrations. We also took note of how much time each forager spent before and after depositing pollen. Likewise, we calculated the total amount of time each non-depositing forager spent inside the nest.

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 order to video record more than one foraging bee per trial, we left the pollen in the foraging arena throughout the trial. We chose 10 intranidal runs for the 50% and 100% pollen concentrations 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 the 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 to determine how clustered the movements are within the nest. 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).

*Statistical Analyses*

Our data conformed to parametric assumptions (we tested for normality and conducted residual analyses). We therefore performed ANOVA using JMP Statistical Software v5.1 (alpha=0.05). Where applicable, we first analyze the full model with interactions for the effects of pollen stores and pollen influx and then perform detailed analyses on significant factors, including post-hoc Tukey HSD tests. We tested for an effect of colony pollen stores (long term effect over 24 hours) by comparing high pollen store trials to low pollen store trials. We tested also for pollen foraging activation within each trial (short term effect) comparing the second 30 min period of the 60 min experimental phase to the 30 min control phase to take into account the baseline number of bees exiting the nest on any given day (unless otherwise noted above). We report averages as mean±SD.

**Results**

*Experiment 1: Pollen foraging activation*

The number of bees exiting the nest significantly increased after a forager encountered pollen in the foraging arena and returned to the nest (as compared to control trials in which a forager returned from visiting an empty dish, $F_{1,421}$=3.88, $p$=0.049). There was a 15.9% increase in the number of bees exiting the nest when pollen was made available to the forager ($mean_{pollen}$=10.6 $\pm$8.3 bees; $mean_{control}$=9.2 $\pm$6.7 bees).

*Experiment 2: Assessing colony pollen stores*

In the full model, pollen stores and pollen influx are significant effects on the number of bees exiting the nest (full model: $F_{2,179}$=11.5, $p$<0.0001; pollen stores: $F_{1,179}$=5.9, $p$=0.017; pollen influx: $F_{1,179}$=17.1, $p$<0.0001; interactions NS). The number of bees leaving the nest was significantly higher (14.3%) when colony pollen stores were low versus high (long term effect, $F_{1,250}$=7.48, $p$=0.0067, Fig 1b). In the short term, adding pollen to the pollen stores did not significantly affect the number of bees leaving the nest during the low pollen store trials ($F_{1,50}$=1.3, $p$=0.26, Fig 1a). However, the number of bees leaving the nest significantly decreased (23%) when pollen was added during the high pollen store trials ($F_{1,128}$=17.7, $p$<0.0001, Fig 1a).

*Experiment 3: Effect of pollen odor on foraging activation*

In the full model, pollen stores and pollen odor are significant effects on the number of bees exiting the nest (full model: $F_{2,164}$=17.0, $p$<0.0001; pollen stores: $F_{1,164}$=24.0, $p$<0.0001; pollen odor: $F_{1,164}$=9.8, $p$=0.002; interactions NS). The number of

bees leaving the nest was significantly higher (40.6%) when colony pollen stores were low versus high (long term effect, $F_{1,165}$=22.9, $p$<0.0001, Fig. 1d). In the short term, adding intranidal pollen odor significantly increases the number of bees exiting the nest when pollen stores are low ($F_{1,82}$=7.6, $p$=0.0073). On average, the number of bees exiting the nest during experimental phases increased by 27.3% as compared to control phases (Fig. 1c). There was no significant effect of added pollen odor on the number of bees exiting the nest when pollen stores were high ($F_{1, 81}$=2.6, $p$=0.11, Fig. 1c).

*Intranidal behavior*. In the full model, pollen stores and pollen odor are significant effects on the number of intranidal fanning bees and pollen pot inspections (full model: $F_{3,115}$=9.3, $p$<0.0001; pollen stores: $F_{1,115}$=10.9, $p$=0.0013; pollen odor: $F_{1,115}$=7.9, $p$=0.0057; interactions NS).

The number of inspections was significantly higher (49.8%) when colony pollen stores were low versus high (long term effect, $F_{1,117}$=5.96, $p$=0.016, $\text{mean}_{\text{low}}=3.5\pm2.8$, $\text{mean}_{\text{high}}=2.3\pm2.3$, Fig. 2b). In the short term, when pollen odor was pumped into the nest, there were significantly more (194.6%) pollen pot inspections under empty conditions as compared to full conditions ($F_{1,22}$=5.9, $p$=0.023, Fig 2a). When no pollen odor was pumped in, there was no significant effect of pollen stores on the number of pollen inspections ($F_{1,93}$=1.7, $p$=0.19, Fig 2a).

The maximum number of bees fanning was significantly higher (133%) when the colony pollen stores were high versus low (long term effect, $F_{1,117}$=19.0, $p$<0.0001, $\text{mean}_{\text{low}}=1.1\pm1.1$, $\text{mean}_{\text{high}}=2.6\pm2.5$, Fig. 2e). In the short term, when no pollen odor was pumped in, there was significantly more fanning (120%) when nest pollen levels were

high versus low ($F_{1,93}$=15.2, $p$=0.0002, Fig. 2d). Similarly, when pollen odor is pumped in, the maximum number of bees fanning was significantly greater (76%) when pollen pots are full ($F_{1,22}$=10.7, $p$=0.0035, Fig. 2d).

Comparing the different time intervals across 24 hours, there is no significant difference in the number of pot inspections or maximum number of bees fanning when pollen stores were low (*Tukey HSD*, $\alpha$=0.050, $Q$=3.29). Equally, there is no significant difference in the number of pot inspections when pollen stores were high (*Tukey HSD*, $\alpha$=0.050, $Q$=3.35). However, under high pollen store conditions, fanning at 13 and 16 hrs (after pollen odor presentation) is significantly greater than fanning at 3 and 10 hrs (Fig. 2).

*Experiment 4: Pollen quality and foraging activation*

In the full model, pollen concentration and pollen availability are significant effects on the number of bees exiting the nest (full model: $F_{3,110}$=7.0, $p$=0.0002; pollen concentration: $F_{2,110}$=6.9, $p$=0.0015; pollen availability: $F_{1,110}$=7.2, $p$=0.0086; interactions NS).

*Foraging arena.* There is no significant difference in the number of bees leaving the nest when 50% pollen is available versus control phases in which no pollen was available ($F_{1,40}$=.078, $p$=0.78, Fig 3a). The number of bees leaving the nest is significantly higher (26.6%) when 75% pollen is available versus control phases ($F_{1,40}$=6.6, $p$=0.014, Fig 3a). The number of bees leaving the nest is significantly higher (30.7%) when 100% pollen is available versus control phases ($F_{1,40}$=7.8, $p$=0.0095, Fig 3a).

The number of bees leaving the nest is significantly higher when 100% pollen is available versus 50% pollen (31.2%) but not significantly different between 50% and 75% pollen availability or 75% and 100% pollen availability (*Tukey HSD*, $\alpha$= 0.050, $Q$=2.38, Fig 3b).

*Intranidal behavior.* Foragers that had collected pollen in their corbiculae either deposited this pollen into pollen pots or moved throughout the nest without depositing this pollen. The quality of the collected pollen positively influences the probability of deposition ($F_{2,13}$=5.1, $p$=0.023). The number of depositing foragers is significantly higher (233.0%) in 100% pollen trials versus 50% pollen trials; there is no significant difference between 75% pollen trials and 100% or 50% pollen trials (*Tukey HSD, $\alpha$=0.05, $Q$=2.64*, Fig. 3c).

In the full model, whether a forager deposits pollen is the only significant effect on the time a forager spends inside the nest (full model: $F_{5,61}$=1.6, $p$=0.16; pollen concentration: $F_{2,61}$=0.45, $p$=0.64; pollen deposit: $F_{1,61}$=6.4, $p$=0.014; interactions NS). Foragers that deposited their pollen loads into pollen pots spent significantly more time in the nest than foragers that did not deposit the pollen they had on their corbiculae ($F_{1,65}$=7.0, $p$=0.01, $\text{mean}_{\text{deposit}}$= 190.4$\pm$114.5s, $\text{mean}_{\text{nodeposit}}$= 114.7$\pm$120.0s, Fig. 4). Pollen concentration has no significant effect on the total time a depositing forager spends inside the nest ($F_{2,33}$=0.015, $p$= 0.98, Fig. 4). There is also no significant effect of pollen concentration on the time spent before ($F_{2,33}$=0.35, $p$= 0.70) or after ($F_{2,33}$=0.50, $p$= 0.61) depositing the collected pollen (Fig. 4). Pollen concentration has no significant effect on the total time a non-depositing forager spends inside the nest ($F_{2,28}$=0.69, $p$= 0.51, Fig. 4).

From the video analysis, pollen concentration did not significantly affect focal forager total path length, average velocity, total time in nest, area covered, or clustering ($F_{1,18} \leq 2.2$, $p \geq 0.15$, Table 1, Fig. 5).

**Discussion**

Our results show that bumble bees, *Bombus impatiens,* can activate colony foraging for pollen alone, tuning the degree of activation to the protein quality of the pollen and to the level of colony pollen stores. The number of bees entering the foraging arena increased by 50% for 100% pollen as compared to 75% pollen. For 100% pollen, the number of bees entering the foraging arena increased by 30.7% when colony pollen stores were low as compared to when they were high. Thus colony pollen stores, the pollen pots, can serve as an information center. Bees may perceive colony pollen stores through direct inspections and through the diffusion of pollen odor inside the colony. Under conditions of colony pollen dearth, the odor of pollen inside the nest stimulated bees to inspect pollen pots and also to fan, perhaps a mechanism for dispersing the pollen odor.

We examined both the long term effect (>24 hrs) of changing colony pollen stores and the short term effect (90 min) of pollen being added to pollen pots during a foraging period. When the colony is exposed for at least 24 hours (long term) to a given level of pollen stores, the number of bees leaving the nest and the number of bees inspecting the pots is significantly higher when colony pollen stores are low versus high. In contrast, the maximum number of bees fanning at one time was significantly higher when colony pollen stores were high versus low. In the short term, the immediate presence of pollen being brought inside the nest significantly increases the number of bees exiting the nest. The number of bees exiting is correlated with pollen quality such that higher quality pollen results in a greater number of bees exiting the nest. The addition of pollen to the colony pollen stores or the addition of intranidal pollen scent significantly increases the

number of bees exiting the nest. The addition of intranidal pollen scent also significantly increases the number of pollen pot inspections.

*Effect of colony pollen levels*

In the short term (over a 90 min trial), adding pollen to pollen pots when the colony had a dearth of pollen stores did not significantly affect the foraging activity. This may occur because the maximum number of pollen foragers are already foraging when colony pollen stores are low (Fig. 1a). According to Free (1955), the number of bumble bee nectar foragers will significantly increase when nectar stores are low because some pollen foragers will switch to nectar foragers; however, nectar foragers do not switch to pollen foragers. Because we fed the colonies sucrose solution *ad libitum*, it is possible the maximum number of pollen foragers were already foraging; therefore, there would not be a significant increase in foraging activity.

In the short term, we found that the number of bees exiting the nest significantly *decreases* upon addition of pollen to the pollen pots when the pollen stores are high. This procedure simulates the natural situation of pollen accumulating as foragers collect it. Under such conditions, one would expect foraging activation for pollen to decrease because the colony already has a high level of pollen stored and because it also accumulating pollen. Foragers may then switch to other tasks such as nectar foraging. In Dornhaus and Chittka's (2005) study, under low nectar stores, addition of sugar solution significantly increased the number of foragers but had no effect when nectar stores were high.

We found a long term effect of pollen stores on a colony's foraging activation. The average number of bees exiting the nest was higher when pollen stores were low. It appears that when pollen stores are low, the allocation of bees foraging for pollen sources is maximized, or at least above the level witnessed when pollen stores are high. For example, *B. flavifrons*, *B. melanopygus*, and *B. occidentalis* foragers switched from pollen foraging to nectar foraging when colony pollen stores were supplemented and thus high (Cartar, 1992).

*Pollen Quality and Foraging Activation*

We hypothesized that bumble bees would be able to activate colony foraging according to the availability and quality of pollen. *Bombus impatiens* foragers that encountered pollen in the foraging arena were able to increase in foraging activity upon their return to the nest. In some cases, these foragers made contact with the pollen in the foraging arena, but did not collect this pollen. Instead, they returned to the nest and activated foraging. Foraging activity significantly increased by 30.7% and 26.0% for 100% or 75% pollen, respectively, as compared to control phases during the same trial. Foraging activity was significantly higher for 100% than for 50% pollen. There was no difference in foraging activity between control and experimental phases during the same trial for 50% pollen, perhaps because 50% pollen was judged too poor. Thus, *B. impatiens* foragers evidently have the ability to assess and respond to pollen quality, increasing foraging activity for higher quality food just as reported for nectar foraging by *B. terrestris* (Dornhaus and Chittka, 2005).

*Intranidal Behavior*

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 40mm/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.

Although colony-level foraging activation is significantly different for 100% vs. 50% pollen, these two pollen concentrations did not significantly affect focal foraging behavior inside the nest. Pollen concentration has 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 (Fig. 4). 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 sufficient to account for the observed changes in foraging activation.

*Effect of pollen odor on foraging activation*

Adding intranidal pollen odor increased the number of bees exiting the nest, but only when pollen stores were low. However, when we added pollen each 5 min to the pollen stores (short term pollen supplementation), the number of exiting bees significantly decreased only under high pollen store conditions. A key difference between

these experiments is that the pollen odor experiment was conducted with no other sources of foraging activation, whereas the short term pollen supplementation experiment occurred while foragers were already activated to forage by the return of a pollen forager. We expect that under low pollen stores the number of exiting bees will increase if the number of foragers is below the maximum (as occurred when we added pollen odor) or stay the same if the number of foragers is already at a maximum (as occurred 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 significant decrease in the number of foraging bees (as found in the short-term pollen supplementation experiment).

Interestingly, the number of pot inspections increased immediately when pollen scent was pumped in (under low pollen store conditions). Under natural conditions, pollen odor in the nest would result from foragers bringing in pollen. Thus, pollen pot inspections would allow nestmates to evaluate the quantity of newly harvested pollen and overall pollen store levels. Such inspections and concomitant increases in pollen foraging would likely be more important under conditions of colony pollen dearth than when the colony is full of pollen.

We pumped a constant amount of air into the nest at all times to acclimate bees to the air flow and exclude the possibility of air flow acting as a disturbance to increase the number of bees exiting the nest during the trials. Pollen odor had no effect on worker fanning behavior inside the nest (Fig. 2f), and the bees rapidly acclimated to the small amount of air constantly being pumped into the nest. However, under full pollen conditions, the amount of fanning significantly increased after pollen odor pumping

ceased (Fig. 2f). The reason for this effect is unclear. Under full pollen conditions, the colony does not need more pollen. Thus, 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 (no need to collect more pollen). The elevated fanning stopped at 16 hrs, after which the colony normally did not continue foraging. Recently Diaz et al. (Diaz et al., 2007) found that honey bee dance followers contacted the legs of waggle dancing pollen foragers (which carry pollen) more often than legs of dancing nectar foragers. Thus, pollen odor may play a role in foraging activation in honey bees and bumble bees.

Multiple cues of pollen quantity and quality may therefore play a role in bumble bee pollen foraging activation. Most importantly, the bees' responsiveness to those cues is based on the level of pollen stores. Thus, the information center in the nest is a pivotal tool used for delegating foraging efforts.

## Figures

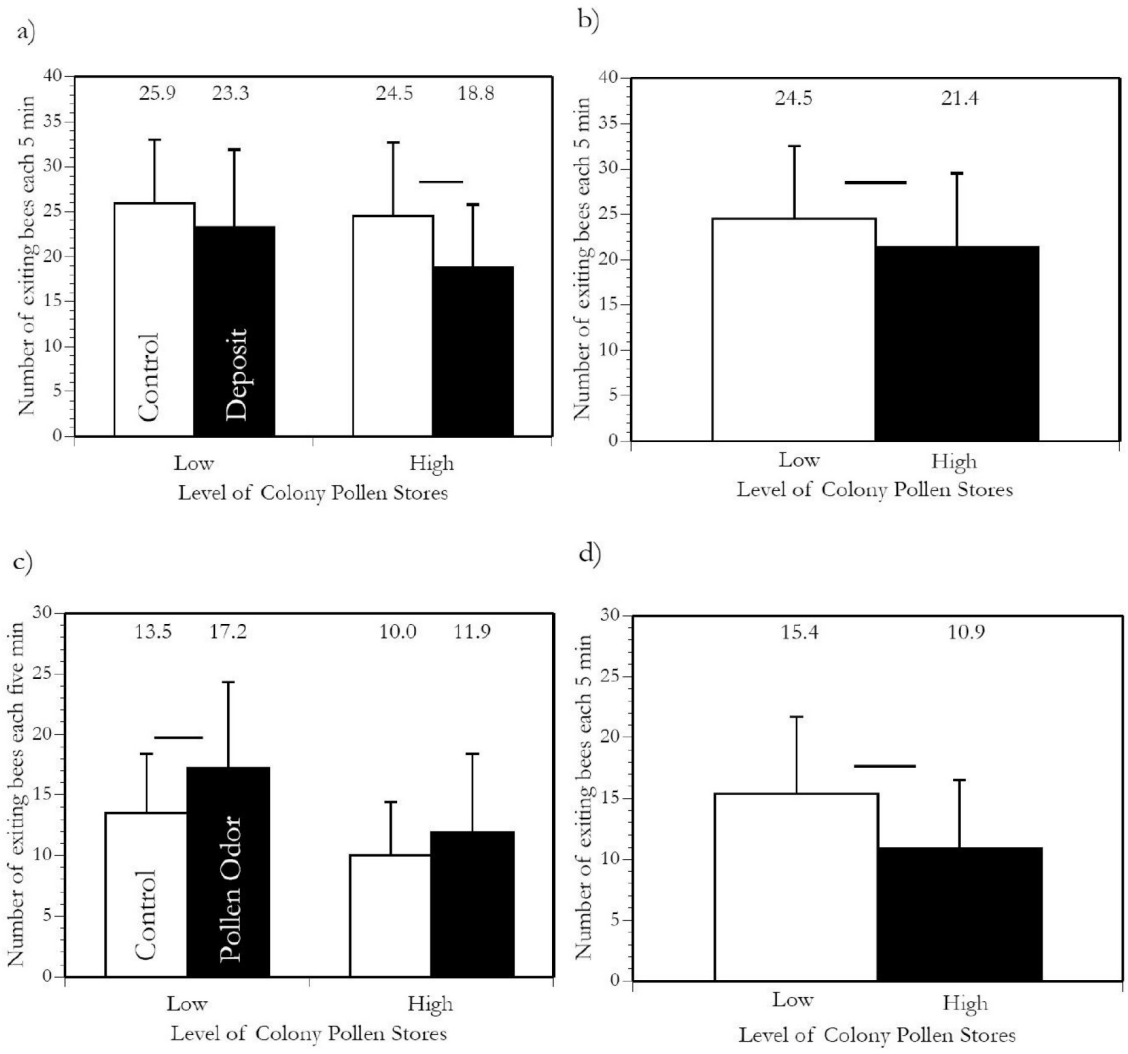



**Fig. 1:** Results of the pollen stores assessment experiment (parts a and b) and the foraging activation through pollen odor experiment (parts c and d). Standard deviation bars are shown. A horizontal line denotes a significant difference ($p<0.05$). a) Open bars show data from control phases (no pollen added to pots) and filled bars show data from experimental phases (pollen added to pots) from 10 high pollen store trials and 4 low pollen store trials. b) Open bars show data from low pollen store trials and filled bars show data from high pollen store trials. c) Open bars show data from control phases (control air) and filled bars show data from experimental phases (pollen odor) from seven high pollen store trials and seven low pollen store trials. d) Open bars show data from low pollen store trials and filled bars show data from high pollen store trials.

**Fig. 2:** Results of the video analysis of the foraging activation through pollen odor experiment. Standard deviation bars are shown. A horizontal line denotes a significant difference ($p<0.05$). a and b) Open bars show data from control phases (control air) and filled bar show data from experimental phases (pollen odor) for seven high pollen store trials and seven low pollen store trials. d and e) Open bars show data from low pollen store trials and filled bars show data from high pollen store trials. c and f) Ethogram of intranidal behavior. Filled circles show data from high pollen store trials, open circles show data from low pollen store trials.

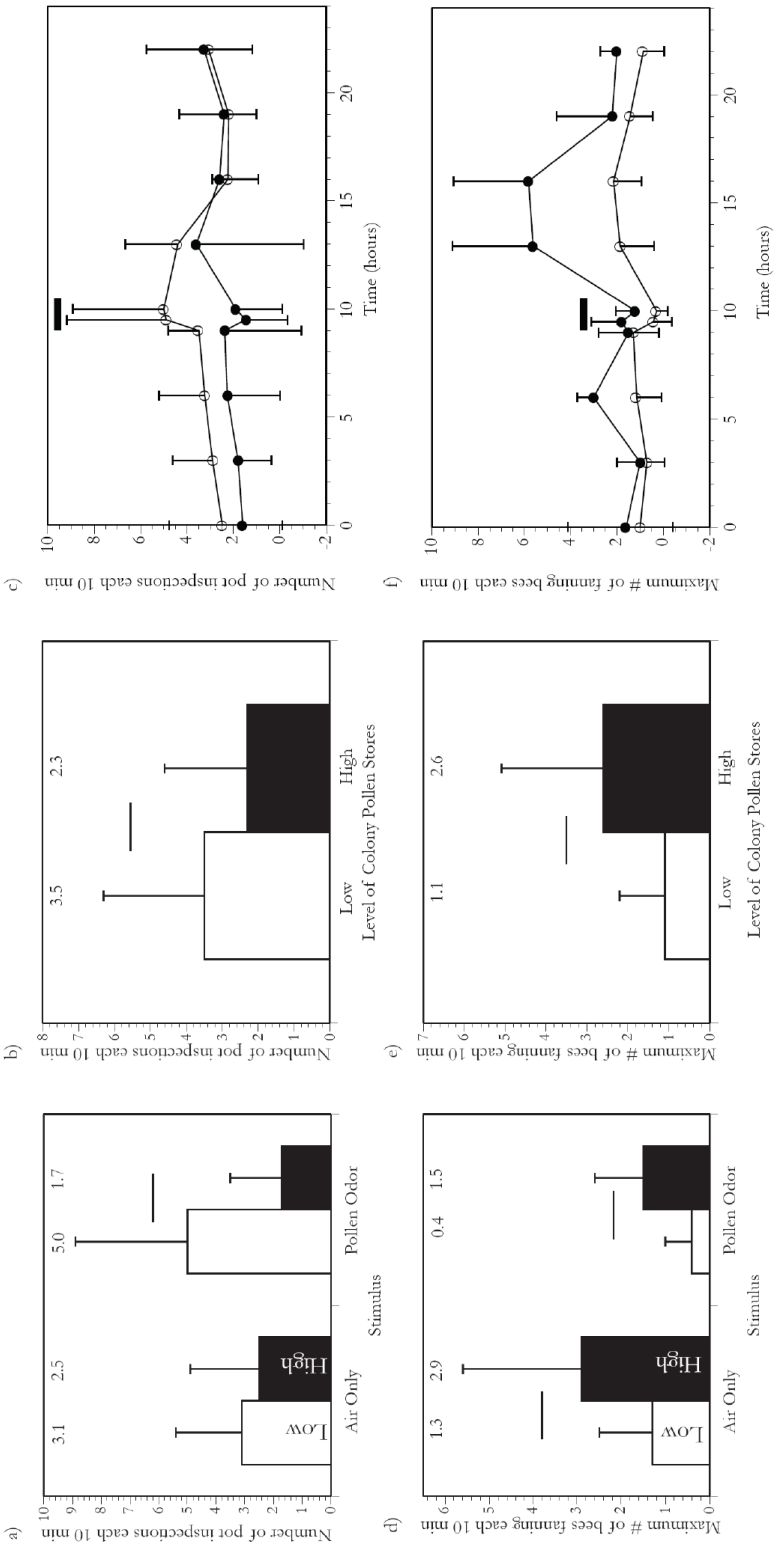
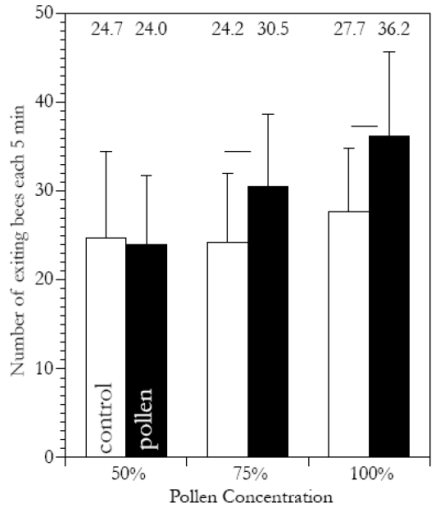
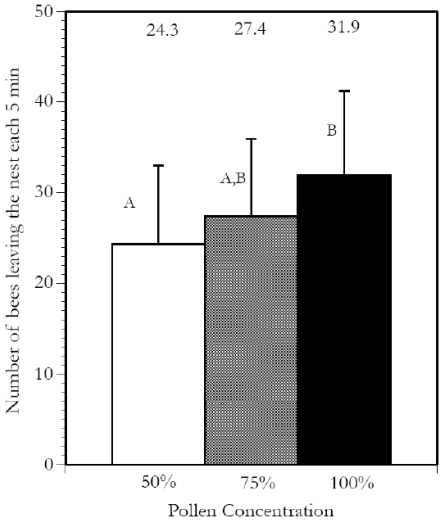

a)
Number of por inspections each 10 min
3.1
2.5
5.0
1.7
Low
High
Air Only
Pollen Odor
Stimulus
b)
Number of por inspections each 10 min
3.5
2.3
Low
High
Level of Colony Pollen Stores
c)
Number of por inspections each 10 min
Time (hours)
d)
Maximum # of bees fanning each 10 min
1.3
2.9
0.4
1.5
Low
High
Air Only
Pollen Odor
Stimulus
e)
Maximum # of bees fanning each 10 min
1.1
2.6
Low
High
Level of Colony Pollen Stores
f)
Maximum # of fanning bees each 10 min
Time (hours)

**Fig. 3:** Results of pollen quality experiment. Standard deviation bars are shown. Open bars show data from control phases (no pollen available) and filled bars show data from experimental phases (pollen available) from seven 50% pollen trials, seven 75% pollen trials, and five 100% pollen trials. In part a, the horizontal line above two bars denotes a significant difference between the two bars. In parts b and c, letters denote significantly different bars.

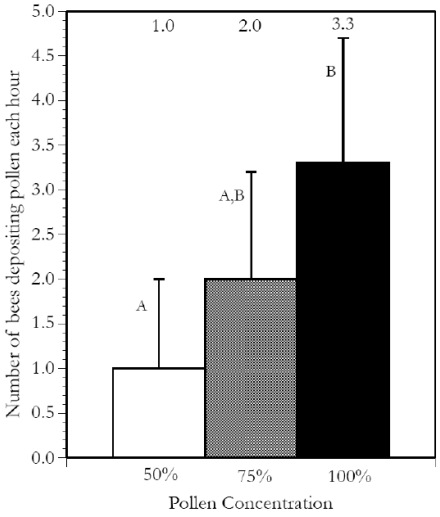
a)


24.7 24.0
24.2 30.5
27.7 36.2
Number of exiting bees each 5 min
control
pollen
50%
75%
100%
Pollen Concentration

b)


24.3
27.4
31.9
A
A,B
B
Number of bees leaving the nest each 5 min
50%
75%
100%
Pollen Concentration

c)


1.0
2.0
3.3
A
A,B
B
Number of bees depositing pollen each hour
50%
75%
100%
Pollen Concentration

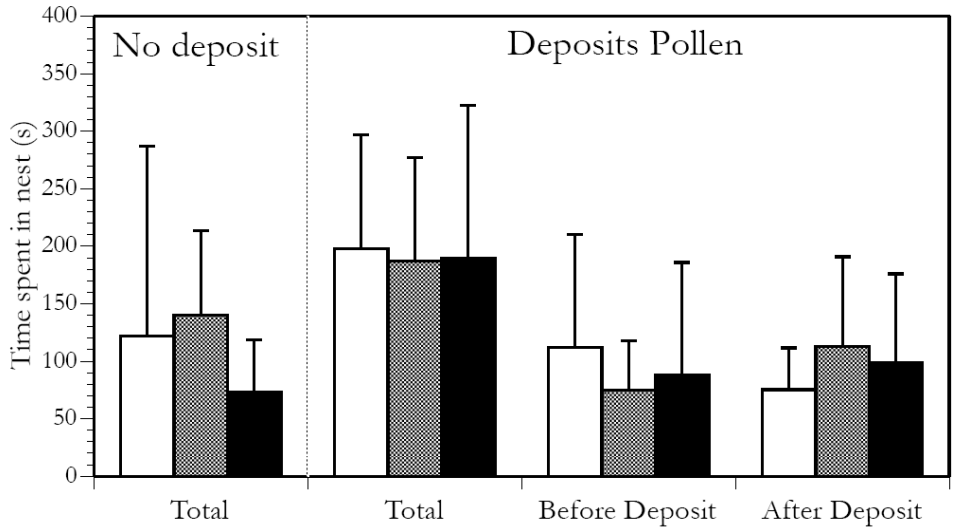



**Fig 4:** Results of the video analysis of the pollen quality experiment. Open bars show data from 20 intranidal runs during 50% pollen trials (6 deposit, 14 no deposit), hatched bars show data from 19 intranidal during 75% pollen trials (10 deposit, 9 no deposit), and filled bars show data from 28 intranidal runs during 100% pollen trials (20 deposit, 8 no deposit). Standard deviation bars are shown. The total time a returning forager stays in the nest is significantly higher when the forager deposits ($F_{1,65}$=7.0; $p$=0.010).

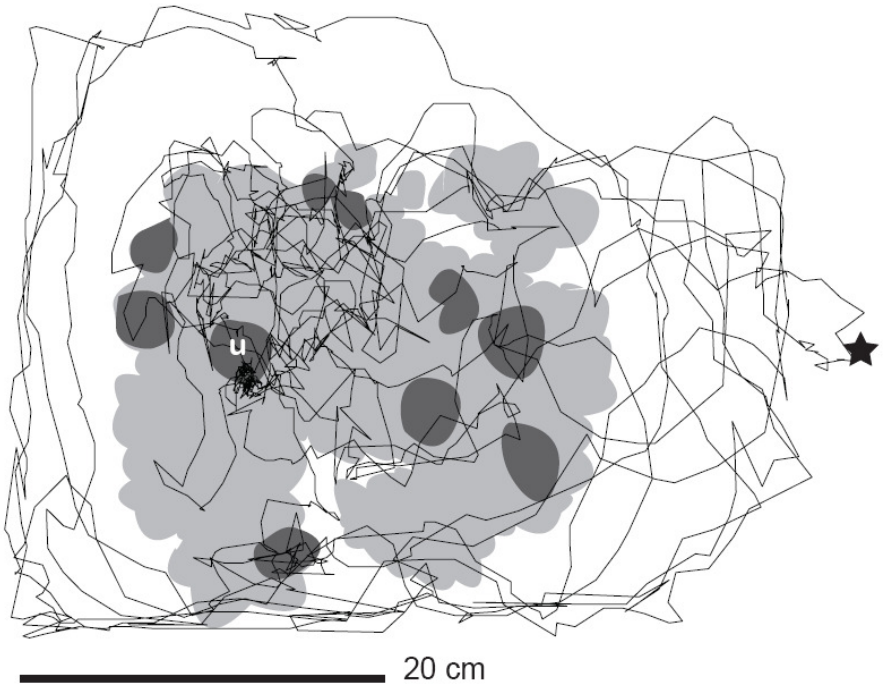



**Fig. 5:** Typical example of the path taken by a focal foraging inside the nest after returning from collecting 100% pollen. The light grey area shows the extent of the nest (colony 4) and the dark grey areas show the location of pollen pots (full in this example). The star denotes the entrance to the colony, and the "u" shows where she unloaded her pollen.

**Table 1.** Intranidal behavior of focal foragers collecting different pollen concentrations. 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. Coverage per cm moved 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.

| *Pollen concentration (by mass)* | *Total path length (cm)* | *Average velocity (cm/s)* | *Total time in nest (s)* | *Area covered (# of 1 $cm^2$ grids)* | *Clustering* | **N** |
|---|---|---|---|---|---|---|
| 50% | 181.6±100.7 | 2.9±1.4 | 103.4±27.0 | 104.5±61.4 | 59.4±13.0 | 10 |
| 100% | 270.7±260.0 | 2.1±0.8 | 139.9±27.0 | 126.3±85.4 | 53.1±12.0 | 10 |

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