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Division of foraging labor in the bumble bee, *Bombus impatiens*: Effect of removing
pollen specialists and colony adaptation.

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

in

Biology

by

Jessica Hagbery

Committee in charge:

Professor James Nieh, Chair
Professor David Holway
Professor David Woodruff

2011

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The Thesis of Jessica Hagbery is approved and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2011

DEDICATION

To my sister, Danielle Hagbery, for her love and support; I wish she were here to celebrate this accomplishment with me.

EPIGRAPH

“We are like dwarfs standing upon the shoulders of giants, and so able to see more and see farther than the ancients.”

- Bernard of Chartres, circa 1130

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ABSTRACT OF THESIS

Division of foraging labor in the bumble bee, *Bombus impatiens*: Effect of removing pollen specialists and colony adaptation.

by

Jessica Hagbery

Master of Science in Biology

University of California, San Diego, 2011

Professor James Nieh, Chair

Foraging specialization plays an important role in the ability of social Hymenoptera to efficiently allocate labor and adapt to environmental changes. However, relatively little is known about whether bumble bees, important social pollinators, can flexibly allocate their foraging. I removed pollen specialists at

different stages in the life of a *Bombus impatiens* colony and recorded the pollen and nectar foraging of every forager on each foraging trip over the lifetimes of five established colonies. Adult bumble bee foragers were defined as pollen specialists ($\geq 90\%$ of all foraging visits on pollen), nectar specialists ($\geq 90\%$ of all foraging visits on nectar) or generalists (all other foragers). The removal of pollen specialists at early and late phases in colony life led to increased pollen foraging (36% and 14% increase, respectively) by generalist foragers. After pollen specialists were reintroduced, generalists decreased pollen foraging to prior levels. A uniform, proportional extraction of all forager types had no effect on the foraging of generalists remaining in the colony. Thus, the specific removal of pollen specialists caused the foraging compensation by generalists. This shows the importance of pollen specialists and the colony's ability to reallocate their foraging labor in response to pollen foraging labor shortages.

I.

Introduction

Foraging specialization can increase the efficiency of resource collection (Rissing, 1981, Cartar, 1992, O'Donnell *et al.*, 2000, Hofstede and Sommeijer, 2006), because specialists are better at their tasks than generalists. In the bumble bee, *B. bifarius nearcticus*, foraging specialists individually contribute more resources and at higher rates than foraging generalists (Cartar, 1992, O'Donnell *et al.*, 2000). For example, individuals who switch from their specialty due to colony need are less efficient at their new task. This has been shown in stingless bees, *Plebeia tobagoensis* (Hofstede and Sommeijer, 2006) and bumble bees, *B. flavifrons*, *B. melanopygus*, and *B. occidentalis* (Cartar, 1992). Specialization definitions vary, but all generally include the concept that a specialist spends a majority of its time and effort on a specialized task. Approximately 40% of honey bee foragers (Ribbands, 1952) and 30-40% of bumble bee, *Bombus bifarius nearcticus*, foragers (O'Donnell *et al.*, 2000) specialized on collecting either pollen or nectar. In stingless bees, approximately 50% of *Melipona beecheii* (Biesmeijer and Tóth, 1998) foragers and 70% of *M. favosa* (Sommeijer *et al.*, 1983) foragers specialized in the collection of one commodity (nectar, pollen, mud, resin or water) during their foraging careers.

Nonetheless, it is unclear if foraging specialization always increases task efficiency. For ants, specialization on plant type may increase efficiency (Rissing, 1981), however task specialists (foragers, nest workers or nest builders) were not more efficient (Dornhaus, 2008). Solitary bee specialist species, which only forage for pollen from one plant species, were more efficient than generalist species, which forage for pollen on multiple plant species (Strickler, 1979). Within stingless bees,

foraging specialists contributed more than generalists for *Plebeia tobagoensis* (Hofstede and Sommeijer, 2006) but not for *M. beechii* (Biesmeijer and Tóth, 1998). Since foraging specialization does not improve foraging efficiency for some species, there may be alternative reasons why foraging specialization exists.

Behavioral flexibility allows a colony to rapidly adapt to changing environmental conditions. Such flexibility has several potential proximate causes, including individuals responding to changes in colony food stores (bumble bees and honey bees, Plowright *et al.*, 1993, Schulz *et al.*, 1998) differences in patterns of gene activation (honey bees, Robinson and Page, 1989) or differences in individual sucrose response thresholds (honey bees, Page *et al.*, 1998).

Behavioral plasticity of foraging and division of labor has been demonstrated in Hymenoptera using two types of manipulation experiments: (1) food store manipulations and nest disturbances and (2) the removal of some individuals. For example, changing food availability or creating a nest disturbance caused ants (*Temnothorax albipennis*) to switch tasks (Gordon, 1989, Dornhaus, 2008). In some ants (*Pheidole*), the worker caste known as “majors” could switch to perform the tasks of “minors” when the ratio between the two fell below 1:1 (Wilson, 1984). In stingless bees (*Plebeia tobagoensis*), 50% of specialists switched to foraging for the opposite food commodity (nectar or pollen) when the food commodity they specialized on was taken away. The remaining 50% stopped foraging altogether (Hofstede and Sommeijer, 2006).

In honey bees, an induced food shortage caused an acceleration of behavioral development: workers became foragers at a younger age (Schulz *et al.*, 1998). In single-cohort colonies, honey bees that switch from nursing to foraging or foraging to nursing will change their pattern of gene expression accordingly (Whitfield *et al.*, 2003). These behavioral changes are not centrally controlled but occur in a self-organizing manner resulting from individual task-switching decisions (Johnson, 2009). In superorganisms such as honey bees, which have, large colony sizes, inactive workers can serve as “backup” if the colony needs change (Seeley, 1995). However, bees with smaller colony sizes (such as most bumble bees and some species of stingless bees) do not have a large number of inactive workers in reserve. In this case, worker flexibility is quite important (Hofstede and Sommeijer, 2006).

Division of labor in bumble bees, unlike honeybees, does not appear to be age based. In bumble bees, juvenile hormone evidently has no effect on worker foraging or nest activities (Cameron and Robinson, 1990). Genetic work in bumble bees has shown various results regarding the foraging gene, which encodes a cGMP-dependent protein kinase. *B. terrestris* exhibits higher foraging gene expression in foragers compared to nest bees (Tobback *et al.*, 2011), while *B. ignitus* exhibits lower foraging gene expression in foragers compared to nest bees (Kodaira *et al.*, 2009). In several species (*B. flavifrons*, *B. melanopygus*, *B. mixtus*, *B. occidentalis*, *B. sitkensis*, and *B. terricola*) foraging will increase to compensate for lost food stores, when colony nectar or pollen is removed (Cartar and Dill, 1990, Cartar, 1992, Plowright *et al.*, 1993). Following the addition of pollen, nectar, or both to colony

stores, fewer foragers left the nest to collect the added food type (Free, 1955, Pelletier and McNeil, 2004, Kitaoka and Nieh, 2009). In addition, bumble bee workers can switch tasks when a large portion of the colony is removed. Nest bees, *B. agrorum* and *B. pratorum*, became foragers when all foragers were removed (Free, 1955), and when half of the workers of a *B. terricola* colony was removed, nest activities, such as larval feeding, was adopted by the remaining bees (Plowright *et al.*, 1993). It is unclear how bumble bee colonies respond to less drastic changes in the workforce.

Body size may play a role in task specialization. Within a bumble bee (*Bombus spp.*) colony there is approximately a 10-fold variation in mass within the working caste of a single nest (Alford, 1975). Foragers tend to be larger than nest bees (Brian, 1952, Free, 1955, Goulson *et al.*, 2002, Spaethe and Weidenmüller, 2002). Among *B. terrestris* foragers, nectar foragers were the largest, followed by generalists (foraged for both nectar and pollen), and the smallest were pollen foragers. This part of the study looked at one foraging day in the colony life rather than an individual's complete foraging history (Goulson *et al.*, 2002). Large bumble bee foragers have higher nectar foraging rates than smaller foragers, but pollen foraging rates were not related to forager size (Goulson *et al.*, 2002, Spaethe and Weidenmüller, 2002). I therefore wanted to see if these trends still held for *B. impatiens* when the complete foraging history of individuals was observed.

In our study, I examined if and how bumble bees (*Bombus impatiens*) can flexibly reallocate labor when pollen specialists are extracted and later

reintroduced. Pollen foraging specialization has been demonstrated in bumble bees, *B. bifarius nearcticus*, *B. terrestris*, as well as stingless bees (Strickler, 1979, O'Donnell *et al.*, 2000, Hofstede and Sommeijer, 2006, Raine and Chittka, 2007) and plays an important role in colony life. Both adults and larvae require pollen as a protein source (Smeets and Duchateau, 2003). All previous studies of bumble bee foraging specialization have been performed on free-foraging colonies. In these studies, the type of food (nectar or pollen) that foragers brought back to the hive was accounted for but the individual foraging decisions a bumble bee made at each flower was not observed. In addition, even the most complete studies done so far, due to time constraints from observing a wild foraging colony, could not obtain each individual's complete foraging history (O'Donnell *et al.*, 2000, Spaethe and Weidenmüller, 2002). To address these issues, I brought the experiment into the lab and monitored all foraging for either pollen or nectar in an enclosed foraging arena. Such laboratory studies have proven to be powerful tools for dissecting and understanding colony foraging behavior (Dornhaus and Chittka, 2004, Kitaoka and Nieh, 2009).

I tested four hypotheses. (H1) The extraction and reintroduction of pollen specialists will cause a change in the foraging behavior of bees remaining in the colony. (H2) A uniform, proportional extraction and reintroduction of all foraging types will not affect the bees remaining in the colony. (H3) Without manipulation foragers will stay consistent in their pollen foraging proportions throughout their lives. (H4) Nectar and pollen foragers will differ in size.

II.

Materials and Methods

Colonies and Study Site

Experiments were conducted at the University of California San Diego in La Jolla, California, USA (32°52.690'N and 117°14.464'W), from April 2009 through January 2011. I sequentially used five lab-raised colonies (size class B) of *Bombus impatiens* obtained from Biobest Biological Systems (Ontario, Canada) in a temperature-controlled room (~21°C) and exposed to a 12-h light cycle illuminated with three 20W halogen bulbs positioned around the foraging arena. I housed the bumble bees in a wooden nest box (32.5x28.4x15 cm) with an opaque lid to keep the nest dark. The nest box was attached to a foraging arena by a plastic tube (30 cm long, 3.5 cm diameter) where they collected food. The arena consisted of a clear plastic box (32x54x27 cm) with a clear plastic lid and two mesh panels on the side to allow ventilation. I weighed each bee, measured its inter-tegular distance (the distance between the wings' attachment on the thorax), and tagged it with a unique color and number (Queen Marking Kit, The Bee Works, Orillia, Ontario, Canada). Daily I marked and measured newly emerged, callow workers (identified by their silvery appearance and unhardened wings).

Pollen Specialist Identification and Extraction

Each colony was fed 1.5 M unscented sucrose solution and ground pollen, collected by honey bees (fresh frozen and then thawed by grinding just prior to feeding) *ad libitum* for one hour (11:00am-12:00pm) per day. To record the behavior and identity of all bees, I used multiple video cameras connected to a Q-See

digital video surveillance system recording onto a hard drive. The field of view for each camera was 6.3 mm by 4.6 mm, allowing the tags to be clearly identified. Video cameras above the food dishes recorded the choice of each forager for the entire time I had the colony. Three video cameras were placed over three feeding dishes (diameter 3.5mm), one camera per dish, in the foraging arena. There were two sucrose dishes and one pollen dish. The camera's field of view limited dish size, and thus I used two sucrose dishes because sucrose foraging rates were much higher than pollen foraging rates. One water dish, which was not video-recorded, was also placed in the flight arena.

Since all foragers were uniquely marked, I were able to determine the foraging decision (pollen or nectar collection) of each forager on every foraging trip over its entire lifetime (for bees born after the colony was received) or for the entire time each forager was exposed to our experimental setup (for bees born before the colony was received). A nectar foraging visit consisted of a bee climbing onto the side of the sucrose dish and drinking from it with its proboscis. A pollen foraging visit consisted of a bee climbing into the pollen dish or positioning itself on the edge of the dish and ingesting the pollen. A foraging visit was finished when the bee removed itself from the dish completely. All video analysts underwent extensive training, and their first analyses were thoroughly verified to ensure uniformity. To ensure accuracy, at the end of each colony I reviewed all foraging visits that looked irregular (data showing sporadic foraging or bees foraging after death). Of approximately 220,000 recorded foraging visits roughly 2-3% of the data collected

were reviewed to ensure accurate identification, and data was corrected accordingly. Analysts viewed videos using Windows Media Player. Each video camera was viewed individually, and 1,572 h of video were analyzed. All bees, including those that arrived with the colony, were included in the overall analysis.

A forager was placed into one of three categories based on its foraging: pollen specialist ($\geq 90\%$ visits for pollen), nectar specialist ($\geq 90\%$ visits for 1.5 M sucrose) or generalist (all other bees). O'Donnell et al. (2000) observed *B. bifarius nearcticus* foragers over their lifetimes and defined a specialist as an individual which performed a given task at a higher probability ($P < 0.05$) than the overall colony-wide performance probability. Spaethe and Weidenmuller (2002) calculated a nectar foraging proportion to determine daily foraging proportions in *B. terrestris*. I decided to establish a stringent cutoff based simply upon the foraging ratio. Thus, I calculated the proportion of visits that forager made to pollen ($P/TL = \# \text{ pollen foraging visits} / \text{total} \# \text{ foraging visits}$). The method used by O'Donnell et al. (2000) did not work well with experimental set-up. Applying this method to find the expected pollen foraging proportions of our colonies and defining specialists as those who foraged for either nectar or pollen with significantly higher probability ($P < 0.05$) than expected would have led us to define a pollen specialist as a bee that spent only $\geq 40\%$ of its time foraging at pollen. A cutoff of at least $\geq 50\%$ pollen foraging specialization agrees better with the general understanding of the term specialist.

I decided instead to use a natural break in the distribution of P/TL ratios in our initial control colony to define pollen specialists. To determine the effect of both early and late extraction in colony life (and allow enough time to evaluate accurately the foraging behavior of all foragers), I observed the P/TL distribution of Control A after two and five weeks (Fig. 1). The natural break for pollen specialists was then defined as $P/TL \geq 0.90$ ($\geq 90\%$ visits for pollen). A P/TL of 0.90 also corresponds to approximately one standard deviation from the mean for both the two and five week distributions, 0.84 and 0.93, respectively (mean \pm SD, after two weeks, 0.56 ± 0.28 ; after five weeks, 0.62 ± 0.31). There was an overall increase in the number of foragers with a $P/TL \geq 0.90$ between week two and week. I focused on foraging bees and therefore required a bee to forage more than once in their life.

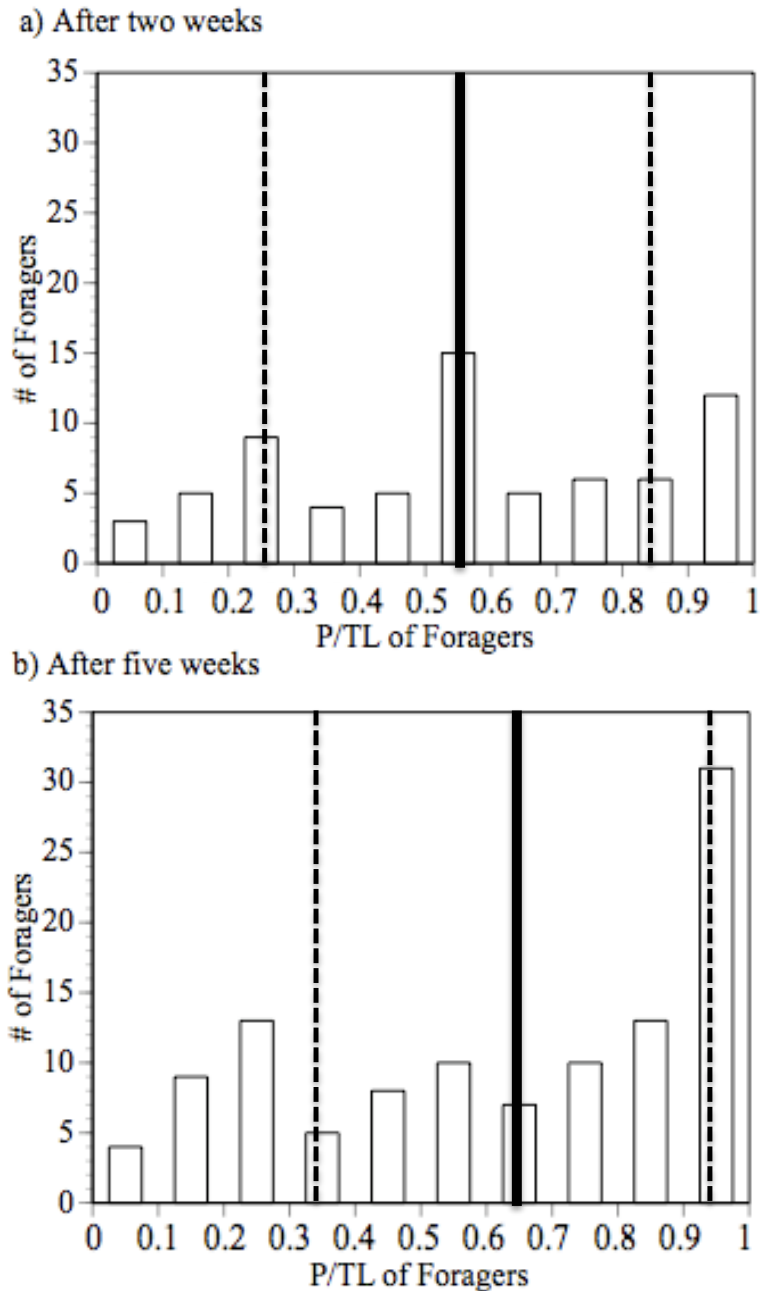


Figure 1: Distribution of the pollen foraging proportions for Control A foragers after two and five weeks.

Histograms showing the pollen foraging proportion (P/TL: # of pollen foraging visits/ total # of foraging visits) of foragers two weeks after colony arrival. Mean (solid line) and SD (dashed line) after two weeks, 0.56 ± 0.28 , and five weeks, 0.62 ± 0.31 .

To test the ability of colonies to modulate foraging specializations, I extracted pollen specialists at different stages of colony life. I extracted bees two and five weeks after colonies arrived. I performed only one extraction per colony and used a different colony for each extraction. Upon arrival, all colonies were at approximately the same age and health (personal communication, Biobest Corporation); colonies contained 185 ± 84.8 bees and lived for 90.6 ± 16.2 days. The day of arrival was defined as day zero. I used two experimental colonies and three control colonies. For the experimental colonies, there was an Early Extraction colony and a Late Extraction colony where all pollen specialists ($\geq 90\%$ visits for pollen, based upon foraging from the day of arrival to the day prior to extraction) were removed at two weeks or five weeks, respectively. These extractions removed 47 and 11 pollen specialists corresponding to 25.3% and 10.1% of the colony's population in the Early and Late Extraction colonies, respectively.

I used the number of extracted bees relative to the colony's entire population because pollen foraging is most strongly correlated with the total number of adult workers in a colony, which is correlated with the amount of brood in a colony (O'Donnell *et al.*, 2000). Only the bees that fit our definition of pollen specialist were removed. Thus, different numbers of bees were removed from the two experimental colonies.

I used one colony at a time and placed extracted bees in an identical but separate nest box with an empty nest from a previous *Bombus impatiens* colony. I provided these bees with pollen and sucrose solution on the same schedule as the

main colony. I did not record the foraging of extracted bees because these had been removed from normal colony and brood cues and because our study focused on how the remaining bees responded to a loss of pollen specialists. After two weeks, I reintroduced the pollen specialists to the main colony.

I had three control colonies: Control A, Control B and the Extraction Control. In Control A and B, no bees were removed. I used these colonies to obtain foraging baselines. To control for the possibility that the extraction of bees alone (irrespective of their foraging specialization) modulated foraging of those remaining in the main colony, I also used an Extraction Control colony. In this colony, I removed an equal proportion of all foraging categories after two weeks of observation and reintroduced these bees to the main colony two weeks later. I chose to extract these bees after two weeks to allow direct comparisons with the Early Extraction colony (specialists removed after two weeks) because this colony had the largest proportion of bees removed. In this Early Extraction colony, 25.3% of the bees in the colony were extracted. I therefore extracted 25.3% (81) bees in the Extraction Control colony.

To uniformly remove an equal proportion of all forager categories, I determined the P/TL of each forager during the first two weeks and generated a histogram in which each bar represented a 10% increment. For example, a forager who spent 12% of its visits collecting pollen fell into the 10-20% category. I then removed an equal proportion of foragers in each category so that the shape of the remaining distribution was identical to the pre-extraction distribution, but with

fewer bees. After verifying the video analysis at the end of the data collection (see methods above), I corrected the assignment of a few individual foraging visits, resulting in a slight change to the foraging preference distribution. However, the correlation coefficient between the numbers of bees in each foraging category that should have been removed versus those that were actually removed is 0.99.

Statistics

Since P/TL is a proportion, it was arcsine transformed for all statistical tests (see below). I define the periods as follows: Pre-Extraction (the two weeks prior to bee extraction), Extraction (the two weeks during bee extraction), and Post-Extraction (the two weeks after the extraction). All removed bees were returned to the colonies at the end of the Extraction period. I reported mean ± 1 standard error and use an alpha level of 0.05.

To test how the extraction of pollen specialists affected the remaining generalists during the early and late portions of colony life, I conducted intra- and inter-colony comparisons. Intracolony comparisons contrasted the control colonies Control A, Control B and the Extraction Control (only Early Extraction colony) with each of the extraction colonies by comparing the behavior of generalists (P/TL) at the same early and late stages as the treatment colonies. Nectar specialists were not used because Control A had only a single nectar specialist for the colony comparisons. I used paired t-tests to compare the arcsine transformed P/TL of the generalists over each time section (Pre-extraction vs. Extraction, Extraction vs. Post-

extraction, and Pre-extraction vs. Post-extraction). I statistically corrected for multiple tests on the same data by applying the Sequential Bonferroni Correction ($k=2$, (Sokal and Rohlf, 1995). For intercolony comparisons, I ran an ANOVAs with post-hoc Tukey tests for the effect of colony on the P/TL of generalist for each time section separately (Lehman *et al.*, 2005). Only generalists that foraged in all three time sections (Pre-extraction, Extraction and Post-extraction) were included in these analyses.

I evaluated the effect of the uniform, proportional forager extraction on the foraging preferences of remaining foragers. I used paired t-tests to compare the arcsine transformed P/TL of the foragers left behind during the extraction over each time section (Pre-extraction vs. Extraction, Extraction vs. Post-extraction, and Pre-extraction vs. Post-extraction). As before, I corrected for multiple comparisons with the same data using the Sequential Bonferroni. Only the bees that foraged in all three time sections were included in this analysis. With this colony, I also examined the effect of extraction on bees that were removed. As before, I used paired t-tests and compared P/TL for Pre-Extraction and Post-Extraction. Only bees that foraged Post-Extraction were included in this analysis.

To find the proportion of specialists in a non-manipulated colony, as well as the size variation among foraging categories, I used Control Colonies A and B. The proportion of foragers that had specialized was calculated using the P/TL a forager obtained for their entire foraging career. I compared the size (inter-tegular distance, IT) of foragers to nest bees (those who never foraged) using an analysis of variance

(ANOVA). The effect of the foraging category (pollen specialist, nectar specialist or generalist) an individual had obtained by the end of their life on size of the bee (IT) was analyzed using an ANOVA with post-hoc Tukey. Virgin queens (IT >5.0 mm) were excluded from this analysis because our study focuses on workers.

I examined the consistency of individual foraging preferences over time by calculating the weekly P/TL for each forager and using a one-way ANOVA, repeated measures design (Lehman *et al.*, 2005). Only foragers born in the lab were used for this analysis because only their entire foraging history was known.

III.

Results

I. Effect of Pollen Specialist Extraction on Generalists

A. Early Extraction Colony (after two weeks)

After the pollen specialists were removed in the early (two week) extraction, generalists significantly increased pollen foraging (Early Extraction colony, $P/TL = 54.4 \pm 0.2\%$ to $74.3 \pm 0.7\%$, Table 1). These generalist bees then significantly decreased pollen foraging after the pollen specialists were returned ($P/TL = 74.3 \pm 0.7\%$ to $53.5 \pm 0.8\%$, Table 1, Fig. 2d), resuming their former Pre-Extraction foraging preferences (Table 1). In Control A, Control B, and the Extraction Control, generalists did not change their levels of pollen foraging over the same periods of colony life (Table 1, Fig. 2a-c).

Comparing pollen foraging of generalist between colonies I found that during the Extraction time section the Early Extraction colony had significantly higher pollen foraging than Control B or the Extraction Control ($F_{3,174} = 4.26$ $P = 0.006$, Tukey HSD, $Q = 2.59$, $P < 0.05$, Fig. 3b). There was no significant difference between colonies for the Pre-Extraction ($F_{3,174} = 1.97$ $P = 0.12$, Fig. 3a) or Post-Extraction time sections ($F_{3,174} = 2.32$ $P = 0.08$, Fig. 3c).

Table 1: Generalist reaction to early pollen specialist (PS) extraction. Results of paired t-tests comparing the pollen foraging proportions (P/TL: # of pollen foraging visits/ total # of foraging visits) of generalists. The time sections are broken into Pre-Extraction (Week 0-2), Extraction (Week 2-4) and Post-Extraction (Week 4-6).

Colony	Pre-Extraction vs. Extraction	Pre-Extraction vs. Post-Extraction	Extraction vs. Post-Extraction
Control A	$t_{20} = 0.12$ $P = 0.91$	$t_{20} = -0.70$ $P = 0.49$	$t_{20} = -1.47$ $P = 0.16$
Control B	$t_{115} = 0.14$ $P = 0.89$	$t_{115} = 0.04$ $P = 0.97$	$t_{115} = -0.09$ $P = 0.93$
Extraction Control	$t_{14} = -0.83$ $P = 0.42$	$t_{14} = -1.44$ $P = 0.17$	$t_{14} = -1.62$ $P = 0.13$
Early PS Extraction	$t_{25} = 3.90$ $P = 0.0006$	$t_{25} = -0.09$ $P = 0.93$	$t_{25} = -2.52$ $P = 0.019$

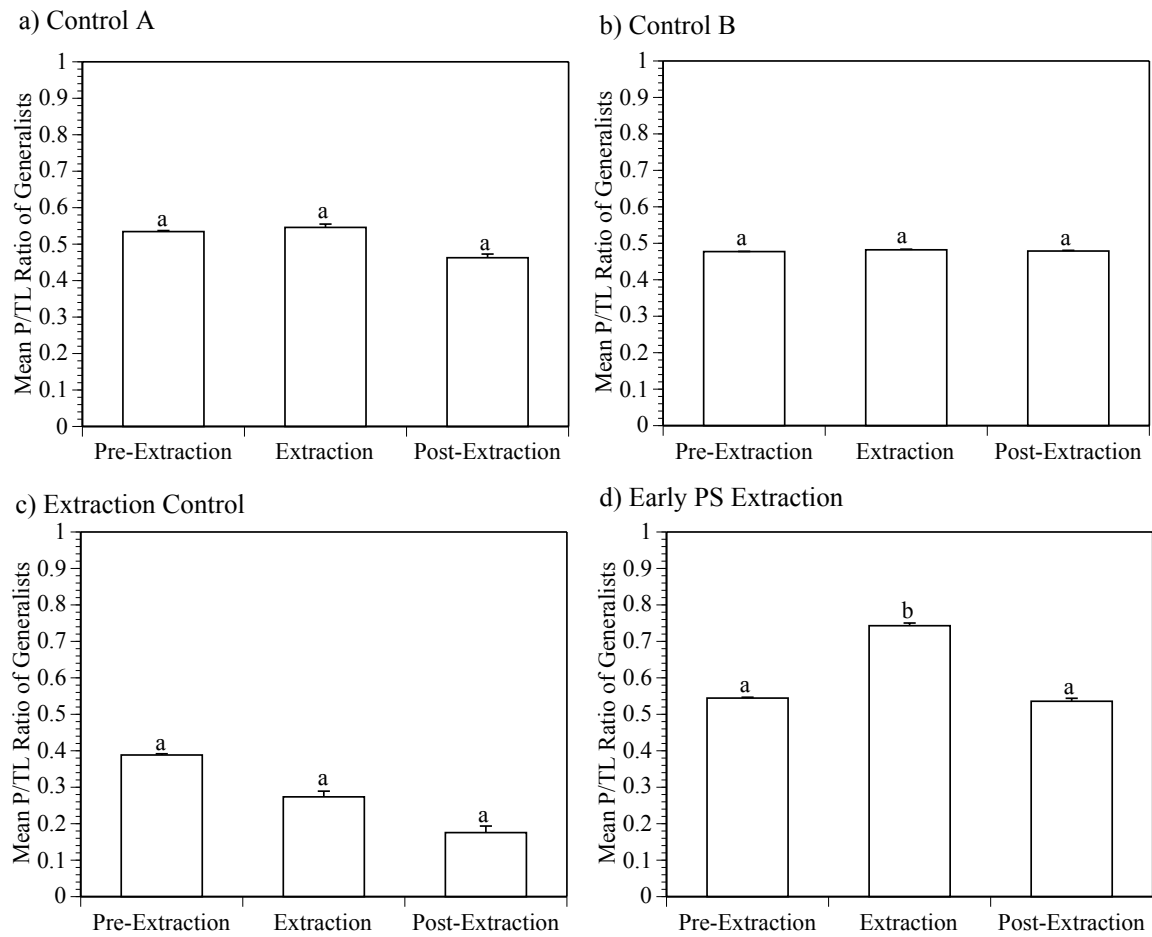


Figure 2: Generalist reaction to early pollen specialist (PS) extraction. Histograms showing the mean (\pm SE) pollen foraging proportion (P/TL: # of pollen foraging visits/ total # of foraging visits) of generalists. The time sections are broken into Pre-Extraction (Week 0-2), Extraction (Week 2-4) and Post-Extraction (Week 4-6). Different lettering designates statistical significance of $P \leq 0.05$.

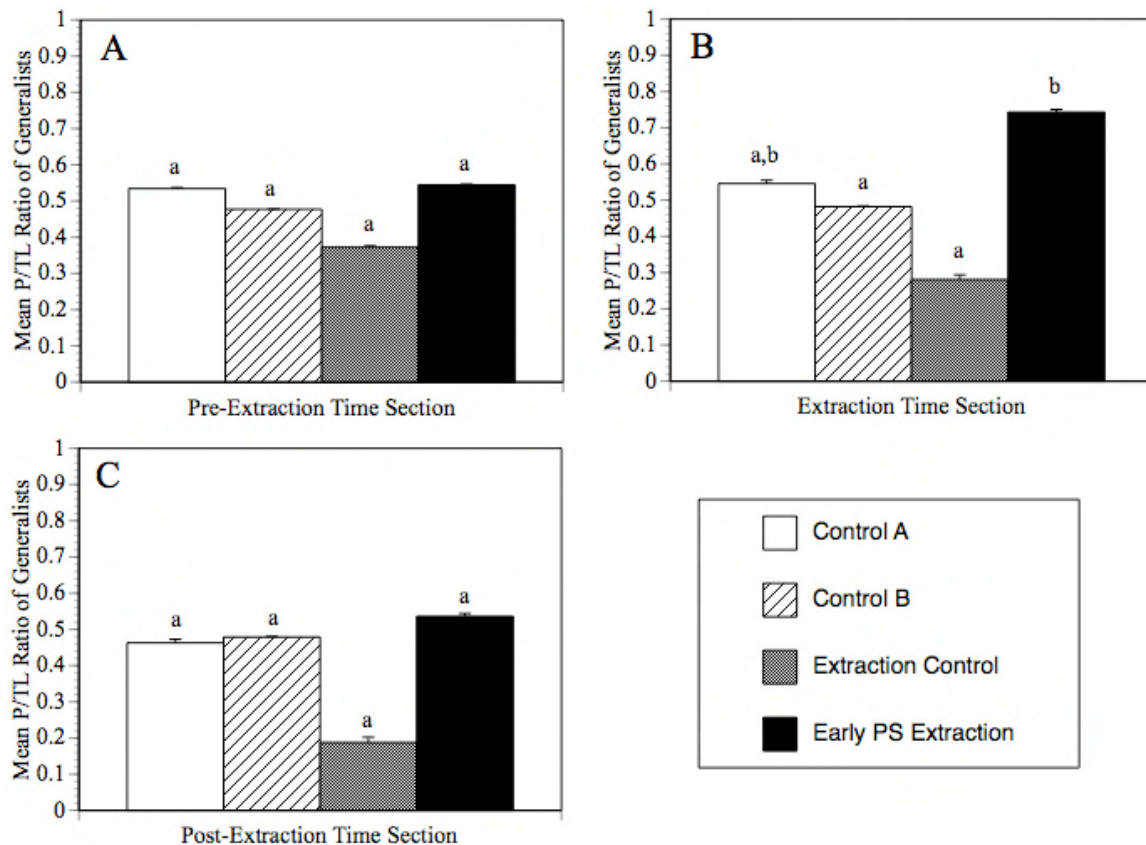


Figure 3: Between-colony comparison to early pollen specialist (PS) extraction. Histograms showing the mean (\pm SE) pollen foraging proportion (P/TL: # of pollen foraging visits/ total # of foraging visits) of generalists for each colony. The time sections are broken into Pre-Extraction (Week 0-2), Extraction (Week 2-4) and Post-Extraction (Week 4-6). Different lettering designates statistical significance of $P \leq 0.05$.

B. Late Extraction Colony (after five weeks)

There was a significant effect of the late (five weeks) pollen specialist extraction on generalists (Late Extraction colony). Generalists significantly increased pollen foraging ($P/TL = 43.8 \pm 0.3\%$ to $50.1 \pm 0.3\%$, Table 2) when pollen specialists were removed. These same generalists then significantly decreased pollen foraging after the pollen specialists were returned ($P/TL = 50.1 \pm 0.3\%$ to $37.9 \pm 0.2\%$, Table 2, Fig. 4c), resuming their former Pre-Extraction foraging preferences (Table 2). There was no significant change in pollen foraging by generalists in Control A between any of the same time sections (Table 2, Fig. 5a). In Control B, pollen foraging by generalists significantly decreased from Pre-Extraction to Extraction ($P/TL = 56.0 \pm 0.1\%$ to $44.3 \pm 0.2\%$, Table 2) and then continued this low level of pollen foraging during the Post-Extraction time section ($P/TL = 39.4 \pm 0.1\%$, Table 2, Fig. 4b).

Comparing pollen foraging of generalist between colonies, I found no significant variation for the Pre-Extraction ($F_{2,163} = 2.16$ $P = 0.12$, Fig. 5a) or Extraction time sections ($F_{2,163} = 1.27$ $P = 0.28$, Fig. 5b). However, the pollen foraging of generalists in the Post-Extraction time period varied significantly among colonies ($F_{2,163} = 6.04$ $P = 0.003$, Fig. 5c). The Control A colony had significantly higher pollen foraging than Control B or the Late Extraction colony (Tukey HSD, $Q = 2.36$, $P < 0.05$).

Table 2: Generalist reaction to pollen specialist (PS) extraction. Results of paired t-tests comparing the pollen foraging proportions (P/TL: # of pollen foraging visits/ total # of foraging visits) of generalists. The time sections are broken into Pre-Extraction (Week 3-5), Extraction (Week 5-7) and Post-Extraction (Week 7-9).

Colony	Pre-Extraction vs. Extraction	Pre-Extraction vs. Post-Extraction	Extraction vs. Post-Extraction
Control A	$t_{13} = -0.13$ $P = 0.90$	$t_{13} = 0.74$ $P = 0.47$	$t_{13} = 0.72$ $P = 0.49$
Control B	$t_{101} = -3.33$ $P = 0.001$	$t_{101} = -5.27$ $P < 0.0001$	$t_{101} = -1.66$ $P = 0.10$
Late PS Extraction	$t_{49} = 2.83$ $P = 0.007$	$t_{49} = -1.84$ $P = 0.07$	$t_{49} = -4.84$ $P < 0.0001$

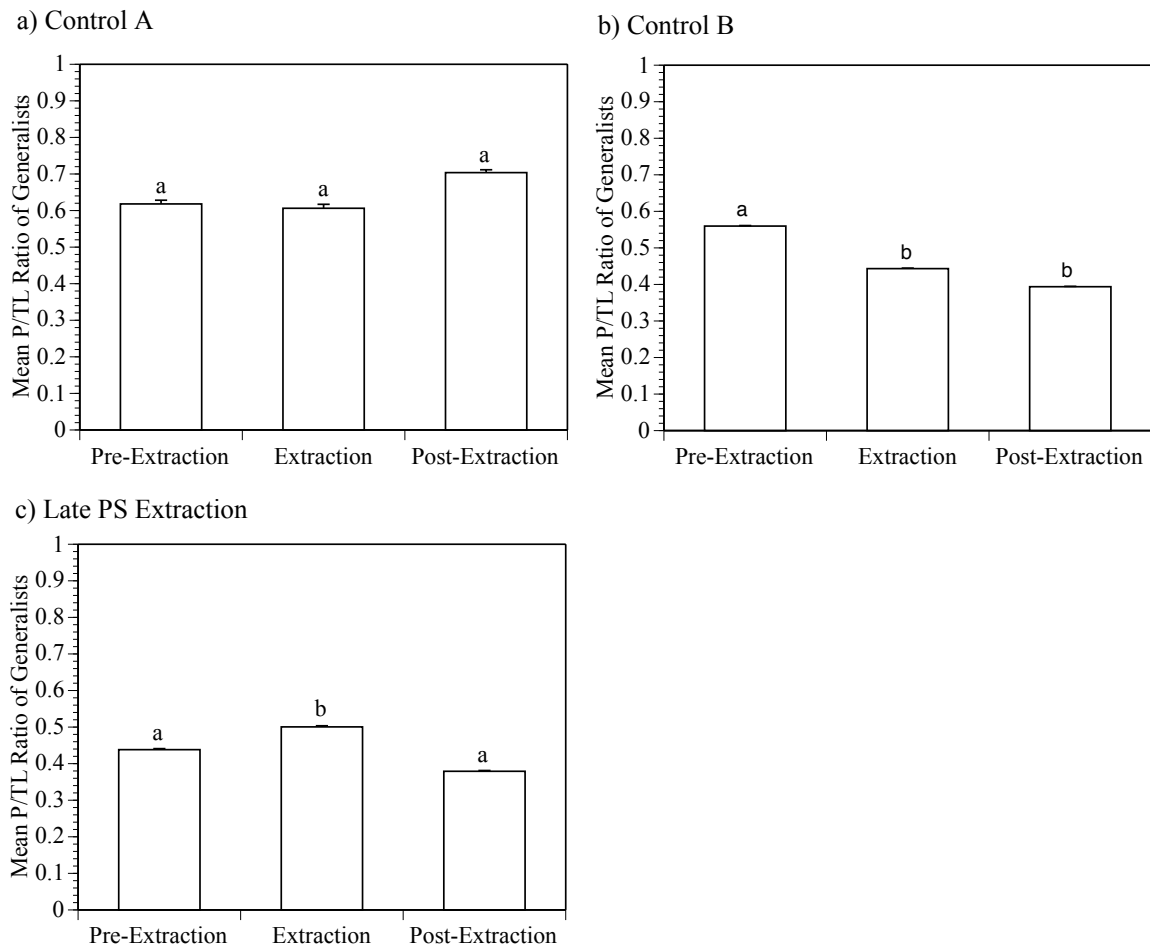


Figure 4: Generalist reaction to pollen specialist (PS) extraction. Histograms showing the mean (\pm SE) pollen foraging proportion (P/TL: # of pollen foraging visits/ total # of foraging visits) of generalists. The time sections are broken into Pre-Extraction (Week 3-5), Extraction (Week 5-7) and Post-Extraction (Week 7-9). Different lettering designates statistical significance of $P \leq 0.05$.

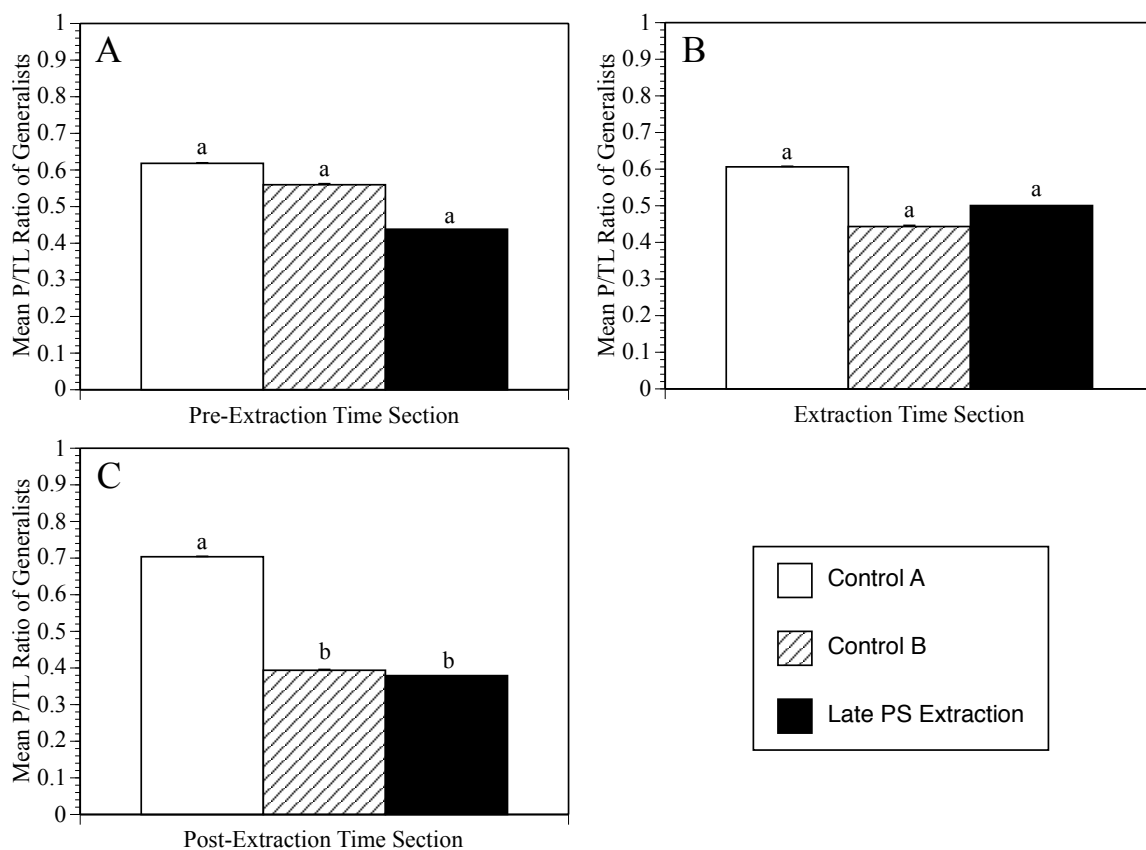


Figure 5: Between-colony comparison to late pollen specialist (PS) extraction. Histograms showing the mean (\pm SE) pollen foraging proportion (P/TL: # of pollen foraging visits/ total # of foraging visits) of generalists for each colony. The time sections are broken into Pre-Extraction (Week 3-5), Extraction (Week 5-7) and Post-Extraction (Week 7-9). Different lettering designates statistical significance of $P \leq 0.05$.

II. Effect of Extraction on the Pollen Specialists

Reviewing the pollen foraging proportions of pollen specialists that were extracted, our data was inconclusive for our experimental colonies compared to Control A and B. In the Early Extraction, there was no significant change in pollen foraging of pollen specialists for Control A ($t_5 = -1.34$ $P = 0.24$); however, there was a significant decrease in pollen foraging proportions for Control B ($t_{19} = -4.55$ $P = 0.0002$) and the experimental colony ($t_{40} = -6.34$ $P < 0.0001$). For pollen specialists in the Late Extraction colony there was no significant change in the pollen foraging for Control A ($t_{13} = -1.20$ $P = 0.25$) or the experimental colony ($t_8 = -0.80$ $P = 0.45$); however, there was a significant decrease in pollen foraging proportions for Control B ($t_{12} = -5.45$ $P < 0.0001$).

III. Effect of a Uniform Proportional Forager Extraction

There was no significant effect of the uniform proportional forager extraction (Extraction Control) on bees that remained in the main colony. For these bees, pollen foraging stayed consistent through all time sections (Table 3). For extracted foragers, pollen foraging significantly decreased between Pre-Extraction and Post-Extraction ($P/TL = 0.47 \pm 0.01$ to 0.28 ± 0.01 , $t_{73} = -2.96$ $P = 0.004$).

Table 3: Reaction of those left behind in the Randomized Control. Results of paired t-tests comparing the pollen foraging proportions (P/TL: # of pollen foraging visits/ total # of foraging visits) of foragers left behind during the extraction. The time sections are broken into Pre-Extraction (Week 0-2), Extraction (Week 2-4) and Post-Extraction (Week 4-6).

Pre-Extraction vs. Extraction	Pre-Extraction vs. Post-Extraction	Extraction vs. Post-Extraction
$t_{51} = -0.09$ $P = 0.93$	$t_{51} = -1.21$ $P = 0.23$	$t_{51} = -1.75$ $P = 0.09$

IV. Results of Control A and Control B

A. Number of specialists

In the Control A colony, pollen specialists, nectar specialists, and generalists made up 32.3%, 3.4%, and 64.3%, respectively, of the foraging population (118 bees). In the Control B colony, pollen specialists, nectar specialists, and generalists made up 2.0%, 13.8%, and 84.2%, respectively, of the foraging population (247 bees).

B. Individual Foraging Consistency Over their Lifetime

Foragers in Control A stayed consistent in their pollen foraging proportions throughout their lifetime, with the effect of time showing no significance ($F_{8,227}=0.85$ $P=0.56$). However, foragers in Control B did not stay consistent throughout their lifetimes, with the effect of time showing significance ($F_{14,627}=0.85$ $P<0.0001$); Control B foragers decreased pollen foraging on average over their lifetimes.

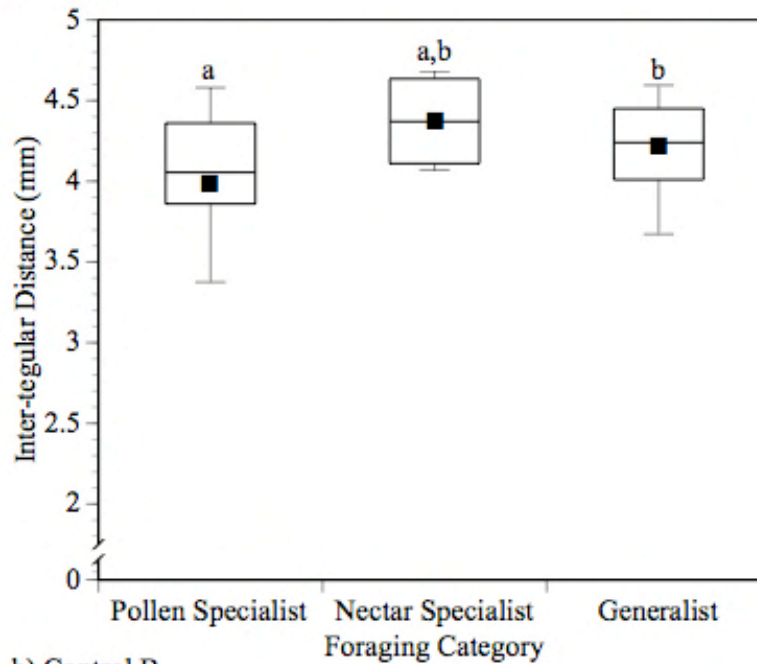
C. Effect of Body Size on Foraging Behavior

I tested for correlations between body size and worker caste in the Control A and B colonies because foragers in these colonies were not manipulated (removed). In both control colonies, bees that foraged (foragers) were significantly larger than bees that only stayed inside the nest (nest bees). Queens were not used in these analyses. On average, forager and nest bee IT was 4.15 ± 0.05 mm and 3.97 ± 0.07 mm, respectively (Control A colony, $F_{1,150}=4.80$ $P=0.030$). In the Control B colony,

average forager and nest bee IT was 3.59 ± 0.04 mm and 3.23 ± 0.13 mm, respectively ($F_{1,262}=6.94$ $P=0.009$).

I found weak evidence for size differences among foraging categories (nectar specialists, pollen specialists, and generalists) for the Control A colony ($F_{2,105}=3.51$ $P=0.033$), but no evidence for the Control B colony ($F_{2,242}=1.72$, $P=0.18$, Fig. 6b). In the Control A colony pollen specialists were significantly smaller than generalists (Tukey HSD, $Q= 2.38$, $P<0.05$, Fig. 6a). Control A and Control B were tested separately because of significant size variation between the two colonies ($F_{1,351}=86.39$ $P<0.0001$).

a) Control A



b) Control B

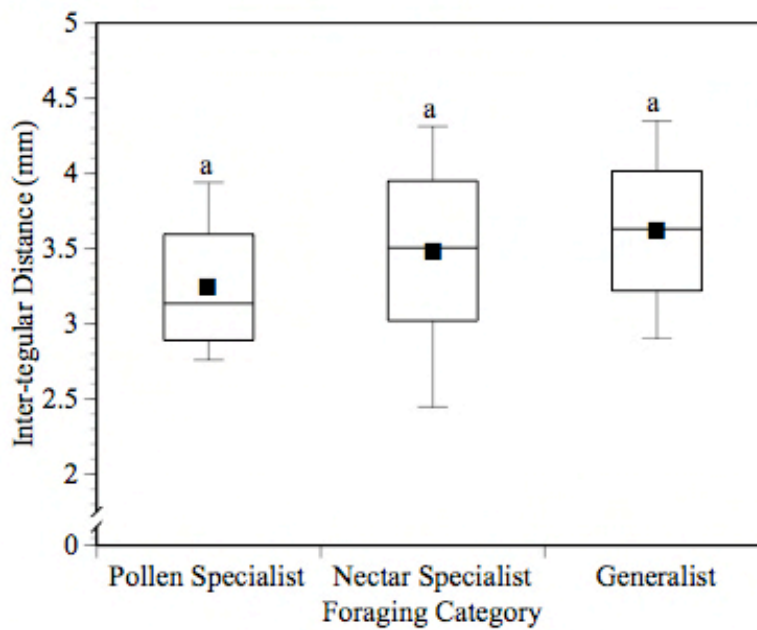


Figure 6: Box plots of the average inter-tegular distance for each foraging category. Pollen specialist ($\geq 90\%$ pollen foraging visits) nectar specialist ($\geq 90\%$ nectar foraging visits), and generalists (all other bees). Different lettering designates statistical significance of $P \leq 0.05$.

IV.

Discussion

Removing pollen specialists significantly increased the pollen foraging of generalists at early and late periods in the life of the colony (one extraction per colony), showing the importance of pollen specialists and a colony's ability to response to pollen foraging labor shortages. Removing pollen specialists significantly increased the pollen foraging of generalists. In the Early and Late Extraction colonies generalists significantly increased pollen foraging on average by 36% and 14%, respectively, while pollen specialists were extracted. After the pollen specialists were returned to the colony, these generalists resumed their lower, pre-extraction levels of pollen foraging. In the Extraction Control, the lack of an increase in generalist pollen foraging during the two week extraction period shows that increased pollen foraging in the experimental colonies is due to the specific extraction of pollen specialists.

Pollen is a vital resource for adults and larvae. Adult bumble bees, *B. terrestris*, need pollen and live shorter lives without it (Smeets and Duchateau, 2003). In past experiments on *B. flavifrons*, *B. melanopygus*, *B. mixtus*, *B. occidentalis*, *B. sitkensis*, and *B. terricola*, when pollen stores were removed, foragers showed an increased foraging effort for pollen (Cartar and Dill, 1990, Cartar, 1992, Plowright *et al.*, 1993). Thus, the extraction of pollen specialist may have led to decreased pollen stores in the nest and stimulated increased pollen foraging in the generalists.

Pollen specialists may have a higher foraging efficiency, which could cause the effect I see in generalist foraging when pollen specialists are extracted. In past studies on *B. bifarius nearcticus*, foraging specialists have been shown to contribute

more to their colonies food stores than expected given their small number (O'Donnell *et al.*, 2000). Also, those who switch tasks in order to compensate for food depletion were said to be less efficient than regular foragers and switched back to their previous activities when food stores were enhanced (Cartar, 1992). I found that generalists returned to their previous foraging habits once the pollen specialists were returned.

In the Extraction Control colony, I removed an equal proportion of foragers (25.3%, 81 bees) from all foraging categories. I found that the foragers left behind did not change their pollen foraging proportions (Table 3). This was what I expected since all foraging categories were equally depleted. As for the response of foragers, which were extracted and then reintroduced our results were inconclusive for both the Early and Late Extraction, as well as, the Extraction control.

On a colony level, I found that 35.7% of Control A and 15.8% of Control B were either nectar or pollen specialists at the end of their foraging careers. Control A is within the range (30-40%) that O'Donnell *et al.* found in 2000 for *B. bifarius nearcticus* but Control B is much lower. This variation may have been because Control A and Control B were active for different lengths of time, 9 weeks and 15 weeks, respectively. Control A and Control B had an unequal number of foraging days after the final newborn emergence, 46 days and 88 days, respectively. Foragers in Control B colony foraged for a longer time without larvae present and therefore may have foraged for less pollen than Control A. Since the foraging categories were

created using an individuals' entire foraging history, this may have caused fewer pollen specialists in Control B (2.0%) than Control A (32.3%).

On an individual level, I found that foragers tended to be larger than nest bees, which agrees with previous studies on various bumble bee species (Brian, 1952, Free, 1955, Goulson *et al.*, 2002, Spaethe and Weidenmüller, 2002). Among foragers the generalists were larger than pollen specialists for Control A. This trend of pollen foragers being the smallest foraging category was also observed in *Bombus terrestris* (Goulson *et al.*, 2002). Size variation among foraging categories in Control B was not significant. Natural variation within a colony may have caused this; future studies are needed to thoroughly address size variation among foraging categories. Regarding the foraging consistency of an individual over the course of its lifetime, I found that the individuals of only one of our two control colonies foraged consistently, indicating that some colonies may forage more consistently than others.

Overall, I found that the extraction of pollen specialists influences the foraging of generalists remaining in the colony, causing them to forage more for pollen in the pollen specialists' absence. Removing equal proportions of all foraging categories does not affect the foraging of bees left behind. The proximate cause of the adaptation I see when pollen specialists were extracted may be either at the individual or colony level. The overall question of how labor is controlled is beyond the scope of this experiment. A limitation of the study was the fact that the foraging environment was not natural. However, this could not be avoided since the only way

to observe every individual decision to forage for either nectar or pollen required an artificial environment where the two are separated. In the future, I would like to explore foraging specialization in more detail and investigate how foragers become specialists.

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