

Individual lifetime pollen and nectar foraging preferences in bumble bees

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Abstract Foraging specialization plays an important role in the ability of social insects to efficiently allocate labor. However, relatively little is known about the degree to which individual bumble bees specialize on collecting nectar or pollen, when such preferences manifest, and if individuals can alter their foraging preferences in response to changes in the colony workforce. Using *Bombus impatiens*, we monitored all foraging visits made by every bee in multiple colonies and showed that individual foragers exhibit consistent lifetime foraging preferences. Based upon the distribution of foraging preferences, we defined three forager types (pollen specialists, nectar specialists, and generalists). In unmanipulated colonies, 16–36 % of individuals specialized (≥ 90 % of visits) on nectar or pollen only. On its first day of foraging, an individual's foraging choices (nectar only, pollen only, or nectar and pollen) significantly predicted its lifetime foraging preferences. Foragers that only collected pollen on their first day of foraging made 1.61- to 1.67-fold more lifetime pollen foraging visits (as a proportion of total trips) than foragers that only collected nectar on their first foraging day. Foragers were significantly larger than bees that stayed only in the nest. We also determined the effect of removing pollen specialists at early (brood present) or later (brood absent) stages in colony life. These results suggest that generalists can alter their foraging preferences in response to the loss of a small subset of foragers. Thus, bumble bees exhibit individual lifetime foraging preferences that are established early in life, but generalists may be able to adapt to colony needs.

Keywords Individual foraging specialization · Bumble bees · Division of labor · Pollen foraging · Nectar foraging

Introduction

Division of labor is an important property of social organization (Beshers and Fewell 2001; Holbrook et al. 2009, 2011; Johnson and Frost 2012) and a fascinating aspect of how social insects organize work. Foraging specialization, the preferential collection of one nutrient resource over others, is particularly interesting because it allows researchers to examine how a higher level of organization, the colony, adapts to perturbations at the lower level of individual labor (Leadbeater and Chittka 2009; Schmickl and Crailsheim 2011). Such flexibility has several proximate causes. Individuals may respond to changes in colony food stores (bumble bees, Plowright et al. 1993; Molet et al. 2008; honeybees, Schulz et al. 1998), differences in individual sucrose response thresholds (honeybees, Page et al. 1998), differences in patterns of gene activation (honeybees, Robinson and Page 1989; Ben-Shahar et al. 2002), or a combination of these and other factors.

Specialization definitions vary, but generally include the concept that a specialist spends a majority of its time and effort on a particular task, while generalists spread their time and effort among a variety of tasks. Foraging specialization is beneficial because it increases the efficiency of resource collection. Specialists are usually (though not always; Dornhaus 2008) more efficient at their tasks than generalists (Rissing 1981; Cartar 1992; O'Donnell et al. 2000; Hofstede and Sommeijer 2006). Pollen foraging specialization occurs in honeybees (*Apis mellifera*), stingless bees (*Plebeia tobagoensis*), and bumble bees (*Bombus bifarius nearcticus* and *Bombus terrestris*) (Strickler 1979; O'Donnell et al. 2000; Hofstede and Sommeijer 2006; Raine and Chittka 2007; Brito

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et al. 2010). Approximately 40 % of honeybee foragers specialize on pollen, nectar, or water (Ribbands 1952). In stingless bees, approximately 50 % of *Melipona beecheii* foragers (Biesmeijer and Tóth 1998) and 70 % of *Melipona favosa* foragers (Sommeijer et al. 1983) specialize in the collection of one commodity (nectar, pollen, mud, resin, or water). O'Donnell et al. (2000) found that 30–40 % of *B. bifarius nearcticus* foragers specialize on collecting either pollen or nectar.

Compared to honeybees, bumble bee foraging specialization is less well studied, but is of interest because bumble bees exhibit differences in colony life cycle and foraging organization from the highly social bees, honeybees, and stingless bees. Bumble bee colonies are annual, not perennial, and therefore, have changing needs for pollen as they senesce. Bumble bee foragers are also solitary, not group foragers like honeybees (Dornhaus and Chittka 2004). Furthermore, unlike honeybees or stingless bees (Hunt et al. 1998; Grüter et al. 2012), bumble bees exhibit high variation in body size, approximately 10-fold differences in body mass (Alford 1975). Thus, it is relevant to ask if such size differences correlate with foraging preferences, as it does in some ant species (Holldobler and Wilson 1990; Muscedere and Traniello 2012). Bumble bees foragers tend to be larger than bees that stay only in the nest (Goulson et al. 2002; Spaethe and Weidenmüller 2002; Kapustjanskij et al. 2007; Jandt and Dornhaus 2009). In *Bombus impatiens*, larger foragers have higher nectar foraging rates compared to smaller foragers, but pollen foraging rates are unrelated to forager size (Goulson et al. 2002; Spaethe and Weidenmüller 2002). Goulson et al. (2002) captured *B. terrestris* foragers returning to nests and showed that nectar foragers were the largest, followed by generalists (bees collecting nectar and pollen). Pollen foragers were the smallest. However, the relationship between size and lifetime foraging preferences is not known.

Finally, behavioral flexibility is important for the colony to meet shifting needs and adapt to a changing environment. Bumble bee colonies can adaptively reallocate labor. Several bumble bee species (*Bombus flavifrons*, *Bombus melanopygus*, *Bombus mixtus*, *Bombus occidentalis*, *Bombus sitkensis*, *B. terrestris*, and *Bombus terricola*) increase (Cartar and Dill 1990; Cartar 1992; Plowright et al. 1993; Molet et al. 2008) or decrease (Free 1955; Pelletier and McNeil 2004; Dornhaus and Chittka 2005; Kitaoka and Nieh 2009) colony foraging when stored nectar or pollen is respectively removed or supplemented.

Presumably, this arises from individuals changing their behaviors. For example, *Bombus agrorum* and *Bombus pratorum* nest bees (workers that had stayed in the nest and were not foragers) became foragers when all foragers were removed (Free 1955). When half of all workers in a *B. terrestris* colony were removed, the remaining bees compensated by increasing the rate at which they individually

fed larvae (Plowright et al. 1993). However, to our knowledge, no studies have directly demonstrated that individual bumble bee foragers can alter their pollen and nectar foraging preferences in response to less drastic changes in workforce number, which colonies may more typically face.

We, therefore, examined foraging specialization in *B. impatiens*, a New World species of bumble bee, in great detail. We documented all foraging visits of every forager over their complete foraging lifetimes in multiple colonies. We investigated five questions: (Q1) Are foraging preferences established early in life? (Q2) Do individual foraging preferences persist over a forager's lifetime? (Q3) Does colony production of specialists change over colony lifetime? (Q4) Is there a relationship between worker body size and foraging specialization? (Q5) Will the removal of pollen specialists cause generalists to increase their level of pollen foraging?

Materials and methods

Study site and species

Experiments were conducted at the University of California, San Diego in La Jolla, CA, USA (32°52.690' N and 117°14.464' W), from April 2009 through January 2011. Prior studies using free-foraging colonies provided valuable information on individual foraging preferences (O'Donnell et al. 2000; Spaethe and Weidenmüller 2002). However, it is not feasible to determine the foraging preferences of every individual on each foraging trip in free-foraging colonies. We, therefore, used laboratory colonies and monitored all foraging inside an enclosed arena. Such arena studies are powerful tools for dissecting and understanding colony foraging behavior (Dornhaus and Chittka 2004).

We sequentially used five *B. impatiens* colonies from Biobest Biological Systems (Ontario, Canada). This species occurs in the eastern USA and Canada, ranging from Ontario and Maine in the north to Florida in the south and as far west as Michigan, Illinois, Kansas, and Mississippi (Heinrich 1979). Colonies were placed in a temperature-controlled room (~21 °C) and exposed to a 12-h light cycle (6:00 a.m.–6:00 p.m.) illuminated with three 20 W halogen bulbs positioned around the foraging arena. We housed the colony in a wood nest box (32.5×28.4×15 cm) with a clear lid normally covered to maintain darkness. The nest box was connected to a foraging arena with a plastic tube (30 cm long, 3.5 cm diameter). The arena consisted of a clear plastic box (32×54×27 cm) and lid with two mesh panels on the side of the box to allow ventilation. Colonies at Biobest Biological Systems are raised in nest boxes in which bees cannot leave the nest (information from Biobest). Therefore, we are able to observe each individual's entire foraging

history because bees could only leave the nest to forage after being established in our setup. Due to the data-intensive nature of this experiment, it was not possible to run more than one colony at a time. We, therefore, studied colonies sequentially. All colonies were at approximately the same age and health (determined by close inspection of all bees and nest material) upon arrival. We defined the day of arrival in the laboratory as day 0. The lifetime production of each of these five colonies was 281.6 ± 85.7 bees (mean ± 1 standard deviation) and each colony lived for 91.0 ± 16.5 days. On average, each bee which eclosed after colony establishment in the laboratory lived for 51.3 ± 27.8 days.

To measure bee body size, intertegular (IT) span was used. This is a standard measurement for body size (Greenleaf et al. 2007; Williams et al. 2010; Hagen et al. 2011), which has been tested on females of 20 bee taxa and shown to be an accurate estimator of bee body mass (Cane 1987). When the colony arrived, we measured the IT span of each bee with digital calipers and used cyanoacrylate adhesive to attach a bee tag with a unique color and number on the thorax (Queen Marking Kit, The Bee Works, Orillia, Ontario, Canada). Each day, we looked for newly emerged, callow workers (identified by their silvery appearance and unhardened wings; O'Donnell et al. 2000).

General methods

We fed each colony 1.5 M unscented sucrose solution and pollen (collected from honeybees, fresh frozen, and thawed by grinding prior to feeding) ad libitum for 1 h (11:00 a.m.–12:00 p.m.) per day. Food was not available to the colony at any other time. To record bee behavior and identity, we used three video cameras connected to a digital video system (model QSDF8204, Q-See, Anaheim, CA, USA) recording onto a hard drive. We viewed videos using Windows Media Player software. We placed one video camera over each of three feeding dishes. There were more bees collecting sucrose than pollen, and thus, we used two sucrose dishes and one pollen dish, each with a diameter of 3.5 cm. A camera's field of view (6.3 by 4.6 mm) allowed the tags on foraging bees to be clearly identified with this dish size. During food presentation, we also placed one water dish in the foraging arena in case the colony needed it. We did not record water visitations because we very rarely observed water collection and our study focused on food foraging. All video analysts underwent extensive training, and their initial several hours of video analyses were independently verified. In total, we analyzed 1,572 h of video. After each colony died, we reviewed all video of visits that appeared irregular (data showing sporadic foraging or bees foraging after death) and corrected the data appropriately. Out of approximately 220,000 foraging visits from five colonies, only 2–3 % of the data collected showed such irregularities.

Because all foragers were uniquely marked, we were able to determine the foraging decision (pollen or nectar collection) of each forager on every foraging visit over its lifetime. We thus recorded the complete foraging history of all bees in every colony. A *nectar foraging* visit consisted of a bee drinking sucrose solution with its proboscis. To ensure that a bee was collecting nectar and not just tasting the food source, a foraging visit was identified by proboscis extension, into the nectar, >2 s, accompanied by thorax pumping. A *pollen foraging* visit consisted of a bee collecting pollen on its legs or ingesting it. We define a foraging visit as a single incidence of food collection from a food dish. Once a bee left the food dish, the foraging visit was considered complete. A bee could forage at nectar, pollen, or both, multiple times before returning to the nest; in this case, each food collection was counted individually. Our goal was to study bees that repeatedly foraged for food; we, therefore, included in our analyses only bees that made more than one foraging visit during their lifetimes.

Foraging specialization

In a different species, *B. bifarius nearcticus*, O'Donnell et al. (2000) defined a specialist as an individual performing a given task with a significantly higher probability ($P < 0.05$) than the overall colony-wide probability of performing the same task. Applying this method to our species would have led us to define a pollen specialist as a bee that spent ≥ 52 % of its time foraging at pollen. Because this cutoff was only slightly above the 50 % expected of a *B. impatiens* generalist, we chose an alternative method based upon the observed distribution of foraging visits (Fig. 1).

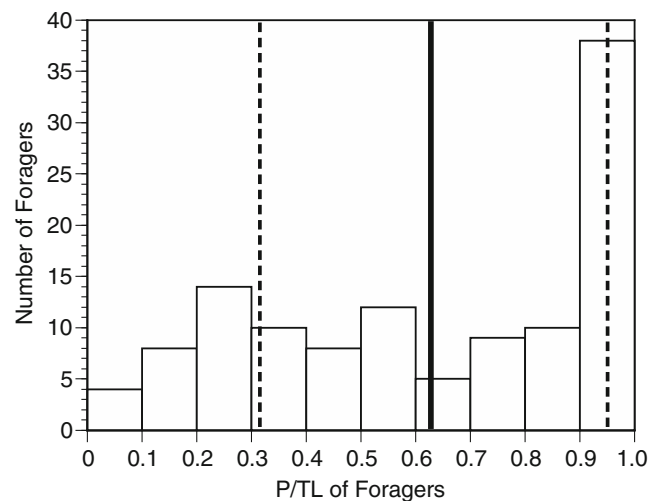


Fig. 1 Distribution of pollen foraging proportions for control A colony foragers. The histogram shows the lifetime pollen foraging proportion (P/TL=number of pollen foraging visits/total number of foraging visits) of foragers. Mean (solid line) and SD (dashed lines) are superimposed (0.63 ± 0.32 , $n=118$ bees)

To determine foraging preferences, we calculated the ratio of the number of pollen visits to total foraging visits (P/TL), following the example of Spaethe and Weidenmüller (2002) who used nectar foraging proportions in *B. terrestris* to monitor daily foraging. We classified each forager as a pollen specialist (≥ 90 % visits for pollen), nectar specialist (≥ 90 % visits for sucrose solution), or a generalist (all other bees). We chose this 90 % cutoff based upon a natural break in the distribution of P/TL ratios in our first colony (Fig. 1), a control colony in which we did not alter the number of foraging specialists. In this control A colony, we measured the lifetime P/TL distribution of foragers (Fig. 1). A natural break in the distribution occurs at a P/TL ratio of 0.90, which is close to 1 standard deviation above the mean (P/TL ratio of 0.95; Fig. 1). We, therefore, choose a P/TL ratio of 0.90 as our cutoff for defining specialists. We defined first-day forager type as the type of food collected by an individual on its first foraging day (nectar only, pollen only, or both nectar and pollen).

To evaluate foraging specialization, we used two colonies, called control A and control B, because no foragers were removed. To determine if the foragers can alter their foraging preferences in response to the removal of pollen specialists, we used two experimental colonies and one additional control colony (removal control in which we removed a random subset of bees). The experimental colonies consisted of an early removal colony and a later removal colony in which all pollen specialists (≥ 90 % visits for pollen, based upon foraging from the day of establishment to the day before removal) were removed after 2 weeks (on day 15) or 5 weeks (on day 36), respectively. These removal dates were chosen based upon brood levels in each colony.

The early removal colony workers were still eclosing at the time of the pollen specialist removal, 2 weeks after colony arrival, and the colony continued producing newly eclosed workers for four additional days. In the later removal colony, the last worker eclosed on the 14th day, 22 days prior to the removal of pollen specialists. These removals respectively extracted 25.3 and 10.1 % of the colony's population in the early and later removal colonies. Colony-level foraging for pollen declines with colony age because pollen foraging is related to the presence of larvae in the nest (Free 1955). Thus, the later removal colony contained fewer pollen specialists. We placed removed bees in an identical, separate nest box containing an empty nest from a previous *B. impatiens* colony and supplied them with pollen and sucrose solution on the same schedule as the main colony. After 2 weeks, we reintroduced the pollen specialists to the main colony to see if the main colony could readapt.

We also performed a removal control to test the possibility that the removal manipulation alone (irrespective of foraging specialization) is sufficient to change the foraging preferences of remaining bees. In the removal control, we,

therefore, removed an *equal proportion* of foragers over the complete range of foraging preferences. We generated a histogram of all foragers based upon their P/TL ratios and uniformly removed the same proportion of foragers in each P/TL bin (a 10 % increment). In the removal control, we extracted bees after 2 weeks to allow direct comparisons with the early removal colony because the early removal colony had the largest proportion of bees removed (25.3 %). We, therefore, removed 25.3 % bees in the removal control colony. After verifying the video analysis at the end of data collection (see the “General methods” section), we corrected the assignment of a few foraging visits, resulting in a slight change to the P/TL distribution. However, there was a 0.99 correlation coefficient (R^2) between the numbers of bees in each foraging category that should have been removed vs. those that were removed. At the time of removal, the removal control colony was still producing newly eclosed workers and continued to do so for seven additional days.

Statistics

Throughout this paper, we report averages as the mean \pm 1 standard deviation, unless stated otherwise, and conducted all statistical analyses with JMP v9.0.2 software. We conducted repeated-measures analyses (Q1–3) and analysis of variance (Q4). We use *t* tests to determine if parameter estimates (coefficients) are significantly different from zero and Tukey's honestly significant difference (HSD) tests to determine significant differences between treatment types. We performed two statistical transformations: arcsine transformation of the ratio P/TL and log transformation of IT span and the number of bee types in a colony (Zar 1984). We statistically corrected for multiple tests ($k=2$) on the same data by applying the sequential Bonferroni correction (Sokal and Rohlf 1995). In the analyses, all transformed and nontransformed data met parametric assumptions as determined by residual analysis. All effects were fixed, except for individual identity (a random effect). To answer Q1–3, we used control A and B colonies exclusively because their workforces were not perturbed. For Q1–3, we examined the effect of age on bee foraging behavior and colony production of foraging specialists, and we, therefore, only analyzed bees that eclosed after colony establishment. For all other analyses, we used all bees in the colonies.

To test Q5, we used two-tailed *t* tests to compare the P/TL ratio of generalists within each colony over three time intervals: pre-removal (the 2 weeks prior to bee removal), removal (the 2 weeks the removed bees were absent from colony), and post-removal (the 2 weeks after removal). We only analyzed the behavior of bees that foraged during all three time intervals. Q5 focuses on how a colony adapts to changes in its workforce. Therefore, we only conducted intracolony comparisons.

Results

Bombus impatiens colonies have foragers that exhibit foraging specialization for pollen and nectar (Figs. 1 and 3). When classified according to lifetime foraging preferences, control A colony contained 32.3 % pollen specialists, 3.4 % nectar specialists, and 64.3 % generalists ($n=118$ bees), while control B colony contained 2.0 % pollen specialists, 13.8 % nectar specialists, and 84.2 % generalists ($n=247$ bees).

Q1 and Q2: are foraging preferences established early in life and do they persist?

We conducted a repeated-measures analysis (REML algorithm) of the effects of first-day forager type (whether bees

collected pollen only, nectar only, or both nectar and pollen on their first foraging day), age, and colony on the lifetime P/TL ratio of individuals. There was no significant effect of colony ($F_{1,1912}=0.58, P=0.45$). The type of food that individuals chose on their *first day of foraging* significantly predicted their foraging choices for the remainder of their lives (Q1: effect of first-day forager type: $F_{2,137}=28.65, P<0.0001$; Fig. 2).

Individual preferences persisted over forager lifetimes (Q2). Individuals within each of the first-day forager types maintained significantly different P/TL ratios over time from the other two types (Tukey’s HSD_{REML} test, $P<0.05$; Fig. 2). However, their rate of foraging changed as they aged, depending upon their foraging preferences. There was a significant effect of bee age ($F_{1,4015}=286.66, P<0.0001$) and a significant

Fig. 2 Mean daily foraging behavior of bees categorized by their first foraging day preference (data from control A and B colonies pooled because of no significant colony effects for these measures, see the “Results” section). Means and standard error (SE) bars are shown

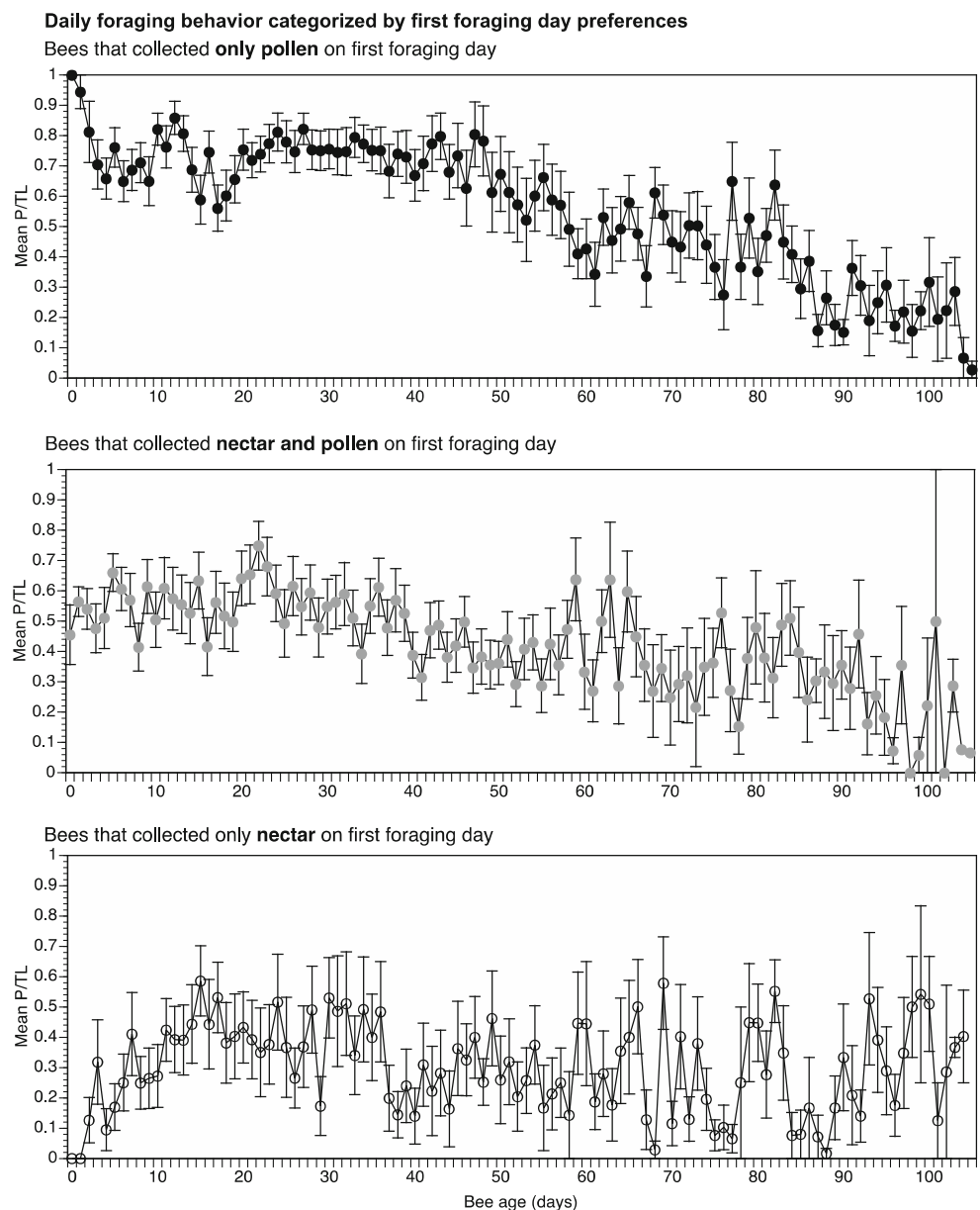
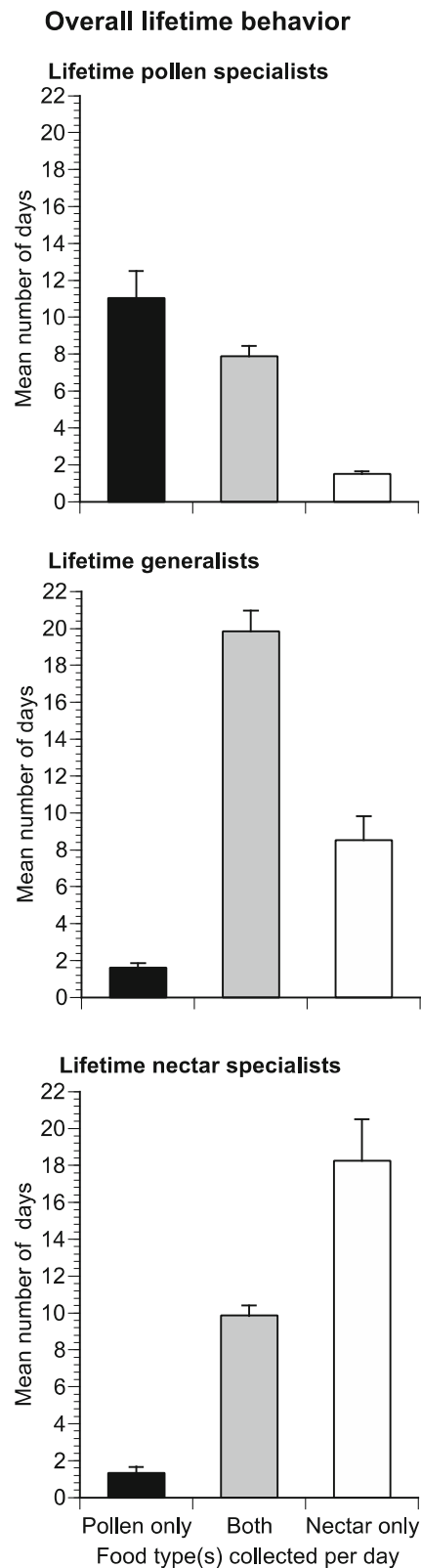


Fig. 3 Mean lifetime foraging behavior of bees classified by their lifetime foraging preferences (data from control A and B colonies pooled because of no significant colony effects for these measures, see the “Results” section). We determined what type of food each forager collected on each foraging day. For example, lifetime pollen specialists spent, on average, 11 days collecting only pollen, 8 days collecting pollen and nectar, and 1 day collecting only nectar. We monitored all foraging visits made by all foragers, but foragers did not collect food every day of their adult lives and usually did not begin food collection immediately after eclosion. Thus, average lifespan is greater than the average number of days spent foraging. Means and SE bars are shown

interaction of bee age \times first-day forager type ($F_{2,4010}=14.18$, $P<0.0001$). Inspection of the parameter estimates reveals that the first-day forager types behaved differently over time (Fig. 2). First-day pollen-only foragers decreased pollen foraging over time (-0.32 coefficient, t ratio= -15.71 , $P<0.0001$). First-day nectar-only foragers slightly, but significantly, increased their pollen foraging over time (0.15 coefficient, t ratio= 4.93 , $P<0.0001$). Bees that collected both nectar and pollen on their first foraging day showed no significant changes in foraging preferences over time (-0.04 coefficient, not significantly different from 0, t ratio= 4.93 , $P=0.20$; Fig. 2). All coefficients are based upon arcsine-transformed P/TL. The same trends are evident when one examines the daily foraging of individuals (whether they collected pollen, nectar, or both on each day that they foraged) according to their lifetime foraging type (Fig. 3). As expected, lifetime specialists spent more days collecting their specialty and generalists spent more days collecting both food types (Fig. 3).

Q3: does colony production of specialists change over time?

Over the life of the colony, production of some forager types (pollen specialists, nectar specialists, and generalists, based upon lifetime foraging preferences) changed. Overall, the number of generalists produced was significantly higher than the number pollen or nectar specialists (effect of forager type accounts for 61.5 % of model sum of squares, $F_{2,101}=14.79$, $P<0.0001$; more generalists than either of the two specialists, Tukey’s HSD, $Q=2.37878$, $P<0.05$). There was an effect of relative forager eclosion date (relative to colony establishment date, accounts for 9.4 % of model sum of squares, $F_{1,101}=4.53$, $P=0.036$) and a significant interaction of forager type \times relative forager eclosion date (accounts for 26.3 % of model sum of squares, $F_{2,101}=6.32$, $P=0.003$) on colony production of forager types. The overall model accounts for 32.2 % of variance in colony production of forager types. Parameter estimates reveal a decrease in colony production of pollen specialists (-0.03 coefficient, t ratio= -2.13 , $P=0.035$) and generalists (-0.05 coefficient, t ratio= -3.17 , $P=0.002$) over time. There was no change in nectar specialist production over time (0.003



coefficient, t ratio= 0.20 , $P=0.84$). All coefficients are based on a log-transformed number of bees. There was no significant effect of colony ($F_{1,101}=1.29$, $P=0.26$).

Q4: is there a relationship between worker body size and foraging behavior?

In this analysis, we classified individuals based upon their lifetime foraging preferences. We did not use virgin queens (defined as $IT > 5$ mm, based upon a distinct gap in our distribution of bee sizes) because our study focuses on workers. In control A and control B colonies, foraging bees were significantly larger (bigger IT) than nest bees ($F_{1,413} = 10.44$, $P = 0.0013$, accounts for 9.6 % of model sum of squares) and there was a significant colony effect ($F_{1,412} = 108.72$, $P < 0.0001$, accounts for 90.4 % of model sum of squares) because control A bees were larger than control B bees ($R^2 = 0.28$ for the overall model). However, foraging bees were consistently larger than nest bees in both colonies. Average forager and nest bee IT spans were 4.15 ± 0.05 and 3.97 ± 0.07 mm, respectively, for the control A colony ($R^2 = 0.03$, $F_{1,150} = 4.51$, $P = 0.035$) and 3.59 ± 0.04 and 3.23 ± 0.13 mm, respectively, for the control B colony ($R^2 = 0.03$, $F_{1,262} = 7.59$, $P = 0.006$). Foragers, therefore, had 4.5 and 11.1 % larger IT spans than nonforagers in control A and B colonies, respectively. In control A, pollen specialists were significantly smaller than generalists (overall: $R^2 = 0.06$, $F_{2,105} = 3.60$, $P = 0.03$; between forager types: Tukey's HSD, $Q = 2.37743$, $P < 0.05$; Fig. 4a). However, in control B, there were no significant size differences based on forager type (overall: $R^2 = 0.01$, $F_{2,242} = 1.90$, $P = 0.15$; Fig. 4b).

Q5: what is the effect of removing pollen specialists?

When pollen specialists were removed from the early removal colony (removal after 2 weeks, 47 bees, 25.3 % of the colony, brood present), generalists significantly increased pollen foraging (from $P/TL = 0.544 \pm 0.002$ to 0.743 ± 0.007 , $P = 0.0006$; Table 1). On average, generalists increased pollen foraging by 37 % in the removal phase as compared to the pre-removal phase. Subsequently, generalist bees significantly decreased pollen foraging after we returned the pollen specialists (from $P/TL = 0.743 \pm 0.007$ to 0.535 ± 0.008 , $P = 0.019$; Table 1, Fig. 5d) and switched back to their former levels of pollen foraging (generalists P/TL were not significantly different between pre-removal and post-removal phases, $P = 0.93$; Table 1). In contrast, control A, control B, and the removal control colony (equal proportion of foragers over all foraging preferences removed after 2 weeks, 81 bees, 25.3 % of the colony, brood present) generalists did not change their levels of pollen foraging over any of the same periods in their colony cycle ($P \geq 0.13$; Table 1, Fig. 5a–c).

We observed the same effects with the later removal colony (removal after 5 weeks, 11 bees, 10.1 % of the colony, brood absent, and reduced pollen foraging). After we removed pollen specialists, generalists significantly increased pollen foraging by 14 % (from $P/TL = 0.438 \pm 0.003$ to 0.501 ± 0.003 , $P = 0.007$; Table 1). After we returned pollen specialists,

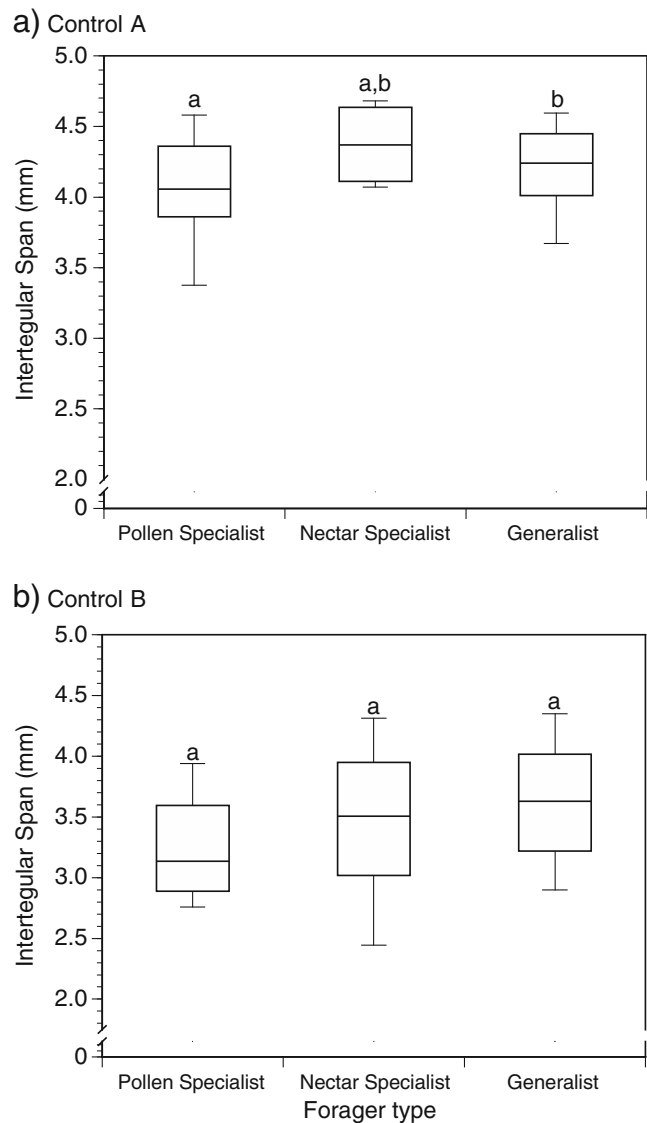


Fig. 4 a, b Box plots showing the average IT span for each foraging category: pollen specialist (≥ 90 % foraging visits to pollen; control A colony, $n = 30$ bees; control B, $n = 4$), nectar specialist (≥ 90 % nectar foraging visits; control A, $n = 4$; control B, $n = 34$), and generalist (all other bees; control A, $n = 74$; control B, $n = 207$). Different letters designate statistically significant differences ($P \leq 0.05$). Data from each colony is shown separately because there is a significant colony effect

generalists significantly decreased pollen foraging (from $P/TL = 0.501 \pm 0.003$ to 0.379 ± 0.002 , $P < 0.0001$; Table 1, Fig. 6c) and switched back to their former levels of pollen foraging. Generalists P/TL were not significantly different between pre-removal and post-removal phases ($P = 0.07$; Table 1). There was no significant change in pollen foraging by generalists in control A between corresponding later removal time intervals ($P = 0.47$; Table 1, Fig. 6a). However, in control B, pollen foraging by generalists significantly decreased during time intervals corresponding to pre-removal as compared to removal (from $P/TL = 0.560 \pm 0.001$ to 0.443 ± 0.002 , $P = 0.001$; Table 1) and then continued this same low

Table 1 Response of generalist foragers to pollen specialist (PS) removal

Colony	Pre-removal vs. removal		Pre-removal vs. post-removal		Removal vs. post-removal	
Early PS removal ^a						
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	$t_{115}=0.14$	$P=0.89$	$t_{115}=0.04$	$P=0.97$	$t_{115}=-0.09$	$P=0.93$
Removal control	$t_{14}=-0.83$	$P=0.42$	$t_{14}=-1.44$	$P=0.17$	$t_{14}=-1.62$	$P=0.13$
Early PS removal colony	$t_{25}=3.90$	$P=0.0006$	$t_{25}=-0.09$	$P=0.93$	$t_{25}=-2.52$	$P=0.019$
Later PS removal ^b						
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 removal colony	$t_{49}=2.83$	$P=0.007$	$t_{49}=-1.84$	$P=0.07$	$t_{49}=-4.84$	$P<0.0001$

Results of paired t tests comparing the pollen foraging proportions (P/TL =number of pollen foraging visits/total number of foraging visits) of generalists are shown. We did not remove any bees from control colonies A and B, but show tests results from these colonies to facilitate comparisons with colonies from which bees were removed

^a The effects of removing pollen specialists *early* in colony life. Time intervals are broken into pre-removal (week 0–2), removal (week 2–4), and post-removal (week 4–6)

^b The effect of removing pollen specialists *later* in colony life. Time intervals are broken into pre-removal (week 3–5), removal (week 5–7), and post-removal (week 7–9)

level of pollen foraging during the post-removal time interval ($P/TL=0.394\pm 0.001$, $P=0.10$; Table 1, Fig. 6b).

Discussion

By examining the complete lifetime foraging preferences of all foragers in multiple colonies, we determined that lifetime foraging specialization for pollen or nectar exists in bumble bees (Figs. 1 and 3), is determined early in life, and manifests as strong, significant, and consistent preferences from the first day of foraging (Fig. 2). As colonies senesced, they significantly decreased the production of generalists and pollen specialists, but not nectar specialists. Pollen foraging specialists tended to be smaller than generalists, but this was only significant for one colony. However, foragers were significantly larger than nonforagers, confirming Jandt and Dornhaus (2009). Removal of all pollen specialists in both removal treatment colonies (early removal with brood present and later removal with brood absent) resulted in significantly elevated levels of pollen foraging by generalists. These generalists then significantly decreased their pollen foraging, returning to former levels, when the pollen specialists were restored (Table 1, Figs. 5d and 6c). Thus, generalist foragers may be a reservoir of unspecialized foraging labor that can adapt to colony needs. All five colonies were run in series, not in parallel due to the time-intensive nature of the study, which requires nearly concurrent video determination of forager preferences for every forager on every foraging visit. This may have introduced time effects or increased variation in colony behavior.

Effect of forager body size

We confirmed (Jandt and Dornhaus 2009) that *B. impatiens* foragers were significantly larger compared to bees that only remained inside the nest (respectively 4.5 and 11.1 % larger IT spans in control A and B colonies). This finding agrees with studies on other bumble bee species (Brian 1952; Free 1955; Goulson et al. 2002; Spaethe and Weidenmüller 2002). However, our evidence for a relationship between forager size and foraging preferences was equivocal. In one colony, pollen specialists were significantly smaller, by 5.5 %, than generalists (control A, among all three forager types, $P=0.03$). For control B, there were no significant size differences between forager types (control B, $P=0.15$), although pollen specialists tended to be smaller. Further studies are needed.

Effect of removing pollen specialists

Behavioral plasticity of foraging and division of labor in Hymenoptera has been demonstrated with food and food store manipulations, nest disturbances, and the removal of colony individuals (ants, Wilson 1980; Wilson 1984; Gordon 1989; Dornhaus 2008; honeybees, Schulz et al. 1998; stingless bees, Hofstede and Sommeijer 2006; wasps, O'Donnell 1998). We examined whether the colony can adapt to changes in its foraging workforce. Generalists significantly increased pollen foraging after pollen specialists were removed, even when this subset of foragers comprised only 10 % of the colony. Due to time constraints, no additional replicates of the pollen specialist removal experiments were performed. Although replicates are needed for any concrete conclusions, we have found trends

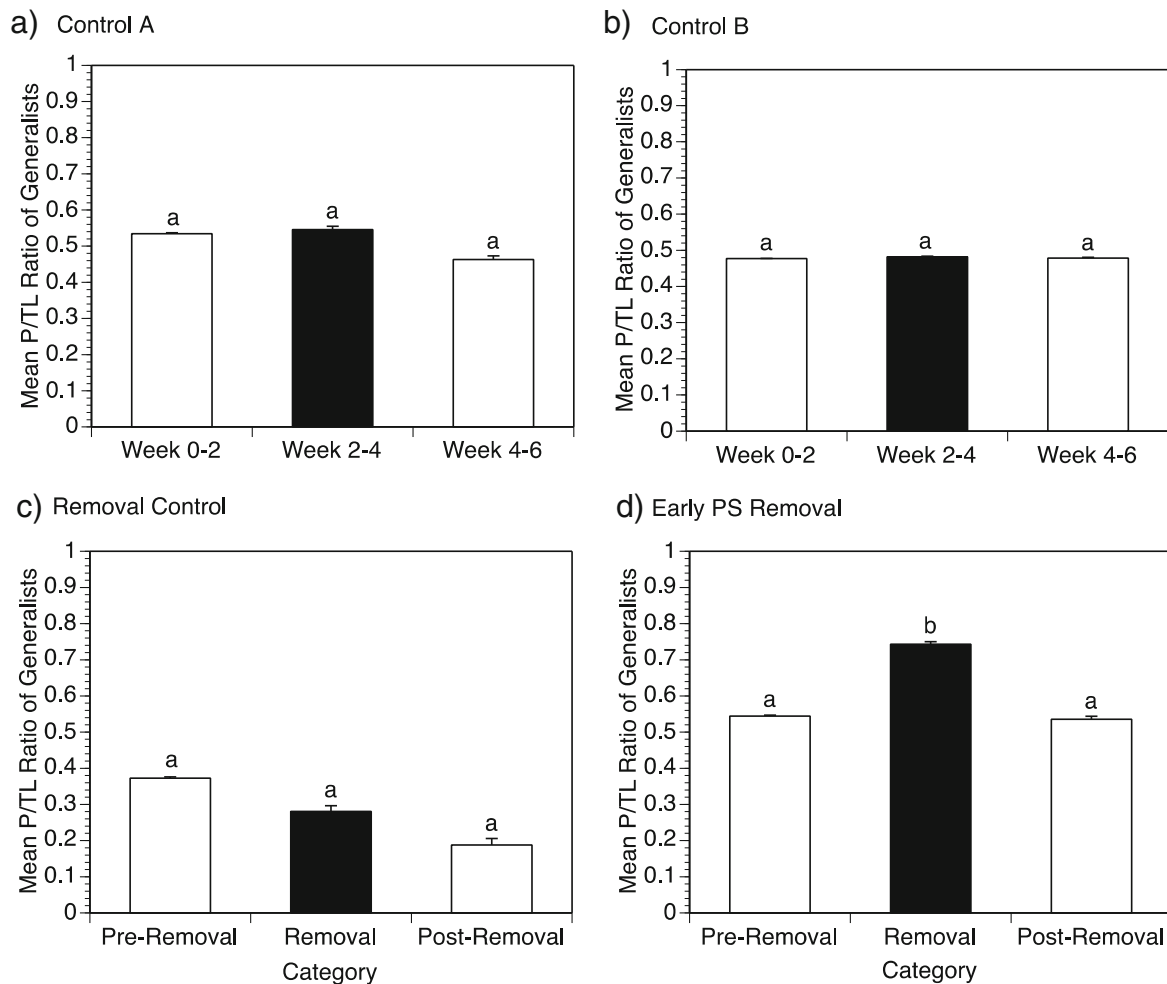


Fig. 5 Response of generalists to *early* pollen specialist (*PS*) removal. Histograms showing the mean (\pm SE) pollen foraging proportion (P/TL= number of pollen foraging visits/total number of foraging visits) of generalists in **a** control A, **b** control B, **c** removal control, and **d** early

PS removal colonies. The time intervals are broken into pre-removal (week 0–2), removal (week 2–4), and post-removal (week 4–6). The removal (week 2–4) time interval is shown in *black*. Different letters above each bar indicate significant differences ($P \leq 0.05$)

that may be used as direction and inspiration for future studies. In both early and later removal colonies, we found the same significant responses with generalists increasing pollen foraging in the absence of pollen specialists and returning to pre-removal pollen foraging levels once pollen specialists were reintroduced (Table 1, Figs. 5 and 6). The colony may have sensed the loss of pollen specialists through changes in pollen collection or pollen stores. Removal of pollen stores in *B. impatiens*, *B. flavifrons*, *B. melanopygus*, *B. mixtus*, *B. occidentalis*, *B. sitkensis*, and *B. terricola* increased colony foraging effort for pollen (Cartar and Dill 1990; Cartar 1992; Plowright et al. 1993; Kitaoka and Nieh 2009). In the removal control colony, we removed an equal proportion of foragers from all foraging categories. This manipulation should have resulted in an equal decrease of stored nectar and pollen. As expected, we found no increased preference for pollen or nectar foraging by generalists (Fig. 5c). We compared the removal control colony with the early removal colony because

we removed 25.3 % of foragers from each of these colonies. Only 10.1 % of foragers were removed from the later removal colony because there were fewer pollen specialists in this colony. Thus, the removal control and the later removal colonies cannot be compared.

Foraging specialization

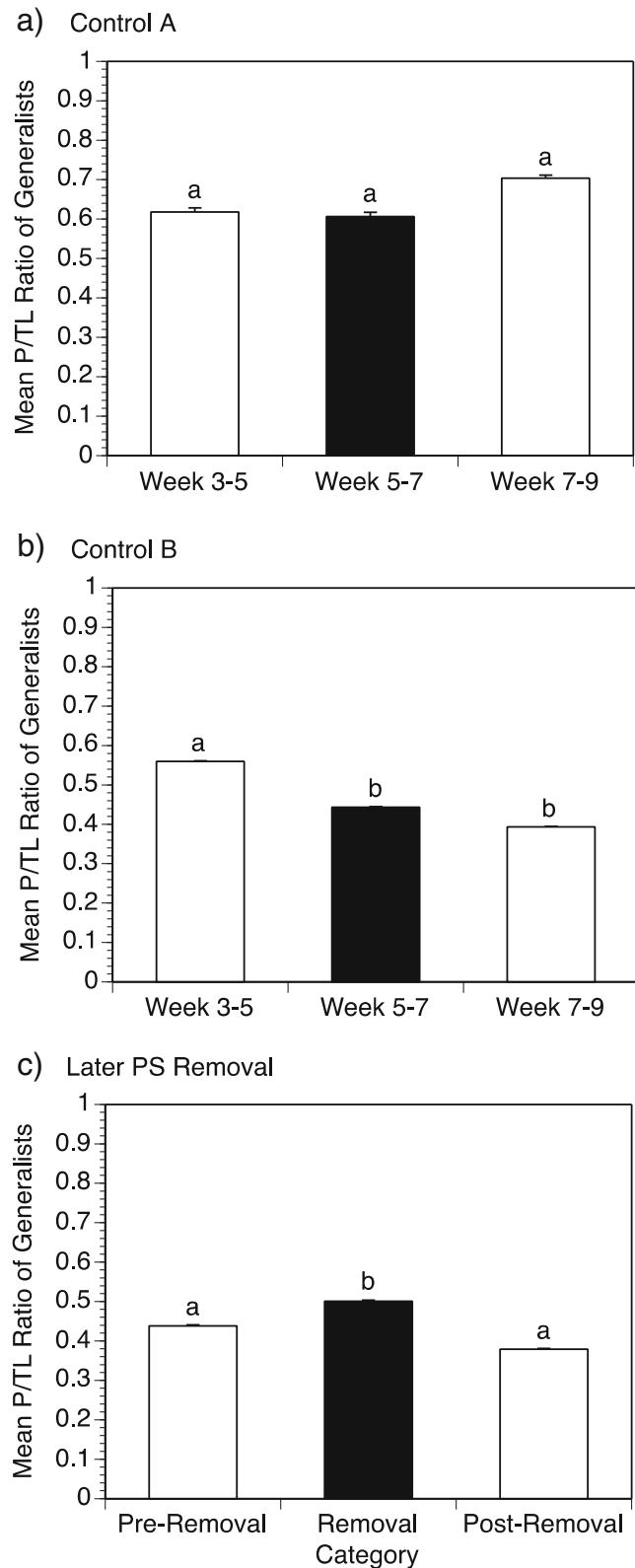
The individual foraging preferences that we found are consistent with a growing body of work demonstrating that bumble bee foragers exhibit consistent individual differences in a variety of behaviors (Weidenmüller 2003; Muller et al. 2010; Muller 2012). In our study, we found that lifetime foraging preferences were evident on the first day of foraging (Fig. 2). Bees that collected both nectar and pollen on their first foraging day did not alter their foraging preferences over time. However, first-day pollen-only foragers decreased their pollen foraging over their lives. Many pollen

Fig. 6 Response of generalists to *later* pollen specialist (PS) removal. Histograms showing the mean (\pm SE) pollen foraging proportion (P/TL=number of pollen foraging visits/total number of foraging visits) of generalists in **a** control A, **b** control B, and **c** later PS removal colonies. The time intervals are broken into pre-removal (week 3–5), removal (week 5–7), and post-removal (week 7–9). The removal (week 5–7) time interval is shown in *black*. Different letters above each bar indicate significant differences ($P \leq 0.05$)

specialists lived nearly the entire lifespan of the colony, and colonies typically require less pollen towards the end of their lives (Heinrich 1979). First-day nectar-only foragers slightly increased pollen foraging over time. The reason for this shift is unclear, but adult bumble bees still consume a small amount of pollen for their own needs (Smeets and Duchateau 2003) and first-day nectar-only foragers may have been foraging for their own needs as the supply of pollen inside the nest decreased over time.

There was no significant effect of colony on (Q1) the predictive power of first foraging day preferences (Fig. 2), (Q2) persistence of these foraging preferences over time, or (Q3) colony production of specialist types over time. However, the percentage of foragers that were specialists varied between colonies: 36 and 16 % of foragers specialized on either nectar or pollen in control A and control B colonies, respectively. The control A colony is within the range (30–40 %) reported by O'Donnell et al. (2000) for the proportion of specialists (nectar and pollen) in a colony of *B. bifarius nearcticus*. However, control B had fewer pollen specialists. Such intercolony variation has also been found in the wasp, *Polybia occidentalis*, with foragers that specialized on a type of foraging materials, either food or nest materials, ranging from 66 to 84 % of foragers (O'Donnell and Jeanne 1992). However, even a small proportion of specialists can still perform a valuable function. In the stingless bee, *Tetragonisca angustula*, guard bees are larger than workers, and although they comprised 1–2 % of the workforce, they significantly contribute to colony defense (Grüter et al. 2012).

Such foraging specialization may be genetically predetermined. In honeybees, there is a strong genetic component to pollen and nectar foraging preferences (Page et al. 1998). However, this does not prevent a bee colony from flexibly adapting to environmental changes (Figs. 5 and 6). For example, food shortages can accelerate behavioral development, causing honeybee workers to become foragers at a younger age (Schulz et al. 1998). In single-cohort colonies, honeybees that switch from nursing to foraging or vice versa have corresponding changes in their pattern of gene expression (Whitfield et al. 2003). The role of genetics in bumble bee foraging preferences and division of labor awaits detailed study. An environmental explanation is also possible: foragers may simply adopt a preference for whatever they first encounter. Other environmental influences such as the colony's nutritional needs and food storage levels may



influence individual foraging preferences, which are labile in generalists (Figs. 5 and 6). Thus, environmental influences and genetics may both affect lifetime foraging preferences and deserve detailed exploration.

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