



# Nectar source profitability influences individual foraging preferences for pollen and pollen-foraging activity of honeybee colonies

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## Abstract

Most foraging honeybees specialize in either pollen or nectar collection, although some do alternate between both resources. Little is known about this behavioral plasticity and the factors that control it. We studied how the profitability of nectar sources influences the transition of bees between nectar and pollen collection at the individual response level, by measuring the number of switches when the productivity of a sugar source (either sugar concentration or solution flow rate) was experimentally decreased or increased. At the social level, we studied whether the quality of the nectar that circulates inside a hive alters the rate of incoming pollen foragers. We then calculated the ratio between pollen and non-pollen foragers, before and after the hives were fed either a 3 or a 50% *w/w* sugar solution. In the first experiment, we showed that bees that persisted in visiting the feeder when offered low-quality solutions were more likely to switch to pollen than those foraging only on highly concentrated solutions. Looking at the collective responses, the ratio of pollen over non-pollen foragers increased after the input of a low-quality sugar solution and decreased after the input of a high-quality sugar solution. We conclude that the profitability of nectar sources interacts with the sugar responsiveness of bees, thus driving foraging preferences for pollen and modifying the pollen foraging activity of the colony. The results also show that bees integrate gustatory information from both rewarding resources based on local cues available either at the foraging site or inside the hive.

## Significance statement

Switching between resource types could be adaptive for honeybees that specialize in either nectar or pollen foraging. This would allow them to react to changes in the foraging environment. Although we observed that switching behavior is constrained by the responsiveness of bees to sugar, the switch from nectar to pollen (and vice versa) is an active decision of the bees in response to the decreasing or increasing profitability of pollen versus nectar sources. Given the ability of some bees to switch between foraging tasks based solely on gustatory cues available at the foraging site, we investigated whether source-related information conveyed inside the hive also affects colony foraging activity towards nectar and pollen resources. We observed that behavioral plasticity of individuals can be integrated into a social response by colonies reallocating their foraging forces according to the food-related information available inside the hive.

**Keywords** Foraging preferences · Pollen collection · Food source profitability · Sugar responsiveness · Task specialization

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## Introduction

Division of labor is an essential feature of insect societies and thought to be responsible for their ecological success (Wilson 1971, 1985; Oster and Wilson 1978; Page et al. 2006) as it enables different activities to be performed simultaneously by different groups of specialized individuals (Jeanne 1986; Oster and Wilson 1978). In honeybees, colony survival depends on an efficient collection of food sources, mainly protein and carbohydrates. This is achieved by the division of labor between pollen and nectar foragers (Page et al. 2006). The regulation of this division is still not well understood

but there is evidence that pollen and nectar foragers differ in how they perceive rewards—pollen foragers tend to have a lower response threshold towards sucrose rewards. Furthermore, predisposition for either nectar or pollen foraging is genetically determined (Robinson and Page 1989; Page et al. 1995, 1998). Greater sensitivity to gustatory stimuli such as sucrose or fatty acids might enable bees to properly assess a pollen resource (Arenas and Farina 2012, 2014). This ability might be hindered in foragers with lower sensitivity.

Some foragers can collect both resources from separate sources during a same foraging trip (Farrar 1944; Fewell and Page 1993). Very little is known about such behavioral plasticity and the factors that control it. Fewell and Page (1993) reported that approximately 24% of bees foraging pollen under high pollen quality conditions switched to nectar foraging when pollen was mixed with 75% brewer's yeast (i.e., low-quality condition). These results lead us to speculate that the transition between collecting pollen and collecting nectar is an active foraging decision made by the bees. It also suggests that foragers can assess food not just within a resource type but across them. This raises the question as to whether or not honeybee foragers can assess and integrate the relative quality of different rewards during a single foraging bout. We hypothesized that the profitability of the food sources interacts with the genetic predisposition of bees and ultimately drives foraging preferences towards either pollen or nectar. The result of this would be that bees visiting sources that offer low concentration nectars would be more likely to switch to pollen foraging than those visiting sources that offer more concentrated nectars. This would maximize the exploitation of the resources offered by the environment with the concomitant evolutionary advantages.

Even less is known about how the pollen foraging activity of the colonies is affected by fluctuations in the profitability of the nectar resource. It is known that colonies reduce the proportion of bees that collect from less profitable nectar sources and increase it for highly profitable ones (Seeley 1986, 1995; Seeley and Towne 1992). Profitability between nectar sources can be indirectly assessed by the time it takes a forager to unload its crop to a receiver bee (Seeley 1986, 1989). The rapid or slow attendance is controlled by the sucrose response threshold (SRT) of receiver bees, which is in turn modulated by the concentrations of the liquid food that circulates inside the colony. Modulation of sugar thresholds (Martinez and Farina 2008) might not only drive re-allocation between nectar sources of different quality but also between nectar and pollen sources. With increasing sugar responsiveness, we would expect more foragers to respond to low-quality nectars but also to pollen-related stimuli (Scheiner et al. 2004) and hence be more easily recruited or activated to forage on pollen sources. Colonies can also adjust their protein demand by

adjusting the number of pollen foragers, mainly by recruiting new foragers to the task and, to a lesser extent, by the transition of nectar foragers to pollen foraging (Rotjan et al. 2002).

Whether honeybees can modulate their foraging activity towards a particular resource type (either pollen or nectar) as a response to fluctuations in the availability or in the profitability of the other resource type (nectar or pollen) is unknown. Comparing the absolute nutritional value of both resource types might at first sight make no sense because both pollen and nectar provide complementary constituents of the diet (Winston 1987), colonies might still make foraging choices between rewarding types that fluctuate in their profitability based on the expectations of the bees (Gil et al. 2007).

We aimed to study how the surroundings influence both individual and collective pollen foraging responses from the dual perspective of behavioral ecology and sociobiology. At the individual level, we studied how fluctuations in nectar source profitability influence the choice between nectar and pollen food sources by foragers. We quantified the percentage of bees that switch collection between resource types. Switches were measured when the productivity of the nectar source (either sugar concentration or solution flow rate) was either decreased or increased experimentally, while the quality/availability of the pollen source remained unaltered (i.e., same amount of pollen and/or intensity of pollen-based cues; 2.1S). We expected the proportion of bees that switched to pollen gathering to increase in proportion to the sugar responsiveness of the foragers that persisted at the nectar feeder despite its ever lower profitability.

Our second aim was to study whether gustatory cues (in this case, sucrose concentration) in the incoming nectar influence foraging activity patterns. We first looked for variations in the rate of incoming pollen and non-pollen foragers before and after offering either a low-quality (3% w/w) or a high-quality sugar solution (50% w/w) inside the hives. We did so to recreate a situation in which the nectar of a suddenly available abundant source was incorporated into the hive. We reasoned that the input of a low-quality sugar solution would increase the ratio of pollen over non-pollen foragers whereas the input of a high-quality sugar solution would decrease such ratio. We also studied the impact of gustatory nectar cues on individual efforts of pollen foragers. Because pollen foragers can boost foraging activity by collecting heavier loads (Fewell and Winston 1992; Eckert et al. 1994) we estimated the weight of pollen loads in the hives fed with 3 and 50% w/w sugar solutions. Finally, we estimated the diversity (Hill 1973) of pollen samples trapped at the entrance of both groups of hives so as to analyze if gustatory information conveyed within the hive biases preferences among the pollen from different plant species available in the surrounding environment.

## Material and methods

### Study site, bees, and hives

The experiments were carried out during the summer seasons of 2016/2017 in the Experimental Field of the School of Exact and Natural Sciences of the University of Buenos Aires (34°32'S, 58°26'W). Colonies of European honeybees, *Apis mellifera*, were used in the different experiments. To obtain individual foraging responses, we trained bees from six different colonies settled in our apiary to visit artificial feeders that offered different resources according to the experiment. The feeders were located approximately 120 m from the hives.

To study the collective foraging response, we used 25 ten-frame Langstroth hives (15,000 worker bees), all containing a mated queen, 4–5 brood frames, and 1–2 frames with food reserves. All these hives were dosed with 500 ml of unscented sugar solution (either 3 or 50% w/w) by pouring it slowly inside the hives and over the central frames. The experiments complied with the animal care guidelines of the National Institute of Health (1985) and the current laws of Argentina.

### Individual foraging choices

#### Testing switching behavior from sugar to pollen feeders

In this experiment, we studied how fluctuations in sugar source profitability influence the decisions of bees to switch from sugar to pollen sources. We then investigated the percentage of foragers that, having been initially trained to visit a sugar feeder (see “Study site, bees and hives”), switched to pollen gathering as the profitability of the sugar source was experimentally impoverished. Switches from a group of color-marked bees were quantified through four concentration-decreasing tests (*T30%*, *T10%*, *T3%*, *T1%*; Fig. 1) in which an *ad libitum* pollen feeder was presented together with the sugar solution feeder. The pollen feeder (Petri dish of 9 cm in diameter) contained 7 g of crushed bee-collected pollen. The pollen and sugar feeders were located side by side at a foraging station that consisted of a wooden platform of 30 cm × 30 cm. Each testing event lasted 40 min. When necessary, the pollen feeder was refilled between consecutive tests so as to always offer the same amount of pollen. Pollen consumption during a 40-min period was never higher than 0.5 g, which suggests little or no variation in the intensity of pollen-based stimuli which could influence switches between resources.

Before the onset of the first test, honeybees previously trained to visit the foraging station (see “Study site, bees and hives”), were reactivated to a sugar feeder offering 30% w/w sucrose solution (Fig. 1). Afterwards, they were color marked on the thorax or abdomen with a hard-bristle brush and acrylic paint (Fig. 1). Marked bees formed the group of foragers that were observed for switching behavior during the four

consecutive tests. Foragers that switched to pollen collection during tests were captured as soon as their baskets revealed incipient loads. Once captured, bees were anesthetized at – 5 °C and checked for color marks to confirm that they did belong to the initial group of bees and had switched from the sugar feeder.

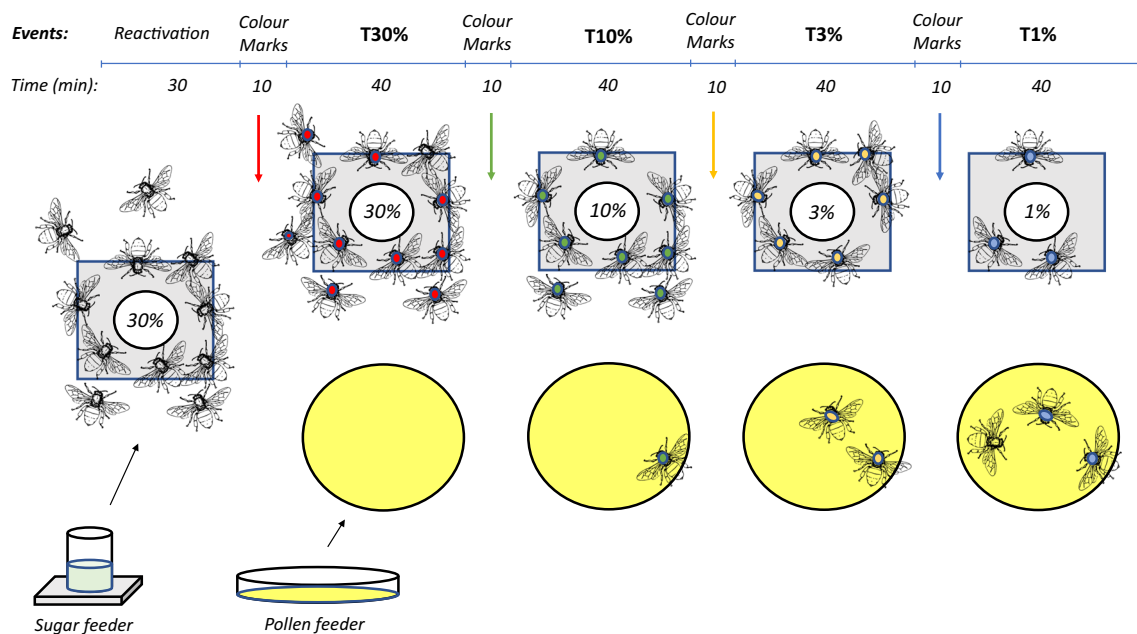
During the first tests (*T30%*), the sugar feeder offered a 30% w/w solution. Once the test was complete, we removed the pollen feeder and replaced the content of the sugar feeder with a 10% w/w sugar solution. Before starting the second test (*T10%*), and for the following 10 min (Fig. 1), we re-labeled (with a second color) those bees that had already been marked at the beginning of the experiment (30%) in order to know how many bees kept foraging under the new reward condition (10%). Once the 10% sugar foraging group was established, the pollen feeder was offered again for the second test (*T10%*). Switches were evaluated in the same manner in the two following tests (*T3%* and *T1%*). The data collected allowed us to calculate (i) the percentage of foragers that abandoned the sugar feeder during each test and (ii) the percentage of foragers that switched to pollen gathering according to the concentration offered at the sugar feeder. The whole procedure (i.e., training a new group of foragers, reactivating, marking, and testing them; Fig. 1) was repeated 8 times to get a mean response for the switching behavior.

In a second experiment, we investigated the transition from nectar to pollen when the profitability of the sugar source was reduced by decreasing the flow rate of the feeder. Switches were evaluated as before, throughout four tests in which the sugar rate-feeder that offered a 30% w/w sugar solution at 5, 2, 0.5, and 0.1 µl/min (*T5*, *T2*, *T0.5*, and *T0.1*) was simultaneously presented with an *ad libitum* pollen feeder. Each test lasted 40 min. Periods of 10 min were established between two consecutive tests to relabel bees that remained foraging in the sugar feeder despite the decreasing profitability.

### Collective foraging response

#### Rate of incoming foragers according to the quality of the sugar solution offered inside the hive

Our aim was to evaluate whether the quality of nectar that circulates inside the hive alters the incoming rate of pollen foragers. We measured the number of incoming bees at the entrance of the hives as an indicator of colony foraging activity. Bee arrivals were recorded for 5 min with a video camera (SONY digital camera) at the entrance of 12 hives (between 10:30 and 11:30 a.m.). Each hive was recorded immediately before and 45 min after being fed either a 3% (6 hives) or a 50% w/w sucrose solution (6 hives). To recreate a situation in which the nectar of an available abundant source was suddenly incorporated into the hive, 500 ml of sugar solution was gently poured over the central frames of each hive. Pollen



**Fig. 1** Schematic schedule of one replicate of experiment 1. A group of foraging workers was first reactivated to visit a feeder offering 30% w/w sugar solution for 30 min. During the following 10 min, they were color marked. Switching behavior of marked bees was evaluated in dual choice tests, where the bees chose to continue foraging from the sugar feeder, switch to the pollen feeders, or abandon the foraging station. Switching behavior was evaluated through four tests (*T30%*, *T10%*, *T3%*, and *T1%*)

foragers were identified as the forager bees that carried pollen loads on their hind legs. Incoming bees without pollen loads were identified as non-pollen foragers (presumably nectar foragers). Rates of pollen and non-pollen incoming bees were calculated from the films (5 min) before and after the offering of sugar solution. Based on these data we first calculated the ratio between pollen and non-pollen foragers and then the “delta” ( $\Delta$ ) for each group before and after the offering of the sugar solutions.

#### Weight of loads and diversity of trapped pollen samples according to the quality of the sugar solution offered inside the hive

With the aim of studying the impact of nectar related cues on individual efforts of pollen foragers, we weighted pollen loads as a measure of the amount of pollen collected during single foraging bouts. Pollen loads were sampled for 1 h using conventional pollen traps fixed at the entrances of 13 hives. Traps consisted of a wooden structure with a removable metal mesh inside and were mounted between 10:30 and 11:30 a.m. for two consecutive days. Six of the 13 hives were fed 500 ml of a 3% w/w sugar solution on the first day and 500 ml of a 50% w/w sugar solution on the second day. The other seven hives were fed a 50% w/w sugar solution on the first day and a 3% w/w sugar solution on the second day. Each colony was

during which the concentration of sugar solution offered at the feeder decreased from 30% up to 1% w/w. The amount of pollen at the pollen feeder remained constant. Bees that switched were captured and those that kept visiting the sugar feeder were relabeled with different colors before the onset of following test. This procedure was repeated eight times on different days and with different bees

sampled twice. First, 45 min after being fed on the first day and then 45 min after it was fed on the second day. We weighted 10 sample units per hive, where each sample unit was defined as the weight of 10 pollen loads, using a Mettler Toledo AG 285 balance. This represented a large share of the trapped loads for most of the hives.

Because nectar-related information circulating within the colony might influence foraging preferences of different types of pollen, for example by altering the perception of the bees to pollen-related compounds and/or inducing recruitment towards a particular pollen source, we studied the diversity of pollen species trapped at the entrance of the hives. To this end, we used the same pollen samples that we used to estimate the weight of the loads (13 hives). Trapped pollen loads were separated according to the different species from which they had been collected. Separation was based on their color and the knowledge of the plants that were blooming at that time. On average, samples contained  $124.38 \pm 11.74$  loads and were identified as having been foraged from up to 6 different plant species. At least 4 pollen types could be easily separated based on their colors. Two pollen types differed in their yellow tones and approximately 10–12% of the yellow loads could not be separated accurately by the naked eye. These loads were processed according to Kearns and Inouye (1993) and identified under a Labomed microscope CXR III. The diversity of pollen samples was estimated based on Shannon’s index (Hill 1973).



## Statistics

The effects of the individual foraging choices were assessed by means of generalized linear mixed models (GLMM; McCullagh and Nelder 1989; Bolker et al. 2009) with binomial distribution. Models were fitted in R (R Development Core Team 2011) using the `glmer` function of the “lme4” package (Bates et al. 2011; R Development Core Team 2011) in which random effects were specified via the model formula. The lme4 package (`glmer` function) uses Wald Z-tests to approximate significant effects and *P* values for GLMMs (Bolker et al. 2009). Binomial distributions were used for proportion data (Crawley 2007). We checked for overdispersion by evaluating whether the dispersion parameter (residual deviance/df) was larger than 1 (Zuur et al. 2009).

Collective responses were assessed by means of a linear mixed-effects model (LMM) with normal distribution. We checked for homoscedasticity and normality assumptions (Levene and Shapiro–Wilk tests, respectively). We also inspected residual plots and verified that the model residuals are not deviating from normality and homoscedasticity. Tukey’s test was used for comparisons using the “lsmeans” package (Lenth 2016).

## Data availability

All data generated or analyzed during this study are included in this published article (and its [supplementary information files](#)).

## Results

### Individual foraging choices

#### Testing switching behavior from sugar to pollen feeders

The percentage of foragers that switched from the sugar feeder to the pollen feeder steadily increased as the concentration of the sugar solution or the solution flow rate decreased. In the experiment where the concentration of the sugar solution was varied, the number of foragers that kept visiting the foraging station steadily declined throughout the tests. The average number of bees at the station decreased from  $97.63 \pm 1.25$  when the feeder offered a 30% w/w sugar solution (*T30%*) to  $81.38 \pm 5.94$  at *T10%*,  $43.88 \pm 3.41$  at *T3%*, and finally to  $24.50 \pm 2.65$  at *T1%*. The number of bees that switched was very low at *T30%* and *T10%*, with no statistical differences between them (Tukey’s test; *T30%* vs. *T10%*:  $Z = 1.973$ ,  $p = 0.1983$ ; Fig. 2a). On the contrary, bees switched to the pollen feeder more often for the lowest concentrations. As a result, the percentage of switches significantly increased in *T3%* and *T1%*, as depicted by the following pairwise contrasts: Tukey’s

test; *T1%* vs. *T3%*:  $Z = 4.106$ ,  $p = 0.0002$ ; *T1%* vs. *T10%*:  $Z = 6.500$ ,  $p < 0.0001$ ; *T1%* vs. *T30%*:  $Z = 5.731$ ,  $p < 0.0001$ ; *T3%* vs. *T10%*:  $Z = 2.976$ ,  $p = 0.0155$ ; *T3%* vs. *T30%*:  $Z = 3.812$ ,  $p = 0.0008$ ; Fig. 2a. Results indicate that bees that persisted at the foraging station when it offered low-quality solutions were more likely to switch than those visiting the station when it offered high-quality solutions.

In the experiment in which we varied the solution flow rate, we found a response pattern similar to that of the previous experiment: the slower the feeder flow rate, the higher the proportion of bees that switched between resources (Fig. 2b). Interestingly, the percentage of bees that switched to pollen sources was much higher than for the *ad libitum* feeders (Fig. 2). As expected, the average number of bees at the station decreased from  $12.4 \pm 1.6$  for 5  $\mu\text{l}/\text{min}$  to  $10.0 \pm 1.1$  for 2  $\mu\text{l}/\text{min}$ ,  $6.3 \pm 1.8$  for 0.5  $\mu\text{l}/\text{min}$ , and  $1.4 \pm 0.5$  for 0.1  $\mu\text{l}/\text{min}$ . However, the number of bees that switched remained constant throughout the tests. Hence, the percentage of switches increased according to the decreasing flow rates (Tukey’s test; *T5* vs. *T2*:  $Z = 1.842$ ,  $p = 0.253$ ; *T5* vs. *T0.5*:  $Z = 4.048$ ,  $p = 0.0003$ ; *T0.5* vs. *T2*:  $Z = 4.048$ ,  $p = 0.0003$ ; *T0.1* vs. *T5*:  $Z = 4.077$ ,  $p = 0.0003$ ; *T0.1* vs. *T2*:  $Z = 3.396$ ,  $p = 0.0038$ ; *T0.1* vs. *T5*:  $Z = 2.484$ ,  $p = 0.0624$ ; Fig. 2b). Taken together, results show that bees that kept on foraging despite the reduction of concentration or flow rate of the sugar feeder were more prone to switch to the pollen feeder. More importantly, results indicate that honeybees were capable of switching not just within but across resource types.

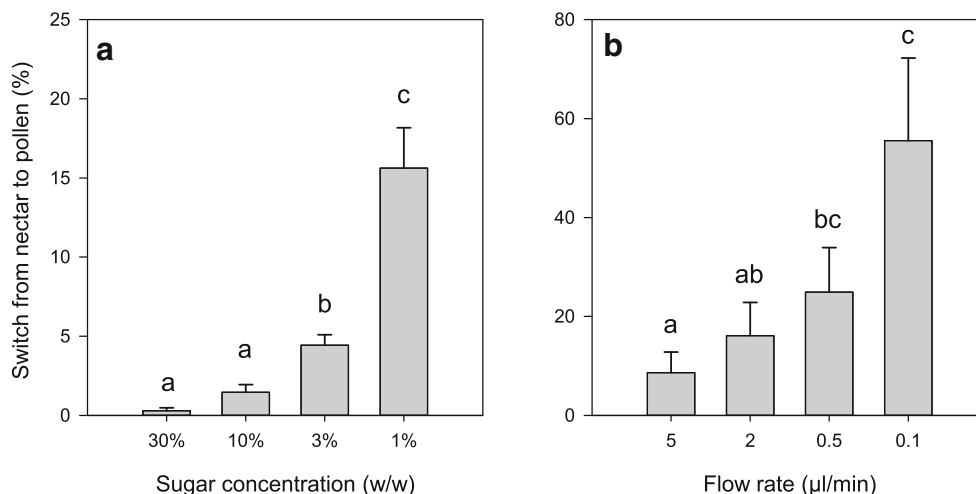
## Collective foraging response

### Rate of incoming foragers according to the quality of the sugar solution offered inside the hive

The ratio of incoming pollen forages over non-pollen foragers increased after the 3% w/w solution was offered. This ratio decreased in colonies fed a 50% w/w sugar solution. Analysis shows that colony foraging activity was affected by the interaction between the *test* and the *concentration* of the sugar solution offered (*test\*concentration*:  $F_{1,10} = 108.9692$ ,  $p < 0.0001$ ; Fig. 3).

Initial foraging ratios between pollen-loaded versus non-pollen-loaded incoming bees were similar in both groups of hives (0.16 for the 3% group and 0.21 for the 50% group) and were not statistically different (simple effects *T0*, 3% vs. *T0*, 50%:  $Z = 1.147$ ,  $p = 0.626$ ; Fig. 4), confirming that hives assigned to each treatment exhibited similar foraging patterns. Interestingly, foraging ratios changed after the sugar solutions were offered. Ratios increased to 0.268 in the 3% group and decreased to 0.120 in the 50% group (simple effects *T1*, 3% vs. *T1*, 50%:  $Z = -5.047$ ,  $p < 0.001$ ; Fig. 4). Within each group of hives, differences were also detected before and after the input of sugar solution (Simple effects *T0*, 3% vs. *T1*, 3%:  $Z =$

**Fig. 2** Switching behavior from sugar to pollen feeders. **a** Percentage of labeled honeybees that changed their foraging preferences to pollen throughout four concentration-decreasing tests. **b** Percentage of labeled honeybees that changed their foraging preferences to pollen when a 30% w/w sugar solution was supplied at decreasing flow rates. Bars show medians ± SE of eight independent groups of bees in each panel. Different letters indicate statistically significant differences ( $p < 0.05$ ; Tukey's test)



7.027,  $p < 0.001$ ;  $T0$ , 50% vs.  $T1$ , 50%:  $Z = 7.736$ ,  $p < 0.001$ ; Fig. 4). In a very similar experiment (2.2S; Fig. 2S) in which we included a group of control hives (hives that were not fed), no differences were detected between the first ( $T0$ ) and the second test ( $T1$ ), suggesting little or no variations in ratios measured at different times of the day. Altogether, our findings indicate that foraging activity patterns were affected by the quality of the liquid food we provided.

To address if changes in foraging activity patterns were due to fluctuations in the activity of pollen foragers, nectar foragers, or both, we compared  $\Delta$  ( $T1 - T0$ ) of each group of bees for colonies that were given a 3% w/w sugar solution against colonies that received a 50% w/w sugar solution. Results show that both groups of foragers contributed to modifying the ratio of pollen over non-pollen incoming bees (Fig. 3). On the one hand, the  $\Delta$  of pollen foragers recorded in hives fed a 3% sugar solution was higher than in hives fed a 50% sugar solution (concentration:  $F_{1,10} = 5.0161$ ,  $p = 0.049$ ; Fig. 4a). We also found differences for non-pollen incomers.  $\Delta$ s from hives

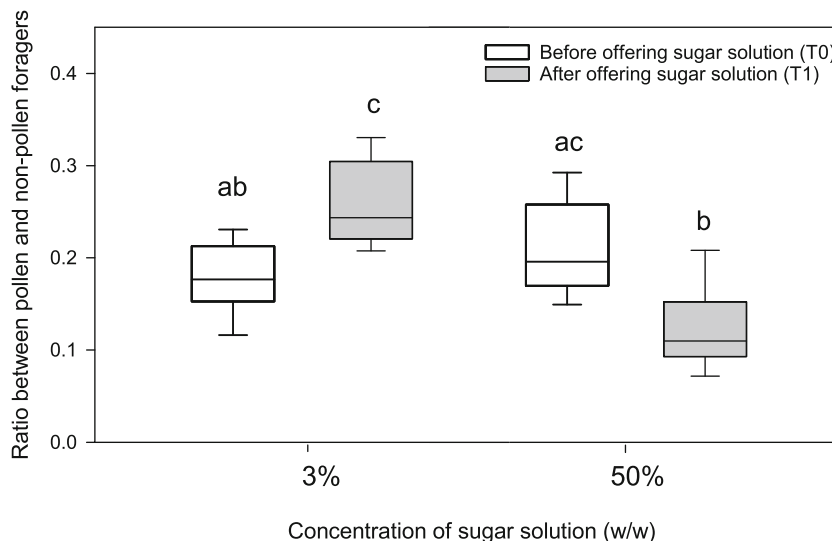
fed a 3% sugar solution were lower than the  $\Delta$ s from hives fed a 50% sugar solution (concentration:  $F_{1,10} = 19.717$ ,  $p = 0.001$ ; Fig. 4b).

**Weight of loads and diversity of trapped pollen samples according to the quality of the sugar solution offered inside the hive**

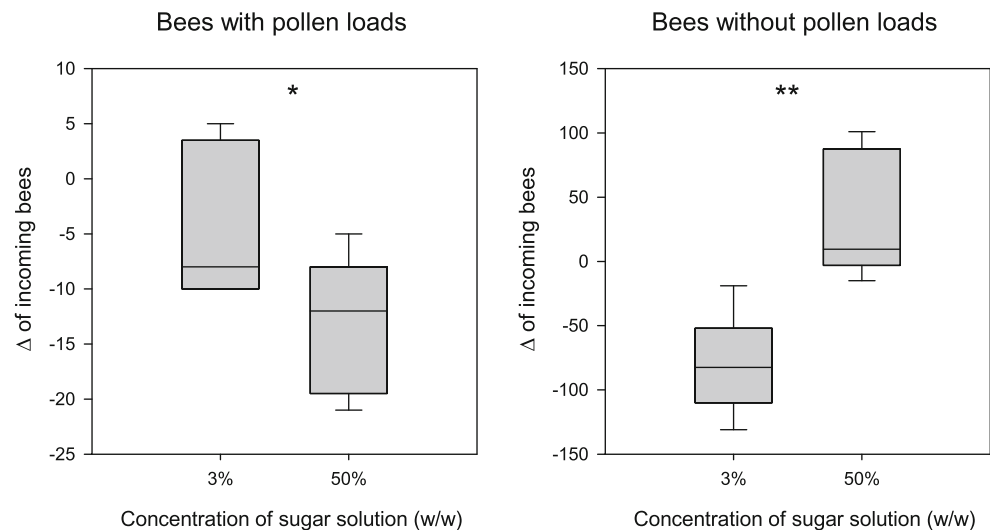
Results show that individual efforts of pollen foragers were not modulated by the offering of sugar solutions of different concentrations (Table 1). The weights of collected loads were independent of the day when the sample was trapped (day:  $t = 0.001$ ,  $p = 0.9987$ ), the quality of the solution offered (concentration:  $t = 0.714$ ,  $p = 0.482$ ), and the interaction between these two factors (day\*concentration:  $t = 0.533$ ,  $p = 0.599$ ).

The diversity of pollen types (i.e., loads from different plant species) neither differed with the sampling day (day:  $t = -0.078$ ,  $p = 0.938$ ) nor with the quality of the solution offered

**Fig. 3** Ratio of incoming pollen foragers over incoming non-pollen foragers according to the quality of the sugar solution offered inside the hive. Ratio of incoming foragers with and without pollen loads measured before ( $T0$ ) and after ( $T1$ ) 3 and 50% w/w sugar solutions were offered into the nests. Box plots show medians, quartiles, and 5th and 95th percentiles from 12 hives. Different letters indicate statistically significant differences among ratios ( $p < 0.001$ ; simple effects)



**Fig. 4**  $\Delta$  (final rate minus initial rate) of incoming bees according to the quality of the sugar solution offered inside the hive.  $\Delta$  of incoming bees with (a) and without (b) pollen loads obtained from colonies fed 3 and 50% w/w sugar solutions. Box plots show medians, quartiles, and 5th and 95th percentiles from 12 hives. Asterisks indicate statistical differences (\* $p < 0.05$ ; \*\* $p < 0.01$ )



(concentration:  $t = 1.430$ ,  $p = 0.166$ ; Table 1). The interaction of these factors was also not significant (day\*concentration:  $t = 0.533$ ,  $p = 0.599$ ). The lack of differences in the diversity of the samples indicates that foraging preferences for any particular type of pollen was not altered by the quality of the gustatory input.

## Discussion

This study addresses the influence of food source profitability on pollen foraging responses. At the individual level, we observed that the higher the sugar responsiveness of the foragers, the higher the rate of bees that switched to pollen gathering. Similarly, more bees foraging on pollen switched to nectar as the side-feeder offered sugar solutions of increasing concentrations (Fig. 1S). These results lead us to conclude that the bees we consider “specialists” are actually capable of switching between resource types and that this switch is influenced by the interaction between the sugar responsiveness of the bees and the foraging environment. At the colony level, we noted that the ratio of pollen to non-pollen incoming bees increased when we recreated a scenario in which only low-quality nectar sources were available in the environment. When we offered a more concentrated sugar solution, indicating the availability of high-quality nectar sources, the ratio declined. Changes in ratios were due to variations in both nectar and pollen foraging activity, demonstrating that colony response was affected by the gustatory information within a particular resource type and also across different types.

### Switching behavior and sugar responsiveness

Foraging efficiency of social insects is commonly related to an improved task performance due to specialization (Wilson, 1971, 1985; Oster and Wilson 1978). Task specialization

between nectar and pollen foragers correlates well with the sensitivity to gustatory stimuli, including sucrose (Pankiw and Page 2000). Our results show differences in the responsiveness of the foragers tested and indicate that switching behavior is positively correlated with it. During every single test, there were foragers that abandoned the food source (returned to the hive or explored alternative sources elsewhere) as they detected that the solution profitability had fallen below a minimum threshold of acceptance. There was also a group of bees that persisted at the foraging site despite the decreasing food quality, possibly because their sugar thresholds had not been exceeded. Bees were more likely to switch in tests with lower concentrations. This indicates that in bees that switch, the expectancy of a reward is not associated with a unique resource and that bees can fulfill it within a range of resources from those providing energy (i.e., nectar) to those that are not even consumed by foragers (i.e., pollen). The transition between resource types indicates that bees can integrate gustatory/olfactory information from both rewarding stimuli based on local cues available at the foraging station. These findings are consistent with previous studies (see Scheiner et al. 2004) and suggest that transitions are carried out by bees with very low sensory thresholds. Furthermore, the transition from nectar to pollen might share the same physiological bases that enable water collection for temperature regulation of the nest (Lindauer 1955; Núñez 1979), regardless of the differences in how solid and liquid resources are collected.

### Switch behavior in ad libitum vs. rate feeders

Switches from nectar to pollen exhibited a similar profile when evaluated in *ad libitum* nectar feeders versus a feeder in which the flow rate of the nectar solution was regulated. However, foragers were approximately three times more likely to switch tasks in the rate-feeder than in the *ad libitum*

**Table 1** Weight of pollen loads and diversity of trapped pollen samples according to the sugar solution quality offered inside the hive

	3%	50%	<i>p</i> value
Weight of 10 the pollen loads (mg)	0.052 ± 0.001	0.050 ± 0.002	0.4824
Shannon's diversity index	2.025 ± 0.010	1.983 ± 0.013	0.1666

feeder. Artificial feeders regulating the flow rates mimic natural sources better, as they can both qualitatively and quantitatively regulate the reward offered (Núñez 1977). At first glance, differences among feeders suggest that the 1% sugar solution at an unlimited flow rate (*ad libitum*) was valued higher by bees than the 30% sugar solution offered at a 0.1 µl/min flow rate, which is indeed very low (Grosclaude and Núñez 1998). Thus, under natural foraging conditions, we might expect transitions between reward types higher than 15–20%, likely up to 55–60%. Apart from the simplicity of controlling the profitability of *ad libitum* sugar feeders by means of the sugar concentration, our results highlight the importance of using rate-feeders when trying to understand how bees respond in natural environments.

### Do bees integrate information of both the carbohydrate and the protein status of the colony?

We observed that colonies increased pollen foraging activity when the quality of the incoming nectar was low but decreased it when the quality was high, indicating that honeybee colonies modulate collection of pollen in response to fluctuations in the profitability of the nectar that circulates inside the hive. Little is known yet about the mechanisms by which colonies re-allocate foragers between pollen and nectar sources in response to changes in the foraging environment. It can be argued that the redistribution of the foraging force among resources of different types requires bees to integrate information about the carbohydrate and the protein status of the colony. Up to now, the mechanisms involved in the assessment of the protein and sugar needs of a colony were thought to operate quite independently from each other (Moeller 1961; Seeley 1995). Nectar-foraging behavior is likely affected by the quality of the nectar that circulates through trophallaxis among nest mates and, to a lesser extent, by cues directly provided by nectar stores (Seeley 1985). In contrast, pollen foraging seems to be regulated by a direct assessment of the quantities of young brood and pollen supplies, as pollen foragers pack their own loads into cells near the brood (Camazine 1993). Empty cells might stimulate pollen foraging, whereas the absence of empty combs may reduce pollen-foraging efforts (Dreller et al. 1999).

Camazine (1993) proposed that foragers also assess pollen requirements through social interactions with nurse bees. Nurses, who convert pollen into glandular secretions to feed

the larvae and also interact with foragers through trophallaxis (Crailsheim 1998; Hrassnigg and Crailsheim 1998), share the surplus of glandular proteins with foragers, which in turn could inhibit pollen collection. If this is the case, foragers could integrate information of both carbohydrate and protein needs from small samples of food shared by nurses.

Alternatively, it is plausible that changes in pollen foraging activity were a side effect of bees adjusting their sugar responsiveness according to the quality of the nectar offered (Pankiw et al. 2001; Pankiw et al. 2004; Martinez and Farina 2008). As expected, SRT of in-hive bees increased after the high-quality sugar solution was offered but decreased in bees from hives fed low-quality sugar solution (Fig. 3S). In a scenario of low-quality nectar input, workers could be discouraged from initiating or resuming nectar-foraging bouts as, according to information transferred inside the nest, sources currently available would be unproductive. Unemployed foragers previously engaged in nectar collection and with low sucrose thresholds (Fig. 3S) could, in turn, be more responsive to pollen-related stimuli. This speculation is supported by both the decrease in the rate of non-pollen foragers and the increase in the rate of pollen foragers after the 3% w/w sugar solution was offered. In the opposite situation (after the 50% w/w sugar solution was offered), naïve or unemployed experienced foragers might initiate/resume foraging for carbohydrates if gustatory cues inside the nest indicate the sudden appearance of a high-quality nectar source. Hence, foragers engaged in pollen collection might switch to nectar and as we show that such behavioral plasticity can occur solely as a result of the increasing concentration of the sugar solution at the foraging site.

### Pollen foraging: individual efforts vs. amount of foragers

Variations in effort by pollen foragers have been documented as the strongest response against an increasing protein demand (Lindauer 1952; Fewell and Winston 1992; Eckert et al. 1994). However, our results show that this response was not relevant under conditions of no or moderate stress. Individual foraging efforts quantified as the weight of loads collected by the bees in colonies fed either a 3% or a 50% w/w sugar solution did not show any differences. Instead, our colonies responded to changing foraging environments by modulating the foraging rates. As rates were calculated during 5 min-periods (bouts need longer times to be completed), differences among treatments cannot be explained by having counted the



same bees twice within a single measurement. Hence, our colonies modulated the number of foragers, most likely by recruiting non-foragers to the foraging tasks and/or by re-allocating foragers that switched between resource types. Therefore, we speculate that foraging response patterns are primarily modulated by foraging environment, whereas variations in individual efforts are mainly induced by nutritional requirements of the colony. Yet, more experiments are needed to confirm that the two responses are controlled by different stimuli.

### Do sugar thresholds affect preferences for different pollen species?

Pollen can strongly differ in chemical composition (such as proteins, amino acids, carbohydrates, lipids and fatty acids, phenolic compounds, enzymes, and coenzymes as well as vitamins and bioelements) according to its botanical origin (Herbert and Shimanuki 1978; Conti et al. 2016). Unfortunately, very little is known about the factors that drive the preferences of bees for different types of pollen (Percival 1955; Free 1963, 1964; Nye and Mackensen 1965; Hanley et al. 2008; Pernal and Currie 2002). However, it would be expected for such composition to affect foraging choices. It might be argued that selection among different pollen species could also be affected by the gustatory responsiveness of foragers, in a similar manner to how foragers choose among nectar sources of different composition. The perception of certain biologically active substances in pollen might determine allocation of foragers among sources of different botanical origin. However, in our experiments, the different inputs of gustatory information did not alter the diversity of the pollen collected: Shannon's diversity index of pollen samples demonstrated that preferences for the different pollen species were very similar between hives fed 3 and 50% w/w sugar solutions (Table 1). More studies are needed to understand how bees select sources of different pollen types.

Collective response patterns of social insects result from decisions made by individual workers that respond to local informational cues within their behavioral constraints. By showing foragers that specialize on different foraging tasks according to differences in their sensitivity, we provide a new case in which information available both at the foraging site and inside the hive is essential to integrate the behavioral plasticity of individuals into a social response.

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