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**By**

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**מרכז לחקר הרציונליות**

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# **The use of numerical information by bees in foraging tasks**

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## ABSTRACT

The ability of invertebrates to perform complex cognitive tasks is widely debated. Bees utilize the number of landmarks en-route to their destination as cues for navigation, but their use of numerical information in other contexts has not been studied. Numerical regularity in the spatial distribution of food occurs naturally in some flowers, which contain a fixed number of nectaries. Bees that collect nectar from such flowers are expected to increase their foraging efficiency by avoiding return visits to empty nectaries. This can be achieved if bees base their flower-departure decisions on the number of nectaries they had already visited, or on other sources of information that co-vary with this number.

We tested, through field observations and laboratory experiments, whether bees adapt their departure behavior to the number of available food resources. Video-recorded observations of bumblebees that visited *Alcea setosa* flowers with five nectaries revealed that the conditional probability of flower departure after five probings was 93%. Visit duration, the spatial attributes of the flowers and scent marks could be excluded as flower-leaving cues, while the volume of nectar collected may have guided part of the departure decisions. In the laboratory the bees foraged on two patches, each with three computer-controlled feeders, but could receive only up to two sucrose-solution rewards in each patch visit. The foragers gradually increased their tendency to leave the patches after the second reward, while the frequency of patch departure after the first reward remained constant. Patch-visit duration, nectar volume, scent marks and recurring visit sequences in a patch were ruled out as possible sources of patch-leaving information.

We conclude that bumblebees distinguish among otherwise identical stimuli by their serial position in a sequence, and use this capability to increase foraging efficiency. Our findings support an adaptive role for a complicated cognitive skill in a seemingly small and simple invertebrate.

## INTRODUCTION

The prevalence of complex cognitive processes in animals is under ongoing debate. One of these capabilities, numerical competence, involves the ability to enumerate objects to determine their quantity (Gelman & Gallistel, 1978, Davis, 1993). Some researchers maintain that animals do not use numerical skills under natural conditions, even though they can do so under some extreme experimental schedules (Davis & Pérusse, 1988). Other workers, on the other hand, stress the potential evolutionary benefits of numerical abilities in animals when performing actions such as foraging, evading nest parasitism or seeking mates (Seibt, 1988, Capaldi, 1993, Gallistel, 1989).

Honeybees are popular models for studying information processing and decision-making in invertebrates (Menzel et al., 2007, Chittka & Niven 2009). Several experiments addressed the ability of honeybees to complete navigation tasks based on numerical information. Chittka & Geiger (1995) trained bees to navigate to a feeder placed along a row of four landmarks, between the 3<sup>rd</sup> and 4<sup>th</sup> ones. When more landmarks were added to the row in front of the feeder, some of the bees shortened their flight distance when searching for it. Conversely, reducing the number of landmarks preceding the feeder led to increased flight distances. These results suggested that the number of landmarks served as a navigation cue. In a later navigation study in a flight tunnel, the number of landmarks preceding the feeders, the distances between them, and their areas were manipulated. The bees learned to search for a feeder after flying by 1, 2, 3 or 4 landmarks using this setup (Dacke & Srinivasan, 2008). An additional set of experiments involved training honeybees to navigate mazes that required a fixed sequence of right and left turns. The bees performed the task successfully even in the absence of regularity in the turn sequences (Zhang et al., 1996, 2000), or when the walls of the mazes were removed (Collett et al., 1993). These findings demonstrate the ability of honeybees to take action sequences of a fixed length when navigating to food sources. A common cognitive basis to this ability and to long-term spatial memory was proposed by Cooper (1984) as part of a discussion of early number development.

Here we ask whether bees use numerical information in foraging situations as well. Bees forage for nectar and pollen in flowers, and typically visit hundreds of flowers within each foraging bout. The probability that a forager would revisit a previously emptied flower within a bout is potentially high, since the presence of

nectar in the flowers cannot usually be assessed from a distance. Several foraging strategies used by bees reduce the frequency of revisits and thereby increase foraging efficiency. These include bottom-to-top movement along vertical inflorescences (Pyke, 1979, Waddington & Heinrich, 1979), foraging along a fixed route (traplining) (Ohashi & Thomson, 2009) and scent-marking of visited flowers (Giurfa & Nunez, 1992, Giurfa, 1993, Goulson et al., 2001). We hypothesized that the number of nectaries visited per flower is also used as an information source for avoiding revisits, when the number of nectaries is constant. A fixed number of nectaries occurs in flowers of several species of families such as the Malvaceae, Brassicaceae, Ranunculaceae, Oxalidaceae, Asclepiadaceae, and Liliaceae, and can be profitably exploited by bees if they use numerical information. We tested whether bumblebees foraging on *Alcea setosa* (Malvaceae) flowers with five nectaries tend to leave a flower after probing it five times. After confirming this foraging pattern, we combined field observations and laboratory experiments to evaluate possible mechanisms that underlie it.

## METHODS

### Field observations

#### Study site and species

A wild population of *Alcea setosa* (Boiss.) was observed in the Judean hills of Israel (altitude 500 m) during six days in May 2009. 70-100 flowers were in bloom during the days of observation. *A. setosa* is an herbaceous perennial that grows mainly in mountain areas of the Mediterranean region and blooms during April and May. The flowers measure 8-13 cm in diameter, are situated vertically on tall stalks of 1-2 m, and produce large amounts of nectar and pollen. The nectar is secreted from five nectaries located behind the petals, and is accessible through five slits situated between the petals (Endress, 1994).

The flowers were visited by workers and young queens of bumblebees (*Bombus terrestris* L.), honeybee workers, and females of solitary bees (mainly

*Eucera spp.*). We report here on the bumblebees, which were the dominant foragers at the study site.

### **Data recording**

Most of the bees' visits were recorded with a hand-held video camera. Each individual was traced since it was first observed on a flower and until it disappeared from view. We recorded the following variables for each flower visit when analyzing the video sequences: (a) the date and hour of visit (b) the number of nectaries probed (c) the location of the first and last nectaries visited, relative to the horizontal plane (d) the direction of movement within the flower (clock- or counter-clockwise) (e) the time spent in each nectary and the duration of the whole flower visit (f) whether the bee turned towards a nectary, but did not visit it, before leaving the flower. Such turns may be interpreted as rejection following inspection for odor marks. A similar criterion for flower rejection (hovering within 1 cm of a flower without subsequent landing) was proposed by Corbet et al. (1984).

Nectar standing crops (the amounts of nectar available in flowers exposed to visitors) were sampled at 0530, 0600, 0930 and 1300 h on one observation day. The 0530 sample was taken before the onset of bee activity, and the 0600 sample was taken shortly after the bees started foraging. Each sample was based on two flowers from each of 5-7 plants. Nectar was collected separately from each nectary, using 5- $\mu$ l micropipettes. Sucrose concentration was determined in samples with sufficient volume with a Bellingham & Stanley hand-held refractometer.

### **Analysis of the field data**

One-way ANOVA followed by Tukey post-hoc tests was used for testing the effects of sampling hour on *A. setosa*'s nectar standing crops. We combined observations of foraging sequences conducted by bumblebees between 0545-0900, 0900-1200, 1200-1500 and 1500-1900 on all observation days. We then tested for the effect of foraging hour on the time spent per flower, time spent per nectary and the number of nectaries visited per flower using one-way ANOVAs followed by Tukey post-hoc tests. We used t-tests to compare the durations of visits that preceded flower departure vs. visits that did not.

To characterize the bees' movement patterns within flowers, we assigned numbers from 1 to 5 to the nectaries within each flower. The first nectary moving

from the 12 o'clock position in a clockwise direction was designated #1, and the subsequent nectaries received consecutive numbers. Nectaries #1 and #5 were thus always located at the top half of the flowers, whereas #3 (and usually also #4) occupied the bottom half. Each foraging path within a flower, in visits that involved 2-6 probings, was characterized by a three-character string. The first two characters (which ranged 1-5) designated the first and last nectaries probed within the flower, respectively. The third character was defined as "+" or "-" for clockwise or counterclockwise movement, respectively. Visits of 7 probings were very rare and hence omitted from this analysis. We calculated the frequency of occurrence of each of the possible 50 paths (5 starting positions×5 end positions×2 movement directions) in the data set. Using a chi-square test, we assessed whether the bees' choices of the first nectary probed conformed to a uniform distribution. We tested whether the frequencies of "+" and "-" paths deviated from a random 1:1 distribution using the binomial test.

Binomial tests were also used to check whether individuals were more likely to retain their starting position and movement direction, in two consecutive flower visits, than expected at random. The random expected probability for probing two consecutive flowers at the same starting position was estimated as

$$\sum_{n=1}^5 (p_n)^2$$

where  $p_n$  is the observed frequency of the  $n^{\text{th}}$  nectary being used as the starting position within a flower. Based on the data set of bumblebee observations, the random expected probability for retaining the starting position in two consecutive flowers is 0.24. The random expected probability for keeping the same movement direction in two consecutive flowers was calculated as  $p(+)^2 + p(-)^2$ .  $p(+)$  and  $p(-)$  are the observed frequencies for clockwise and counterclockwise movement, estimated from the data. Based on these estimates, the expected probability that a bee would take the same direction of movement in two consecutive flowers, under a random null model, is 0.54.

We calculated the expected probabilities for probing 1-7 nectaries per flower under a logarithmic null model. The frequency of leaving ( $p_l$ ) a flower after probing one nectary was estimated from the data. The predicted probability of leaving after probing two nectaries is  $p_l(1 - p_l)$ , the leaving probability after three nectaries is  $p_l(1 - p_l)(1 - p_l)$  etc. We compared the observed and expected distributions of visits of 1-7

and  $\geq 8$  nectaries per flower using a chi-square test with  $df=6$ , since the parameter of the expected distribution was estimated from the observed data.

## **Laboratory experiments:**

### **The lab setup:**

The experiments were conducted in a 3×4 m flight room on the Hebrew University campus in 2007-2009. The temperature in the room ranged 26-29° C, and the relative humidity was 35-65%. The room was constantly illuminated with 12 200-Hz fluorescent bulbs. Young *B. terrestris* colonies, comprising a queen and 2-10 workers, were obtained from Polyam Inc., Kibbutz Yad Mordechai, Israel. They were used for experiments for ca. 4 weeks, and typically grew to about 100 workers during this time. The bees were first allowed to forage outside their nest box after they arrived at the lab. Pollen was supplied without restriction, directly into the colony. Newly-arrived colonies were supplied with 75% w/v sucrose solution inside the nest box. The bees were then gradually pre-trained to feed on a 35% solution supplied in the flight room (see below). This solution was provided *ad-lib* in Petri-dish feeders between experiments, and was used as reward in the electronic feeders during experiments.

The electronic feeders are described in detail elsewhere (Keasar, 2000). Briefly, each feeder consisted of a cylindrical container that held the sucrose solution and was topped by a horizontal landing surface, and a miniature cup that was refilled when programmed to dip into the sucrose-solution container. Cups of either 0.7  $\mu$ l or 1.5  $\mu$ l were used, according to the experimental schedule, as detailed below. Only foragers that landed on the top part of the flowers and probed them were able to access this cup and feed. Each feeder was equipped with a photodetector that was activated when the foraging bee inserted its proboscis. The photodetector signals were computer-recorded, allowing tracking of feeder visitation sequences for each forager. The feeders were placed on a green wooden table, 2.5 m away from the bee colony, in two patches. Each patch consisted of three feeders that were covered between experiments, invisible and inaccessible to the bees.

### **Pre-training**

At the start of pre-training, the within-colony feeder was replaced with a transparent Petri-dish feeder with 35% sucrose solution outside the colony. This Petri



dish was gradually moved away from the colony until it was placed on the table, between the two patches of electronic feeders. Workers that fed on the dish were marked with numbered tags at this stage.

Petri-dishes with sucrose solution were next placed on top of each of the six electronic feeders. After the group of bees learned to visit the Petri dishes regularly, the dishes were removed and the bees were allowed to access the electronic feeders. The feeders were first programmed to provide a sucrose reward each time they were visited. As soon as at least one bee probed the feeders repeatedly, the number of rewards per patch was gradually reduced to encourage patch shifts. The first worker that shifted between the patches was used in the next experiment, and the remaining foragers were caged outside the colony. Thus each replicate of the experiments involved only one individual.

## **Experiment 1**

The electronic feeders within each patch were arranged in a triangle with sides of 10 cm. The distance between the patches was 170 cm. The landing surfaces of all feeders were marked with a yellow round plastic disc of 35 mm diameter. Spectral reflectance data for the landing disc are provided in Keasar et al. (1997). Each of eight focal bees was allowed to visit all six feeders, which provided a 0.7  $\mu$ l reward according to the following rules:

- (a) All feeders were filled before the start of the experiment, and were refilled each time the forager returned to the colony to unload the nectar it had collected.
- (b) Only two rewards are provided in a sequence of visits within one patch. In other words, while the forager conducted its second visit within a patch, the rewards in the other two feeders were made inaccessible.
- (c) All feeders within a patch were refilled immediately after the forager shifted to the second patch.

According to this schedule, bees received a sucrose reward on their two first visits to each patch. After patch shifts, the counting of visits was reset. Each forager was allowed 1600 feeder choices, or 500 patch shifts, whichever came later. To exclude possible scent-marking of flowers as a patch-leaving cue, we also noted feeder rejections. These were cases where bees flew towards feeders or landed on them, but eventually left without feeding. When rejection of the same feeder occurred on three consecutive patch visits, all three feeders in this patch were replaced. This

was done to rule out the possibility that the bee has established positive scent marks associations with feeders that it had not rejected.

## **Experiment 2**

This experiment included two phases, and aimed to test the possibilities that either the volume of nectar collected at a patch, or the time spent in it are used as a departure cue. The first phase was identical to Experiment 1 in feeder arrangement and reward schedule, but the number of visits or patch shifts allowed per bee during this phase was not fixed in advance. After a focal bee reliably learned to visit two feeders per patch (i.e., when two-feeder visits per patch became more common than one- or three-feeder visits), the second phase was set up. This typically occurred after 1000-1500 visits to the feeders. While the forager was in the colony after completion of a foraging bout, we replaced the cups in the feeders of one of the patches with 1.5- $\mu$ l cups. We also replaced the cups of the second patch with a new set of 0.7- $\mu$ l cups. We allowed the forager 100 additional visits to the feeders. We compared the number of visits per patch, and the time spent in a patch, between the low- and high-volume patch during phase 2. We also calculated the time that bees spent visiting two feeders per patch during the 100 last visits of phase 1. We tested whether two-feeder visit sequences that were followed by patch shifts differed in duration from sequences that were followed by an additional visit to the same patch.

## **Analysis of the laboratory data**

The optimal foraging pattern (which maximized the bees' energy intake rate) under our experimental conditions would be to visit two feeders out of three in each patch, and then switch to the other patch. We scored a visit as optimal only if it conformed to this pattern. We also scored each visit as either rewarded or unrewarded, depending on whether the forager obtained a sucrose reward (which could be obtained even when switching patches after a single visit). Repeated-measures ANOVAs were used to test for changes in the bees' performance along experiment 1 (the use of the repeated-measures procedure follows Stach et al., 2004). The arcsine-transformed proportions of optimal and rewarded probings were calculated for each bee in 8 blocks of 200 consecutive visits, as the dependent variables. The number of the visit block (1-8) was treated as the repeated measure, and bee identity was the between-subject factor. We used additional repeated-

measures ANOVAs to test for the effect of experience and bee identity on the frequencies of leaving a patch after one rewarded visit, two rewarded visits, one non-rewarded visit or two unrewarded visits.

In experiment 2, we employed two-way ANOVAs to test for the effects of nectar volume and bee on the frequencies of patch departure. We also used ANOVA to test for differences in patch times prior to departing the patch or staying in it during the last 100 visits of the experiment's first phase. This calculation was not performed for experiment 1, since three bees in this experiment did not attain the learning criterion, namely two-feeder visits per patch as their most frequent sequence. The first phase of experiment 2, on the other hand, was continued until all foragers achieved the learning criterion. These individuals were therefore more suitable for analysis of patch-leaving strategy after completion of the learning task.

## RESULTS

### Field observations

We observed 516 visits to *A. setosa* flowers by bumblebees. Probing of five nectaries per flower was by far the most common event (48% of all cases). Revisits to emptied nectaries (i.e., a sixth or seventh probing in a flower) occurred in only 1.1% of all nectary visits. We calculated the conditional probability of departing a flower after  $n$  nectary probings by dividing the number of flower departures after the  $n$ th nectary by the number of probings of this nectary. This analysis shows that the frequency of flower departure after feeding on four nectaries is rather low (25%), and that it increases sharply (to 92%) after five probings (Fig. 1).

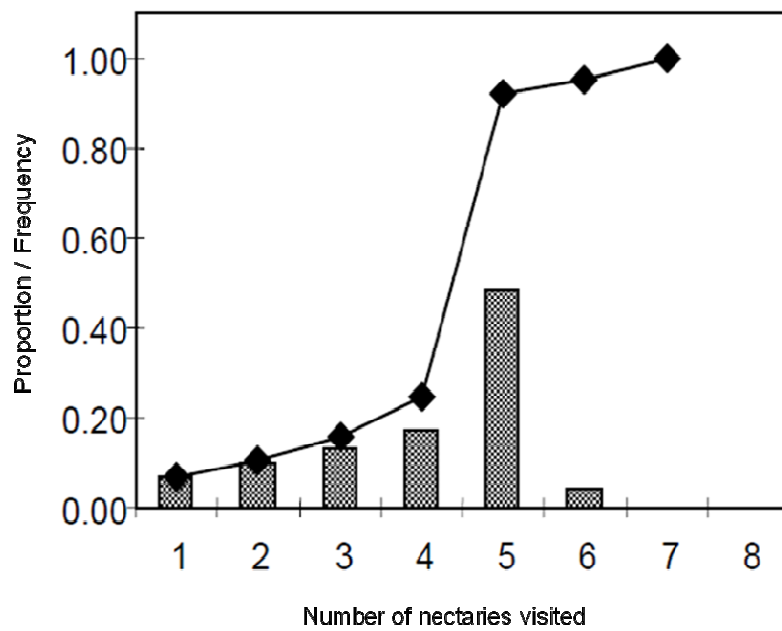


Fig. 1: The relative frequency of probing 1-7 nectaries in *A. setosa* flowers by bumblebees (bars), and the conditional probability for departing the flowers after varying numbers of probings (line). Conditional probabilities were calculated as the ratio of the number of flower departures after the  $n$ th nectary and the number of probings of this nectary.

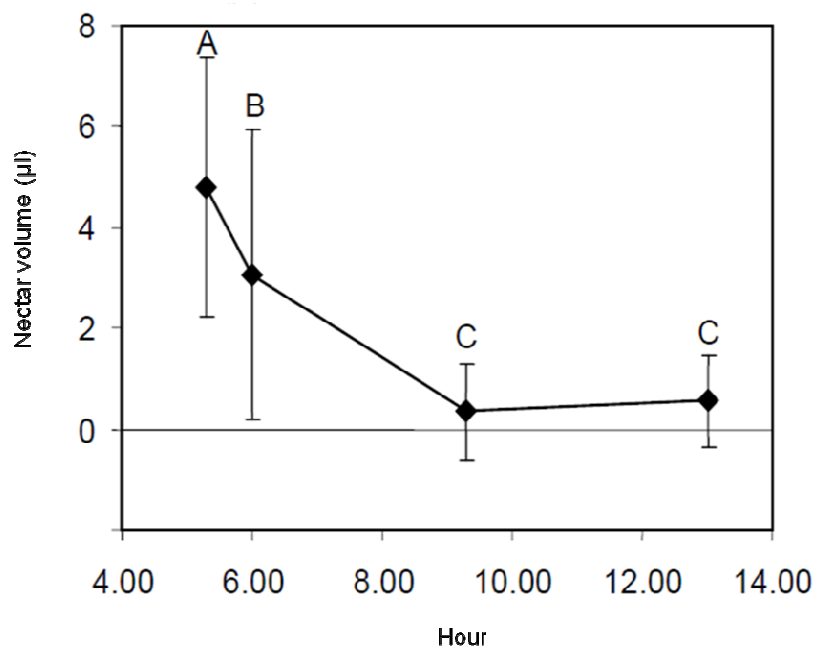


Fig. 2: Mean $\pm$ SD nectar standing crops in *A. setosa* flowers at different hours. Different letters indicate significantly different means in post-hoc tests.

We considered non-numerical signals that may be used by the bees as flower-departure cues, and thus could allow them to reduce revisits to depleted nectaries. We first asked whether the volume of nectar ingested by the bees could provide a flower-leaving signal. Nectar standing crops were highest in the early morning, decreased rapidly from the onset of the bees' foraging activity until 9 am, and remained low afterwards (Fig. 2). The mean $\pm$ SD sucrose concentration in the nectar was 19 $\pm$ 5% at 0530 and 0600. During later hours nectar volumes were insufficient for measurement of concentrations. Analyses of Variance revealed significant effects of observation hour on the bees' visit time per nectary ( $F_{3,427}=22.144$ ,  $p<0.001$ ) and per flower ( $F_{3,419}=6.804$ ,  $p<0.001$ ), as well as on the number of nectaries visited per flower ( $F_{3,427}=3.401$ ,  $p=0.018$ ). These effects were due to longer durations spent per flower and per nectary, and fewer nectaries visited per flower, during 5-9 am than later in the day (Fig. 3). One possible interpretation of these observations is that the bees responded to the high nectar standing crops available in early morning by leaving the flowers sooner. Alternatively, the cue for performing fewer probings in early morning could have been the longer handling time of the flowers, rather than their higher nectar volumes. We investigated the first interpretation in the laboratory experiments, by testing whether doubling the nectar volume collected in a food patch accelerates patch departure (see below). To examine the second interpretation, we first excluded visits recorded before 9 am from the data set to eliminate possible confounding effects of the nectar standing crops on foraging patterns. Next we tested whether a time-based departure rule (i.e. that the tendency to leave increases with time on the flower) is compatible with the bees' behavior. To this end we calculated the durations of visits that involved flower departure after 1, 2, 3, 4 or 5 probings, compared to cases where a forager visited the same number of nectaries and stayed on the flower. Contrary to the prediction of time-based departure rules, foraging durations on the flowers prior to departure were not significantly shorter than prior to staying for any number of probings (Fig. 4).

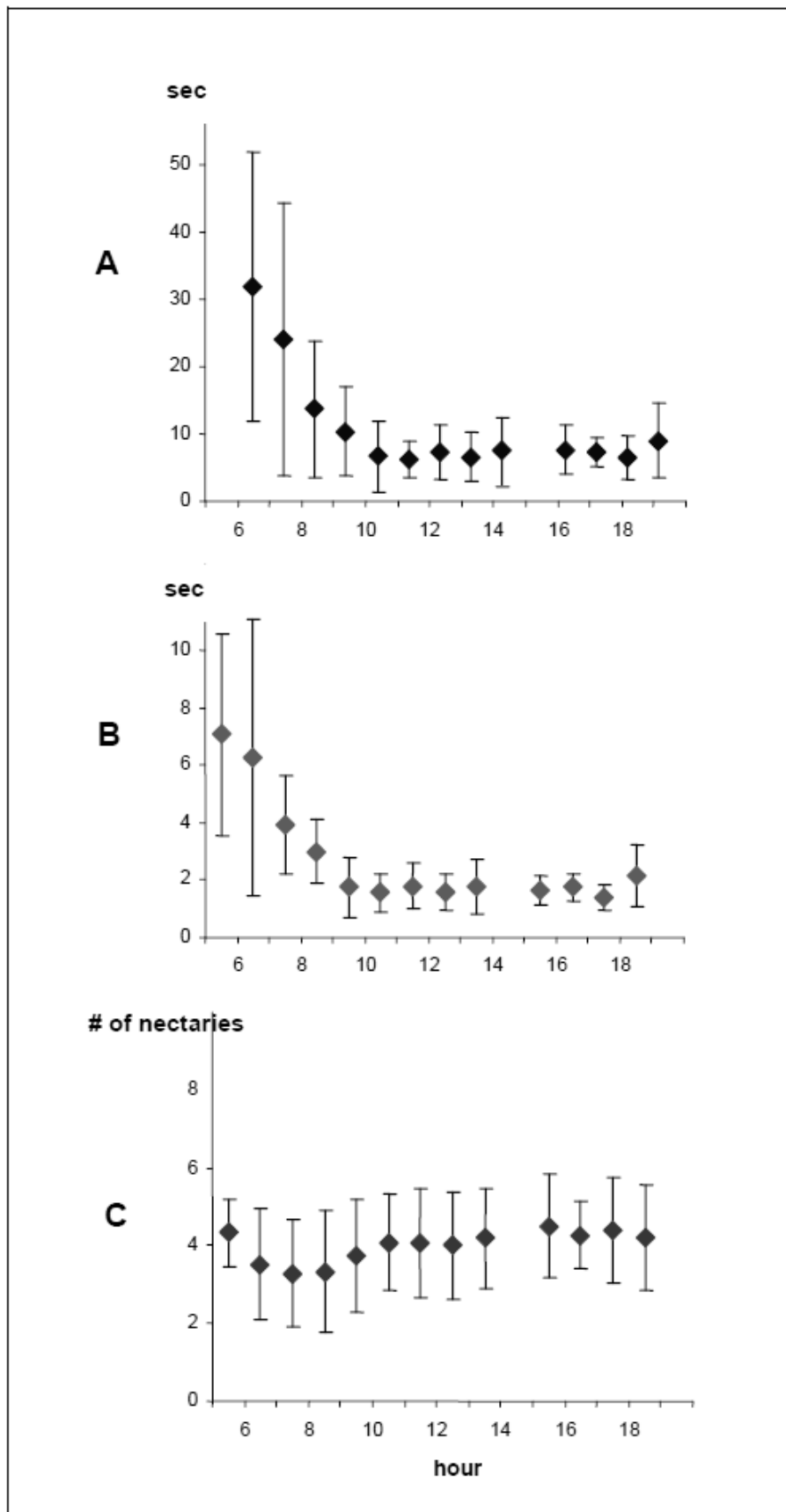


Fig. 3: Mean $\pm$ SD flower visit durations (A), nectary visit duration (B) and number of nectaries visited per flower (C), at different hours of the day.

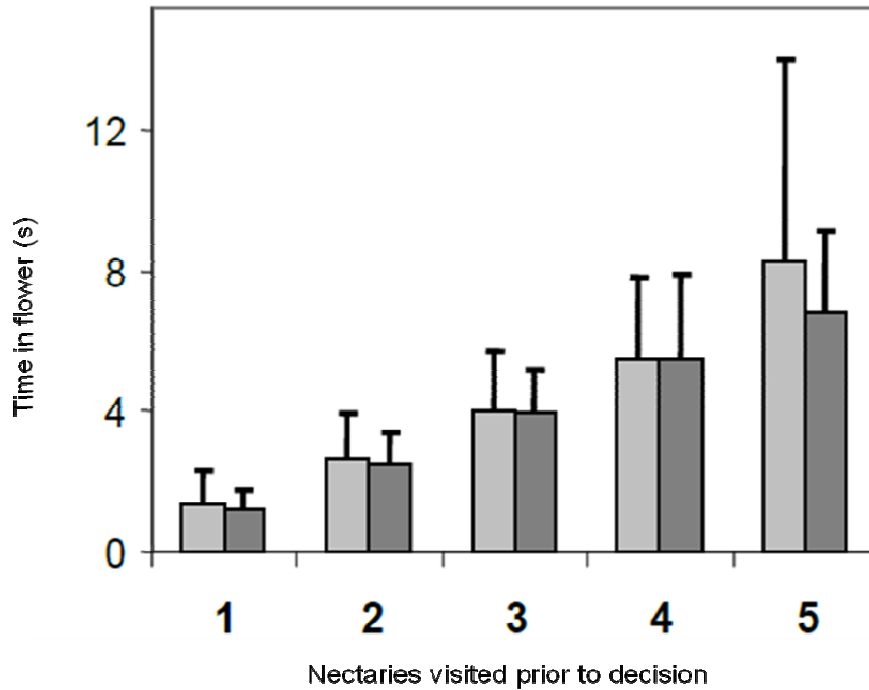


Fig. 4: Mean $\pm$ SD time spent by bumblebees on *A. setosa* flowers while probing 1-5 nectaries, prior to staying on a flower (light bars) or departing it (dark bars). Flower visits that involved probing of more than one nectary contribute more than one data point to this figure. For example, probing of five nectaries in a flower generated time records regarding staying decisions after nectaries 1, 2, 3 and 4, and a departure decision after nectary 5. One-tailed t-tests for comparison of mean probing time prior to staying or departing the flower yielded the following statistics: 1-nectary visits -  $t_{33}=1.24$ ,  $p=0.11$ ; 2-nectary visits -  $t_{51}=1.00$ ,  $p=0.16$ ; 3-nectary visits -  $t_{83}=0.29$ ,  $p=0.39$ ; 4-nectary visits -  $t_{121}=0.18$ ,  $p=0.38$ ; 5-nectary visits -  $t_{19}=1.09$ ,  $p=0.15$ .

An additional feasible departure cue could involve the spatial attributes of the flowers (Dyer, 1994). Bees may tend to land and depart from the flowers at constant locations, and to move along fixed routes or directions within a flower or inflorescence (Kells and Goulson 2001). This could enable foragers to avoid revisits even if they do not use numerical information while foraging. Indeed, most of the bees' paths within flowers started at nectary #4 (30% of 404 paths) or #5 (25% of the paths). Thus the distribution of the path starting points significantly differed from uniform ( $\chi^2_4=58.2$ ,  $p<0.001$ ). In addition, most (64%) of the paths were directed

clockwise, revealing a non-random choice of movement directions (binomial test,  $p < 0.001$ ). Landing on one of the two nectaries on the left side (#4 or #5), followed by clockwise movement, occurred in 50.7% of the flower visits. This proportion is remarkably high, since eight additional combinations of landing position and movement direction were available to the bees.

Whether or not the bees followed one of their preferred paths, they usually departed the flowers after probing five nectaries. Table 1 shows that the five-nectary path was the most frequent in 9 out of the 10 possible combinations of starting position and movement direction. Thus, probing of five nectaries was not limited to the cases where bees followed a fixed trajectory within the flowers.

Start point:		1			2			3			4			5			total		
nectaries visited		path	Relative frequency		path	Relative frequency		path	Relative frequency		path	Relative frequency		path	Relative frequency		frequency		
clockwise	2	{12}	3	0.7%	{23}	0	0.0%	{34}	2	0.5%	{45}	9	2.2%	{51}	9	2.2%	20	5.7%	
	3	{13}	3	0.7%	{24}	0	0.0%	{35}	3	0.7%	{41}	14	3.5%	{52}	12	3.0%	32	7.9%	
	4	{14}	7	1.7%	{25}	0	0.0%	{31}	7	1.7%	{42}	18	4.5%	{53}	25	6.2%	53	14.1%	
	5	{15}	11	2.7%	{21}	1	0.2%	{32}	15	3.7%	{43}	69	17.1%	{54}	37	9.2%	133	32.9%	
	6	{11}	0	0.0%	{22}	0	0.0%	{33}	0	0.0%	{44}	10	2.5%	{55}	2	0.5%	23	3.0%	
clockwise	Counter-	2	{-15}	4	1.0%	{-21}	5	1.2%	{-32}	3	0.7%	{-43}	0	0.0%	{-54}	6	1.5%	18	4.5%
		3	{-14}	6	1.5%	{-25}	7	1.7%	{-31}	6	1.5%	{-42}	1	0.2%	{-53}	2	0.5%	22	5.4%
		4	{-13}	3	0.7%	{-24}	9	2.2%	{-35}	7	1.7%	{-41}	0	0.0%	{-52}	1	0.2%	20	5.0%
		5	{-12}	12	3.0%	{-23}	21	5.2%	{-34}	40	9.9%	{-45}	4	1.0%	{-51}	3	0.7%	80	19.8%
		6	{-11}	1	0.2%	{-22}	0	0.0%	{-33}	5	1.2%	{-44}	0	0.0%	{-55}	1	0.2%	7	1.7%
total			50	12.4%		43	10.6%		88	21.8%		125	30.9%		98	24.3%	404	100%	

Table 1: The frequency distribution of the possible foraging paths that involved probing of 2-6 nectaries in *A. setosa*. Each path is characterized by its starting point (nectary #1-5, top row), direction of movement (leftmost column) and number of nectaries visited (second column). The most frequent path length, for each of the 10 possible combinations of starting points and direction, is highlighted. Frequencies are reported as numbers of observations, and as their relative proportions out of all observed paths.



A possibility that should be considered at this point is that individual foragers used different paths within flowers, and that they varied in their use of spatial attributes as landing and departure cues. According to this hypothesis, the observed variety of five-nectary paths results from variability among individual bees, but each bee has established its own regular visiting path. To evaluate this hypothesis, we analyzed 326 observations of two consecutive flower visits by the same individual. We first calculated how often a bee used the same starting point and movement direction in both flower visits. Next we asked whether using the same path in both flowers increased the frequency of 5-nectary visits, compared to cases where different paths were taken in each flower.

The bees were significantly more likely to keep their starting position and movement direction between consecutive visits than expected at random (binomial tests,  $p < 0.001$  for both). However, retaining or changing the starting position did not significantly affect the frequency of 5-nectary visits in the second flower (52% when the starting point was retained, 55% when it was changed, test for independence:  $\chi^2_1 = 0.15$ ,  $p = 0.70$ ). Similarly, keeping or changing the direction of movement did not influence the proportion of 5-nectary visits (56% when the direction was retained, 49% when it was changed,  $\chi^2_1 = 0.51$ ,  $p = 0.48$ ). Thus, individuals did tend to use similar paths in consecutive visits, but this tendency did not account for the observed prevalence of five-nectary visits.

Finally we investigated whether scent-marking of previously visited nectaries may have helped the bees avoid revisits. Bees that turned towards a nectary, but subsequently left the flower rather than actually probing it, were scored as potentially inspecting repellent scent marks. The frequency of turns increased with the number of nectaries probed (Fig. 5). Out of the 294 visits that did not include a turn towards a nectary (and hence no inspection of odor marks), 132 (44.9%) involved five probings. Visits with five probings were significantly more frequent than expected under a logarithmic null model, both in the complete data set ( $\chi^2_6 = 1082.09$ ,  $p < 0.001$ ) and in the subset of visits that did not involve inspection turns ( $\chi^2_6 = 538.38$ ,  $p < 0.001$ ). We conclude that five-nectary visits are common even in the absence of any evidence for possible inspection of scent-marks.

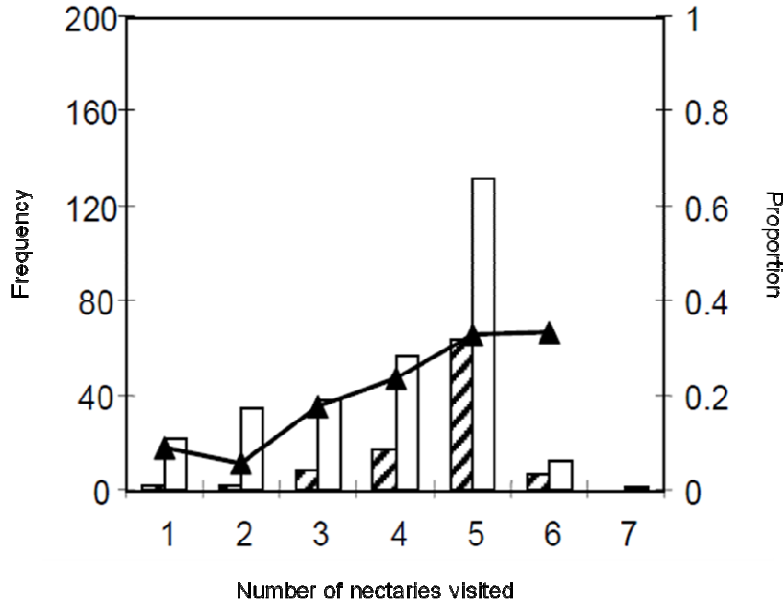


Fig. 5: The number of probings of 1-5 *A. setosa* nectaries that were followed (striped bars) or not followed (light bars) by turns towards an additional nectary. Such turns possibly indicate inspection of scent marks, as a means of detecting previously visited nectaries. The line shows the proportion of turns towards an additional nectary after varying numbers of nectary probings.

## Laboratory experiments

### Experiment 1

The proportion of optimal patch visits (i.e., two feeders probed per patch) significantly increased during the experiment (repeated-measures ANOVA:  $F_{7, 49}=19.84$ ,  $p<0.001$  for visit number,  $F_{1,7}=3788.895$ ,  $p<0.001$  for differences among bees, Fig. 6). The proportion of rewarded visits increased as well ( $F_{7, 49}=18.38$ ,  $p<0.001$  for visit number,  $F_{7, 63}=3267.85$ ,  $p<0.001$  for differences among bees). This improvement was mediated by a decrease in the number of visits per patch as the experiment progressed ( $F_{7, 63}=18.34$ ,  $p<0.001$  for visit number,  $F_{7, 63}=2.24$ ,  $p=0.047$  for differences among bees). As a conservative measure, we also compared the proportions of optimal and rewarded patch visits during the first and last 200 visits by each bee using paired t-tests. These tests confirmed that frequency of rewarded and optimal visits significantly increased as the bees became more experienced ( $t_7>5.17$ ,  $p<0.001$  for both tests). Fig. 7 summarizes the frequencies of 1-4 feeder visits per patch along the experiment. It shows an increase in the frequency of two-feeder visits,

and a decrease in the frequency of four or more visits per patch. Paired t-tests for comparisons between the 1<sup>st</sup> and 5<sup>th</sup> block of patch visits indicate that these trends were statistically significant ( $t_7=5.42$ ,  $p<0.0002$  for two-visit frequencies,  $t_7=7.26$ ,  $p<0.0001$  for four-visit frequencies.)

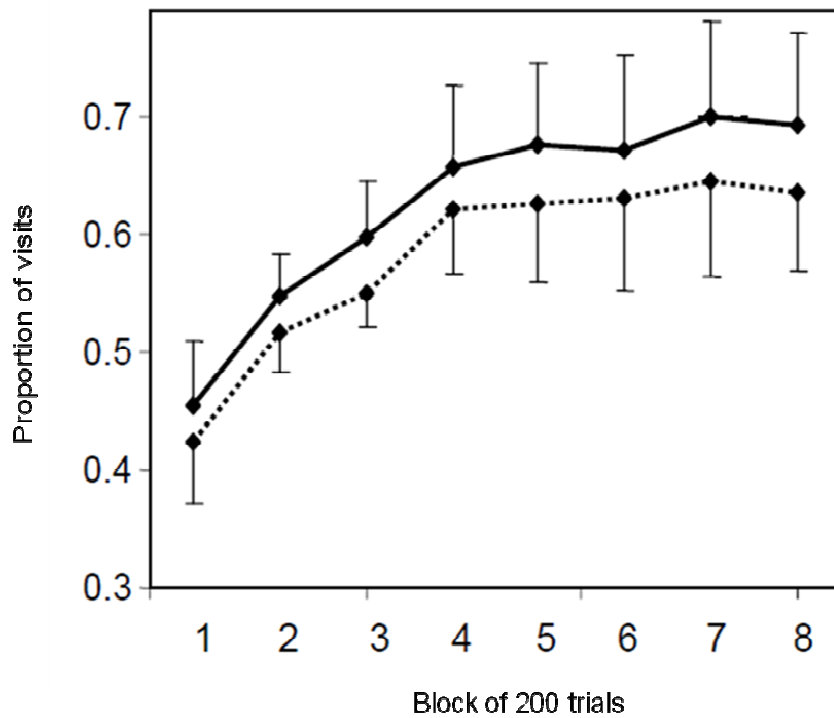


Fig. 6 top: Mean $\pm$ SD proportions of rewarded feeder visits (solid line) and optimal visits (dashed line) along laboratory experiment 1. By *rewarded* visits, we mean that the bees ( $n=8$ ) obtained a sucrose reward from any rewarding feeder they visited. By *optimal* visits, we mean that the bees obtained a reward from the *closest* rewarding feeder.

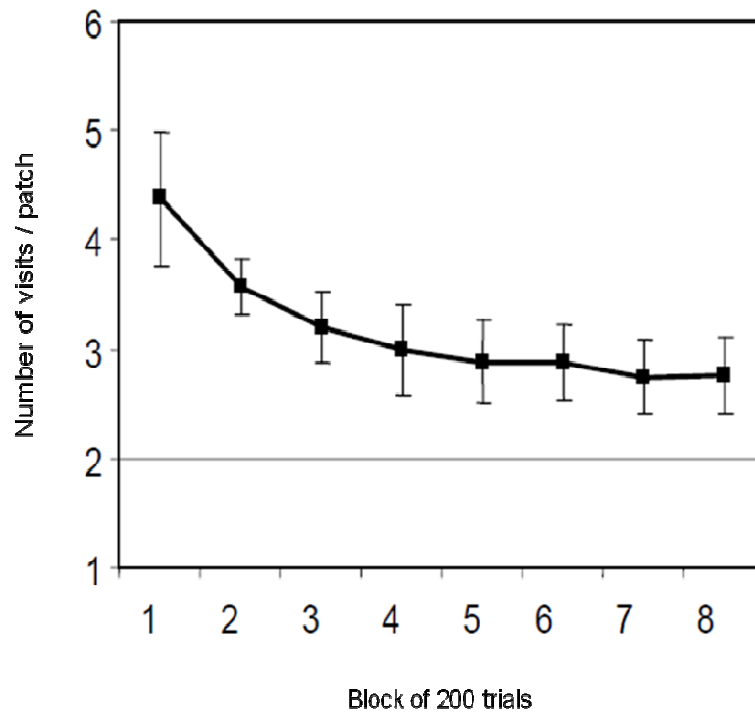


Fig. 6 bottom: Mean $\pm$ SD of feeders visited per patch along the experiment. The horizontal line depicts the optimal number of visits per patch.

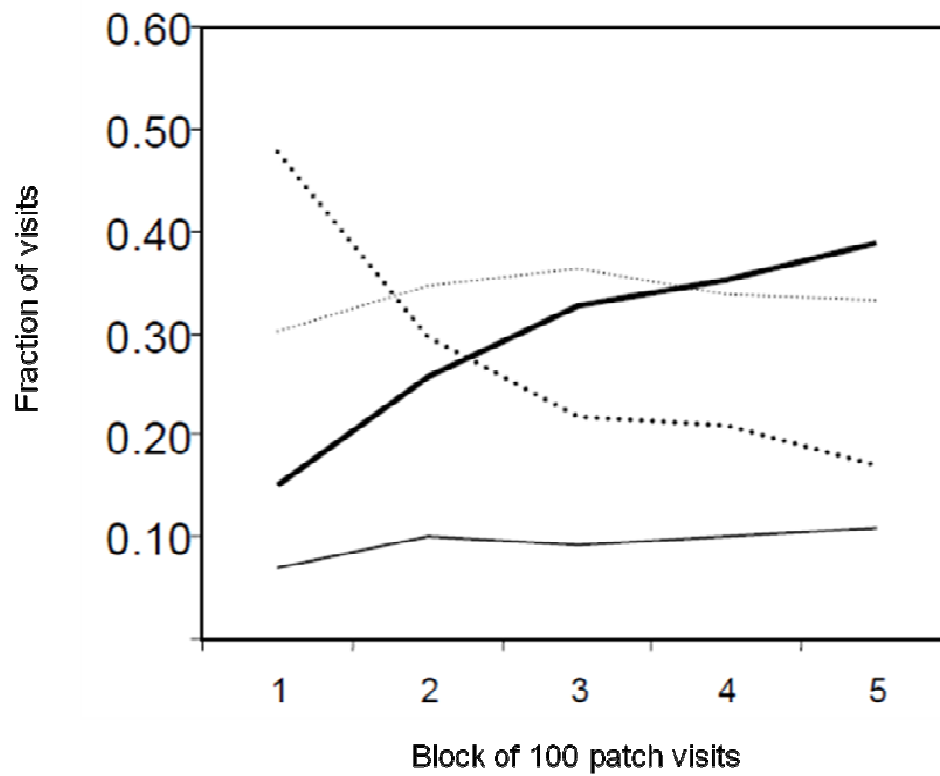


Fig. 7: Relative frequencies of visits to 1 (thin continuous line), 2 (thick continuous line), 3 (thin dashed line) and  $\geq 4$  (thick dashed line) feeders per patch along laboratory experiment 1.

We explored the bees' patch departure rules during the course of the experiment by calculating the frequencies of patch leaving after they visited (a) a single rewarding feeder (b) two rewarding feeders in succession (c) a single non-rewarding flowers and (d) two non-rewarding feeders in succession. This calculation was performed separately for each forager, for consecutive blocks of 200 feeder visits (Fig. 8). The frequency of patch-leaving after one rewarded visit was low and rather constant along the experiment ( $F_{7, 63}=1.57$ ,  $p=0.17$  for visit number,  $F_{7, 63}=2.34$ ,  $p=0.04$  for differences among bees). The frequencies of patch leaving after two rewarded visits, one unrewarded visit and two unrewarded visits increased significantly during the experiment ( $F_{7, 63}>4.7$ ,  $p<0.001$  for visit number in all three ANOVAs). These frequencies were also significantly affected by differences among bees ( $F_{7, 63}=4.92$ ,  $p<0.001$  for departure after two rewards,  $F_{7, 63}=2.38$ ,  $p=0.04$  for departure after one non-reward,  $F_{7, 63}=2.81$ ,  $p=0.02$  for departure after two non-rewards).

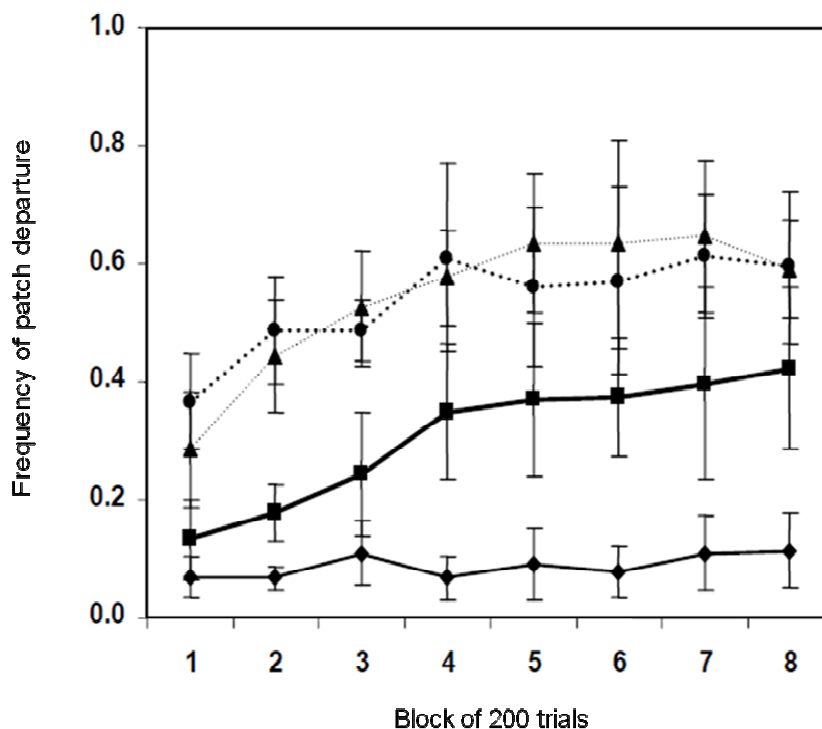


Fig. 8: Mean $\pm$ SD frequencies of patch departure in laboratory experiment 1, following one rewarded feeder visit (thin continuous line), two rewarded visits (thick continuous line), one unrewarded visit (thin dashed line) or two or more unrewarded visits (thick dashed line).

The increase in the frequency of two feeder visits per patch can potentially be attributed to the formation of specific preferences for certain feeders, or certain visit sequences in each patch. A forager might, for example, preferentially visit feeders A and B within a patch that contains A, B and C. Such visits could occur without any particular order (A-to-B and B-to-A being equally frequent), or mostly according to a particular sequence (such as B-to-A). Either way, memorizing two specific feeders per patch, and consistently ignoring the third, may provide bees with an optimal patch-leaving mechanism that does not require numerical competence. We tested for this possibility by finding the preferred two-feeder combination and visit sequence for each of the foragers, in each of the patches, during the last 150 visits of the experiment. The frequency of visits to the favorite feeders was  $0.64 \pm 0.13$ , while the expected frequency for randomly visiting two specific feeders out of three is  $1/3$ . Similarly, the frequency of visits along the bees' favorite two-feeder sequences was  $0.55 \pm 0.16$ , while the expected random frequency for any of the six possible sequences is  $1/6$ . These results indicate that the foragers did form preferences to specific feeders and visit sequences during the experiment. However, patch departure after a two-feeder visit was as frequent in cases where the favorite feeders were visited ( $0.35 \pm 0.25$ ) as in cases where they were not ( $0.36 \pm 0.20$ ). Likewise, departures following the bees' preferred two-feeder visit sequence were similar in frequency ( $0.34 \pm 0.26$ ) to departures following non-preferred two-visit sequences ( $0.37 \pm 0.18$ ).

## *Experiment 2*

Doubling the nectar volume provided by the feeders in one of the patches significantly increased the bees' residence time in the patch ( $3.24 \pm 0.62$  s in the low-volume patch,  $5.17 \pm 1.78$  s in the high-volume patch, paired t-test:  $t_7 = 4.04$ ,  $p = 0.002$ ). However, the proportions of patch departures after one- or two-feeder visits were not affected by the feeders' nectar volume (one-feeder visits:  $F_{1,15} = 0.002$ ,  $p = 0.97$  for nectar volume,  $F_{7,15} = 0.779$ ,  $p = 0.625$  for differences among bees; two-feeder visits:  $F_{1,15} = 0.069$ ,  $p = 0.80$  for nectar volume,  $F_{7,15} = 1.941$ ,  $p = 0.201$  for differences among bees). Moreover, the durations of two-visit sequences in the patches, during the 100 visits prior to the change in nectar volume, were similar whether or not they were followed by a patch shift ( $F_{1,15} = 1.23$ ,  $p = 0.16$  for patch leaving/staying,  $F_{7,15} = 13.58$ ,  $p < 0.001$  for differences among bees). These findings, in line with the field

observations, suggests that time in the patch was not used as a departure cue by the foragers.

## DISCUSSION

Our results show that bees adapt their foraging behavior to numerical regularity in the spatial distribution of their food sources in ways that enhance their feeding efficiency. Bumblebees that collected nectar from *A. setosa* foraged efficiently by avoiding revisits to nectaries that they had previously depleted. In the lab, the bees learned to anticipate two rewards per patch visit, and avoided unrewarded visits even though these did not involve returns to a just-depleted feeder. We were able to exclude time at the nectar source, the volume of nectar collected, spatial characteristics of the nectar source and repellent scent-marking as possible signals for patch-switching in the lab. Most of these cues could be ruled out as guiding flower departure in the field as well. We therefore suggest that reliance on the number of nectar rewards received (i.e. numerical competence) provides the most likely explanation for the bees' behavior.

The foragers in the laboratory experiment required an exceptionally high number of trials to learn to visit only two feeders in each patch. Associative learning of other reward-related cues, such as odor, color and location usually occurs much faster in bees (e.g. Menzel & Muller, 1996, Keasar et al., 2002, Burns & Thomson, 2006). This may hint that the learning process involved in processing of numbers is more complex than the associative learning of simple sensory cues. Numerical processing by bees could possibly resemble learning of a sequence of motor actions, such as probe-stay-probe-leave-the-patch in the lab experiment. The ability of bees to memorize and repeat a fixed activity sequence, even in the absence of external stimuli at each decision point, has been described in several navigation studies (Collett et al., 1993, Zhang et al., 1999, 2000, Menzel, 2009). The bees in the present study treated the first four nectaries of *A. setosa* differently from the fifth one in the field, and also foraged differently on the first vs. the second feeder per patch in the lab. Thus, similar events (probing) within the action sequence were perceived as different by the bees, according to their position in the sequence. Such differential perception incorporates the principles of ordination (tagging items by a fixed order) and cardination (using the

last tag to determine the number of items), which underlie numerical ability (Gelman & Gallistel, 1978).

A complementary interpretation of the possible processing of numerical information by bees involves an accumulator model (Meck & Church, 1983). According to this model, nervous signals are generated upon each encounter with a food source (nectary or feeder). The signals are then summed and compared to a threshold value. The bee leaves the food source when the accumulated signal exceeds the threshold. According to this interpretation, the learning phase in the laboratory experiment may have involved gradual reduction of the threshold value, which led to an increase in the frequency of patch departure after two visits.

Our results suggest that bees can learn to leave a foraging patch after collecting a fixed number of food items. Such a "fixed number rule" has previously been proposed, in theoretical models, as a possible patch departure cue for foragers (Green, 1980, Iwasa et al., 1981, Pyke, 1984). As far as we know, the present study provides the first empirical support for this rule from a natural foraging situation. On the other hand, our observations are incompatible with other rules-of-thumb for patch departure that were suggested in the literature. First, reliance on numerical information in foraging tasks caused bees in the present study to depart a food source after a sequence of rewarded visits, rather than after unrewarded ones (win-stay-lose-shift). Win-stay-lose-shift behavior was shown to be an Evolutionarily Stable foraging Strategy (Motro & Shmida, 1995). It has been repeatedly described in foraging bees under laboratory and field conditions that patch departures usually follow unrewarded visits (Pyke, 1978, Giurfa & Nunez 1992a, Kadmon & Shmida 1992, Dukas & Real 1993a, Keasar et al., 1996, Chittka et al., 1997). Our experiments, however, demonstrate the bees' ability to learn a patch departure pattern that contradicts their widely used lose-shift rule. In addition, several theoretical models use time at the patch, or rates of prey capture, to predict patch departure behavior of foragers (Gallistel & Gibbon, 2000, Bateson, 2003). Other models combine an increased tendency to leave a patch as search time increases, and a reduction in this tendency each time a food item is discovered (Waage 1979, Iwasa et al., 1981). The bees' behavior in our study does not conform to these models either, since the frequency of departure increased sharply after a fixed number of nectaries/feeders probed, regardless of the time at the flower/patch.



We conclude that bumblebees enhance their foraging efficiency by taking advantage of numerical regularities in the distribution of their food sources. This improved foraging may provide the selective drive for the evolution of a complex cognitive capability in the bees. This may, in turn, select for the evolution of floral reward schedules that attract numerically competent pollinators, which possibly provide high-quality pollination services. Testing this possibility requires comparison of pollinator species that differ in numerical capabilities. These tests are currently underway.

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