

The use of numerical information by bees in foraging tasks

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The ability of invertebrates to perform nonelemental cognitive tasks is widely debated. Bees utilize the number of landmarks en-route to their destination as navigation cues, but their use of numerical information in other contexts is unknown. Numerical regularity in the spatial distribution of food occurs naturally in some flowers, which contain fixed numbers of nectaries. Nectar foragers on such flowers can increase their foraging efficiency by avoiding return visits to empty nectaries. This can occur if bees base their flower-departure decisions on the number of nectaries they had already visited. We tested, through field observations and laboratory experiments, whether bumblebees adapt their departure behavior to the number of available food resources. In bees that visited *Alcea setosa* flowers with 5 nectaries in the field, the conditional probability of flower departure after 5 problings was 92%. Visit duration, the flowers' spatial attributes, and scent marks could be excluded as flower-leaving cues. In the laboratory, bees foraged on 2 patches, each with 3 computer-controlled feeders, but could receive only up to 2 sucrose-solution rewards per patch visit. The foragers gradually increased their frequency of patch departure after the second reward. 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 forage efficiently. Our findings support an adaptive role for a complicated cognitive skill in a small invertebrate. *Key words:* artificial flower, *Bombus terrestris*, cognition, counting, numerical competence. [*Behav Ecol*]

The prevalence of nonelemental cognitive processes in animals arouses widespread interest (Menzel et al. 2007). One of these capabilities, numerical competence, involves the ability to enumerate objects to determine their quantity (Gelman and 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 and Perusse 1988). Other authors, 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; Gallistel, 1989; Capaldi 1993).

Honeybees are popular models for studying information processing and decision making in invertebrates (Menzel et al. 2007; Chittka and Niven 2009). Several experiments addressed the ability of honeybees to complete navigation tasks based on numerical information. Chittka and Geiger (1995) trained bees to navigate to a feeder placed along a row of 4 landmarks, between the third and fourth 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 (Menzel et al. 2010). 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 and 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 results demonstrate the ability of honeybees to take action sequences of a fixed length when navigating to food sources. These findings are compatible with a common cognitive basis to numerical ability and to long-term spatial memory, as proposed on theoretical grounds by Cooper (1984).

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 because 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 and Heinrich 1979), foraging along a fixed route (trapping) (Ohashi and Thomson 2009), and scent marking of visited flowers (Giurfa and 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 occur in several species from 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 5 nectaries tend to leave a flower after probing it 5 times. After confirming this foraging pattern, we combined field observations and laboratory experiments to evaluate possible mechanisms that underlie it.

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METHODS

Field observations

Study site and species

A wild population of *A. setosa* (Boiss.) was observed in the Judean mountains of Israel (altitude 500 m) during 6 days in May 2009. Seventy to 100 flowers were in bloom during the days of observation. *Alcea setosa* is an herbaceous perennial that grows mainly in mountain areas of the Mediterranean region and blooms during April and May. The flowers are shaped as wide funnels, 8–13 cm in diameter, are situated vertically on tall stalks of 1–2 m, and produce large amounts of nectar and pollen (Figure 1). The nectar is secreted from 5 nectaries located behind the petals and is accessible through 5 slits situated between the petals (Endress 1994). The flowers' size and shape do not allow insects to see the entire flower, nor the area surrounding it, while visiting.

The flowers were visited by honeybee workers, females of solitary bees (*Eucera spp.*), and workers and young queens of bumblebees (*Bombus terrestris* L.). 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 followed from the time it was first observed on a flower until it disappeared from view. We recorded the following variables for each flower visit when analyzing the video sequences: 1) the date and hour of visit, 2) the number of nectaries probed, 3) the location of the first and last nectaries visited, relative to the horizontal plane, 4) the direction of movement within the flower (clockwise or counter-clockwise), 5) the time spent in each nectary and the duration of the whole flower visit, and 6) whether the bee turned toward a nectary, but did not visit it, before leaving the flower. Such turns may be interpreted as inspection for odor marks (Corbet et al. 1984).

Nectar standing crops (the amounts of nectar available in flowers exposed to visitors) were sampled at 0530, 0630,



Figure 1
Alcea setosa.

0930, and 1300 h on 1 observation day. The 0530-h sample was taken before the onset of bee activity, and the 0630-h sample was taken shortly after the bees started foraging. Each sample was based on 2 flowers from each of 5–7 plants. Nectar was collected separately from each nectary, using 5- μ l micropipettes. Sucrose concentration was determined in samples with sufficient volume (>1/3 μ l) with a Bellingham and Stanley hand-held refractometer.

Analysis of the field data

One-way analysis of variance (ANOVA) followed by 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 h 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 1-way ANOVAs followed by Tukey's post hoc tests. We used *t*-tests to compare the durations of visits that preceded flower departure versus 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 topmost nectary relative to the horizontal plane was designated as #1 and the remaining nectaries received consecutive numbers, clockwise from #1. 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 3-character string. The first 2 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 counter-clockwise movement, respectively. We calculated the frequency of occurrence of each of the possible 50 paths (5 starting positions \times 5 end positions \times 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 2 consecutive flower visits, than expected at random. The random expected probability for probing 2 consecutive flowers at the same starting position was estimated as

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

where p_n is the frequency of the n 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 2 consecutive flowers is 0.24. The random expected probability for keeping the same movement direction in 2 consecutive flowers was calculated as $p(+)^2 + p(-)^2$. $p(+)$ and $p(-)$ are the frequencies for clockwise and counter-clockwise movement, estimated from the data. Based on these estimates, the expected probability that a bee would take the same direction of movement in 2 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 1 nectary was estimated from the data. The predicted probability of leaving after probing 2 nectaries is $p_l(1 - p_l)$, the leaving probability after 3 nectaries is $p_l(1 - p_l)(1 - p_l)$, etc. We

compared the observed and expected distributions of visits of 1–7 and ≥ 8 nectaries per flower using a chi-square test with degrees of freedom = 6 because the parameter of the expected distribution was estimated from the observed data.

Laboratory experiments

The laboratory 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 approximately 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 laboratory. 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 pretrained 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 (Kesar 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 or 1.5 μ l were used, according to the experimental schedule, as detailed below. Only foragers that landed on the top part of the feeders 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 2 groups of 3 feeders (henceforth called patches). Feeders were covered between experiments, invisible and inaccessible to the bees. The feeders within each patch were arranged in a triangle with sides of 10 cm. The distance between the 2 patches was 170 cm (Figure 2). The landing surfaces of all feeders were overlaid with a yellow round plastic disc of 35-mm diameter. Spectral reflectance data for the landing disc are provided in Kesar et al. (1997).

Pretraining

At the start of pretraining, 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 2 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 6 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 1 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 1 individual.

Experiment 1

Each of 8 focal bees was allowed to visit all 6 feeders, which provided a 0.7 μ l reward according to the following rules:

- 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.
- Only 2 rewards are provided in a sequence of visits within 1 patch. In other words, while the forager conducted its second visit within a patch, the rewards in the remaining 2 feeders were made inaccessible.
- 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 2 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 toward feeders or landed on them, but eventually left without feeding. When 3 consecutive rejections within a patch were scored, all 3 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 or negative associations with the rejected ones (Saleh et al. 2007).

Experiment 2

This experiment included 2 phases and aimed to test the possibility that the volume of nectar collected at a patch is 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 2 feeders per patch (i.e., when 2-feeder visits per patch became more common than 1- or 3-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- μ l cups. We also replaced the cups of the second patch with a new set of 0.7- μ 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. The time that bees spent visiting 2

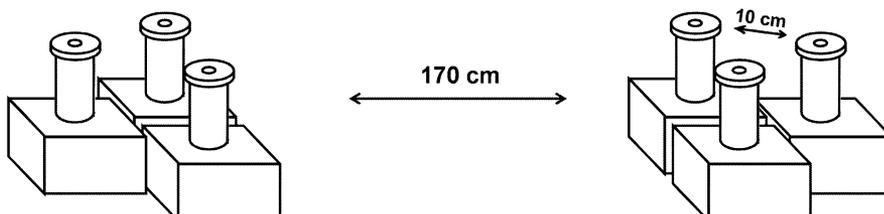


Figure 2
A scheme of the feeder setup in the laboratory experiments.

feeders per patch during the 100 last visits of phase 1 was also calculated. We tested whether 2-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 2 feeders out of 3 in each patch and then switch to the other patch. We scored each 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 number 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 1 rewarded visit, 2 rewarded visits, 1 nonrewarded visit, or 2 unrewarded visits.

In experiment 2, we employed 2-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 because 3 bees in this experiment did not attain the learning criterion, namely 2-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 5 nectaries per flower was by far the most common event (48% of all cases). Revisits to emptied nectaries (i.e., probing of a sixth nectary 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 4 nectaries is rather low (25%) and that it increases sharply (to 92%) after 5 probings (Figure 3).

We considered nonnumerical 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 afterward (Figure 4). The mean \pm standard deviation sucrose concentration in the nectar was $19 \pm 5\%$ at 0530 and 0600 h. 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

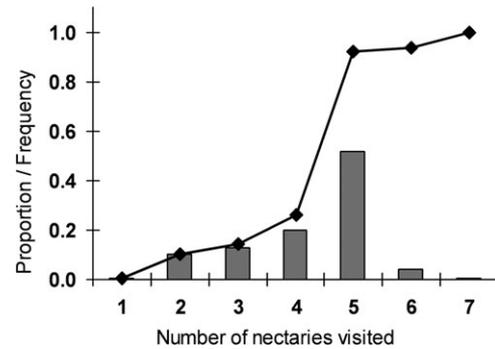


Figure 3

The relative frequency of probing 1–7 nectaries in *Alcea 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.

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 (Figure 5). 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 with 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 (Figure 6).

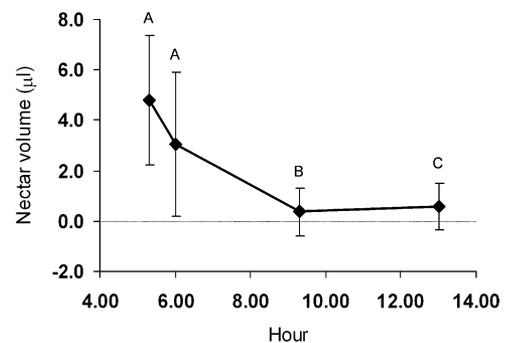


Figure 4

Mean \pm standard deviation nectar volumes in *Alcea setosa* flowers at different hours. Different letters indicate significantly different means in post hoc tests.

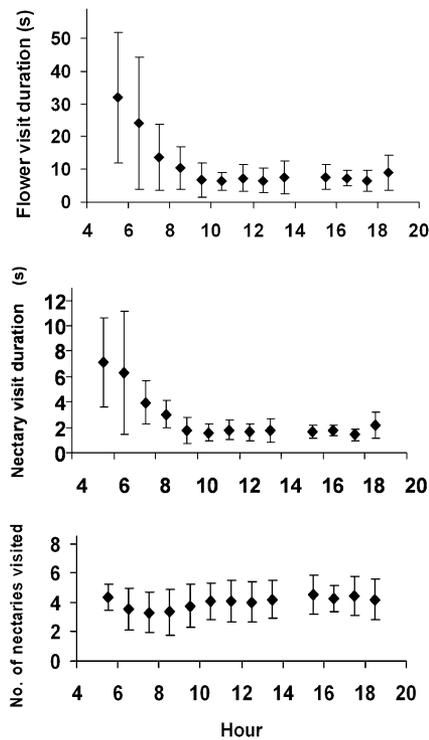


Figure 5
Mean \pm standard deviation flower visit durations (A), nectary visit duration (B), and number of nectaries visited per flower (C) at different hours of the day.

An additional feasible departure cue could involve the spatial attributes of the flowers (Dyer 1994). Bees may tend to land on and depart from the flowers at constant positions and move along fixed routes or directions within a flower. 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

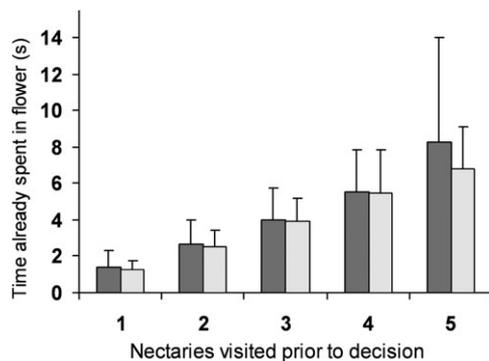


Figure 6
Mean \pm standard deviation time spent by bumblebees on *Alcea setosa* flowers while probing 1–5 nectaries, prior to staying on a flower (light bars) or departing it (dark bars). Flower visits that involved probing of more than 1 nectary contribute more than 1 data point to this figure. For example, probing of 5 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— $t_{19} = 1.09$, $P = 0.15$.

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 nonrandom choice of movement directions (binomial test, $P < 0.001$). Landing on one of the top-left nectaries, followed by clockwise movement, occurred in 50.7% of the flower visits. This proportion is remarkably high because 8 additional combinations of landing position and movement direction were available to the bees. This indicates that foragers indeed use some trajectories in preference to others.

Nevertheless, whether or not the bees followed one of their preferred paths, they usually departed the flowers after probing 5 nectaries. Table 1 shows that the 5-nectary path was the most frequent in 9 of the 10 possible combinations of starting position and movement direction. Thus, probing of 5 nectaries was not limited to the cases where bees followed a fixed trajectory within the flowers.

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 5-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 2 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 with 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 and 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 and 49% when it was changed, $\chi^2_1 = 0.15$, $P = 0.48$). Thus, individuals did tend to use similar paths in consecutive visits, but this tendency did not account for the observed prevalence of 5-nectary visits. Finally, we investigated whether scent marking of previously visited nectaries may have helped the bees avoid revisits. Bees that turned toward 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 (Figure 7). Of the 294 visits that did not include a turn toward a nectary (and hence no inspection of odor marks), 132 (44.9%) involved 5 probings. Visits with 5 probings were significantly more frequent than expected under a logarithmic null model, both in the complete data set ($\chi^2_6 = 0.8209$, $P < 0.001$) and in the subset of visits that did not involve inspection turns ($\chi^2_6 = 538.38$, $P < 0.001$). We conclude that 5-nectary visits are common even in the absence of any evidence for possible inspection of scent marks.

Laboratory experiments

Experiment 1

The proportion of optimal patch visits (i.e., 2 feeders probed per patch) significantly increased during the experiment (repeated measures ANOVA: $F_{7, 49} = 19.84$, $P < 0.001$ for visit number and $F_{1, 7} = 3788.895$, $P < 0.001$ for differences among bees). The proportion of rewarded visits increased as well ($F_{7, 49} = 18.38$, $P < 0.001$ for visit number and $F_{7, 63} = 3267.85$, $P < 0.001$

Table 1

The frequency distribution of the possible foraging paths that involved probing of 2–6 nectaries in *Alcea setosa*

Start point: nectaries visited	1		2		3		4		5		Total Frequency	
	Path	Relative 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%	
Counter-clockwise												
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%	

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.

for differences among bees; Figure 8). This improvement was mediated by a decrease in the number of visits per patch as the experiment progressed (Figure 9; $F_{7, 63} = 18.34$, $P < 0.001$ for visit number and $F_{7, 63} = 2.24$, $P = 0.047$ 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 frequencies of rewarded and optimal visits significantly increased as the bees became more experienced ($t_7 > 5.17$, $P < 0.001$ for both tests). Figure 10 summarizes the frequencies of 1–4 feeder visits per patch along the experiment.

We explored the bees' patch departure rules during the course of the experiment by calculating the frequencies of patch leaving after they visited 1) a single rewarding feeder 2) 2 rewarding feeders in succession 3) a single nonrewarding flower, and 4) 2 nonrewarding feeders in succession. This calculation was performed separately for each forager, for consecutive blocks of 200 visits (Figure 11). The frequency of patch leaving after 1 rewarded visit was low and rather constant along the experiment ($F_{7, 63} = 1.57$, $P = 0.17$ for

visit number and $F_{7, 63} = 2.34$, $P = 0.04$ for differences among bees). The relative frequency of patch leaving after 2 rewarded visits increased significantly during the experiment. Similar increases were observed when we considered the relative frequency of patch departure after 1 unrewarded visit and after 2 unrewarded visits (Figure 11; $F_{7, 63} > 4.7$, $P < 0.001$ for visit number in all 3 ANOVAs). These frequencies were also significantly affected by differences among bees ($F_{7, 63} = 4.92$, $P < 0.001$ for departure after 2 rewards; $F_{7, 63} = 2.38$, $P = 0.04$ for departure after 1 nonreward; and $F_{7, 63} = 2.81$, $P = 0.02$ for departure after 2 nonrewards). Individual differences among bees were also evident in choice performance. We exemplify this variation in Figure 12, which plots the proportion of 2-feeder patch visits (i.e., optimal ones) for each of the 8 foragers.

The increase in the frequency of 2 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

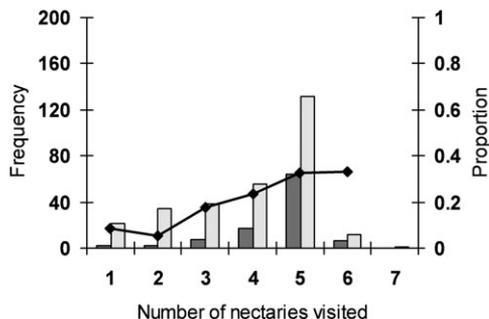


Figure 7

The number of probings of 1–5 *Alcea setosa* nectaries that were followed (striped bars) or not followed (light bars) by turns toward 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 toward an additional nectary after varying numbers of nectary probings.

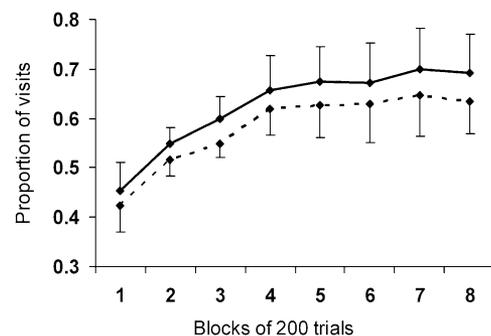


Figure 8

Mean \pm standard deviation proportions of rewarded feeder visits (solid line) and optimal patch visits (dashed line) along laboratory experiment 1. In rewarded visits, the bees ($n = 8$) obtained a sucrose reward from the feeder they visited. In optimal visits, the bees visited 2 feeders and then switched to the second patch of feeders.

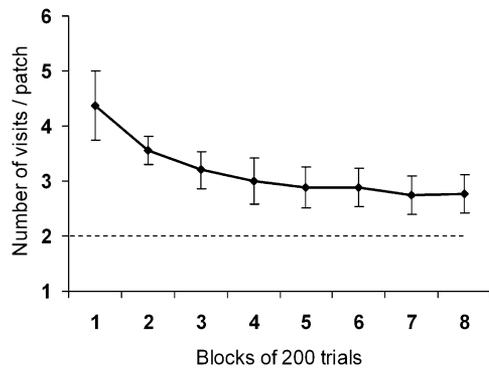


Figure 9
Mean \pm standard deviation of feeders visited per patch along laboratory experiment 1. The horizontal line depicts the optimal number of visits per patch.

and B-to-A being equally frequent) or mostly according to a particular sequence (such as B-to-A). Either way, memorizing 2 specific feeders per patch may provide bees with an optimal patch-leaving mechanism that does not require numerical competence. We tested for this possibility by finding the preferred 2-feeder combination and visit sequence for each of the bees, in each of the patches, during the last 150 visits of the experiment. The frequency of visits to the favorite feeders was 0.64 ± 0.13 , whereas the expected frequency for randomly visiting 2 specific feeders of 3 is $1/3$. Similarly, the frequency of visits along the bees' favorite 2-feeder sequences was 0.55 ± 0.16 , whereas the expected random frequency for any of the 6 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 2-feeder visit was as frequent in cases where the favorite feeders were visited (0.35 ± 0.25) as in cases where they were not (0.36 ± 0.20). Likewise, departures after the bees' preferred 2-feeder visit sequence were similar in frequency (0.34 ± 0.26) to departures after nonpreferred 2-visit sequences (0.37 ± 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 ± 0.62 s in the low-volume patch and 5.17 ± 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 1- or 2-feeder visits were not affected by the

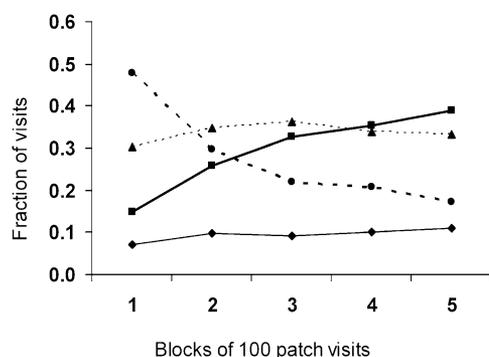


Figure 10
Relative frequencies of visits to 1 (thin continuous line), 2 (thick continuous line), 3 (thin dashed line), or 4 (thick dashed line) feeders per patch along laboratory experiment 1.

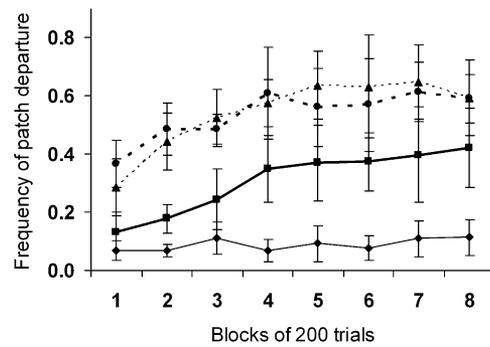


Figure 11
Mean \pm standard deviation frequencies of patch departure in laboratory experiment 1, after 1 rewarded feeder visit (thin continuous line), 2 rewarded visits (thick continuous line), 1 unrewarded visit (thin dashed line) or 2 or more unrewarded visits (thick dashed line).

feeders' nectar volume (1-feeder visits: $F_{1, 15} = 0.002$, $P = 0.97$ for nectar volume and $F_{7, 15} = 0.779$, $P = 0.625$ for differences among bees; 2-feeder visits: $F_{1, 15} = 0.069$, $P = 0.80$ for nectar volume and $F_{7, 15} = 1.941$, $P = 0.201$ for differences among bees). Moreover, the durations of 2-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 and $F_{7, 15} = 13.58$, $P < 0.001$ for differences among bees). These findings, in line with the field observations, suggest 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 laboratory, the bees learned to anticipate 2 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

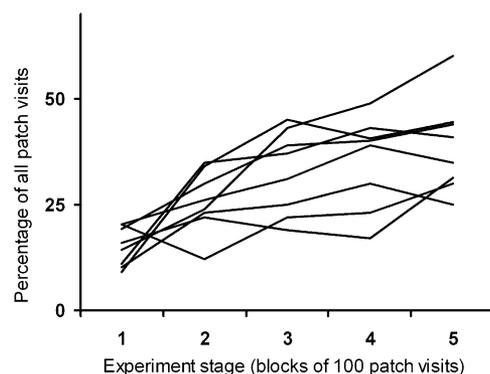


Figure 12
Differences between individual bees in learning performance, measured as proportions of 2-feeder patch visits (i.e., optimal visits) throughout laboratory experiment 1. Each line depicts 1 individual. Lines are arranged in an increasing order of performance after 500 patch visits.

patch switching in the laboratory. Most of these cues could be ruled out as guiding flower departure in the field as well. Another possible flower-departure cue in the field, which we did not investigate, is that that bees memorized their view of the first-visited nectary and used it as a signal to leave. We regard this as unlikely because it would have required bees to return to an already-depleted nectary on their sixth nectary visit, and our observations show that this was very rare. Furthermore, the radial symmetry of the flower, and the fact that only one nectary can be viewed at a time, may make it difficult for the bees to visually distinguish between nectaries.

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. These findings suggest that numerical regularity may serve bees as a source of information to improve foraging efficiency, in addition to other sources underlying previously described foraging strategies. Such sources include the presence of rewards in previous visits (e.g., Motro and Shmida, 1995; Keasar et al. 1996), spatial (e.g., Pyke 1979; Ohashi and Thomson 2009), and chemical information (e.g., Saleh et al. 2007).

The foragers in the laboratory experiment required an exceptionally high number of trials to learn to visit only 2 feeders in each patch. Associative learning of other reward-related cues that have been studied in the same experimental system, such as size, color, and location, occurred much faster (e.g., Keasar et al. 1997; Blarer et al. 2002). Because identical feeders and conditions were used in all of these studies, this may hint that the learning process involved in processing of numbers is more complex than the associative learning of simple sensory cues.

A related issue is whether the complex task of numerical processing can be learned by bees in the field within reasonable time. The high number of trials that was required for naive bees foraging on artificial feeders does not seem to pose a problem for bumblebees foraging in the wild. Our field observations show that a foraging bee visits on average 5–6 *A. setosa* flowers per minute, probing approximately 4.5 nectaries in each. This would amount to 800 probings in less than 1 h of foraging time. Because bumblebee workers are active for several weeks (Goulson 2003), the time required to complete the learning task is rather short.

Numerical processing by bees could possibly resemble learning of a sequence of motor actions, such as probe-stay-probe-leave-the-patch in the laboratory 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; Chittka et al. 1995; Zhang et al. 1999, 2000; Menzel 2009). The bees in the present study treated the first 4 nectaries of *A. setosa* differently from the fifth one in the field and also foraged differently on the first versus the second feeder per patch in the laboratory. Thus, different events 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 cardinality (using the last tag to determine the number of items), which underlie numerical ability (Gelman and Gallistel 1978).

A complementary interpretation of the possible processing of numerical information by bees involves an accumulator model (Meck and Church 1983). According to this model, nervous signals are generated on each encounter with a food source (nectary or feeder). The signals are then summed and compared with 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 involved gradual reduction of the

threshold value, which led to an increase in the frequency of patch departure after 2 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 to depart a food source after a sequence of rewarded visits (win-shift), rather than after unrewarded ones (lose-shift) in the present study. Win-stay-lose-shift behavior was shown to be an evolutionarily stable foraging strategy (Motro and Shmida 1995). It has been repeatedly described in foraging bees under laboratory and field conditions (Pyke 1978; Giurfa and Nunez 1992; Kadmon and Shmida 1992; Dukas and Real 1993; Keasar et al. 1996; Chittka et al. 1997). Our experiments, however, demonstrate the bees' ability to learn to win-shift when this increases their foraging success. In addition, several theoretical models use time at the patch, or rates of prey capture, to predict patch departure behavior of foragers (Gallistel and 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 because 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 exploiting 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 may vary in numerical capabilities, such as solitary versus social bees that are considered to differ in cognitive abilities (Willmer and Stone 2004). These tests are currently underway.

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