Facultative use of the repellent scent mark in foraging bumblebees: complex versus simple flowers

NEHAL SALEH*†, KAZUHARU OHASHI*, JAMES D. THOMSON* & LARS CHITTKA†

*Department of Zoology, University of Toronto

†School of Biological and Chemical Sciences, Queen Mary College, University of London

Bumblebees leave scent marks on flowers, and use these marks to avoid recently depleted resources. We tested whether the response to such scent marks is fixed, or whether bees can adjust their responses flexibly, depending on floral complexity. Complex flowers require longer handling times and, when foraging on these flowers, bees show spatial foraging patterns that make revisits more likely. Therefore, we examined whether bees responded to scent marks more strongly if they were found on complex flowers, in order to reduce these revisits. To do this, we used two types of artificial flowers that differed in handling time. Bees preferred foraging on short flowers, but accepted both types. However, when they approached flowers, bees were more than twice as likely to reject scent-marked long flowers than short ones, and the effect of scent marks lingered for 60% longer in long flowers. Bees most often rejected long flowers in flight, without direct access to tactile cues indicating floral handling time. Therefore, they solved the task by using the current visual input to recall a memory of floral handling time, and they combined this information with a current olfactory cue, that is, the scent mark.

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Scent marking can be advantageous for animals that encounter potential resources more than once (Kruuk 1992). Numerous animals ease demands on memory by tagging depleted food sources with easily detected scent marks. The two animal groups best represented in the literature are canids and bees, although many other animals probably share this behaviour (Henry 1977; Harrington 1981, 1982). Bees, which forage in a complex and unpredictable environment, scent-mark emptied flowers with tarsal gland secretions (Schmitt et al. 1991; Williams 1998; Goulson et al. 2000). This behaviour allows animals to avoid time-consuming inspections of unprofitable food sources.

Bees must sometimes handle a wide range of flowers. Because most floral rewards are very small, bees have to forage on hundreds of flowers per foraging bout to fill their honey crops (Ribbands 1949). Floral nectar is usually a renewing resource, but the time it takes a flower to refill with nectar typically exceeds the duration of a foraging bout or visits within a patch; therefore, bees should avoid returning to flowers that they have recently visited. One way of doing this would be to remember all individual flowers they have already exploited. Although bees have impressive memory capacities (Chittka et al. 1999; Menzel 1999; Giurfa et al. 2001; Menzel & Giurfa 2001; Giurfa 2003a, b), it is probably not feasible for them to store the locations of several hundred individual flowers (Goulson 2000). Yet bees do have some alternative behaviour patterns that are thought to help decrease revisits to flowers. One method is maintaining flight directionality, that is, preferentially moving between flowers along one predominant compass direction (Waddington & Heinrich 1979; Corbet et al. 1981; Pyke & Cartar 1992; Kells & Goulson 2001). However, when foraging from flowers that have equal rewards but differ in access time, bees will sometimes abandon this directionality when the flowers require a long handling time (Schmid-Hempel 1984), or when the number of rewarding flowers they encounter increases (Pyke 1978; Schmid-Hempel 1984). Bees also show less directionality when flowers are aggregated within small spatial scales (Ohashi & Yahara 2002).

A potentially more efficient strategy is to tag all visited flowers with scent marks to avoid revisiting recently probed flowers when foraging (Frankie & Vinson 1977; Giurfa & Nunez 1992; Giurfa 1993; Giurfa et al. 1994;

Goulson et al. 1998, 2000; Stout et al. 1998; Williams 1998; Stout & Goulson 2001, 2002). Bees, indeed, do this by using transient marks that are composed of volatile compounds secreted from the tarsal glands (Schmitt et al. 1991; Goulson et al. 2000). Stout & Goulson (2002) have shown that bees can adjust their reliance on scent marks depending on the nectar secretion rates of the flower species, thereby probing the flowers when they should have refilled with nectar. This suggests that use of scent marks when foraging is a learned behaviour. Relying on such marks has also been shown to reduce time spent probing unprofitable artificial flowers (Giurfa & Nunez 1992). Scent marks have an additional advantage of allowing a bee to track visits made by other bees (Stout et al. 1998). This provides information not present in the other spatial foraging tactics that help individuals avoid returning to flowers that have just been visited.

In theory, we would expect bees to avoid visiting any scent-marked flower, regardless of the costs associated with gaining access to rewards. There are conditions, however, when a strict rejection of all scent-marked flowers may be maladaptive. Bees do not always remove all the nectar from flowers (Hodges & Wolf 1981; Wetherwax 1986). Most individuals may find extracting this remaining nectar more costly than moving to the next flower to imbibe the reward there. However, the nectar left behind may still be a valuable resource for another bee that has a longer proboscis, better handling ability, is foraging for a starving colony or has low metabolic needs. Flowers may also get an excess deposition of tarsal scent if, for example, the bee pauses there to groom. So, although the flower is marked, it may already contain sufficient reward. Thus, scent-marked, recently visited flowers may still be worth probing if the costs associated with such visits do not exceed the gains from the possible rewards. This may be the case for simple, quickly handled flowers, but is less likely to be true for complex flowers with long handling times. We suspect, therefore, that bees can respond flexibly to scent marks. We investigated whether bees can use the scent marks facultatively, by relying on scent marks more when they encounter flowers with long handling times, such as those with a complex morphology, than flowers of simple morphology where probing costs are relatively low.

Handling costs can differ by a factor of 10 between simple and complex flowers (Laverty 1994; Ohashi 2002). However, it is often advantageous for bees to forage on complex flowers, because they are exploited by fewer individuals and thus may contain more reward than simple flowers (Heinrich 1975). As stated earlier, bees show less directionality when foraging from flowers with long handling times (Schmid-Hempel 1984) and highly rewarding flowers (Pyke 1978; Schmid-Hempel 1984). Although this loss of directionality increases the probability of visiting other such highly rewarding flowers nearby, it also increases the risk of revisiting flowers that have just been drained. Therefore, bees foraging from complex flowers might respond especially strongly to scent marks. Evidence suggests that bees can indeed perform this feat in field conditions (Goulson et al. 2001), but, given how difficult it is to manipulate handling time while keeping all other factors equal in the field, we conducted a controlled laboratory experiment to confirm these observations.

METHODS

Test Animals and Flight Arenas

We obtained colonies of *Bombus impatiens* from Biobest Canada Ltd. (Leamington, Ontario, Canada). Each colony was connected to a flight arena by means of a transparent Plexiglas tube. This tube contained movable plastic flaps (henceforth 'doors') to allow only selected individual foragers into the flight arena. Pollen was fed directly into the nest.

Two flight arenas were used in parallel for all experiments. In one arena (henceforth, the scent-marking arena), bees foraged freely from artificial flowers, and in the process left scent marks on them. Flowers with such scent marks were then offered to foragers in the other arena (henceforth, the test arena), where we studied the responses of individual foragers. In the course of the experiments, we used a variety of flight arenas of various sizes: 100×40 cm and 70 cm high, $75 \times 75 \times 75$ cm, $105 \times 72 \times 30$ cm and $103 \times 71 \times 30$ cm. The flower array presented to bees, however, was identical in all cases (see below). A green Bristol board was taped to the entire floor of each arena.

Artificial Flowers

Two types of flowers were designed from 5-ml Polypropylene Round-Bottom Tubes (12×75 mm style; Falcon, Becton Dickinson Labware, Franklin Lakes, New Jersey, U.S.A.). The flowers were designed to allow us to change the handling time by adjusting the length of the tubes. The 'simple' or short flowers were cut to 2 cm in depth. Unmodified 7.5-cm test-tubes served as the 'complex' or long flowers. Each flower was inserted into a block of light blue extruded Styrofoam measuring $2.5 \times 2.5 \times 2$ cm, to allow these flowers to stand upright. The long flower penetrated the Styrofoam block at a $45°$ angle to facilitate access for the bees. Each flower had a white filter paper collar of 2.4-cm diameter (3MM Qualitative, Whatman, W & R Balston Ltd., Maidstone, U.K.) around the top (Fig. 1a, b). Three short and three long flowers were placed 15 cm apart on a rectangular array (Fig. 1c). This array was placed on a piece of cardboard (20×32 cm) that was covered with green Bristol board, henceforth called a tray. Velcro patches, placed on the trays 15 cm apart, were used to hold the flowers in place on the boards.

In preliminary trials, we determined the mean handling time of empty flowers. Short flowers took a mean $\pm$ SD of 1.66 ± 0.58 s to probe and long flowers took 6.50 ± 1.18 s ($N = 17$ bees in both cases). Therefore, access to the information that a flower is empty entailed ca. four times the temporal costs in long flowers than in short flowers. Thus, our artificial flower types are well suited to explore how bees respond to scent marks depending on handling
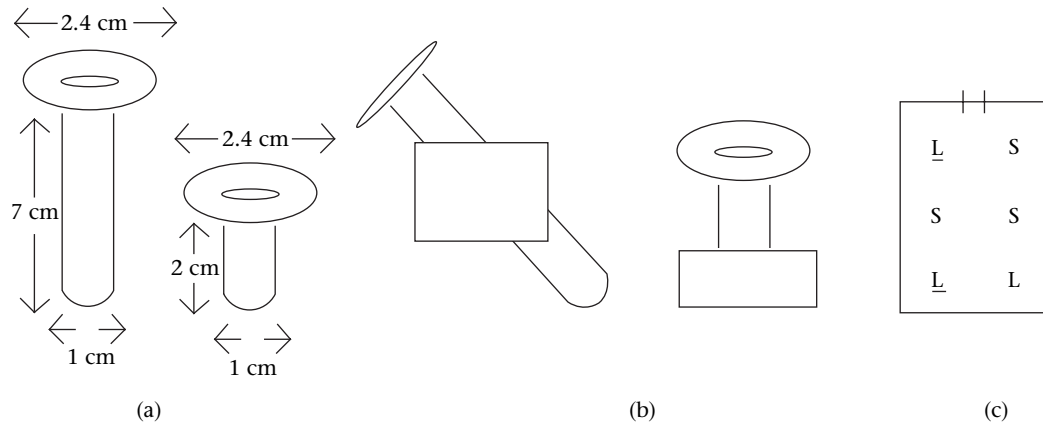
Figure 1. (a) Long and short flower dimensions. (b) Manner in which flowers were presented to the bees: long flowers penetrated the Styrofoam at a 45° angle to facilitate access for the bees; short flowers were placed on top of a light blue Styrofoam base to allow these flowers to stand upright. (c) Tray with flowers on it: L: long flower; S: short flower; underlined flowers indicate test flowers that replaced regular flowers in experiment 1. In experiment 2, the last row of flowers was not presented. The two lines at the top indicate the entrance to the arena. The example is for long flowers, but the same set-up was used for short test flowers. All flowers were moved randomly between bouts.

costs. All experiments were videotaped and all bees foraged from both flower types.

Experimental Procedures

Training: familiarization with set-up

Whether the response of bees to tarsal scent marks on flowers is innate is unknown. Therefore, we introduced an experimental phase to allow bees to familiarize themselves with the experimental set-up and, if they needed to, make the appropriate associations between empty flowers and scent marks. The forager in the test arena was offered short and long flowers (on trays as described above), with each flower containing 13 µl of 30% sucrose solution. Flowers were refilled only between, not during, the bouts. Thus, returns to previously drained flowers within a bout did not yield nectar. The type of flower at each position was changed randomly for each foraging bout, to exclude the possibility of spatial learning between bouts. Each bee foraged on this set-up for 15 bouts before she was moved into the experimental phase.

Experiment 1: effect of flower type

The experimental phase differed from the training phase trials in that one flower among the six was scent marked. The position of this flower was randomly chosen and the bee's behaviour towards it compared to another randomly chosen unmarked flower (Fig. 1c). These 'test' flowers were either both long or both short. All six flowers used in each bout for this phase were brand new, never previously touched by bees and touched only by powder-free latex gloved hands (SafeSkin PFE, Kimberly-Clark Worldwide Inc., Roswell, New Mexico, U.S.A.).

After her 15th bout, the test forager was kept outside of the arena until a scent-marked flower was ready. Meanwhile, live bees from the scent-marking arena were used to scent-mark the test flowers. These bees were allowed to forage from two flowers (one long and one short) ad libitum. Before allowing them to mark the test flowers,

we filled the flowers with 13 µl of 30% sucrose solution, to ensure that the bees went all the way into them. After they left the flower, we immediately added 13 µl of sucrose solution to the marked flower, placed it in its position on the tray, and put the tray into the test arena. The test forager was then allowed to enter the experimental arena, and her responses to each test flower were monitored. We repeated this procedure six times, three times with long test flowers and three with short. We tested 20 experimental bees in total and each bee was tested only once.

See Appendix for data analysis.

Experiment 2: memory for flower type

If bees do reject scent-marked flowers with different probabilities, depending on their complexity, then one possibility is that they act upon stored information about the handling costs of these flowers. Alternatively, more scent mark may be left on the long flowers, because the marking bees spent more time on them or because evaporated scent molecules were slower to diffuse out of the deeper tubes. To tease these possibilities apart, we used scent marks left on filter papers of short flowers from the scent-marking colony and placed them on clean flowers of either short or long design in the other arena, where they were presented to the experimental bees. With this procedure, bees were confronted with flowers of unequal handling time, but paired with (on average) equal amounts of scent mark.

In the test colony, a bee underwent the same training as described above. However, in this experiment, bees were tested with four unrewarding scent-marked flowers (two long and two short), instead of six, to hasten the transfer of flowers from one arena to the other. Each test trial lasted 5 min, but was terminated prematurely if the experimental bee did not interact with the flowers more than once in 1 min. This was to avoid any negative reinforcement that may have caused the significance of the scent marks to change or caused the bee to lose her foraging

motivation because she was unrewarded for a long period. Between test bouts, bees were allowed to forage for two nontest bouts, with four rewarded flowers (two short and two long) that contained 13 μl of 30% sucrose solution. We conducted five test sessions for each bee.

After the test forager's 15th bout she was held between the two doors while the filter paper was being marked. Bees of the scent-marking colony foraged continuously from six flowers. The top of these flowers consisted of test-tubes 2 cm long (same design as short flowers used throughout). Filter paper was placed around the entrance of the test-tubes as described above. Sucrose was dispensed at 1.2 μl/min into a syringe needle in the middle of the test-tube by means of a motor. After one or more bees landed and probed the flowers in the scent-marking arena, we removed the filter paper with forceps. Bees were never forced off the flowers to ensure that they did not leave any possible alarm pheromone or distress cues that may have disrupted the experiment. When all flowers had marked filter paper we moved the tray to the test arena and released the experimental bee. While she was foraging, a new set of filter papers was placed on the flowers in the scent-marking arena. Experimenters wore gloves throughout the test phase. The type of flower at each position was randomized for each bout. We used 10 bees in total.

See Appendix for data analysis.

RESULTS

Experiment 1: Effects of Flower Type

Bees were more likely to accept unmarked flowers than scent-marked flowers (scent-marked: mean percentage acceptance ± SD= 45.8 ± 29.9%; unmarked: 94.2 ± 12.8%; sign test on selectivity index: $N = 40$, $P < 0.0001$; Fig. 2a). There was no significant difference in acceptance between short and long unmarked flowers (long: 93.3 ± 13.7%; short: 95.0 ± 12.2%; sign test on selectivity index: $N = 40$, $P = 1$; Fig. 2a), but there was a significant difference in acceptance of scent-marked flowers depending on flower type. The short scent-marked flowers were more likely to be accepted at first approach than the long scent-marked flowers (short: 56.7 ± 30.8%; long: 35.0 ± 25.3%; sign test on selectivity index: $N = 40$, $P = 0.013$; Fig. 2a). This was also the case when we took into account the ratio of marked to unmarked flowers accepted within each flower type (sign test on selectivity differential: $N = 40$, $P = 0.021$). This confirms that bees indeed respond differently to scent marks, depending on whether they encounter them on flowers with short or long handling times.

Bees took longer to accept marked flowers than flowers that were not marked (scent-marked: mean time to accept ± SD = 139.4 ± 59.9 s; unmarked: 58.7 ± 22.6 s; Mann–Whitney U test: $W = 2221.5$, $N = 40$, $P < 0.00001$; Fig. 2b). There was no significant difference between time to acceptance of long and short unmarked flowers (short: 61.7 ± 26.4 s; long: 55.9 ± 17.6 s; $W = 445.5$, $N = 20$, $P = 0.34$). However, there was a significant difference between time to acceptance of scent-marked long
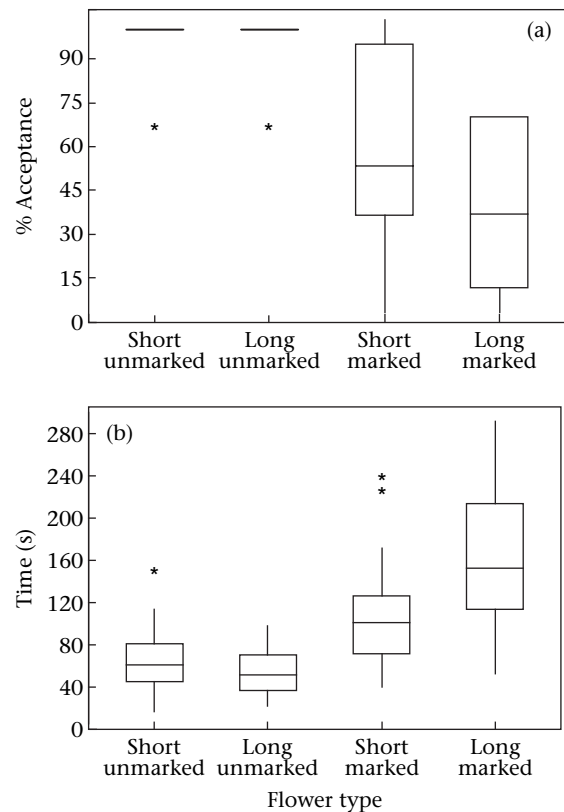

Figure 2. (a) Percentage of short and long flowers accepted by bees in experiment 1. (b) Time to first acceptance of flowers in experiment 1. Horizontal lines inside the boxes are medians; the boxes indicate the interquartile range and the whiskers indicate the range of the data; asterisks signify outliers ($N = 20$ for each column).

and short flowers ($W = 315$, $N = 20$, $P = 0.01$), where short marked flowers were more likely to be accepted at first approach than long marked flowers (short: 106.9 ± 48.6 s; long: 173.9 ± 49.8 s; Fig. 2b).

Experiment 2: Memory for Flower Type

In this experiment, bees were still more likely to accept short flowers than long flowers, when both flowers had equal amounts of scent mark (short: mean percentage acceptance ± SD = 23.0 ± 15.0%; long: 11.1 ± 7.10%; sign test on selectivity index: $N = 10$, $P = 0.002$; Fig. 3a). Time to acceptance was still significantly longer for long flowers than for short flowers (long: mean time to accept ± SD = 267.1 ± 102.5 s; short: 191.8 ± 120.3 s; Mann–Whitney U test: $W = 77.0$, $N = 10$, $P = 0.037$; Fig. 3b). Overall, short flowers received a higher visit rate (i.e. total approach behaviours) than long flowers (short: mean number of visits ± SD = 70.8 ± 24.5 visits; long: 44.0 ± 22.2 visits; $W = 135.5$, $N = 10$, $P = 0.023$). The fact that bees preferred to forage on short flowers within a bout does not influence the results for the acceptance of unmarked flowers because we took only the first approach to each test flower within each bout when analysing the data.
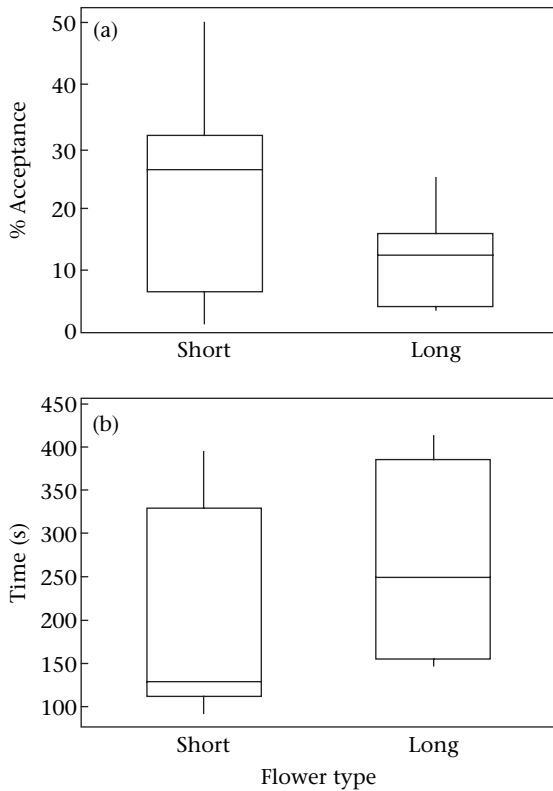
68.5 ± 16.3%; sign test on selectivity index: $N = 10$, $P = 0.002$; Fig. 4).

Figure 3. (a) Percentage of short and long flowers accepted by bees in experiment 2. (b) Time to first acceptance of flowers in experiment 2. Horizontal lines inside the boxes are medians; the boxes indicate the interquartile range and the whiskers indicate the range of the data ($N = 10$ for each column).

Rejection of Flowers in Flight

Compared to short flowers, long flowers were more likely to be rejected in flight before landing (long: mean percentage of in-flight rejection ± SD = 30.1 ± 13.3%; short: 8.1 ± 5.11%; Fig. 4). Rejection of short flowers occurred more commonly after landing (long: mean percentage of landing rejections ± SD = 38.7 ± 15.9%; short:
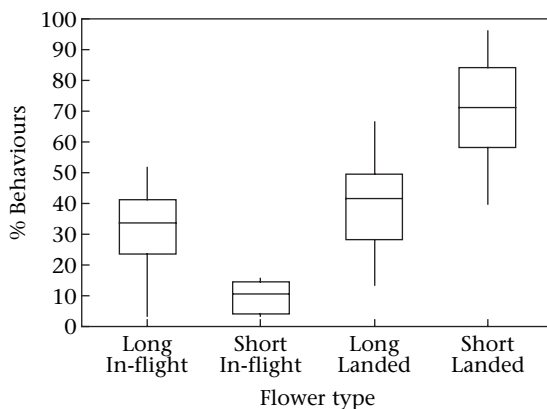

Figure 4. Percentage of total behaviours that were in-flight or landing rejections. Horizontal lines inside the boxes are medians; the boxes indicate the interquartile range and the whiskers indicate the range of the data ($N = 10$ for each column).

DISCUSSION

In deciding to accept or reject flowers, bumblebees were more likely to rely on scent marks when the flowers took longer to handle. The bees rejected more of the long scent-marked flowers, and when they accepted them the acceptance took longer. We also found that bees approached short flowers more frequently than long flowers. Thus, as predicted, bees appear to be minimizing the energy and time they spend probing flowers by selectively rejecting marked flowers with long handling times, as well as selectively approaching flowers with short handling times. The bees were also able to reject flowers without landing on them.

In experiment 1, the scent-marking bees were allowed to crawl into the flower to mark it; therefore, the shorter flowers presumably contained less scent (fewer footprints) than the longer flowers. This experiment did not allow us to distinguish whether the bees were relying more heavily on the scent marks found on long flowers because more mark was present or because they were relying on memory of the handling costs associated with each flower type. In experiment 2, however, the bees still rejected more long flowers, even though they bore equal amounts of scent mark. The quantitative trends in acceptance rates and time to acceptance in the two experiments were very similar despite the differences in the amount of mark deposited. This indicates that the bees relied on previously stored information about floral complexity rather than the concentration of the scent on the flowers.

Such facultative use of scent marks would place no small demands on cognitive ability. If foragers do selectively reject scent-marked complex flowers more consistently than scent-marked simple flowers, they need to identify the flower type ('species') from some cues, such as its visual appearance, and that appearance must help them to retrieve the memory of its handling costs. With this, they have to combine the chemosensory information of whether a scent mark exists. The retrieval of this memory can be done in several ways. The bees may have to perform part of the motor pattern in order to trigger this memory or they may be able to do it while hovering near the flower. Distinguishing between these two modes of memory retrieval will provide insight into how sensory cues are stored in the bee's brain.

In previous studies, bees have been shown to reject flowers while hovering in front of them, and this is thought to be the result of the bees detecting scent marks (Corbet et al. 1984; Wetherwax 1986; Kato 1988). In our study, bees rejected flowers without actually alighting on them. To make such decisions, bees need to be able to use the visual appearance of the flower to recall the memory of the flower handling time, and integrate this with the current olfactory cue, that is, a scent mark. It is important that the bees did not necessarily need to perform part of the motor pattern in order to trigger the memory of the flower type and its association with a scent mark. They

were able to retrieve this information with only the visual input, the image of the flower, acting as the memory trigger (Chittka et al. 1997).

There may be many scenarios in which such facultative reliance on scent marks can support adaptive foraging. Consider the motivational state associated with hunger or starvation. For example, hungry wasps, *Microplitis croceipes*, maintained significantly higher proboscis extension responses to a conditioned odour without reward than did well-fed wasps (Tertuliano et al. 2004), suggesting that hunger state can influence reliance on sensory cues. Bees foraging in a resource-poor, highly competitive environment (or from a starving colony) may want to probe as many flowers as they can because they will find some reward in these flowers. Bees do not always remove all the nectar from flowers, so even recently drained flowers might still contain residual rewards, even though they are scent marked (Hodges & Wolf 1981; Wetherwax 1986). Thus, we may find, when food is scarce, that these bees rely on scent marks less when foraging from both complex and simple flowers because of the minimal rewards that they can obtain from probing recently visited flowers.

Bees have versatile abilities to make associative memories depending on the context involved (Giurfa 2003a). Our study has shown that bees rely on scent marks differently when they are found on flowers that differ in handling time. They are more likely to reject flowers with a long handling time, if these have recently been visited by other bees, thereby reducing revisits. However, this is not the case with flowers that have short handling times. Bees foraging on simple flowers in the wild are expected either to revisit more, or rely on other cues or behavioural tactics to avoid revisits (such as the ability to maintain directionality). Bees were also able to reject flowers with long handling times without landing on them, indicating that they rely on a visual memory of the flower type to retrieve information of the handling time associated with flowers. They then use this information in conjunction with the presence of scent marks to decide whether or not to visit flowers. This complex use of information further underlines the complex behavioural abilities of bees.

Acknowledgments

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Appendix

Data analysis

In scoring the videotapes, we recorded an approach if a bee either landed on a flower or came to within 1 cm of the flower while hovering in front of it. Approaches were subdivided into acceptances or rejections based on criteria that were specific to the flower type. We scored acceptances only when a bee entered a flower and her head reached the halfway point for a short flower, or the 3/5 point for a long flower. Approaches that did not meet the appropriate criterion were scored as rejections. For certain analyses, we further subdivided rejections into those in which the bee landed on the flower with all six legs (landing rejections) or in which she hovered for ≥ 1 s, less than 1 cm away from the flower (in-flight rejections). In-flight rejections may sometimes have involved antennal contact with the flower in flight. Distances were determined from videotape recordings.

To ensure that bees were not relying on spatial memory of the flowers within bouts, we looked only at a bee's first approach to the scent-marked test flowers in each bout in each experiment. We determined the bees' preferences by calculating selectivity indices for each bee, which were determined from the sum of the bee's acceptances for each treatment. These indices were then analysed with a sign test. The selectivity indices were calculated as follows. A value of zero meant no preference in all cases examined.

Experiment 1

When looking at the differences between marked and unmarked flowers regardless of flower type we used the formula $1 - M/U$ where M is the total number of acceptances of marked flowers by the bee and U is the total number of acceptances of unmarked flowers. A positive value indicates that the bee had a preference for unmarked flowers and a negative value indicates that the bee had a preference for marked flowers. We also looked at the number of acceptances in relation to flower type. Thus, we compared acceptances of marked long and short flowers as well as acceptances of unmarked long and short flowers. These were calculated with the following formulae: $1 - (LM/SM)$ and $1 - (LU/SU)$ where LM is the total number of acceptances of long marked flowers, SM is the total number of acceptances of short marked flowers, LU is the total number of acceptances of long unmarked flowers, and SU is the total number of acceptances of short unmarked flowers. In both of these cases a positive value for the selectivity index indicates that bees preferred foraging on short flowers and a negative value indicates that bees preferred foraging on long flowers.

Next we wanted to look at what bees did when we took into account that they were foraging in a mixed array of marked and unmarked flowers. We did this by calculating selectivity indices for each flower type. Thus, we used the following formula: $(SM/SU) - (LM/LU)$, to yield a selectivity differential; a positive value indicates a preference for marked short flowers and a negative value indicates a preference for marked long flowers.

In addition to examining patterns of decisions (above), we also measured the time it took a bee to make those decisions. For experiment 1, the time to first acceptance was measured from the time the test flowers (one marked and one unmarked) were placed in the test arena until the bee landed on them. For experiment 2, this was the time the filter paper was placed on the test flowers. The method used in experiment 2 allowed us to measure the time from when the scent mark was placed on the flower to when the bee accepted the flower more accurately. Specifically, we asked whether a bee took longer to accept marked flowers and if this time to acceptance differed for short and long flowers. If the bee did not accept a flower during the entire foraging bout, then the time to acceptance was recorded as the time from the beginning of the trial to when the bout ended. Note that this is conservative, since bees might have taken even longer to have accepted such flowers. We compared time to acceptance for the two flower types by taking the median value of each bee for each flower type (recall that each test was repeated three

(experiment 1) or five (experiment 2) times; see above) and using this median for Mann–Whitney U tests with bees as the unit of replication.

Experiment 2

We calculated a selectivity index for the acceptance rate of long and short flowers by using the following equation $1 - (L/S)$ where L is the total number of acceptances of long flowers and S is the total number of acceptances of short flowers (recall that all flowers in this part of the study were marked). A positive selectivity index means that the bee prefers short flowers and a negative value that she prefers long flowers.

To determine how the bees retrieved their memories of the association of flower types, handling time and presence or absence of scent marks, we analysed the number of rejections that took place in-flight and compared them to the number of rejections that took place when the bee landed on the flower. Thus we calculated the selectivity index for each behaviour with the equation (LL/LS) − (IL/IS), where LL is the total number of landing rejections towards long flowers and LS is the total number of landing rejections towards short flowers, IL is the total number of in-flight rejections towards long flowers and IS is the total number of in-flight rejections towards short flowers. This gave a selectivity differential. If this differential is positive it means that in-flight rejections occur more often with long flowers, and a negative value indicates that landing rejections were more common with long flowers. Times to acceptance were analysed as described above for experiment 1.