Honey bees are predisposed to win–shift but can learn to win–stay

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Abstract. The ability of honey bees, *Apis mellifera*, to avoid returns to locations recently depleted of sugar solution (win–shift) or to return to locations recently depleted of sugar solution (win–stay) was tested. Bees collected sugar solution from a small matrix of six cells. During each of a series of trials, they first visited a randomly determined set of three cells. They were then allowed to freely choose between the six cells, with the contingencies encouraging either win–shift or win–stay behaviour. Previous research indicates that honey bees use spatial working memory to discriminate previously visited cells from unvisited cells in this experimental preparation (Brown & Demas 1994, *J. comp. Psychol.*, **108**, 344–352). In the present experiment, bees in the win–shift condition tended to choose previously unvisited cells throughout the experiment. Bees in the win–stay condition learned to choose previously visited cells over the course of the experiment. These results indicate that bees choose locations based on previous visits, either being attracted to, or repelled from, locations recently depleted of forage.

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Although a food reward typically increases the probability of a behaviour, in the context of spatial food-gathering tasks many animals tend to search elsewhere for food after finding it and gathering it from one location. This avoidance of revisits to locations where food has recently been found (and typically depleted) is referred to as a win–shift tendency.

A win–shift tendency has been found in a wide range of vertebrate animals, but has been studied in detail using the radial-arm maze, a laboratory task in which rats gather food from a set of locations (usually eight or 12), arranged in a circle (Olton et al. 1981). Olton & Schlosberg (1978) showed that young laboratory rats (with no natural foraging experience) readily learned to choose locations following a win–shift rule, but slowly or never learned to choose locations following a win–stay rule. They concluded that rats have a natural win–shift predisposition, but that choices are also sensitive to the pattern of food distribution in the environment.

In the present study we examine the win–shift or win–stay tendencies of honey bees foraging in a

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semi-naturalistic laboratory situation for sugar solution. In nature, honey bees forage for both nectar and pollen from a wide variety of flowers (e.g. Seeley 1985; Winston 1987), with nectar foraging much more common during the late summer, which is when we conducted the present experiment. Most flower species exploited by bees produce nectar at a sufficiently low rate that the flower is depleted of nectar during a single visit (Pleasants 1981). Thus, the contingencies operating in the natural environment of bees appear to encourage a win–shift tendency. On the other hand, flowers vary widely in nectar production rate, across both flower species and environmental conditions (e.g. soil and rainfall). Thus, it may be advantageous for honey bees to be somewhat flexible in terms of win–stay versus win–shift tendencies.

Brown & Demas (1994) recently developed a task for the study of spatial choice in honey bees that is analogous to the radial-arm maze task used with rats and other vertebrates. We showed that bees avoided revisits to recently visited locations in accordance with a win–shift tendency, and did so using memory for the identity of previously visited locations. As indicated above, however, honey bees might also behave according to a win–stay tendency if the contingencies encouraged doing so. The present experiment used the basic

procedure developed by Brown & Demas to examine this possibility. Two groups of honey bees were exposed to two different experimental conditions. In both conditions, bees visited three randomly selected locations and were then allowed to choose freely between all six locations. In the win–shift condition, only the three locations not previously visited were baited during these free choices. In the win–stay condition, only the locations that had been previously visited were baited during the free choices. The ability of bees to locate baited cells during the free choices was examined in each condition.

METHOD

Subjects

We used 20 honey bees, *Apis mellifera ligustica*, that were presumed to have been residents of one of two standard hives maintained in a secluded meadow on the campus of Villanova University. These hives had large stores of honey and pollen when the experiment was conducted, during late July and August 1993. Bees were recruited as subjects from a feeder located approximately 4 m from the hives and containing 50% (v/v) sugar solution.

Apparatus

The apparatus, which was identical to one used by Brown & Demas (1994), consisted of a 2 × 3 matrix of holes in a 28 × 28-cm plywood surface (6 mm thick) that was painted violet. Underneath each hole was a clear polystyrene cylindrical cell (one cell in a Corning tissue culture tray no. 25820; 18 mm deep and 15 mm in diameter), open at the end facing the bottom of the plywood. Six differently coloured and shaped children's blocks, intended as spatial landmarks (Cartwright & Collett 1982; Gould 1987; Couvillon & Bitterman 1992), were located on the surface.

This apparatus was located inside a clear Nalgene container (measuring 52·5 × 34·4 × 18·8 cm deep), with a removable clear Plexiglas lid. The lid allowed us to control access to the apparatus, such that only one bee interacted with the apparatus at a time. The apparatus was situated approximately 20 m from the two hives. We baited cells of the apparatus using a micro-

dispenser. We videotaped trials using a camcorder (Panasonic AG-160).

Procedure

Bees were initially recruited to the apparatus by transporting them to the apparatus from the feeder in a plastic vial. There, we opened the vial and inverted it over one of the cells, which was baited with sugar solution. When the bee entered the cell and commenced collection of the solution, we removed the vial. During this first visit to the apparatus, we marked each bee with model paint (Testor's) on its abdomen and/or thorax to allow identification. Most bees treated in this manner returned to the apparatus, thereby becoming subjects in the experiment. Among those that did not return, several were located at the feeder and the recruitment procedure was repeated. Typically, while the experiment was being conducted, sugar solution was not available at the feeder.

We randomly assigned each successfully recruited bee as a subject to either the win–shift or win–stay condition, until there were 10 subjects in each condition. We conducted 30 trials per subject. Prior to each trial, we baited cells with 2 µl of 50% sugar solution. When the subject bee approached the apparatus, we opened the lid of the apparatus container, allowing it to enter the apparatus container. We defined a cell visit as the bee entering a cell through the corresponding hole, such that at least its head and thorax were inside the hole. When a trial was completed, we opened the lid and the bee left the apparatus. Typically, bees left the area of the apparatus for at least 5 min before returning for the next trial.

Win–shift condition

We baited all six cells prior to each trial. Access to three randomly chosen cells was blocked, however, by placing bolts (with round heads 1·5 cm in diameter) in the holes corresponding to each of the three cells. The bee was allowed to visit the three cells to which access was not restricted. While it was in the third of these cells, we removed the bolts, thereby allowing free access to all six cells. We then allowed the bee to visit cells until it had visited all the baited cells and flew into the lid of the container, or until 2 min elapsed without a visit.

Win–stay condition

Three randomly chosen cells were baited prior to each trial. We blocked access to the remaining

three cells using the bolts. We then allowed the bee to visit the three cells to which access was not restricted. While it was in the third of these cells, we removed the bolts and rebaited the two visited cells. We rebaited the third cell to which access had been allowed by the bolts while the bee was in the first cell visited following bolt removal. As in the win–shift condition, the bee was allowed to visit cells until it had visited all the baited cells and flew into the lid of the container, or until 2 min elapsed without a visit.

Probe trials

In a series of probe trials following the primary experiment, we tested any ability of the bees to perceive the presence of solution in cells prior to visiting the cell (e.g. by sight or odour), as follows. We allowed a subset of eight bees that had completed the primary experiment to return to the apparatus for five additional trials. Prior to each of these probe trials, we baited three randomly chosen cells. None of the cells was restricted by the bolts, however, so bees could choose freely between the six cells throughout the trial. We considered a probe trial to be complete when 2 min elapsed without a visit, or when all six cells had been visited.

Data Analysis

We analysed data from the primary experiment in three blocks of 10 trials each. Choice accuracy was measured in terms of the number of correct choices (visits to baited cells) during the first three choices following removal of the bolts. We chose this measure because it is analogous to the measure of choice accuracy most commonly used in the radial-arm maze task. In addition, the measure restricts the analysis to choices made immediately following bolt removal. This restriction is important because, in the win–stay condition, choices made late in the choice sequence are subject to both an intended win–stay contingency and an unintended win–shift contingency. The latter contingency stems from the fact that cells visited following bolt removal are not rebaited. Thus, the first three choices made after removal of the bolts represent behaviour that is subject to a relatively purer win–stay contingency.

As in our earlier experiments (Brown & Demas 1994), we compared the choice behaviour of individual bees to a modified estimate of chance, designed to control for the possibility that preferences for specific cells or systematicity in the cell-to-cell movement pattern of the bee may affect the probability of revisits. We used the data from individual bees for each block of trials to construct matrices of transition probabilities. Given that a bee was in each of the six cells, we determined the probability of moving from that cell to each of the six cells. We only included cell-to-cell transitions during the free choices (i.e. after the bolts were removed, allowing free access to all six cells) in these transition probability matrices. We then used the probability matrices in a Monte Carlo simulation, which chose cells using the same transition probabilities as individual bees, but without regard to the identity of previously visited cells.

For each simulated trial, the algorithm used to implement the Monte Carlo simulation first randomly chose three alternatives from a set of six, emulating that the first three cells visited by the bees were chosen randomly. The algorithm then chose between all six alternatives, using the cell-to-cell transition probabilities of individual subjects during individual trial blocks. The simulation was run 1000 times using each bee's transition matrix during each of the three trial blocks. This simulation allowed an estimate of chance that controlled for any cell preferences or cell-to-cell movement biases of individual bees over the course of the experiment.

RESULTS

Figure 1 shows the mean choice accuracy of the bees and the simulations in the two experimental groups during the three trial blocks. We conducted a 3 (trial block) × 2 (empirical versus simulated data) × 2 (experimental condition) analysis of variance (ANOVA), with repeated measures on the first two variables. The bees performed more accurately than the simulations ($F_{1,18}=61\cdot6$, $P<0\cdot001$). There was no main effect of trial block ($F_{2,36}=3\cdot0$) or experimental condition ($F_{1,18}<1\cdot0$). However, there was a significant triple interaction between the effects of the three variables ($F_{2,36}=3\cdot45$, $P<0\cdot05$).

To understand the form of the triple interaction, we analysed the data using separate ANOVAs for the two experimental conditions. In the win–shift condition, empirical performance
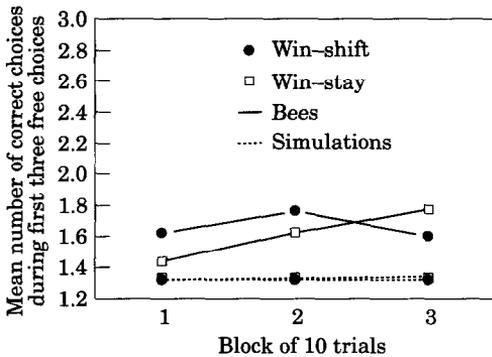
Figure 1. Choice accuracy during the first three free choices of the bees and simulations in the win–shift and win–stay conditions.

was superior to that of the simulations ($F_{1,9}=69.2$, $P<0.001$), but neither the effect of trial block ($F_{2,18}<1.0$) nor the interaction term ($F_{2,18}=1.2$, $P>0.05$) was significant. In the win–stay condition, empirical performance was also superior to that of the simulations ($F_{1,9}=16.9$, $P<0.01$). In addition, the main effect of trial block ($F_{2,18}=8.3$, $P<0.01$) and the interaction between the effects of these variables ($F_{2,18}=13.3$, $P<0.001$) were significant. The interaction found in the win–stay condition can be understood by comparing empirical and simulation performance during each of the three trial blocks. There was no difference between empirical and simulation performance in the win–stay condition during the first trial block ($F_{1,9}=1.3$, $P>0.05$), but the corresponding values were significantly different during the second ($F_{1,9}=13.8$, $P<0.01$) and third ($F_{1,9}=36.1$, $P<0.001$) blocks. These results confirm the apparent effects seen in Fig. 1. The choice accuracy of bees in the win–shift condition was slightly but significantly superior to the estimate of chance provided by the simulations throughout the 30 trials of the experiment. The performance of bees in the win–stay condition was also superior to chance performance, but it improved (relative to chance) over the course of experimental trials. Initially, win–stay accuracy was equivalent to chance, but during the last 10 trials, it was equivalent to the accuracy shown by bees in the win–shift group.

Probe Trials

We ranked cells visited during each probe trial according to the ordinal position of the initial visit

to that cell (i.e. we assigned the cell visited first a rank of 1, the cell visited second a rank of 2, etc.). If cell visits were controlled by an ability to discriminate the presence of sugar solution perceptually, then the mean rank for baited cells should be lower than the mean rank for unbaited cells. The mean ranks for baited and unbaited cells were 3.33 and 3.68, respectively, which were not significantly different (Wilcoxon rank sum test: $W=10.5$, $P>0.10$).

DISCUSSION

The behaviour of bees in the win–shift condition was very similar to that of bees in the earlier experiments of Brown & Demas (1994). Specifically, bees had a small but reliable tendency to avoid revisits to locations recently depleted of sugar solution. In the context of this replication under win–shift contingencies, the win–stay results represent a degree of flexibility in the spatial food-gathering behaviour of bees. When the contingencies encourage it, bees can learn to return preferentially to locations recently depleted of sugar solution. These win–shift and win–stay abilities cannot be explained in terms of systematic cell-to-cell movement patterns or a perceptual cue.

Honey bees in the present experiment were predisposed to win–shift, but capable of learning to win–stay. Of course, these subjects probably had experience foraging for nectar prior to participating in the experiment. Given that most flowers from which these honey bees previously collected nectar are depleted during a single visit and do not quickly replenish, the predisposition found in this experiment could have been produced by this prior experience. Alternatively, honey bees may be predisposed to win–shift regardless of previous foraging experience. Experiments controlling the foraging experience of bees are necessary to address this issue.

Other factors may have worked against the win–stay contingencies in this experiment. First, the win–stay condition in fact included both win–stay and win–shift contingencies, because cells visited following bolt removal remained empty for the remainder of the trial. Thus, the win–stay contingency was in force only during the initial visit to each cell following removal of the bolts.

Second, bees completely depleted a cell of solution during each visit. In rats, the ease of learning win–shift and the difficulty of learning win–stay are amplified when locations are depleted of food when visited (Olton et al. 1981; Hermann et al. 1982). It is possible that bees would likewise learn the win–stay rule more readily if they were not allowed to deplete the solution in visited cells. These considerations suggest that bees may not always be predisposed to win–shift over win–stay. As the present results make clear, however, bees are capable of choosing according to either contingency, and are predisposed to win–shift under the present conditions.

These results show that honey bees can flexibly adapt to the contingencies controlling the outcome of revisits to locations recently depleted of food. Such locations can be visited either more often than expected by chance or less often than expected by chance. The ability of bees in nature to choose flowers contingent on the identity of recently visited flowers would have obvious adaptive value. We (Brown & Demas 1994) have shown that memory for previous visits allows previously visited locations to be discriminated from those not yet visited. The present results show that honey bees can use this memory flexibly, to allow behaviour to be directed either towards previously visited locations or away from them. Whether this ability is used under conditions of natural foraging remains to be determined.

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