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Investing in learning: studies of learning flights in honeybees,
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INVESTING IN LEARNING: STUDIES OF LEARNING FLIGHTS IN HONEYBEES, APIS MELLIFERA

By

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ABSTRACT

INVESTING IN LEARNING: STUDIES OF LEARNING FLIGHTS IN HONEYBEES, APIS MELLIFERA

By

Cynthia A. Wei

Upon discovering new sources of food, honeybees and other insects perform learning flights to memorize visual landmarks that can guide their return. Learning flights are longest following initial visits to the food and subsequently decline in duration, suggesting that investment in learning results from an active decision modulated by a bee's accumulating experience. In Chapter 1, I document various factors that influence this decision: 1) Learning flights reappear when experienced bees encounter a delay in finding food at a familiar place and the durations of such "reorientation flights" increase with the length of the delay. 2) The decay in learning flight duration over visits following such reorientation flights is more rapid than following initial discovery of the food. 3) Learning flight duration increases with the visual complexity of the scene surrounding the food, and when spatial relationships among landmarks are unstable. 4) Durations of learning flights at a new feeding place are influenced by the sucrose concentration in the food. Taken together, these experiments suggest that bees can adjust their learning efforts in response to changing needs for visual information and that both sources of spatial uncertainty and the quality of the food influence the value of such information. Implicit in this assertion is the assumption that longer learning flights allow bees to return to a goal with greater spatial accuracy. In Chapter 2, I found that: 1) longer learning flights performed at a new location increase the probability and accuracy of a bee's return

to the departure point. 2) Over time, bees shift to the use of information learned on arrival for guidance (see Lehrer and Collett 1994), but departure information is retained and can be used when arrival cues fail. 3) After performing reorientation flights in response to uncertainty at a familiar location, bees continue to rely on information learned prior to reorientation flights. 4) However, information is learned during the reorientation flight, and longer reorientation flights increase the likelihood that bees will use this updated information when old information fails. These results verify our assumption and also show that foraging choices following a reorientation flight are influenced by past experience, but that new information about landmarks can be encoded during the reorientation flights and used in subsequent searches. In Chapter 3, we expand upon our previous findings that learning flights are modulated by sucrose concentration by demonstrating that bees foraging on real flowers in an open field also perform learning flights after feeding on a new, sweeter food source. Using a more natural distribution of food in controlled environment, we also show that longer reorientation flights, performed in response to changes in food distribution and profitability, *decrease* the likelihood that a bee returns to the departure point. This results contrasts our findings in Chapter 2, where reorientation flights were triggered in response to uncertainty. Taken together, these results suggest that by performing longer learning flights, bees obtain information that can help them more accurately relocate a goal, but that this information is utilized differently depending on the circumstances prompting the learning flights in the first place. Chapter 4 concludes by discussing directions for further study. I discuss questions that arise from the results of the experiments presented here, as well as some unexpected findings that suggest interesting new directions for research.

To my parents, who never failed to ask, “how are the bees?”

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CHAPTER 1

DECIDING TO LEARN: MODULATION OF LEARNING FLIGHTS IN HONEYBEES, *APIS MELLIFERA*

INTRODUCTION

As central place foragers, honeybees must quickly learn how to navigate between their nests and feeding places remote from the nest. For long-distance navigation, honeybees rely on celestial cues and large-scale features of the terrain (reviewed by (Dyer, 1998). Once near the goal, odors and local landmarks help guide them to a food source. Although odors provide powerful cues, the ability of honeybees and other insects to return to these pinpoint locations relies critically upon their use of visual landmarks (Cartwright and Collett, 1982; Tinbergen, 1932; Tinbergen and Kruyt, 1938; von Frisch, 1967). The acquisition of such landmark information occurs through specialized learning flights, during which an insect intensively examines the location to which it will return (Iersel and Assem, 1964; Lehrer, 1991; Lehrer, 1993b; Opfinger, 1931; Tinbergen and Kruyt, 1938; Zeil, 1993a). In honeybees, when the goal involves a food source, this learning flight has been called a "turn back and look" (TBL) (Lehrer, 1991), reflecting the observation that departing bees turn around and face the direction of the goal and nearby landmarks. The flight then progresses in an arcing, circling pattern that increases in radius and height over the span of several seconds, ending when the bee flies back towards the hive. Previous studies have shown that the pivoting structure of these flights is likely to provide motion cues that allow for identification and learning of navigationally useful nearby landmarks (Cheng et al., 1987; Collett, 1995; Collett and Zeil, 1997; Lehrer, 1991; Lehrer, 1993b; Lehrer and Collett, 1994).

A particularly interesting feature of this learning behavior is that the learning follows receipt of a reward, and can thus be considered an example of "backwards conditioning" (Lehrer 1993b). In classical conditioning terms, the occurrence of the conditioned stimulus (CS), which in this case is the visual features that predict the location of food, follows that of the unconditioned stimulus (US), the presence of food. Although some learning of the visual features near the goal also occurs on arrival (Bitterman and Couvillon, 1991; Couvillon et al., 1991; Lehrer, 1993b; Opfinger, 1931), learning on departure is critical. This is demonstrated by the fact that without the performance of a learning flight, bees are unable to find their way back to a particular location (Lehrer 1993b).

Given that bees do learn upon arrival, why do they learn on departure? Lehrer (1993b) has suggested that the TBL might reinforce learning that takes place upon arrival or that it is necessary to learn specific cues that are only needed during the first few trips to a location. A more general functional explanation for learning after feeding is that it allows bees to be more efficient in the allocation of the time, energy and memory capacity required for learning. By postponing learning until after feeding, these resources are used only when the information acquired during learning is likely to be valuable.

How then, does a bee determine when and for how long to turn back and look? Because the information acquired by this learning behavior comes with an energetic cost, we might predict that the behavior is more likely to be performed when information is needed that will impact foraging success and ultimately the fitness of the colony. As would be expected, the duration of these flights gradually declines with repeated visits to the same food source until the behavior is no longer performed (Lehrer, 1993b), presumably because sufficient information has been learned. Furthermore, in various species, learning flights at a nest reappear when the insect returning to home encounters difficulty finding its goal (reviewed by (Wehner, 1981). This initial decline in learning flight duration, and the subsequent reinstatement of the behavior following a delay, suggest that a bee's investment in learning may be modulated by her level of uncertainty about the location of the food. Another situation in which learning flights are seen is during the process of recruitment when bees are taken to a new location and given a more rewarding sucrose solution (Lehrer, 1993a). This suggests that learning flights may also be modulated by the value of the food reward, and, in natural flower patches, by the discovery of new foraging areas.

To better understand how a bee's investment in this learning behavior is controlled, we investigated several factors that may influence the decision to perform learning flights and their duration. Our premise is that the degree of investment in the learning flight might correlate with the value of the information that the learning flight would provide. We would therefore expect learning flights to be performed with greater duration when the bee's uncertainty about the location of food relative to landmarks is greater, and when the payoff provided by the food is greater. To explore these ideas, we examined the following questions:

1. *Does the duration of learning flights correlate with the extent of delay between arrival at a familiar feeding place and receipt of the sucrose reward?* Previous work has shown

that delay or confusion in reaching a familiar goal causes experienced insects to do orientation flights (van Iersel and van den Assem 1964). Situations in which a bee must search for an extended period of time for a familiar food source might be caused by a change in visual information or by inadequate memories of where the food should be. Thus, the increased search time caused by the delay may correlate with spatial uncertainty and hence the performance of learning flights. Here we ask whether the duration of such "reorientation flights" (Wehner, 1981) is modulated by the extent of the delay.

2. *Given that learning flight duration decays over successive departures from a goal, are the initial durations and rate of decay affected by whether bees have prior experience at a site?* More specifically, we asked whether the decay tends to be faster following a reorientation flight induced in experienced bees than it is following the initial discovery of the food by the same bee. Such a difference might be expected if learning during a series of reorientation flights builds upon previously acquired information that is still present in memory.

3. *Does the duration or rate of decay of learning flights correlate with scene complexity?* Lehrer (1993b) found that the duration of learning flights and the number of visits over which they are performed is greater for bees learning the "shape" of landmarks (i.e.vertically and horizontally striped patterns) than for bees learning their color, which was presumed to be an easier task. Here, we asked whether an increase in the complexity of the visual scene, as reflected in the number and variety of landmarks, also changes the

duration or rate of decay, or both, of learning flights. Such an effect might be expected if scene complexity influences the ease of learning spatial information.

4. *Does the duration or rate of decay of learning flights correlate with the spatial stability of landmarks that mark the position of the food source?* Changes in the locations of landmarks encountered on successive visits to a goal have been shown to influence spatial localization and exploratory behavior in rats (Biegler and Morris, 1996; Thinus-Blanc and Gaunet, 1999). Such shifts in the positions of landmarks presumably also influence the ability of honeybees to learn the location of food sources (Collett and Kelber, 1988). We asked whether such changes in spatial relationships among landmarks in turn affected the performance of learning flights. By moving local landmarks near the food between visits by a bee, we disrupted the link the local cues and the external frame of reference provided by global landmarks. We expected that this would create a more difficult learning situation, which we expected would in turn result in longer learning flight durations.

5. *Is the duration of learning flights affected by changes in the sucrose concentration of the food source?* In addition to sources of spatial uncertainty, the profitability of resources gained at a site might also influence the value of performing learning flights. Sucrose concentration is a highly motivating factor in honeybee foraging decisions (Nunez, 1970; Nunez, 1982); hence we expected that learning flights would be longer when food rewards were greater.

MATERIALS AND METHODS

General Methods

Bees

A colony of *Apis mellifera* was kept in an observation hive inside a large flight cage on the campus of Michigan State University, East Lansing, MI. The enclosed space measured 19.5 m (l) x 5.6 m (w) x 2.3 m (h) and was covered with a mesh fabric (30% shade cloth, designed to block 30% of incident light). The honeybees' only source of food was sucrose solution provided in feeders, and pollen introduced directly into the hive. Preventing bees from flying to natural food sources guaranteed high levels of motivation for foraging in the experiments.

Depending upon hive conditions and seasonal factors, we set up a "stock feeder", with scented or unscented sucrose solution ranging in concentration from 0.15-1.0 M. Initially, we used scented solutions to facilitate the training of bees to the stock feeder. Once the bees were familiar with the feeder, however, we found scented solutions to be unnecessary and hence switched to using unscented solutions in order to reduce the effect of odors on recruitment of bees to the test apparatuses. We filled the feeder before experiments in order to establish a population of bees available for use in the experiments.

Scent-gland plugging

Our studies required us to be able to observe individual bees and record the durations of their departures from the test apparatus. This was difficult to do in the presence of recruited bees that inevitably followed our experimental bees to the test apparatus. In order to reduce this recruitment of other foragers to the feeders, we sealed the Nasanov glands, which produce recruitment pheromones, of all bees used in the experiments (Towne and Gould, 1988). We accomplished this by capturing individual bees from the stock feeder and chilling them for a few minutes until they were immobilized. A layer of a rosin and beeswax mixture was then applied using a fine tipped soldering iron to the dorsal side of the posterior-most two segments of the abdomen, effectively covering the Nasanov gland, which is the source of the recruitment pheromone. These individuals were identified by colored paint marks on the abdomen and thorax. This procedure was done a few hours to a few days before testing. The success of the procedure could be visually determined by observing whether the wax remained on the bees' abdomens. Only bees with intact scent-gland plugs were used in the experiments, with the exception of twelve bees in the delay experiment (see Results). The flight behavior of the bees did not appear to be affected by this procedure. Learning flights performed by treated bees showed the same characteristics as those observed in other studies (Lehrer 1991, 1993b, Capaldi and Dyer, unpublished data) and in our pilot studies where bees did not have their Nasanov glands sealed.

A second reason for plugging the scent glands was to reduce the influence of recruitment pheromones on the bees' behavior. Specifically, we wanted to ensure that the effects we observed on learning flights in our experiments were not influenced by odor cues left by bees. As a further precaution to reduce the influence of odors, we also wiped the feeder and the floor of the test apparatus where the feeders rested with a damp paper towel following each visit by a bee. Despite this procedure, familiar odors on the feeder or on the test apparatus may have been available to the bees. We doubt that the presence of such odors significantly influenced the performance of learning flight behavior primarily because learning flights are performed to learn visual information (Lehrer, 1993a) rather than olfactory information. It remains possible that there are indirect effects of odors. For example, bees might decrease the duration of their learning flights over successive visits because the build up of recruitment odors on the feeder over time lessens their reliance on visual information to help pinpoint the feeder. However, this is unlikely to be true because of the following observations. First, recruits that followed scent-gland plugged test subjects to the test apparatus were unable to find the cryptic feeder unless there was a bee on the feeder already. Thus, any odors that might have accumulated on the feeder were not strong enough to guide an inexperienced bee to the food. Second, during one of our experiments in which the food and associated landmarks were moved to a new location between visits by a bee, bees that arrived to find the feeder removed still searched at the precise location relative to the local landmarks where the feeder should have been. This location was not the same exact location visited in the previous few trips and hence should not have accumulated odors. Thus, bees must have relied on visual cues to locate the feeder.

Apparatus

We used two types of experimental apparatus. In most of the experiments, bees were recruited to an oblong arena measuring 4.9 m x 1.0 m in the 1999 season and 3.73 m x 1.0 m in the 2000 season. The walls surrounding the arena were 0.5 m high, which blocked most external landmarks from the bee's view once inside the arena. The floor of the arena was covered with wallpaper bearing a textured beige pattern to minimize disorientation inside the otherwise white arena. Food was provided in a clear, inconspicuous microcentrifuge cap (1 cm diameter). Cylinders made of PVC pipe were arranged near the feeder to provide landmarks that bees could use to pinpoint the location of the food. The surfaces of these cylinders were covered with different patterns (black, vertically striped, horizontally striped and checkered), and their number, size and arrangement was varied depending on the experimental condition (Figure 1.1). In most experiments, a triad of black cylinders, each 31.6 cm tall and 9 cm in diameter marked the location of the food. We used this *simple* condition to investigate the effects of delay and past experience on learning flight durations (Questions 1 and 2). In the *moving* condition, which we used to investigate the influence of landmark stability on learning flight duration (Question 4), the triad of black landmarks, along with the feeder, was moved between each visit by a bee to a different location in the arena. Thus, the location of the food was constant relative to the three black cylinders but varied between visits in relation to external landmarks. Finally, in the *complex* condition, which we used to investigate the influence of scene complexity (Question 3), numerous cylinders of various sizes and surface patterns were placed in the arena in addition to the triad of black cylinders nearest the food.

A second type of apparatus, used in investigating the effect of sucrose concentration on learning flights (Question 5), consisted of a wooden box 32 cm x 32 cm x 31 cm with one side open. A feeder, consisting of a vial cap with wire mesh (2.5 cm diameter), was placed inside the box. A pattern of colored tape was placed underneath the feeder to help guide bees. The box was placed on top of a small workbench about 50 cm high. Two identical setups were placed approximately 3 m apart, each 6 m from the

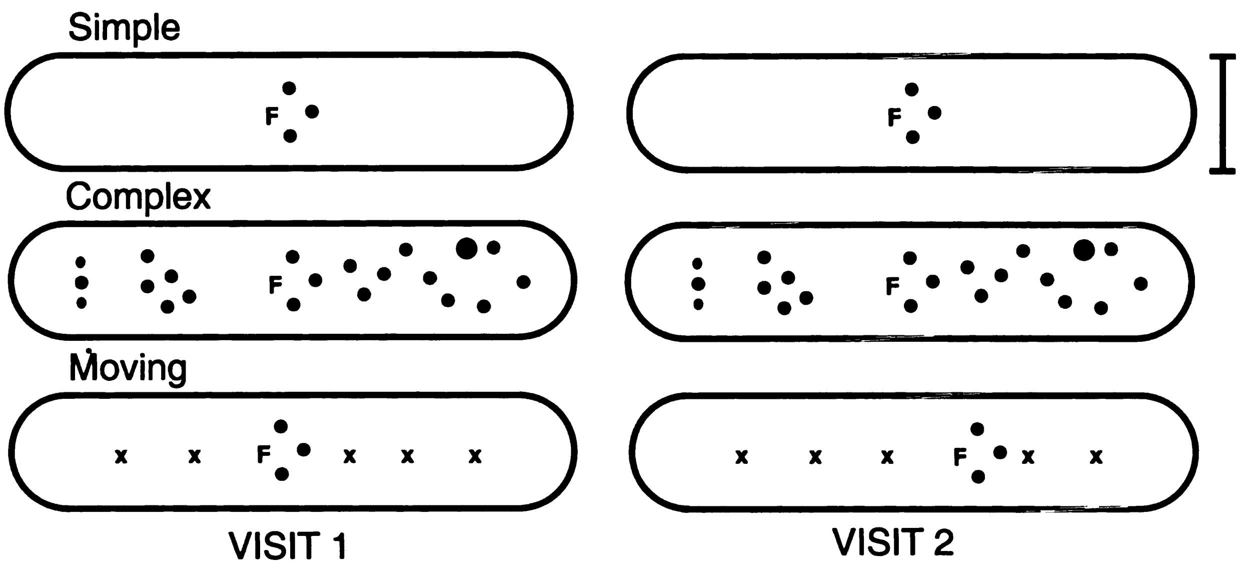


Figure 1.1. Diagrams represent the positions of landmarks and feeder in the arena (drawn approximately to scale) for each condition: simple, complex, and moving. Landmarks and feeder remain stationary in the simple and complex conditions. The spatial relationships among the additional landmarks (which varied in size and pattern) in the complex condition are approximate. During this experiment, the array of landmarks was kept exactly the same. In the moving condition, the three landmarks and feeder move to different positions within the arena. These positions are marked with a *cross*, denoting the location of the feeder. *Black circles* represent the landmarks and the letter F marks the feeder location. Scale bar corresponds to 1m.

hive. This was done for purposes of efficiency; with two boxes, we could continue to train a few bees at one box while testing another bee in a separate but similar box nearby (see Specific Experimental Methods below).

Recruitment to experimental apparatus

Honeybees are known to transfer their allegiance to a food source if the quality is higher than that of the food on which they were previously feeding (Lehrer 1993a). In all of our experiments, individual bees feeding on low concentration sucrose (0.25M) at the stock feeder were transferred to the test apparatus by using sucrose solutions of higher concentrations (generally 2.25M) in the test feeders. We accomplished this by first introducing the concentrated solution to the bee via a pipette while she was feeding at the stock feeder. When the bee began feeding, she was placed onto the test feeder and carried by hand to the test apparatus. Some bees returned immediately to the new food source on subsequent trips, while others needed the recruitment process to be repeated from 1-5 times.

Measurement of Learning Flights

Following each bee's visit to the experimental apparatus, the duration of learning flights was recorded using stopwatches. For experiments in the boxes, the criteria were similar to those used in Lehrer's study (1993b) where the duration of the flight was recorded from the moment the bee departed from the feeder until she turned around to head back to the hive. The point at which she turns around to head to the hive is accompanied by an easily observed shift in flight speed and direction.

For experiments in the arena, we used a different criterion: two observers started their stopwatches at the moment the bee lifted off from the feeder and stopped timing when she flew above the level of the 0.5 m wall. The recorded duration included the intensive examination time described by Lehrer as the Turn Back and Look, but also included a significant amount of circling in the vicinity of the food (see also (Lehrer, 1993b). We included this circling behavior because bees often turned away from the landmarks soon after departure, but well before they left the vicinity of the landmarks. Using video recorded from above the arena, we found that in a sample of 15 departures, bees spent an average of 6.16 ± 0.74 (mean ± SEM) seconds longer investigating the landmarks using our criterion compared to the 180° turn criterion. Since bees spent a longer time investigating the landmarks than would be measured with the 180° turn criterion, our measure seemed a reasonable indicator of time spent in learning. Also with video analysis, we tested other criteria for timing the learning flights. These included time spent in a defined area around the food source and time elapsed until the last moment where the bee faced the landmarks. All methods yielded similar results. Therefore, as a matter of convenience, we report the change in flight durations as measured by hand timing.

Averages of the two hand recorded times were used in our analyses. The recorded times of each observer were consistently similar; in a sample of 308 recorded learning flight durations from the delay experiment, the mean difference between the two times was 0.19s with a standard deviation of 0.2s and a range of 0-1.16s. A sample of 67 recorded durations in the sucrose concentration experiments, the mean difference between the two times was 0.31s with a standard deviation of 0.34s and a range of 0-

1.94s. Given that the effect sizes of the differences in learning flight durations were in the order of seconds (see Results), these measurement differences are relatively small.

To characterize the change in learning flight duration over repeated visits, we fitted a nonlinear regression using the function $y=y_0 + A \bullet e^{-visit \bullet \tau}$ to the data using the JMP statistical program (SAS Institute). The fitting of this function allowed us to compare how quickly bees' flight durations decayed by comparing the slope parameter, "τ", obtained for each bee's fitted curve (Figure 1.2).

Some of our experiments require us to assess whether the duration of learning flights changed following an experimental manipulation, such as a delay or change in sucrose concentration. To do this, we established a baseline by averaging the departing flight durations of the three visits prior to the manipulation. At the point at which the manipulations were performed, these durations had stabilized such that learning flights did not show patterns of increasing or decreasing duration. Twelve visits were generally sufficient to reach this stable point. In the data from the arena, this baseline duration included the time required to clear the wall of the arena, but very little time circling. In the data from the boxes, the baseline duration was very nearly zero, because it included only the time needed for the departing bee to turn by 180° to head homeward. The difference between the baseline value and the recorded flight duration following the experimental manipulation yielded the change in flight duration, which was used as the dependent variable in most experiments.

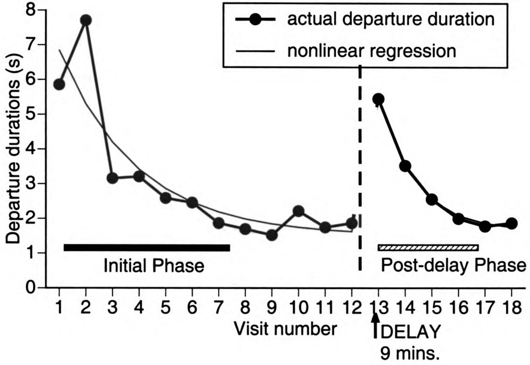


Figure 1.2. Change in learning flight duration over a series of visits by a single bee feeding in the arena. The initial phase, marked by the *black bar*, is the period during which learning flights are performed at a new location. The post-delay phase, marked by the striped bar, is the period during which learning flights are observed after a delay has been imposed. The fitted curves shown for each phase (thin line) are the nonlinear fits for the equation: y= yo + A*e –visit* τ (see text for details).

Specific Experimental Methods

Delay experiments (questions 1 and 2)

We observed that learning flights reoccurred when bees arrived at a familiar feeding place and were required to search for an extended period of time before finding the goal (Figure 1.2), a phenomenon also observed by many other authors (Wehner, 1981). Our first aim was to determine whether the length of such a delay influences the duration of the subsequent learning flight. To do so, we subjected bees that had been feeding at the test arena to delays of varying duration, and assessed whether the duration of the learning flight on the departure following the delay was correlated with the length of the delay.

Bees were trained and tested individually. Each bee was recruited from the stock feeder to the arena with a microcentrifuge cap filled with 2.25 M unscented sucrose solution. The arena contained the simple triangular array of black cylinders (Figure 1.1-*simple* condition), and the feeder was placed 30 cm away from the middle of the triangular configuration. We then recorded the duration of each departure over multiple successive visits. We waited until the bee's latency to depart the arena stabilized, and then we imposed a delay of approximately 3, 6, 9 or 12 minutes by removing the food. During the delay period, the bee would arrive at the arena and persistently search for the feeder near the familiar location. Although bees often searched areas outside of the arena as the delay progressed, they usually returned to search more at the precise location. When the appropriate time elapsed, the feeder was very quickly replaced, and soon after, the bee would find it and feed. The act of replacing the feeder never interfered with the flight path of the bee, and in fact, the bees were often outside the arena at the moment the feeder was replaced. Thus, the brief presence of the experimenter's arm in the arena itself is not the cause of the reoccurrence of learning flights. The actual delay was usually a little longer than the intended delay due to the lag until the bee discovered the replaced food. The bee's departure following this delay was recorded, as were the departures following the ensuing 4-6 visits. These data were later used to compare the decay in learning flight duration before and after the delay period in order to determine whether past experience influences the performance of learning flights.

Visual scene complexity and landmark stability experiment (questions 3 and 4):

To assess whether features of the visual scene itself might affect learning flight durations, we created foraging environments, the *simple, moving*, and *complex* conditions described earlier, and observed the learning flights performed in each of these conditions. In this experiment, we assumed that the *moving* and *complex* conditions might be more difficult to learn. Although we could not determine a priori whether the *complex* condition would be more difficult for the bees to learn, we reasoned that the addition of more landmarks would cause difficulty in extracting the relevant information (see Discussion). In the *moving* condition, we suspected that a reduction in the total number of cues that remain stable and predictive of the location of the food would create a more difficult situation for bees to learn the visual and spatial information. We expected that the increase in difficulty of learning in these environments would be reflected in learning flights that are longer or slower to decay than *simple* condition.

Using similar procedures as previously described, we recruited a test bee to the arena and recorded her learning flight departures over multiple visits. Bees were assigned randomly to either the *simple*, *complex* or *moving* conditions, and each bee was removed from the population after she had participated in a trial. Longest learning flight durations and the decay rate in durations (see General Methods) for bees in the *simple* scene were compared to that of bees in *complex* and *moving* scenes.

Effect of sucrose concentration (question 5):

To determine whether the performance of learning flights is influenced by sucrose concentration, we trained bees to a feeder of a constant concentration and then compared their learning flight durations before and after a switch to a higher concentration. In these experiments, scent-gland plugged bees were trained to feed at the box apparatus (see General Methods), to which they returned repeatedly. During the training period, sucrose concentrations remained the same and learning flight durations following each departure were recorded. When these durations were no longer decreasing, which usually took between 10-15 visits, the sucrose concentration in the feeders was increased. Increases in sucrose concentration were manipulated in two ways. In the first experiment, bees in all three treatment groups fed on solutions with the same initial concentrations, and were then switched to new feeders containing either the same concentration or two higher concentrations. In the second experiment, bees were trained to varying concentrations, and were then switched to the same extremely high concentration solution (see Results for further detail).

Following the switch in sucrose concentration, a bee would then return to box apparatus to find an identical feeder containing solution of the same or higher concentration on which she would feed. While feeding, the bee was moved to the identical test box 3 m away, and her subsequent learning flight duration was recorded.

The treatment assignments were randomized among bees, and equal numbers of bees were transferred in each direction between the boxes. Each tested bee was selected from a group of 3-5 bees, the remainder of which were allowed to continue feeding at the training box. The observers also moved to the new apparatus, and recorded the learning flight duration of the test bee as it flew away after finishing feeding. Following the test trial, observers returned to the original box where the remaining bees were training.

RESULTS

Delay experiments

Effect of a delay in receipt of food reward on performance of learning flights

As shown in Figure 1.3, we found that increasing the period of delay, during which bees had to search for a familiar food source, led to an increased duration of the subsequent learning flight. There is a positive correlation between length of the delay and the measured change in departure flight duration ($R^2 = 0.373$, $p < 0.05$, $n = 25$). In this analysis, we pooled both scent-gland plugged and non plugged bees. Each condition had approximately equal numbers of bees (Non scent-gland plugged: $n = 12$, Scent-gland plugged: $n = 13$) and at least three data points in all groups (3, 6, 9, 12 minutes). We used ANCOVA to investigate whether scent-gland plugging influenced the bees' behavior. This analysis revealed a significant effect of delay ($F = 18.676$, $p = 0.0003$), but no effect of condition (scent-gland plugged and non scent-gland plugged) ($F = 0.949$, $p = 0.34$) and no interaction between condition and delay ($F = 0.661$, $p = 0.42$). Thus, there was no difference in the scent-plugged and non plugged bees in terms of learning flight durations following the periods of delay.

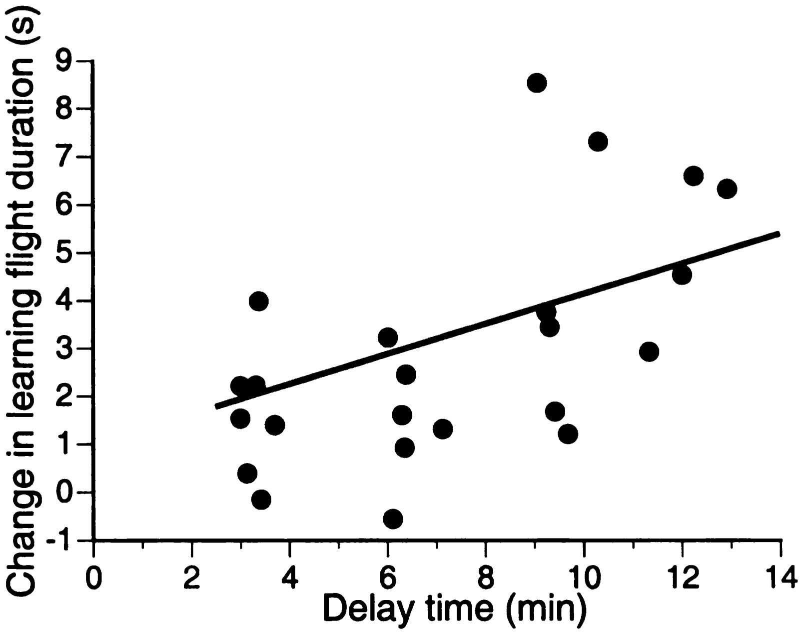


Figure 1.3. Relationship between the length of delay in receiving food reward and increase in duration of subsequent learning flight (R2 = 0.34, $p < 0.05$, n = 25.) This increase in duration was measured relative to the baseline flight duration established by the bee prior to the delay. Each data point represents a single bee subjected to a delay.

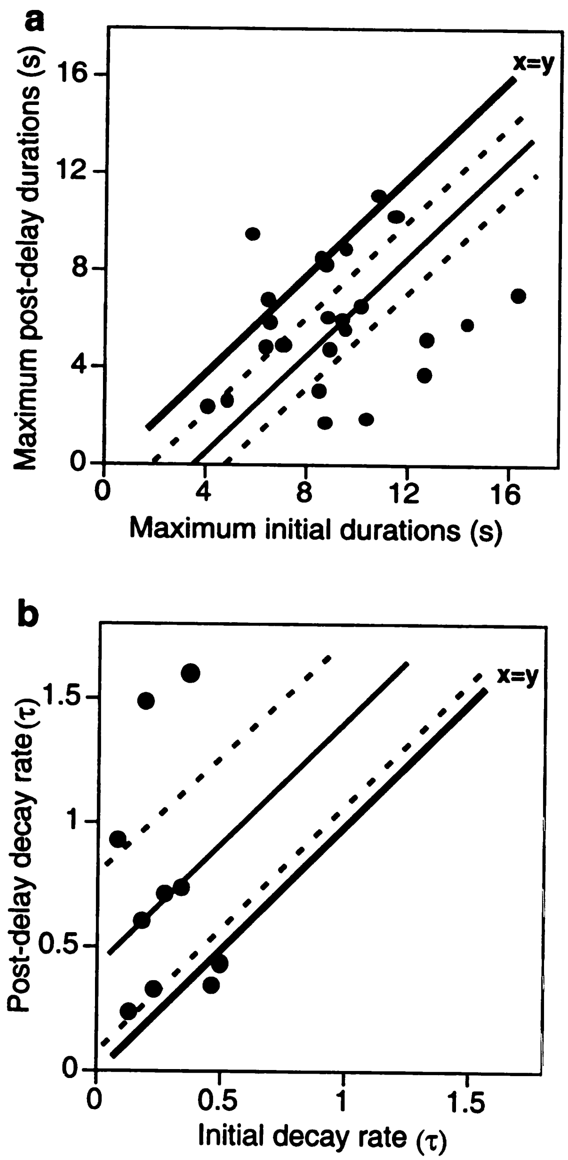
Effect of prior experience on decay of learning flight duration

If the modulation of learning flight duration is affected by how well a bee already knows the landmarks around the target, then one might expect learning flights following a delay to be shorter and to decay faster than those following the initial discovery of the food. Our data support both of these expectations. First, we compared the longest learning flight during the initial learning phase with that of the phase following the delay for each individual bee. The longest flight by each bee was typically one of the first few flights following recruitment to the new feeding place or following a delay. In all but one case, the longest flight following the delay was shorter than the longest one during initial period of learning in the arena (paired *t*-test: $t = 4.621$; $p < 0.001$, $n = 25$) (Figure 1.4a).

Next, we considered how the rate of decay of learning flight duration following a delay differs from the initial rate of decay. Specifically, we compared the decay rate, τ, of the exponential decay curves fitted to each bee's data before and after the delay. The decay of learning flight duration was more rapid following a delay than during the initial phase (paired *t*-test: $t = -2.871$, $p < 0.01$, $n = 11$) (Figure 1.4b), which suggests that bees are returning more quickly to baseline.

The pattern shown in Figure 1.4 led us to ask whether the duration of the first few flights influenced the subsequent decay rate such that bees would return more quickly to baseline when initial learning flights were shorter. This would suggest that the more rapid decay rate in learning flight durations following a delay is simply a result of starting with shorter initial durations, rather than being influenced by past experience as we suggest. If decay rates are determined by the duration of the longest initial flights, we might expect to find a steeper decay associated with shorter initial learning flights even

Figure 1.4. Comparison of learning flight parameters for individual bees following initial discovery of food (initial phase) and following an imposed delay (post-delay phase). **a.** Comparison of the longest learning flights performed by individual bees during initial and post-delay phases (paired t-test: t=4.62; P<0.001, n=25). **b.** Comparison of the rate of decay (t) for learning flight durations during initial and post-delay phases (paired t-test: t=-2.87, P<0.01, n=11). The *thick solid line* represents the point at which initial and post-delay departure times or parameters are equivalent. The *thin solid line* is displaced by an amount equal to the difference in the means between the two departure times or slopes. The *dashed lines* indicate the 95% confidence intervals.


a
Maximum post-delay durations (s)
0
4
8
12
16
x=y
0
4
8
12
16
Maximum initial durations (s)
b
Post-delay decay rate (τ)
0
0.5
1
1.5
x=y
0
0.5
1
1.5
Initial decay rate (τ)

during the initial series of learning flights following discovery of the food. A regression analysis, however, revealed no such relationship between initial durations and subsequent decay rates, τ ($R^2 = 0.054$, $p = 0.31$, $n = 21$) (Figure 1.5). Therefore, the observation that bees show both shorter initial learning flights and steeper decay rates during the post-delay phase reflects the influence of prior learning on the modulation of these learning flights.

Effects of visual scene complexity and landmark stability

If learning flights allow for the collection of visual and spatial information about landmarks, then features of the visual scene itself might affect learning flight durations. Presuming that the decay of learning flight duration reflects the learning of visual and spatial features of nearby landmarks, we might expect the decay rates to be slower when the visual and spatial features of landmarks are more difficult to learn. Consistent with this expectation, we found that learning flights are longer in two situations in which visuospatial information might be expected to be harder to encode: when the visual scene is complicated with many landmarks and when spatial relationships among landmarks are unstable. Learning flight duration in a *simple* scene, consisting of a stable triad of black cylinders near the food, was compared to that in *complex* and *moving* scenes. In the *complex* condition, we added a diverse array of landmarks to the basic triad of black cylinders near the food. In the *moving* condition, the food and the triad of black cylinders were moved to a different position in the arena between each visit by the bee. Thus, nearby landmarks remained predictive of the location of the food, but more distal landmarks were made unreliable. Maximum learning flight duration differed among the

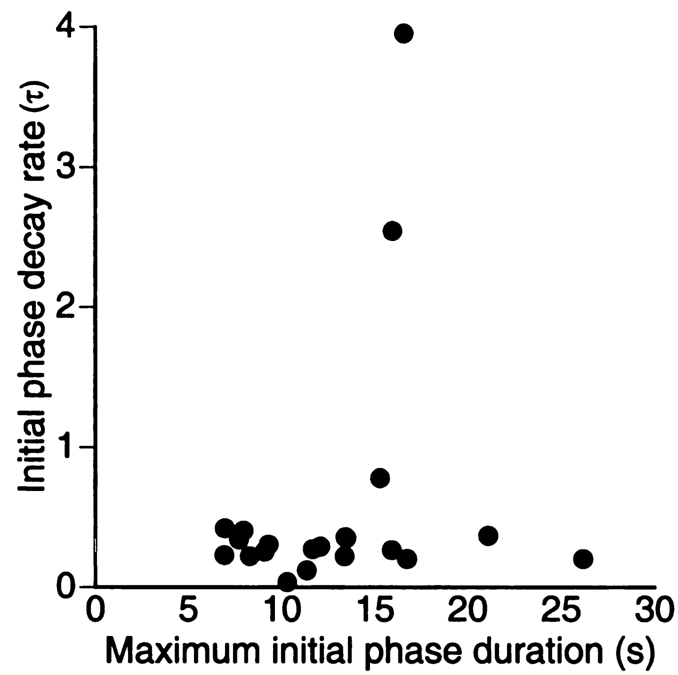


Figure 1.5. Relationship between the durations of the longest initial learning flights and the slopes of the subsequent decay in learning flight durations (τ) (R2= 0.05, p=0.31, n = 21). Each data point represents one bee. All bees were subjected to the *simple* condition.

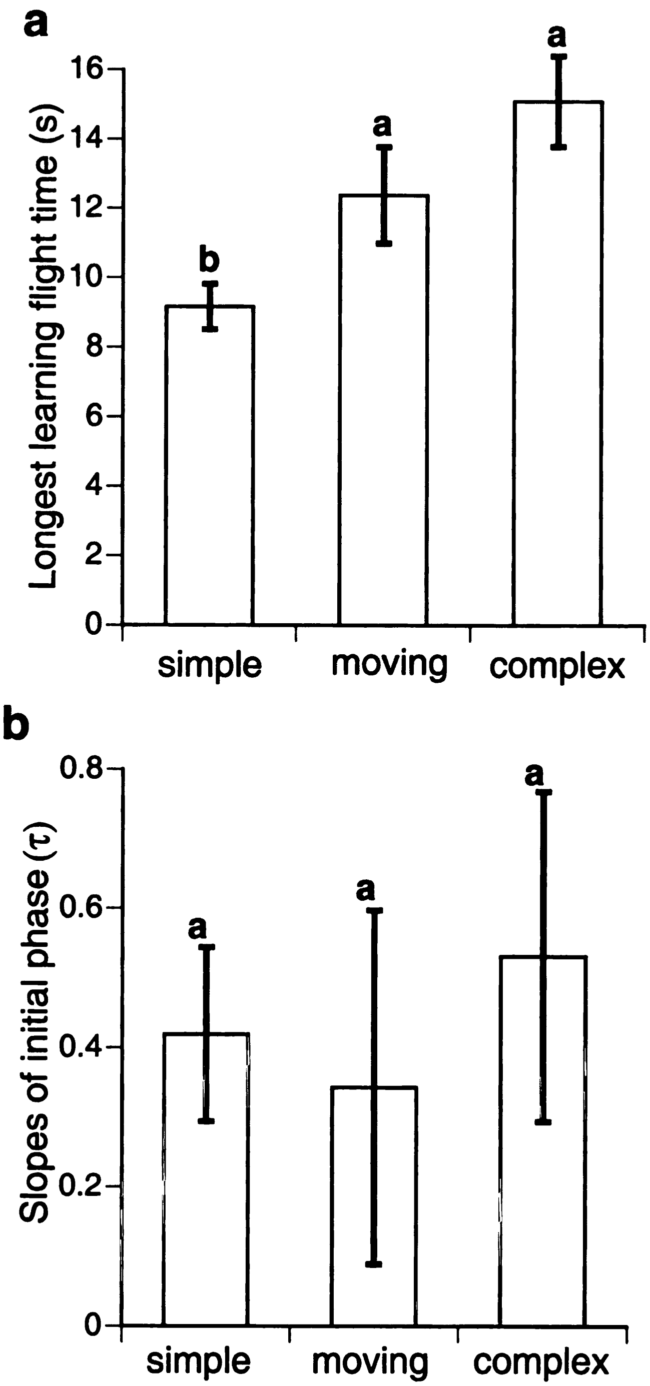
three conditions (One-way ANOVA, $F_{2,44} = 9.606$, $p < 0.05$, $N = 47$) (Figure 1.6a): bees in the *complex* and *moving* conditions performed longer learning flights than the bees in the *simple* condition (Tukey-Kramer pairwise comparisons, $p<0.05$).

Although the durations of the longest learning flights differed among the three conditions, the rates of decay (as estimated by the τ parameter from the fitted exponential decay curves) did not differ significantly (One-way ANOVA: $F_{2,42} = 1.222$, $p = 0.305$, $N = 45$) (Figure 1.6b). The similarity of the decay functions in all the conditions suggests that the rate of decay of flight duration does not depend on the magnitude of the longest initial flight. This is consistent with our earlier regression analysis, illustrated in Figure 1.5, which revealed that there is no relationship between duration of the longest flights in the initial discovery phase and the t parameters derived from the decay functions.

Effect of sucrose concentration

We observed an effect of sucrose concentration on learning flight duration in two sorts of experiments, both involving an increase in sucrose concentration accompanying a move to a new feeding place. While the question of whether an increase in sucrose concentration alone is sufficient to obtain the same results was unanswered in this experiment, our method of moving bees to a second box may be a closer approximation of natural foraging experiences where changes in sucrose concentration are encountered. In the first experiment, scent-gland plugged bees that had been regularly foraging on 0.5 M sucrose from the training box returned to an identical feeder containing either the same concentration (0.5 M) (control group) or one of two higher ones (1.0 M or 2.25 M). In all three groups, learning flight duration increased from baseline durations prior to the

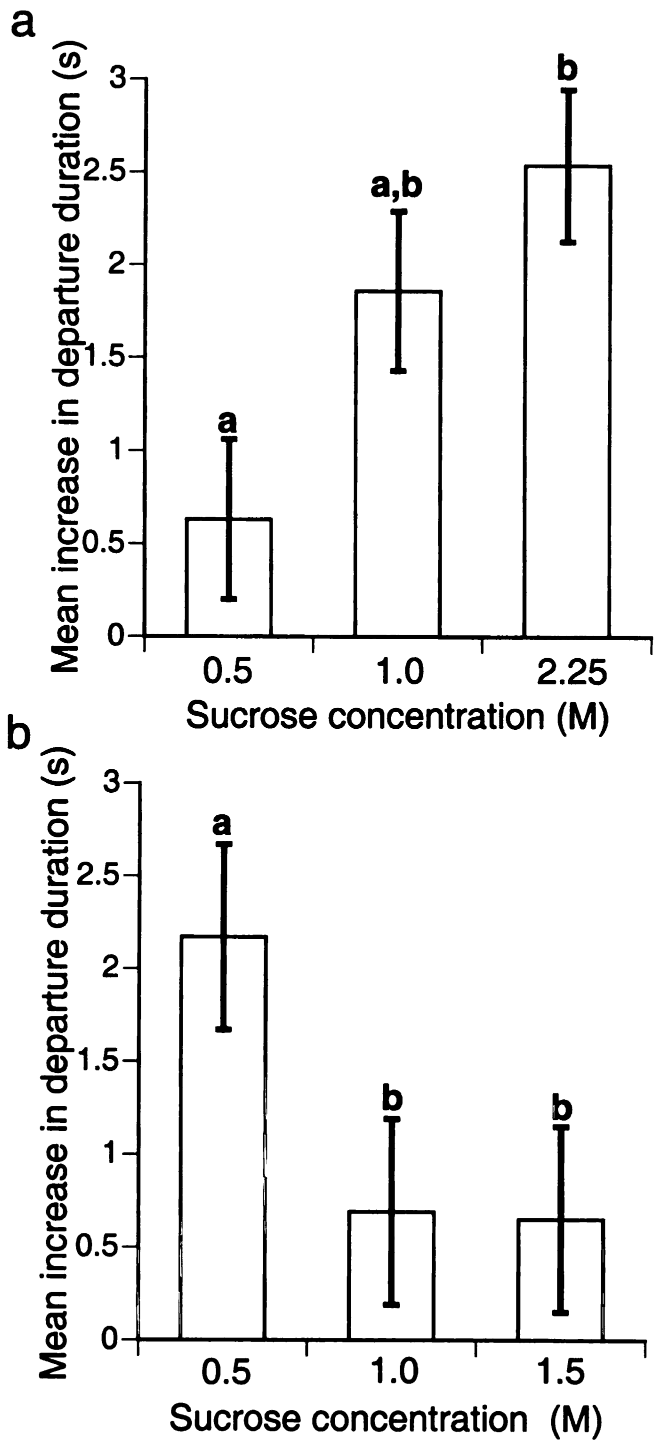
Figure 1.6. Effects of three different visual conditions on learning flights. **a.** Comparison of maximum learning flight durations of bees initial visits ($F_{2, 44}$ =9.61, P<0.05, n=47; simple n=32, moving n=7, complex n=8). **b.** Comparison of decay rate ( ) of learning flight duration during the initial phase ($F_{2, 42}$ =1.22, P=0.31, n=45; simple n=30, moving n=7, complex n=8). (Note: to increase the power of the test, data from bees in the delay experiment, which experienced simple conditions, were pooled with the data from the simple condition in this experiment: 25 bees **a**, and 23 in **b**.) In each graph, pairs sharing the same letter are not significantly different from each other according to Tukey-Kramer pairwise comparisons (P<0.05). Bars represent mean±1 SE.


a
Longest learning flight time (s)
0
2
4
6
8
10
12
14
16
b
a
a
simple
moving
complex
b
Slopes of initial phase (τ)
0
0.2
0.4
0.6
0.8
a
a
a
simple
moving
complex

transfer, but the magnitude of increase differed among groups ($F_{2,43} = 5.243$, $p<0.05$, $N = 46$) (Figure 1.7a). Tukey- Kramer pairwise comparisons revealed a significant difference between the control group and the 2.25 M group ($p < 0.05$). Because we used a different feeder when switching concentrations, there also remains a remote possibility that the bees are responding to changes in level of recruitment odors on the feeder rather than changes in the sucrose concentration. Recruitment odors, however, cannot explain our results. If bees were responding to changes in levels of recruitment odors and not to changes in sucrose concentration, we should have seen significant increases in learning flight duration in the control group as well since a new feeder was used in that condition as well.

These results support our premise that learning flights are modulated according to the payoffs provided by a food source. How learning flights may be modulated according to the quality of the food may be interpreted in two ways. First, transferred bees might modulate their learning flight durations according to the absolute value of the sucrose concentration in the new feeder, such that a given sucrose concentration always elicits a specific learning flight duration. Alternatively, bees might modulate flights according to the magnitude of the difference in sucrose concentrations between the new food source and the previous one (Raveret Richter and Waddington, 1993). The second experiment was designed to distinguish between these two possibilities. In this case, bees were initially trained to one of three different sucrose concentrations (0.5 M, 1.0 M, or 1.5 M), and were then all switched to 2.25 M. Thus, the end values were the same for all bees, but their prior experiences were different. Comparisons of the three groups again yielded significant differences ($F_{2,39} = 8.9797$, $p<0.05$) (Figure 1.7b). Tukey-Kramer

Figure 1.7. Effects of different sucrose concentrations on learning flights. **a.** Comparison of bees that all began feeding upon the same concentration (0.5 M) and were then moved to identical-looking feeders with different levels of increased sucrose concentration ($F_{2, 43}$ =5.243, $P<0.01$, n=46; 0.5 M, n=15, 1.0 M, n=15, 2.25 M, n=16). **b.** Comparison of bees that initially fed on different sucrose concentrations (0.5 M , 1.0 M , or 1.5 M) and were then all moved to feeders with a high (2.25 M)-sucrose solution ($F_{2, 39}$ =8.979, $P<0.01$, n=41; 0.5 M, n=15, 1.0 M, n=13, 1.5 M, n=14): Pairs sharing the same letter are not significantly different from each other according to Tukey-Kramer pairwise comparisons. Bars represent mean±1 SE.


a
Mean increase in departure duration (s)
3
2.5
2
1.5
1
0.5
0
a
a,b
b
0.5
1.0
2.25
Sucrose concentration (M)
b
Mean increase in departure duration (s)
3
2.5
2
1.5
1
0.5
0
a
b
b
0.5
1.0
1.5
Sucrose concentration (M)

pairwise comparisons ($p<0.05$) again revealed significant differences only between the group that started at 0.5 M and each of the other two groups, which experienced smaller changes in sucrose concentration. This suggests that the mechanism governing the modulation of learning flights in relation to sucrose concentration involves the magnitude of the change in food quality and not the absolute value itself.

An additional experiment, which subjects bees to decreases in sucrose concentration and observes subsequent learning flight durations would have complemented these data well. Unfortunately, however, bees arriving at a familiar location will not land and are generally very disturbed and reluctant to feed if the value of the food reward is decreased. Also, since we have shown that delay in receiving an expected reward influences the performance of learning flights, the time elapsed while the bee flies around in search of the expected food source instead of feeding would add a confounding factor.

DISCUSSION

In these experiments, we have identified a number of factors that affect the occurrence and duration of learning flights performed by honeybees in a new feeding place. Specifically, we found that learning flight duration is modulated by the extent of delay in receiving expected food, the extent of past experience at the site, the complexity of the visual scene, the stability of landmarks, and the value of the food. We discuss each of these factors in turn.

Effect of a delay in receipt of food reward

As mentioned before, bees and other insects will resume performance of learning flights at a familiar location following periods of delay and searching for the goal (van Iersel and van den Assem 1964). Our results build on this by showing that the durations of these reorientation flights correlate with the length of the delays. These results suggest that a bee's uncertainty about the location of a goal may control learning flight behavior, and that uncertainty accumulates as a function of search time. We also found that learning flights following a delay are shorter in duration and faster to decay than are learning flights following initial discovery of the food. This is consistent with the idea that the bees' uncertainty about the location of a food source is influenced by prior learning experiences and in turn influences investment in learning following a delay.

Taken together, these findings from delay experiments show that bees behave as if they can gauge the state of their knowledge about the location of food, and can then adjust their investment in learning accordingly. A relatively simple mechanism could account for these effects, as well as for the decay of learning flight durations across multiple visits. For example, bees might use search time in the vicinity of the goal, which would be correlated with their uncertainty about food location, as an indicator of how long the learning flight should be. The latency between arrival at the location of the food and the receipt of reward may be measured in several possible ways: by measuring energy expenditure, by summing optic flow during the search for the food, analogous to the way optic flow is used to judge the distance of flight to food (Srinivasan et al., 2000; Srinivasan et al., 1997), or by measuring the output of a clock-like interval timer (Gallistel, 1990).

Alternatively, the effect of a delay on the performance of learning flights might be influenced by the dynamics of memory formation. Previous studies have shown that memories in honeybees are formed in phases that begin with fragile short term memories (early and late STM), pass through a consolidation process through the mid-term memory phase (MTM), and are then stored in long term memory (LTM) (Menzel, 1999). Before the consolidation process is completed, memories are highly susceptible to interference (Menzel, 1999). Thus, a possible explanation for the effect of delays on the performance of learning flights might be that during delays of several minutes, the process of forming late STMs, which is thought to correlate with foraging between patches and to operate on the order of minutes, may be disrupted. This disruption in the consolidation process of memories for the location of the food could somehow trigger longer learning flights. Decay of stored information during unrewarded time intervals on the scale of minutes does explain foraging decisions within visits, such as decisions by bees whether to change foraging locations or to switch floral species on which to forage (Chittka et al., 1997; Greggers and Menzel, 1993). However, in our studies, the delay is imposed after multiple visits, and usually over an hour after the bee's first visit to the test arena. This time span would imply that the bees have formed MTMs and such memories are less prone to interference, hence it seems unlikely that they would be affected by a delay in receiving a reward. However, whether disruptions in MTM could explain the results we obtained in the delay experiments remains an intriguing possibility.

Our delay results implicate a role for search time in determining subsequent learning flight duration. This correlation was observed when the food was removed and bees were forced to search for several minutes. We have also considered the possibility that the variation in search time over the much shorter time intervals on typical arrivals at the food may also influence the duration of subsequent learning flights. For example, the decay in learning flight duration seen over a series of visits may be determined by a decrease in search time as bees become more familiar with the location of the food. To examine this possibility, we attempted to correlate arrival times with subsequent learning flight durations. However, we found that search time was often confounded by scenting behaviors by the bees upon arrival. Specifically, bees returning to the food hovered in the vicinity of the feeder trying to expose their sealed Nasanov gland to release recruiting pheromone, which delayed their landing. We found it difficult to discriminate between bees that were searching for food and those that spent time scenting before landing. Perhaps because of this ambiguity, the search times on arrival seemed to vary greatly and did not correlate well with subsequent learning flight durations. Thus, although we have shown that search times of several minutes, as seen during imposed delays, can influence subsequent learning flight durations, the question still remains whether the shorter search times seen on arrivals may also modulate learning flights.

While the nature of the mechanism by which delay, and hence search time, influences learning flight durations is unclear, this influence has an important functional implication. When a bee is unable to locate the goal and spends time and energy searching, the benefit of performing a learning flight after finally locating the food may ensure that the information encoded in her memory is current and that she is better able to locate the food source in subsequent visits. In a natural context, this need to update spatial information in response to a delay in finding a familiar food source might occur if landmarks surrounding the food source have changed.

Effect of visual scene complexity and landmark stability on learning flights

Our results suggest that the visual features and spatial stability of the landmarks themselves have a strong effect on learning flights. The finding that initial learning flight durations are longer in the *complex* condition in comparison with the *simple* one suggests that the *complex* condition may be more difficult for a bee to learn, hence requiring a greater investment in learning. The addition of landmarks in the *complex* scene may make it more difficult to obtain the necessary information by increasing the total amount of visual information present. One important type of information learned during learning flights is the distance between the food source and nearby landmarks (Lehrer 1991, 1993b), and this information is extracted through motion cues. The addition of several landmarks to the visual scene would increase the number of moving edges that move across the bee's field of vision during a learning flight. Such an increase might in turn increase the difficulty of determining distances. In humans also, the difficulty of a search task is also increased by adding visual "distracters" (Treisman, 1993).

The influence of scene complexity on learning flight duration may also, as discussed previously in the delay experiments, involve a timing mechanism. For example, if search time on arrival is greater in the presence of a more complex scene, then delay effects could lead to longer learning flights. Alternatively, the increased learning flight durations in the complex condition may be the result of changed optic flow patterns created by the additional landmarks. During orientation flights, wasps have been shown to adjust their flights speeds so that they move across distances that allow for equal visual angles (in relation to the nest) to be traversed in equal time (Zeil, 1993a). Zeil suggests the possibility that this flight control may be mediated visually by image motion created by ground texture patterns (Zeil, 1997). Although the learning flights of wasps and bees are not precisely the same, they likely operate on similar principles, as their functions are very similar. Perhaps texture patterns, such as those created by the addition of landmarks in the *complex* scene, also influence the duration of learning flights in insects.

The greater duration of the learning flights in the *moving* condition, as compared with the *simple* condition, may also reflect the increased difficulty of the learning task. In our experiment, the bee returns each time to find a slightly altered scene. She must distinguish between cues that are reliable (proximal landmarks that were moved with the feeder) and those that are not (distal landmarks). The shifting reliability in the cues may cause a greater need for information in subsequent trips. This idea is supported by our results, which show that the longest learning flights in the *moving* condition tend to be during the second or third departure, whereas they occur on the first departure in the *simple* condition. This suggests that bees do a longer learning flight after encountering a change in the scene.

An earlier study by Lehrer (1993a), also suggests that learning flights are modulated in response to the difficulty of the learning task. Specifically, bees experiencing different cues upon arrival and departure performed learning flights over a greater number of visits following discovery of the food. While the duration of flights was not compared specifically, the data suggested that bees that experienced differing landmarks performed longer learning flights than bees that saw the same landmarks. Learning of various features of the landmarks takes place both during arrival and departure; color is learned primarily upon arrival, whereas spatial information, such as shape and spatial relationships among landmarks, is learned primarily upon departure (Lehrer, 1993b). Hence, bees receiving conflicting cues on arrival versus departure face a more difficult learning task and it is not surprising that they would consequently invest in longer and more learning flights.

Our work on the learning flights of bees complements previous work in rats which has shown that landmark instability increases the difficulty of locating of a food source, and influences exploratory search behavior (Biegler and Morris, 1996; Thinus-Blanc and Gaunet, 1999). In rodents, exploratory activity functions to update spatial information when an animal is exposed to a novel spatial environment (Thinus-Blanc and Gaunet, 1999). As in the learning flights of bees, this exploratory behavior is likely to be modulated by extrinsic landmark cues. Thus, evidence from both vertebrates and invertebrates suggests that more difficult learning situations elicit greater investments in active learning behaviors.

Effect of changing sucrose concentrations on the performance of learning flights

In addition to the influence that sources of spatial uncertainty have on learning flight duration, as suggested by the experiments on delay, scene complexity, and landmark stability, we found that learning flights are also modulated by motivational variables related to the quality of the food source. A honeybee's motivation in collecting nectar varies with colony conditions (Schmid-Hempel et al., 1993). On an individual level, a bee's motivation is also influenced by sucrose concentration (von Frisch, 1967), flow rate of sucrose (Farina and Nunez, 1991; Nunez, 1970), and perception of profitability (Waddington and Gottlieb, 1990). We have shown that learning flights performed when bees are moved to a new, better food source are longer when the increase in concentration is greater. Furthermore, we have also found that learning flight duration is modulated by the magnitude of change in sucrose concentration rather than by the absolute value of the new food source. These results suggest that a bee compares the concentration of a new food source to that which she has recently experienced. This in turn influences the behavioral decision of how long learning flights will be.

This result is reminiscent of another study by Raveret-Richter and Waddington (1993) involving the influence of the assessment of food quality on the performance of waggle dances, which signal profitability. In their study, bees that had previously foraged on solutions of lesser concentration danced to indicate a more profitable source than bees that had been constantly feeding at the higher concentration food source. Similarly, (De Marco and Farina, 2001a) also found that dance behaviors and trophallactic behaviors (mouth-to-mouth food exchange contacts)(Wainselboim et al., 2001) were influenced by changes in the profitability of food sources and not just by absolute profitability. Thus, in these studies and in ours, a behavioral decision (how long a learning flight will be, or how many circuits a waggle dance will have) is modulated by an assessment of the value of a food source. In both cases, this assessment is determined by comparison of concentrations across visits rather than by the most recent visit alone.

The modulation of learning flights by sucrose concentration in this manner makes sense in an ecological context. While individual flowers will vary in nectar concentration due to evaporation over the day, most flowers stay relatively constant (reviewed by (Barth, 1985). Thus, when a bee perceives a substantial increase in sucrose concentration between visits, the corresponding event is likely to be the landing on a different flower or in a different, more profitable patch. By performing a learning flight every time she feeds on a source with higher sucrose concentration, a bee effectively ensures that she learns her way back to this new and superior source. In other words, an increase in sucrose concentration serves as an indicator that she has found a new food source that is worth learning. This interpretation is consistent with the fact that increasing sucrose concentrations and flow rates of a flower increases the probability that bees will perform a "stay flight" and return to that flower (Greggers and Menzel, 1993). These repeated visits to a flower within a foraging bout may also allow a bee to update or collect new information about a valuable food source during the foraging trip. Beyond this, the fact that learning flight duration increases in proportion to the magnitude of the increase in sucrose concentration implies that the advantages of learning a new location increase in proportion to the energetic payoff that the new food would provide. This assumes that increasing learning flight durations directly enhance foraging performance, for example by improving the accuracy of learning or the strength of memories.

Conclusions

Taken together, our results suggest that the mechanisms by which learning flights are modulated allow bees to adjust their learning efforts in response to changing needs for visual information. The value of such information is influenced by motivational variables related to food quality and by sources of spatial uncertainty in the environment.

We have shown that the study of learning flights is a valuable way to quantify the investment a bee makes in learning, and hence study learning as an active decision-making process. Our results have demonstrated the remarkable flexibility with which bees are able to respond to their environment. By adjusting the duration of their learning flights, bees are able to react to changes in informational demands; bees are able to adjust their learning so that they have enough information to allow them to return to a food source. In addition to responding to informational demands, we have seen how bees might adjust their learning in response to changes in the value of a food source such that they might learn the location better. In our studies, we have looked at the effect of changing informational demands and changing quality of food sources separately. Natural situations, however, involve changes along both axes simultaneously, and thus the modulation of learning flights is likely to be influenced by both factors. While many questions about the mechanisms modulating learning flight durations remain open, our results pave the way for deeper investigations of such mechanisms and their role in allowing bees to respond efficiently to changes in the foraging landscape.

CHAPTER 2

INVESTING IN LEARNING: BENEFITS OF PERFORMING LEARNING FLIGHTS FOR HONEYBEES, *APIS MELLIFERA*

INTRODUCTION

To successfully navigate a dynamic environment, animals need to possess information that is current and relevant. In some species, animals actively explore their environment to learn features of the landscape that will help them return to a food source or find their way home (Boal et al., 2000; Joubert and Vauclair, 1986; Nicholson et al., 1999; Thinus-Blanc and Gaunet, 1999; Zeil et al., 1996). Such exploratory behavior in rats has been noted to occur particularly when novel landmarks are introduced into a familiar landscape, and to diminish over time as the rat presumably learns (Thinus-Blanc and Gaunet,1999). Similarly, when bees discover a new, rewarding foraging site, a specialized behavior called a ' learning flight', or a ' turn back and look' (TBL) (Lehrer 1991, 1993; Wei et al. 2002) is performed upon departure from the site to learn information about nearby landmarks that will guide their return. These learning flights are akin to the 'orientation flights' performed by bees and wasps upon departure from their nest or hive to learn visual information that will guide them back home (Tinbergen, 1932; von Frisch, 1967; Wehner, 1981; Zeil, 1993b). Learning flights, like the exploratory behavior in rats, are most intensive at the onset of the behavior, and diminish over time. Unlike the exploratory learning behavior in other species, learning flights occur *after* the discovery of a rewarding location (Lehrer 1991,1993). Because this behavior occurs in response to the discovery of food, the learning flights of honeybees and other insects provide a convenient window through which we may quantify the investments actively made in learning a specific location.

Investments in learning flights are necessary for a bee to efficiently relocate a profitable food source that she has discovered. Given that such a food source may be a small patch of flowers within a large foraging range, up to 14 km in some cases (Seeley, 1995), this is not an easy task. While celestial cues and large-scale features of a landscape can guide a bee to an approximate area, they are insufficient to guide her to a precise location, such as a small flower patch or an artificial feeder. Yet honeybees are remarkably adept at using visual information to pinpoint a location, such as a nest of food source. Such precise navigation is accomplished using information about landmarks located near the goal (Iersel and Assem, 1964; Lehrer, 1991; Lehrer, 1993b; Tinbergen, 1932; Tinbergen and Kruyt, 1938; Wehner, 1981; Zeil, 1993b). When the goal is a food source, bees use features of nearby landmarks such as color and shape, which are primarily learned during arrival (Bitterman and Couvillon, 1991; Couvillon et al., 1991; Gould, 1988; Opfinger, 1931) to identify the area, but spatial information about the food source and nearby landmarks is also required for a bee to accurately locate the goal (Lehrer, 1991; Lehrer, 1993b; Lehrer and Collett, 1994; Zeil, 1993b). The acquisition of such information occurs through learning flights. During learning flights, bees fly in an arcing, circling pattern that expands in radius and height as they depart from a food source. This flight pattern has been shown to provide motion parallax cues that allow for absolute distances between the food source and landmarks to be learned (Brunnert et al., 1994; Cartwright and Collett, 1979; Zeil et al., 1996). These characteristic flight patterns are not generated upon arrival to a food source, which helps explain why absolute distances between landmarks and feeder are only learned upon departure. However, absolute distance is only one type of information about spatial relationships; the two-dimensional apparent, or retinal, size of a landmark can provide information about relative distances between the food source and nearby landmarks (Cartwright and Collett, 1979). Unlike the three-dimensional (3-D) information learned through learning flights, this two-dimensional (2-D) relative distance information can be learned upon arrival (Lehrer and Collett 1994, reviewed in Lehrer and Bianco, 2000). Either type of spatial information can be used to guide a bee's return to a goal, yet a bee uses both. In the initial visits following the discovery of a new foraging site, bees rely on absolute distance cues to guide their return. Over time, as learning flight durations diminish, bees switch their preference to the use of retinal size cues (Lehrer and Collett 1994).

In their 1994 study, Lehrer and Collett suggested explanations for why bees use both cue types. They suggested that learning flights are critical for allowing bees to choose which features of the scene surrounding the goal are most important to learn; bees are known to weight landmarks closest to the goal more heavily in importance (Cheng et al., 1987), and the motion parallax cues generated during learning flights would allow bees to distinguish the proximity of landmarks. Once a bee has divided the scene into near and far regions, she shifts towards a reliance on apparent size cues and "knowledge of distance may be allowed to decay" (Lehrer and Collett 1994). One reason why bees may shift to reliance on 2-D information learned on arrival is that it is likely easier to obtain and to use. In comparison, 3-D information is more costly to obtain given the energy expenditure required to perform learning flights. Some authors have also suggested that 3-D information may be less efficient to use for small-scale navigation, as bees would likely need to repeat similar motion patterns upon arrival in order to assess landmark distances and match them to memory (Lehrer and Collett 1994, Zeil 1993b, reviewed in Zeil 1996, reviewed in Zeil, 1997).

If the use of absolute distance information for local navigation is indeed costly in terms of energy and efficiency, one might expect bees to minimize this cost by performing learning flights when and for as long as necessary. One characteristic of learning flights that seems to be consistent with this idea is the modulated nature of learning flight durations: learning flights are longest in the first few visits to a new food source, and over successive visits, they decay in duration until they are no longer performed. Previously, we found (Wei et al. 2002) that the duration of learning flights is adjusted in response to uncertainty about the location of food relative to landmarks, and to payoffs provided by the food. Specifically, learning flights varied as a function of: 1) the extent of delay in receiving expected food, 2) the extent of past experience at the site, 3) the visual complexity of the scene, 4) the stability of landmarks, and 5) the increase in sucrose concentration relative to recently visited sources. Given these results, we proposed that the degree of investment in the learning flight might correlate with the value of the information that the learning flight would provide, which is determined by its impact on foraging success and ultimately the fitness of the colony. Implicit in this assertion is the assumption that bees modulate learning flights in order to maximize a trade-off between the information gained by performing learning flights and their associated costs in terms of energy expenditure and memory formation and storage. In this study, we explore this issue in two parts. First, we test the idea that the information gained through learning flights is valuable because it allows bees to relocate a rewarding food source with greater spatial accuracy. Thus, we predict that bees performing longer learning flights will return to the departure location with greater probability and accuracy. Alternatively, longer learning flights may allow a bee to remember a location for a longer period of time, but may not necessarily improve the accuracy or efficiency of her foraging.

Second, we ask whether the information gained during learning flights performed at a familiar location, also called "reorientation flights", allows bees to relocate a rewarding food source with greater spatial accuracy. Here, we use the term "reorientation flight" to refer specifically to learning flights that reappear after a bee has been returning successfully to a familiar site, and has ceased performing learning flights. The phenomenon of reorientation flights is somewhat puzzling considering that they are often performed at a site that is already familiar. However, they have been noted to occur after a bee or wasp has experienced some confusion or difficulty in locating a goal (Iersel and Assem, 1964; Wehner, 1981; Wei et al., 2002; Zeil, 1993a). It has been suggested that reorientation flights serve to update visual memories when landmarks surrounding the goal have changed and difficulty in finding the goal signals the need for an update (reviewed in Wehner 1981). Supporting this notion is the finding from wasps (van Iersel and van der Assem 1964) and bees (Wei et al. 2002) that the duration of reorientation flights is correlated with time spent searching for a familiar goal.

MATERIALS and METHODS

General methods

Bees

A colony of *Apis mellifera* was kept in an observation hive inside a large flight cage on the campus of Michigan State University, East Lansing, MI. The enclosed space measured 19.5 m (l) x 5.6 m (w) x 2.3 m (h) and was covered with a mesh fabric (30% shade cloth, designed to block 30% of incident light.) The honeybees' only source of food was sucrose solution provided in feeders, and pollen introduced directly into the hive. Preventing bees from flying to natural food sources guaranteed high levels of motivation for foraging in the experiments.

At a distance of approximately 7m from the hive, we set up a "stock feeder" containing unscented sucrose solution at a concentration of 0.25M. Unscented solutions were used in order to reduce the effect of odors on recruitment of bees to the test apparatus. We filled the feeder before experiments to establish a population of bees available for use in the experiments. Experiment 1 was performed in the summer of 2002, and experiment 2 was performed the following summer, in 2003. In each season, the general methods were the same, although a different colony of bees was used in the two experiments.

Scent-gland plugging

Our studies required us to be able to observe individual bees and record the durations of their departures from the test apparatus. This was difficult to do in the presence of recruited bees that inevitably followed our experimental bees to the test apparatus. To reduce this recruitment of other foragers to the feeders, we sealed the Nasanov glands of all bees used in the experiments (Towne and Gould, 1988) to prevent the release of recruitment pheromones. Methods are described in Wei et al. 2002. Each of these individuals was identified by colored paint marks on the abdomen and thorax. This procedure was done a few hours to a few days before testing. The success of the procedure could be visually determined by observing whether the wax remained on the bees' abdomens, and only bees with intact scent-gland plugs were used in the experiments. The flight behavior of the bees did not appear to be affected by this procedure; learning flights performed by treated bees showed the same characteristics as those observed in other studies (Lehrer, 1991; Lehrer, 1993b; Wei et al., 2002).

A second reason for plugging the scent glands was to reduce the influence of recruitment pheromones on the bees' behavior. Specifically, we wanted to ensure that the effects we observed on learning flights in our experiments were not attributable to odor cues left by bees. As a further precaution to reduce the influence of odors, we also replaced feeders that bees had fed on with fresh ones to eliminate odor marks bees may have deposited on the feeder. Additionally, we wiped each panel of the test apparatus where the feeders rested with a damp paper towel following each visit by a bee, and we rotated the position of the panels between visits, eliminating the ability of any odor cues to predict the location of the food. During each test, the panels used during the training phases were replaced with fresh panels.

Apparatus

In all experiments, bees were recruited to an octagonal shaped rotating table located 13.5m from the hive. The table, with a 122cm diameter, was mounted onto a platform, which was raised 40cm off the ground (Figure 2.1). The table was divided into 8 sectors, and a triangular foam panel, covered with matte textured white paper, was placed in each sector. A spare set of panels was kept out of sight for use during testing. Landmarks consisted of a single black cylinder, located in the center of the 8 panels, and two additional cylinders were placed on edges of the video camera stand, which stood behind the table. In experiment 1, the landmarks were a blue and a yellow cylinder, whose locations were alternated to reduce color biases. In experiment 2, the landmarks were both black cylinders. Each cylinder landmark was 31.6 cm tall and 9 cm in diameter. From the feeders, the landmarks subtended an angle of 29.9 degrees in height and 9.35 degrees in width. The camera stand supported a 2m pole from which a video camcorder (SONY DCR-TRV103) [1] was mounted and centered over the table.

On each panel, we placed a feeder centered approximately 5 cm from the edge of each panel base. In experiment 1, the feeders were red candle holders (0.7 cm height, 0.8 cm diameter) that held 140 ml of liquid. In experiment 2, we switched feeders to transparent microcentrifuge caps (1 cm diameter and 0.5 cm height), with a capacity of 400 ml. Both changes in landmark color and feeder type for experiment 2 were made to increase a bee's reliance on spatial information rather than the color of landmarks and feeders to locate the feeders. We initially used colored landmarks and feeders because we were unsure how difficult the task would be for the bees.

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[1] Footage of arrivals, departures and search patterns during water tests were recorded; however, the data reported here were based on real time observations. We occasionally used video archives to reconfirm the accuracy of our field notes.

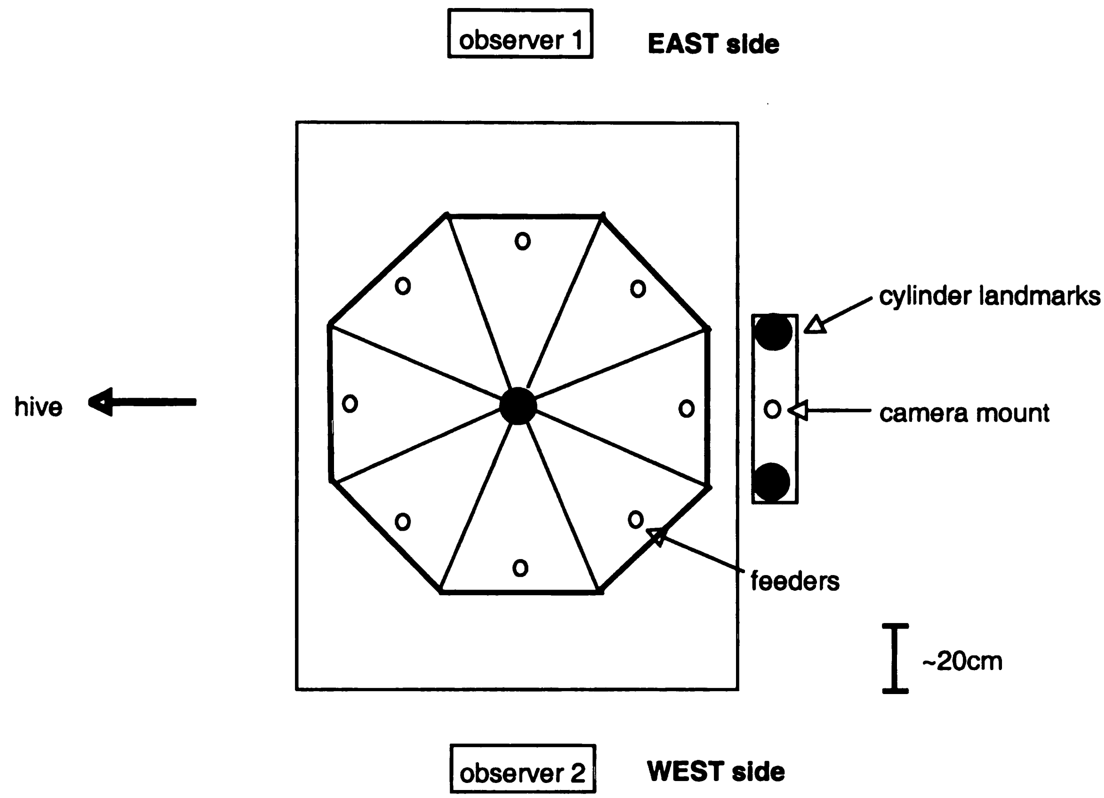

Figure 2.1. Diagram of experimental apparatus (drawn approximately to scale). Small circles indicate where feeders were located in each panel. Large, black circles represent cylinder landmarks.

Recruitment to experimental apparatus

Honeybees are known to transfer to a new food source if the quality is higher than that of the food on which they were previously feeding (Lehrer, 1993b; Wei et al., 2002). In all of our experiments, individual bees feeding on low concentration sucrose (0.25 M) at the stock feeder were transferred to the test apparatus by using sucrose solutions of higher concentrations in the test feeders. We accomplished this by first introducing the concentrated solution to the bee via a pipette while she was feeding at the stock feeder. When the bee began feeding, she was placed onto the test feeder and carried by hand to the test apparatus. Some bees returned immediately to the new food source on subsequent trips, while others needed the recruitment process to be repeated from 1-5 times before returning to the test apparatus and finding the food without assistance.

Measurement of Learning Flights

Following each bee's visit to the experimental apparatus, the duration of learning flights was recorded using stopwatches. Two observers started their stopwatches at the moment the bee lifted off from the feeder and stopped timing when she either stopped flying in obvious arcing, circling patterns, or when she flew approximately one meter beyond the edge of the table. Because there was not a precise way to measure the exact duration of the learning flights, our measures include some variation. Although our measurements included a significant amount of circling in the vicinity of the food (see also (Lehrer, 1993b), it also includes, more importantly, the intensive face forward examination time described by Lehrer as the Turn Back and Look. We included this circling behavior because bees often turned away from the landmarks soon after departure, but well before they left the vicinity of the landmarks. We found in a previous study (Wei et al., 2002) using video recorded from above the arena, that in a sample of 15 departures, bees spent an average of 6.16 ± 0.74 (mean ± SEM) seconds longer investigating the landmarks using our criterion compared to the 180° turn criterion[2] used by Lehrer in her studies of the TBL behavior (Lehrer, 1993b). The 180° turn criterion stopped timing at the first moment a bee had turned 180° away from the feeder and landmarks. Because the structure of learning flights depends upon the visual scene, this criterion is appropriate in cases where the circling portion of learning flights is minimal, as in the Lehrer study and one of our earlier experiments (Lehrer 1993, Wei et al. 2002). Since bees spent a longer time investigating the landmarks than would be measured with the 180° turn criterion, our measure seems a reasonable indicator of time spent learning.

Averages of the two hand recorded times were used in our analyses. The recorded times of each observer were consistently similar; in a sample of 373 recorded learning flight durations from the Turn experiment, the mean difference between the two times was 0.088s with a standard deviation of 0.467 s and a range of 0-3.64s. In a sample of 426 recorded durations in the delay experiment, which was recorded by a different observer than one of the observers in the turn experiment, the mean difference between the two times was 0.33s with a standard deviation of 0.5s and a range of 0-5.05s. Given that differences in learning flight durations were in the order of seconds (see Results), these measurement differences are relatively small.

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[2] The 180° turn criterion stopped timing at the first moment a bee had turned 180° away from the feeder and landmarks. Because the structure of learning flights depends upon the visual scene, this criterion is a good one in cases where the circling portion of learning flights is minimal, as it was in the Lehrer study and one of our earlier experiments (Lehrer 1993, Wei et al. 2002).

Experiment 2 (Delay) required assessment of whether the duration of learning flights changed following an experimental manipulation. To do this, we established a baseline by averaging the departing flight durations of the three visits prior to the manipulation. At the point at which the manipulations were performed, these durations had stabilized such that learning flights did not show patterns of increasing or decreasing duration. Twelve visits are generally sufficient to reach this stable point. In most cases, the baseline is not zero. Even after several visits, some bees might perform one or two loops to orient themselves, but this behavior is distinctly different from the learning flights performed in initial visits, where several loops are made. The durations of reorientation flights were defined as the difference between the baseline value and the recorded flight duration following the experimental manipulation.

Measurement of spatial foraging patterns

In order to quantify the choices bees were making in terms of their spatial foraging patterns, we also recorded the following information for each bee on arrival: 1) the panel position of the first feeder where she landed and extended her proboscis onto the feeder (first landing), and 2) the sequence, position, and number of contacts on the feeders (used to calculate biases). The first landing data were presumed to reflect a bee's initial prediction about the location of food, and is a good indicator of the bee's accuracy in foraging. Such data was quantified using a scoring paradigm described in the next section.

However, a bee's first prediction was often incorrect, and her subsequent search patterns reflect additional predictions about where food might be. Thus, biases were also measured as an indication of a bee's foraging accuracy. Finally, after the last rewarded visit was made, a water test was conducted. During this test, bees returned to find all feeders filled with water, which forced bees to contact the feeders before determining whether or not they were rewarding. We recorded each panel position where the bee landed and made contact with the feeder as she searched for the food. The "water test" ended when bees gave up searching at the table and returned either to the stock feeder or the hive. Without rewarding feeders to end a bee's search, the water test enabled us to observe the full extent of a bee's foraging decisions, and represents the most complete measure of foraging accuracy. The water test could be performed only once for each bee, and we were therefore limited to comparisons at a single time point.

Calculation of biases and scores

The probability that a bee would first return to the departure panel was quantified by a score, which was assigned depending upon where the bee first landed (Figure 2.2a): 2 points for landing on the departure panel; 1 point for the departure side; 0 for the north or south panels; -1 for the arrival side; and –2 points for the arrival panel, where food was located when the bee arrived at the table. We summed learning flight durations of the first visit where a bee finds the food without recruitment and the learning flight durations of the following one, two or three visits. Scores were summed for the visit immediately following the first independent visit and the subsequent one, two or three visits (Figure 2.2b). We used these sums to increase the range in values, which might allow a pattern that was otherwise masked to be revealed. Although an alternative analysis might have been to correlate learning flight durations to scores for all visits, we did not do this

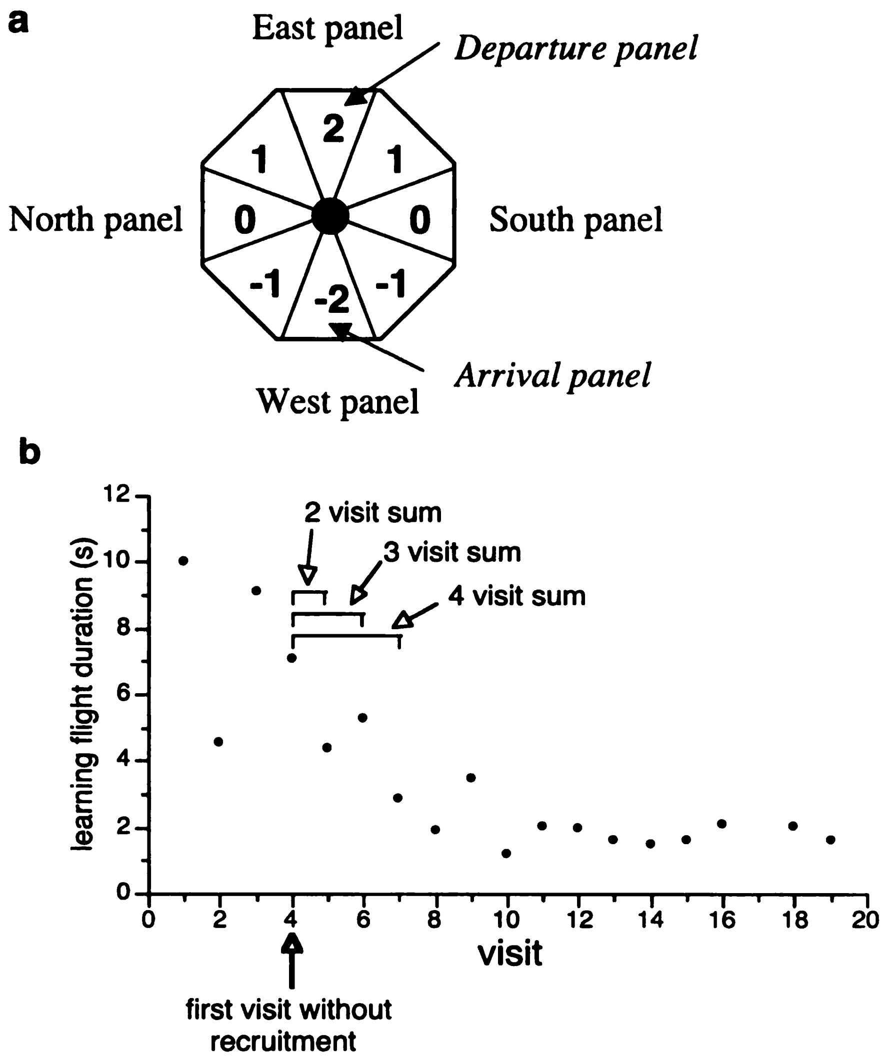


Figure 2.2. **a**. Octagon represents the experimental apparatus. Scores are calculated based on the panel where the bee first lands upon arrival, as marked in the diagram. "Departure panel" indicates the panel from which the bee departs and performs a learning flight. "Arrival panel" indicates the location of the food upon a bee's arrival to the table. **b**. Diagram depicts learning flight durations of a single bee over a series of repeated visits, and shows how sums are calculated. Sums include the learning flight duration of the first visit where a bee finds the food without re-recruitment and the learning flight durations of the following one, two or three visits. Scores are summed for the visit immediately following the first independent visit and the subsequent one, two or three visits.

because learning flight duration and visit number are highly correlated (Figure 2.3). Thus, any effect of learning flight in such an analysis would be confounded by the influence of experience and visit number. With our method of summing across a small number of visits, the confounding influence of experience is minimized.

To measure bias on a trip -by -trip basis, we calculated the difference in the number of contacts with a feeder made on either side of the table divided by the total number of contacts. Further details are given in the results.

To measure bias in the water tests, we calculated the difference in the number of contacts made on either side of the table (A-B) divided by the total number of contacts (A+B). For this calculation, we excluded the north and south panels (Figure 2.2a). A equals the side where food was located upon arrival during training (*post-change* phase in experiment 2), and B equals the opposite side.

During each water test, the total number of contacts varied because we tracked each bee until she left the table. In order to account for the effect of the total number of contacts on the variance of the bias calculation, we included only those bees that made a total number of 20 or more contacts. This conservative approach did not change the significance of our results. The criterion of 20 contacts was determined by choosing a sample size such that the variance of the bias was less than or equal to 5% (alpha = 0.05). In calculating these criteria, we assumed that the data followed a binomial distribution, where the probability of choosing either side is 50%.

We used two-tailed tests in all of our statistical analyses.

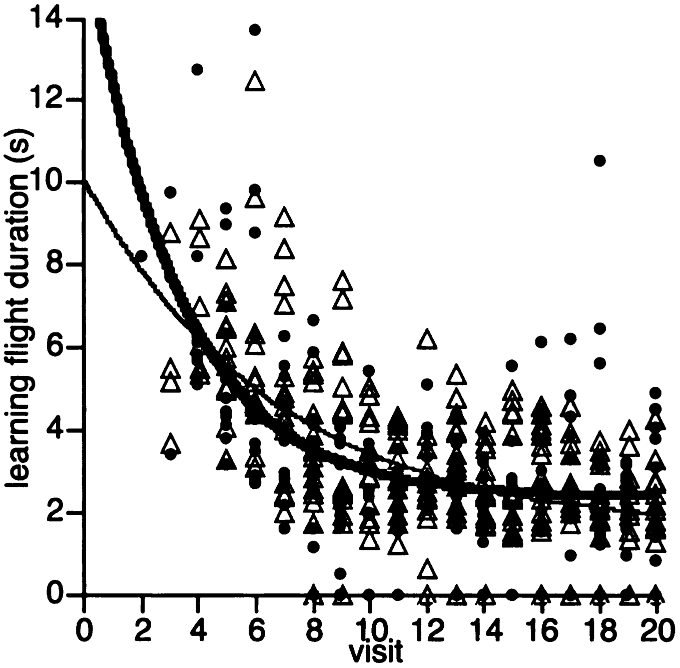


Figure 2.3. Relationship between visit number and learning flight duration. Each triangle represents a single visit for one bee in the Turn condition, and each black circle represents the same for bees in the No Turn condition. Each bee made 20 visits. The *thin* black line represents the nonlinear fit of the equation: y = yo+A*Exp(-visit*t) for the Turn group, and the *thick* black line represents the same for the No Turn group (No Turn: t= 0.31; turn: t = 0.15).

Specific Experimental Methods

EXPERIMENT 1 (TURN)

Our aim in this experiment was to investigate how the duration of initial learning flights performed upon departure from a new location influences a bee's foraging accuracy on her subsequent returns to the table. Since honeybees can learn both during arrival and departure (Bitterman and Couvillon, 1991; Couvillon et al., 1991; Lehrer, 1993b; Lehrer and Collett, 1994; Opfinger, 1931), we needed a way to separate the information learned upon arrival from that learned on departure. To accomplish this, in one group, we trained bees to food on one side of the table and while an individual bee was feeding, we rotated the table so that she would depart from the opposite side (Turn condition). This rotation of the table during feeding did not seem to disturb the bees. In fact, they rarely even stopped drinking while the table was being turned. In another group, bees (No Turn condition) arrived and departed from the same side. We assessed whether the durations of initial learning flights performed upon departure were correlated to the likelihood of returning to the location of departure. This is of particular interest in the turn condition, because the ability to return to the location of departure would have to be based upon information acquired during the learning flight.

For each training and testing session, we used a single bee recruited from the population of marked, scent-plugged bees from the stock feeder. Once bees were used in a session, they were released outside the flight cage; thus all bees used in all four experiments were different individuals. In this experiment, we recruited a bee to a red candle holder containing 140ml (full volume) of 2.25M sucrose solution, and then placed the feeder on either the panel facing directly east or the one facing directly west (Figure

2.4a). Feeders filled to full volume contained ample sucrose solution to allow a bee to fill her crop, which has a maximum capacity of around 50ml (Dyer et al., 2002; Nunez, 1970). Therefore, a bee could complete her entire foraging bout at a single feeder. Bees in each condition returned to the table 20 times and experienced an unrewarded final water test on the 21st visit. By this time, bees have finished the 'TBL phase' (Lehrer, 1993b), or the '*initial* phase' (Wei et al., 2002), the phase during which bees initially perform learning flights. Although the duration of the TBL phase varies by individual, it is usually complete around six visits and always completed by twelve (Wei et al., 2002).

EXPERIMENT 2 (DELAY)

When bees are unsuccessful in finding their goal, they will search the vicinity for an extended period of time. When the goal is eventually located, reorientation flights are frequently observed (Wei et al., 2002). To determine how reorientation flights influence a bee's foraging patterns in subsequent visits, we induced the occurrence of these flights by imposing a delay in receipt of the food on arrival for one group of bees (Delay). To obtain a spread in reorientation flight durations, a second group of bees (No Delay) did not experience a delay.

In the *initial* phase of this experiment, bees were recruited to a single feeder fully filled with 2.25M sucrose solution. This feeder was either on the panel directly east or the one directly west of the table center and remained in this position throughout the 12 visits in the *initial* phase (Figure 2.5). The remaining seven feeders were filled with water. The side chosen for training was alternated between bees to control for natural biases towards one side or the other. After 12 visits, for bees in the Delay group, we

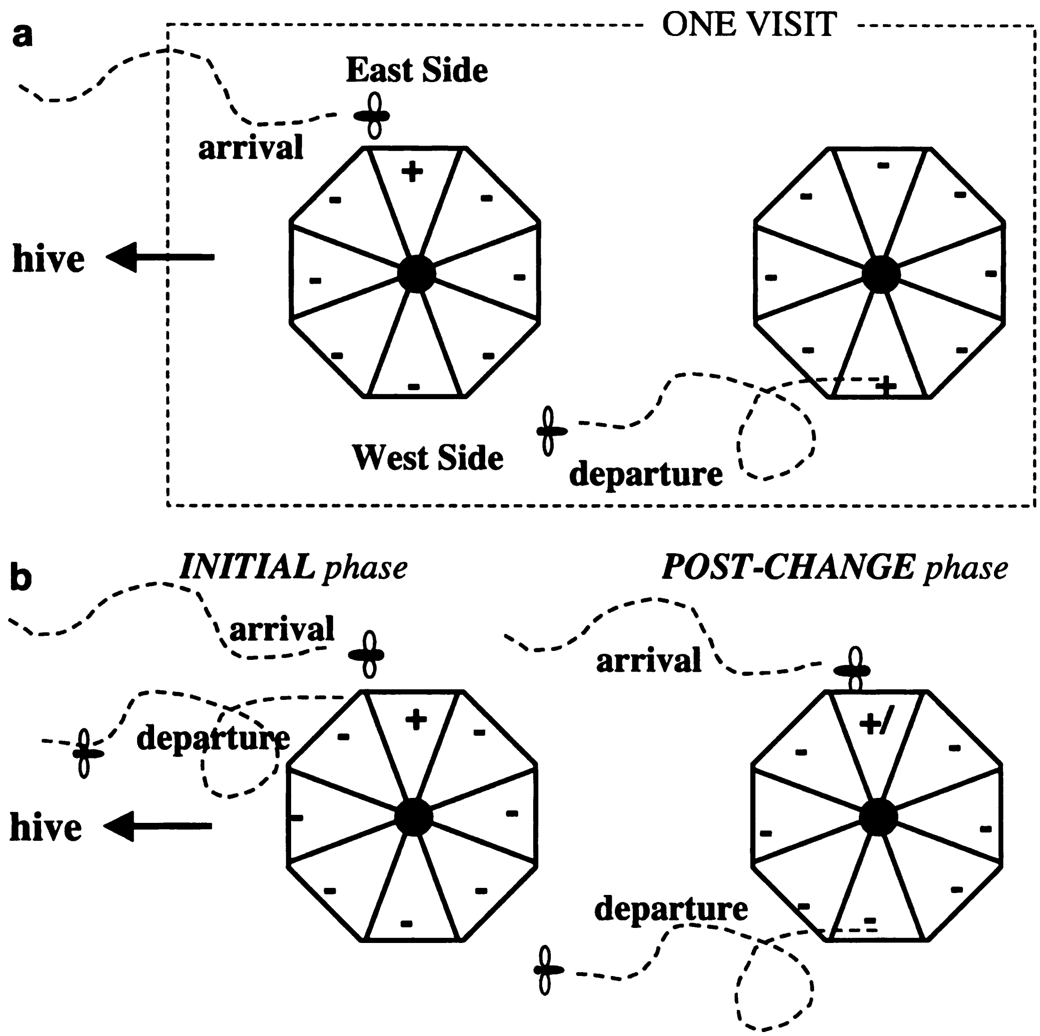


Figure 2.4. Diagram of experimental conditions. Octagons represent the experimental apparatus (Figure 2.1), black circles represent landmarks, "+" symbols indicate panels where food is located, and "-" indicates feeders with water. "+/-" or "-/+" indicates presence or absence of food on arrival/departure. **a**. A single visit for a bee in the Turn condition of experiment 1 (Turns) trained to arrive at the food on the east side. Some bees experienced the mirror image of this pattern and arrived on the west side. Bees in the No Turn condition were recruited to either the east or west side, as in the Turn condition, but departed from the same side as they arrived. **d**. *Initial* and *post-change* phases of experiment 2 (Delay); in the *initial* phase, bees arrive and depart from the same side, as in the No Turn condition of experiment 1. In the *post-change* phase, bees arrive and depart from opposite sides, as in the Turn condition of experiment 1. For each bee, the arrival side in both phases is kept the same.

induced a delay by removing all feeders from the table and replacing them after eight minutes. Based on previous studies (Wei et al., 2002), we knew that a delay of eight minutes usually resulted in reorientation flights, yet was short enough that most bees would persist in searching the experimental apparatus for the entire duration. After the food was replaced and the bee had begun feeding, the table was rotated so that the bee departed from the opposite side from where she landed (Figure 2.4b), as in experiment 1. For the next four visits of this *post-change* phase, we repeated this pattern; arrival side was kept the same as in the *initial* phase, but the departure side was now different. The No Delay group did not experience a delay, but the table was rotated starting on the 13th visit. A water test was conducted on the 18th visit for all bees. In the third group, the Initial condition, bees made 5 rewarded visits to the table where they arrived on one side and departed from the opposite side before a water test was given on the 6th visit. Thus these bees' experience was the same as the *post-change* phase experience of bees in the Delay and No Delay groups. The addition of this group allowed us to investigate the influence of the 12 visits in the *initial* phase on foraging patterns in the *post-change* phase by providing a comparison to bees that did not have this experience.

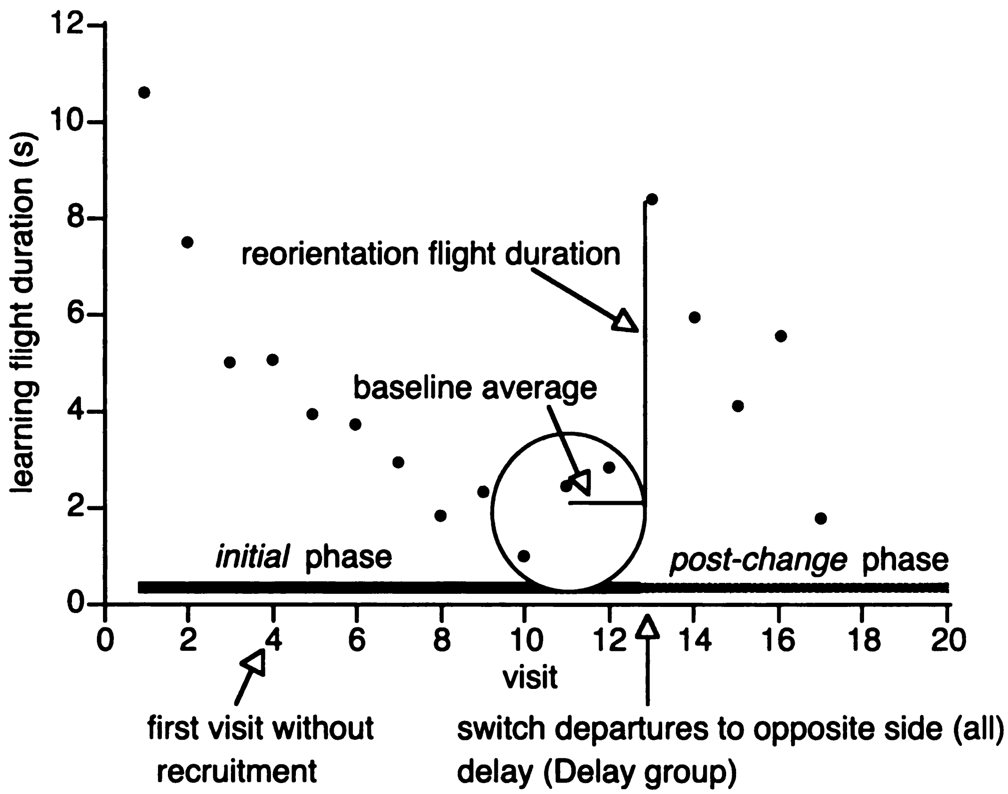


Figure 2.5. Change in learning flight duration over a series of visits by a single bee in experiment 2. The *initial* phase, marked by the *black bar*, is the period during which learning flights are performed at a new location. The *post-change* phase, marked by the *gray bar*, is the period during which learning flights are observed after the change in departure location, similar to the Turn condition of experiment 1, and for Delay group bees, an eight minute delay. The duration of a reorientation flight is calculated as the difference between the learning flight duration following the switch and the baseline average of the three previous visits.

RESULTS

EXPERIMENT 1 (TURNS)

Effects of initial learning flights on spatial patterns of foraging

Given that learning flights are modulated in response to uncertainty about the location of food relative to landmarks, and to payoffs provided by the food (Wei et al. 2002), we expected longer learning flights to be correlated with better foraging performance in subsequent visits. Here, we measure such performance in terms of spatial accuracy in pinpointing the location of sucrose filled feeders.

Patterns of foraging during water tests

During the water tests, a bee returning to the table would usually land on a feeder and extend her proboscis into the feeder. Sometimes, a bee would hover and then dip her legs into the feeder before flying off. After making contact with an unrewarding feeder, bees would investigate other feeders in a similar manner. We recorded each instance where a bee made contact with a particular feeder. Repeated visits to the same feeder were only counted as separate contacts if the bee flew off the boundaries of the panel and then returned; multiple contacts made by a bee walking around the vicinity of the feeder were counted as a single contact. Most bees were quite persistent in their searches; the 21 bees in this experiment made an average of 48.3 contacts with the feeder with a SD =23.75 and a range from 13-91. We excluded 3 bees that failed to meet the criterion of making more than 20 contacts.

We found that bees in the two conditions differed significantly from each other in their preferences for sides of the table during the water test (Figure 2.6). In the No Turn

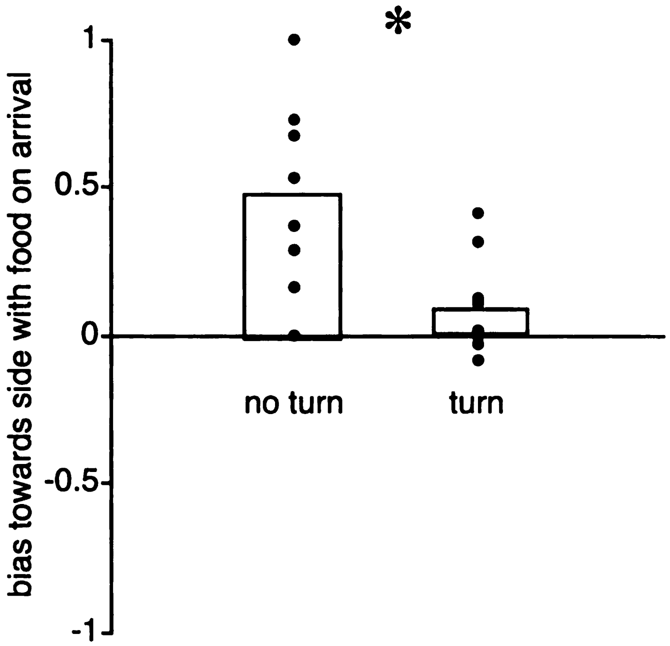


Figure 2.6. Effects of dissociated arrival and departure locations on side biases during water tests. Biases were calculated as the difference in the number of touches or landings made on the side where food was located on arrival during training and the number of stops made on the opposite side. Positive biases indicate a greater number of stops on the side where food was present on arrival, which in the No Turn condition is also the departure side, during the training period. Each data point represents a single bee, and boxes indicate means of each group. In the No Turn condition, bees depart from the same side as the location of the food on arrival. In the Turn condition, they depart from the side opposite that of arrival. Differences in the biases of the two groups are significant (Wilcoxon: $Z = 2.58$, $p < 0.01$, $n=18$). Mean of the No Turn group is significantly different from zero, while the Turn group is not (No Turn: student's t-test, $t= 4.05$, $p < 0.01$, $n = 8$, Turn: signed-rank test, $t= 8.5$, $p = 0.36$, $n= 10$).

condition, bees had a strong preference for the side where the food was located during training, which was the arrival and departure side, and the mean bias of this group was significantly different from zero. Bees in the Turn condition, however, did not have a preference for either side and the mean bias did not differ significantly from zero. This suggests that information gathered during departure influences the development of side preferences during searches for food.

From this analysis, we cannot determine whether bees in the Turn condition did not demonstrate a bias for a side because they were searching at random or because they were choosing between conflicting biases, one for the arrival side and the other for the departure side. If bees were searching at random, we would expect that they would choose the north and south panels equally as often as the east and west side panels during the water test (Figure 2.7a). We found that in all thirteen water tests, each bee made a greater number of contacts with feeders on the east panel and west panel than with feeders on the north and south panels (Figure 2.7b). While this is consistent with the idea that bees are choosing between conflicting pieces of information, one learned on arrival and the other on departure, it is possible that bees may just have a natural preference for foraging on the east and west side of the table compared to the north and south panels. To rule out this possibility, we analyzed data from experiment 2 in Chapter 3. In that experiment, bees were trained in a situation where food was evenly dispersed throughout the table, so that food was equally likely to be found in the east and west panels as the north and south panels. Following twelve rewarded visits, we performed a water test. During this test, bees did not significantly differ in the number of contacts they made on the east and west panels compared to the north and south panels (Figure 2.7c).

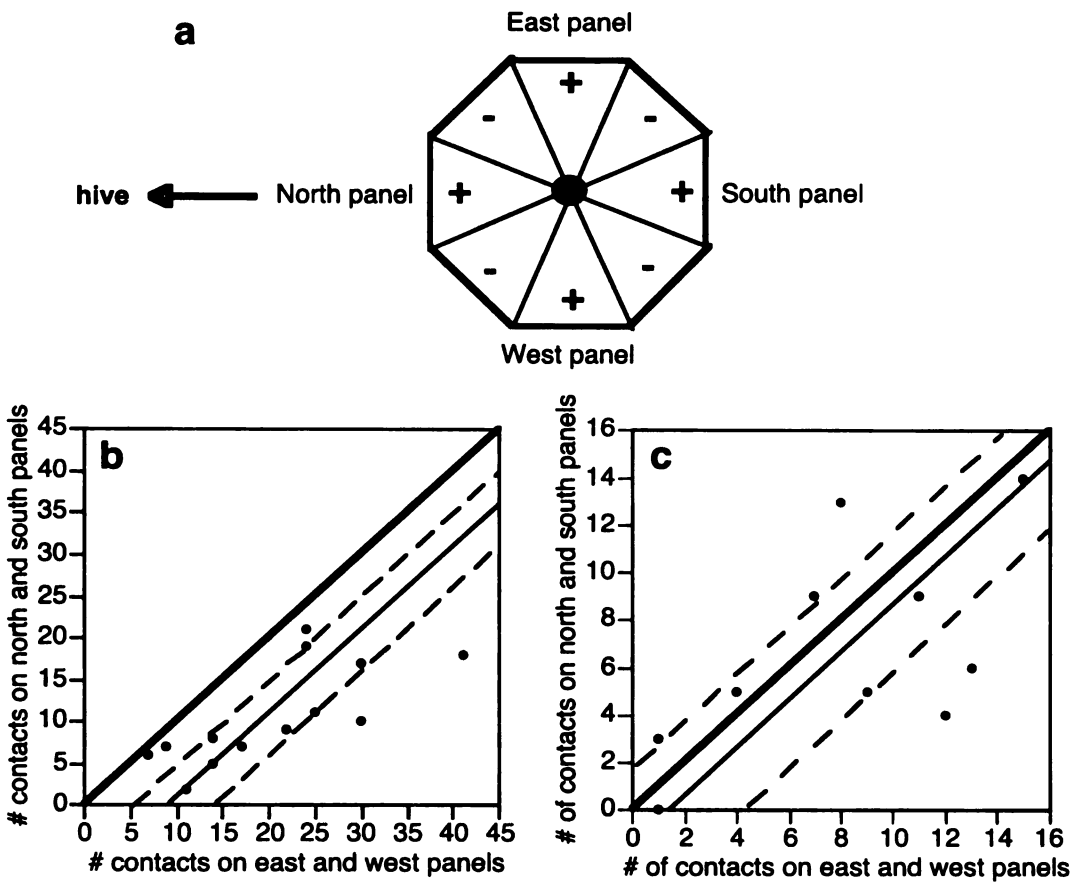


Figure 2.7. Comparison of the total number of contacts made on east and west side panels versus north and south panels during water tests. a. Diagram of table depicting positions of east, west, north, and south panels. "+" indicates panels used in this comparison. b. In the water test for the Turn condition, bees always made a greater number of contacts on the side panels (east and west) than the north and south panels (paired *t*-test: $t = 5.29$, $p < 0.001$, $n = 13$). c. In the water test for a group trained the table with food distributed evenly across the table (part of an experiment not described in this paper), we found that bees did not significantly differ in the number of contacts they made to the east and west panels compared to the north and south panels (paired *t*-test: $t = -1$, $p = 0.34$, $n = 10$). Each point represents a single bee. The *thick solid line* represents the point at which initial proportions and post-change proportions are equivalent. The *thin solid line* is displaced by an amount equal to the difference in the means of the two proportions, and the *dashed lines* indicate 95% confidence intervals.

This confirms that in situations where food is evenly distributed, bees search equally in all areas of the table. Thus, our results suggest that bees in the Turn condition are choosing between their memories of two locations, one learned on arrival and the other on departure, rather than searching at random.

Patterns of foraging across visits

Beginning with the first visit where a bee returned to the table and found the food on her own, without recruitment, we recorded the location of the feeder where she first landed and extended her proboscis. For each of the twenty visits, we calculated the proportion of bees in each condition that landed on the departure side first. We took the number of individual bees that first landed on the departure side of the previous visit (for the No Turn condition, this is also the arrival side), and divided this by the total number of bees in the group. Looking at these proportions of departure side first landings over time, we found that as bees made more visits in the Turn condition, the less likely they were to return first to the departure side. However, there was no significant correlation between visit number and proportion of first landings on the departure side in the No Turn condition (Figure 2.8). For these analyses, we did not include data from the first few visits where the total number of bees that returned on their own for that visit was low (visits #2,3 and 4 for the Turn group, and visits #4 and 5 for the No Turn group). Thus, our results are conservative. The lack of a significant relationship in the No Turn condition (Figure 2.6) is likely explained by a ceiling effect: since arrival and departure information are the same for No Turn bees, they were always very good at finding their way back to the departure/arrival panel. For the Turn bees, the panel containing food on

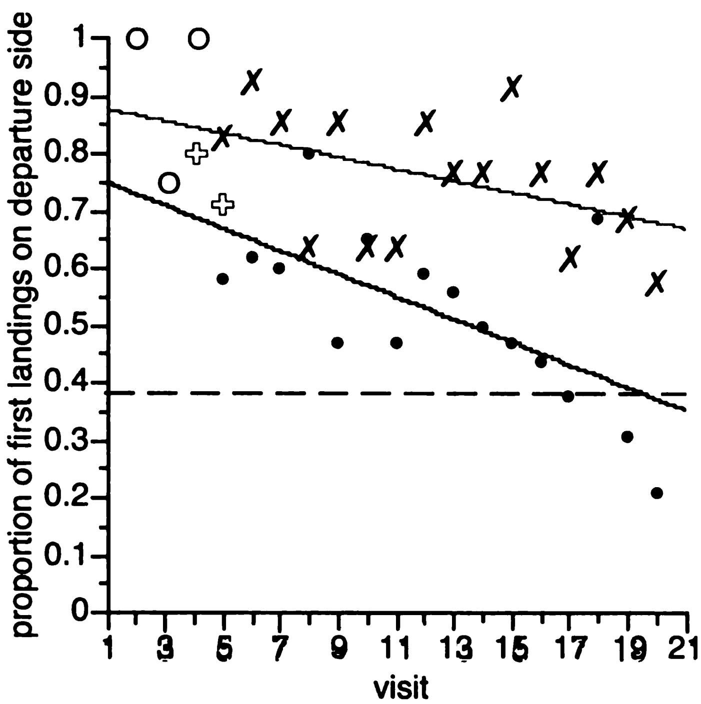


Figure 2.8. Relationship between visit number and the proportion of first landings on departure side. Each point represents the proportion of all bees on a particular visit number that returned first to the departure side, from which they performed a learning flight on the previous visit. "X" symbols represent bees in the no turn condition, and dots represent bees in the Turn condition. Open circles represent data from the turn group that was not included in the regression analysis (see text for explanation), and open crosses represent data points in the no turn condition that were also not included in the analysis. Points included in the analysis begin on the visit where all bees have finished recruitment. The dashed line indicates the proportion of first landings on the departure side that would be expected by chance (three out of eight panels). Linear regressions of these points, as shown by the solid lines, indicate a significant relationship for the turn condition, but not the no turn condition (turn: R2= 0.40. $p < 0.01$, $n = 16$; no turn: R2= 0.19. $p = 0.09$, $n = 16$). ANCOVA analysis revealed a significant effect of visit and condition, but not of interaction (visit: $F = 12.35$. $p < 0.01$; condition: $F= 36.65$, $p < 0.001$; visit*condition: $F_{\,}= 1.24$, $p = 0.28$).

arrival and the panel from which they depart were on opposite sides, and thus arrival and departure information conflicted. These results suggest that bees initially use information learned on departure to guide their return, but over time, their preference on approaching the table shifts away from the departure side.

Using another measure of foraging performance, we looked at the number of contacts a bee made with feeders on each visit before finding the one rewarding feeder. Looking across visits, we found that the more visits a bee made to the table, the faster she was at finding the feeder containing food (Figure 2.9). This analysis adds to the previous one by demonstrating that bees not only shift away from their preference for the departure side, but that they are shifting towards the arrival side and are able to find the food more quickly. No relationship was found in the No Turn condition between the visit number and the total number of contacts made with feeders; this is likely explained by the fact that early on, bees were able to find the food relatively quickly.

Effect of initial learning flight duration on foraging patterns

Further evidence that longer learning flights increase a bee's likelihood of returning to a location is provided by our data from the *initial* phase of the Turn condition. We found a positive correlation between the duration of learning flights and the probability of returning to the panel from which the learning flight was performed (departure panel). In this analysis, we used a score to quantify the probability of returning to the departure panel (see Methods) (Figure 2.10a). When we summed across three and four visits, we found significant positive correlations (Figure 2.10 d,e). These

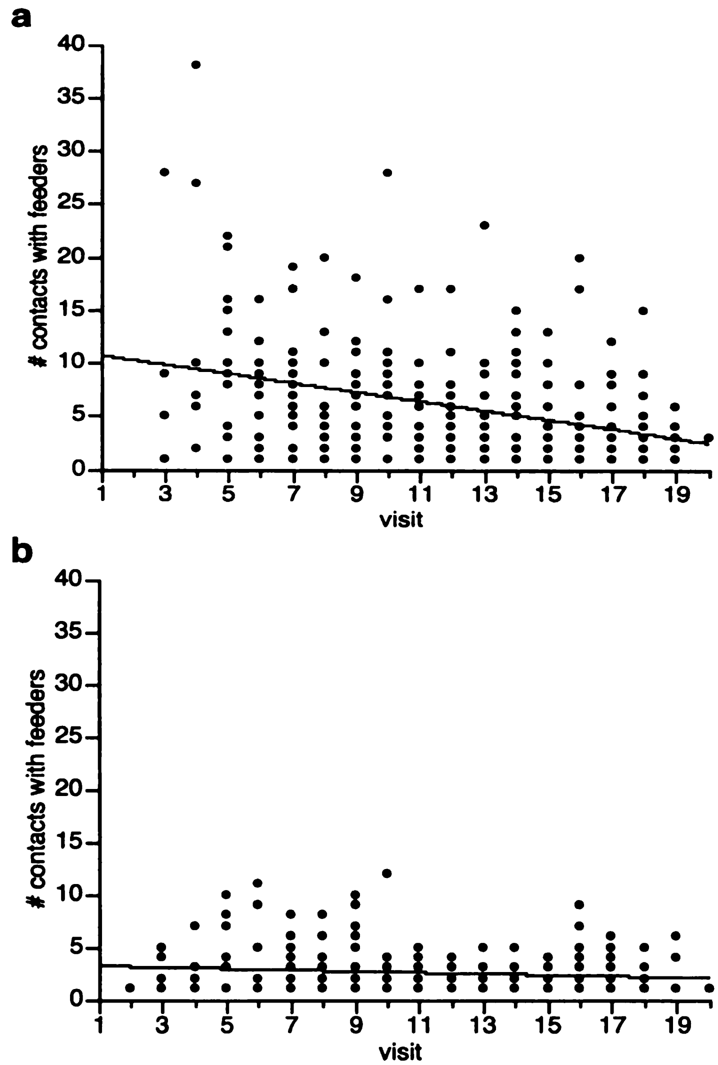
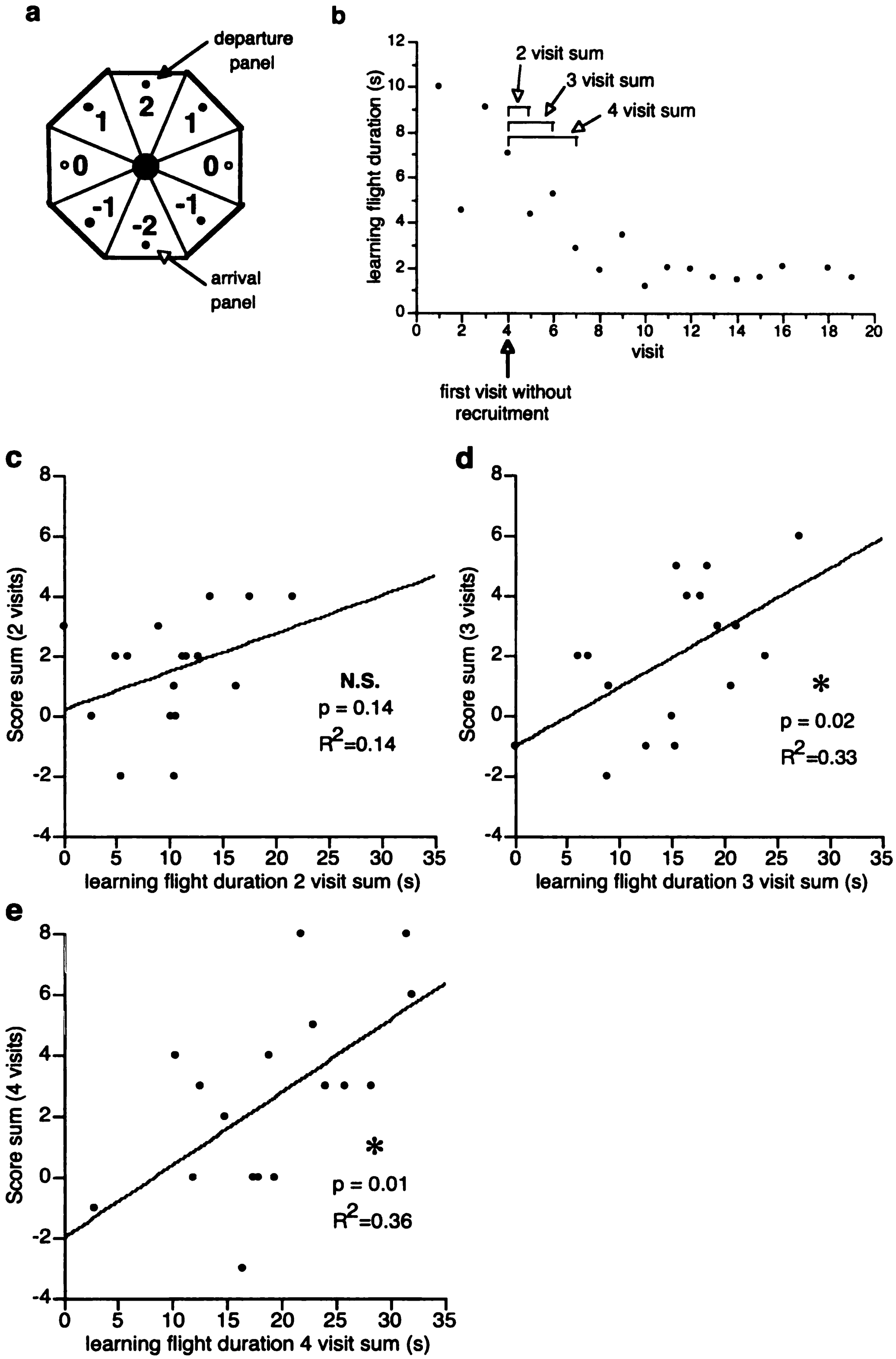


Figure 2.9. Relationship between number of contacts made with feeders and number of visits. Each point represents a single bee on a single visit. In earlier visits, bees require additional recruitment, so few data points are included in the first few visits. Only visits where the bee finds the food without recruitment are included here. **a**. Turn condition (R^2= 0.12, p< 0.001, n = 246) **b**. No turn condition (R^2= 0.02, p= 0.07, n = 217).

Figure 2.10. Relationship between sums of learning flight durations on consecutive visits and sum of scores on correlated return visits in experiment 1. All bees used in this analysis were subjected to the Turn condition, where the location of arrival and departure are on opposite sides of the table. **a**. Scores are calculated based on the panel where the bee first lands upon arrival, as marked in the diagram. 'Departure panel' indicates the panel from which the bee departed and performed a learning flight. 'Arrival panel' indicates the location of the food upon a bee's arrival to the table. **b**. Learning flight durations of a single bee over a series of repeated visits, with indication of how sums were calculated. Scores are summed for the visit immediately following the first independent visit and the subsequent one, two, or three visits. **c.** For two visits (R^2= 0.14, p = 0.14, n=17) **d.** For three visits (R^2= 0.33, p = 0.02, n=17) **e.** For four visits (R^2 0.36, p = 0.01, n=17).


a
departure panel
2
1
1
0
0
-1
-1
-2
arrival panel
b
2 visit sum
3 visit sum
4 visit sum
learning flight duration (s)
visit
first visit without recruitment
c
Score sum (2 visits)
N.S.
p = 0.14
R^2=0.14
learning flight duration 2 visit sum (s)
d
Score sum (3 visits)
*
p = 0.02
R^2=0.33
learning flight duration 3 visit sum (s)
e
Score sum (4 visits)
*
p = 0.01
R^2=0.36
learning flight duration 4 visit sum (s)

analyses suggest that the more learning flight time a bee accumulates over a few visits, the more likely she is to return to the departure location.

We also looked at the relationship between learning flight durations and another measurement of foraging pattern, 'bias', for bees in the Turn condition. As in water tests, biases were calculated as the number of contacts made on the departure side minus the number of contacts made on the arrival side, divided by the total number of contacts. Positive values thus indicate preferences for search on the departure side. This measure gives a sense of the bee's search pattern following the first landing. As in the previous analysis, we summed learning flight durations and subsequent biases over two, three and four visits, beginning with the first visit without recruitment. Although the trends are in the direction of results in the previous analysis (Figure 2.10), we did not find any significant correlation between learning flight durations and subsequent biases (Figure 2.11).

We were unable to account for the effect of the total number of stops on the variance of the bias as we had in the water test (see Methods) because bees made fewer than twenty stops before finding the food. Because the food was located on the arrival side, bees that had a preference for searching the arrival side made fewer stops before finding the food. Thus, the measure of their biases would be inflated compared to bees with an equivalent preference for the departure side. However, this issue would not influence the overall directions of the relationships between learning flight durations and biases (Figure 2.10). Thus, these results, although not significant, are consistent with the results using score measures for first landing data, and support the idea that longer

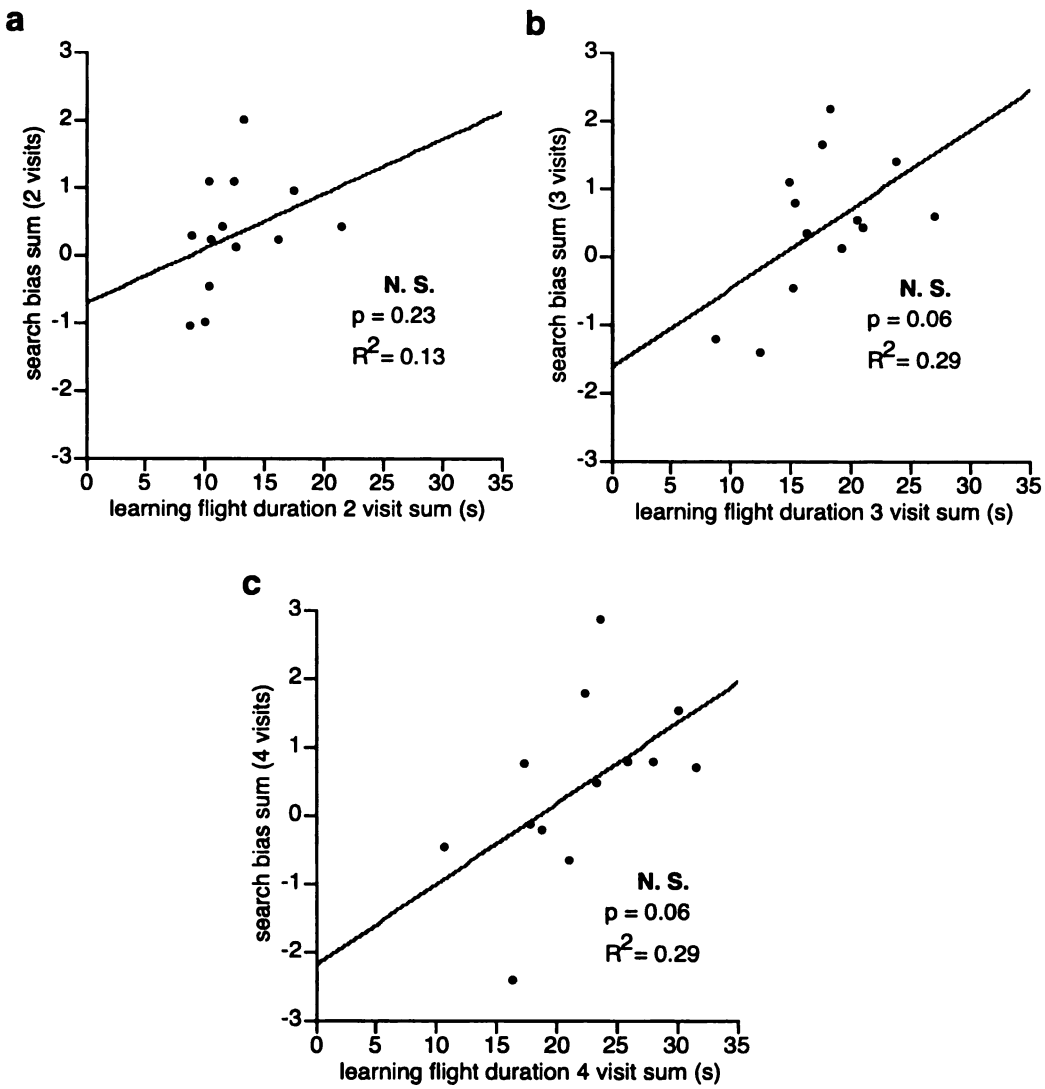


Figure 2.11. Relationship between sums of learning flight durations on consecutive visits and sum of biases on correlated return visits in experiment 1. All bees used in this analysis were subjected to the Turn condition, where the location of arrival and departure are on opposite sides of the table. Sums include the learning flight duration of the first visit where a bee finds the food without recruitment and the learning flight durations of the following one, two or three visits. Biases are summed for the visit immediately following the first independent visit and the subsequent one, two or three visits. Biases are calculated as the number of contacts made on the departure side minus the number of contacts made on the arrival side, divided by the total number of contacts. Positive values thus indicate preferences for search on the departure side. **a.** For two visits ($R^2 = 0.13$, $p = 0.23$, $n = 13$ **b.** For three visits ($R^2 = 0.29$, $p = 0.06$, $n = 13$). **c.** For four visits ($R^2 = 0.29$, $p = 0.06$, $n = 13$).

learning flights increase a bee's likelihood of returning to the specific location where she had performed the flights.

EXPERIMENT 2 (DELAY)

Effects of reorientation flights on spatial patterns of foraging

To investigate the influences of reorientation flights on performance, we subjected bees to a task in which they had to learn to shift their foraging to the opposite side of the table from where they initially learned to find food. To produce variation in reorientation flight durations, we imposed a delay on one group on the visit when the food location was changed. Comparison of the reorientation flight durations between the two groups, Delay and No Delay, confirm that this manipulation had the desired effect; bees subjected to a delay had longer reorientation flights following the manipulation than those in the No Delay group (Figure 2.12). Although the duration of reorientation flights for bees in the No Delay group were significantly shorter, the means were still different from zero, suggesting that a shift in location alone may trigger some reorientation flights.

Effect of reorientation flight duration on foraging patterns

We found a significant positive relationship between reorientation flight durations (for departure flights immediately following the switch on the 13th visit) and biases towards the departure side (defined by the *post-change* phase) during the water test when combining data from the Delay and No Delay groups (Figure 2.12). However, we cannot rule out the possibility that this relationship is influenced by a difference in the two groups, namely the delay experience. Using an ANCOVA analysis, we found no

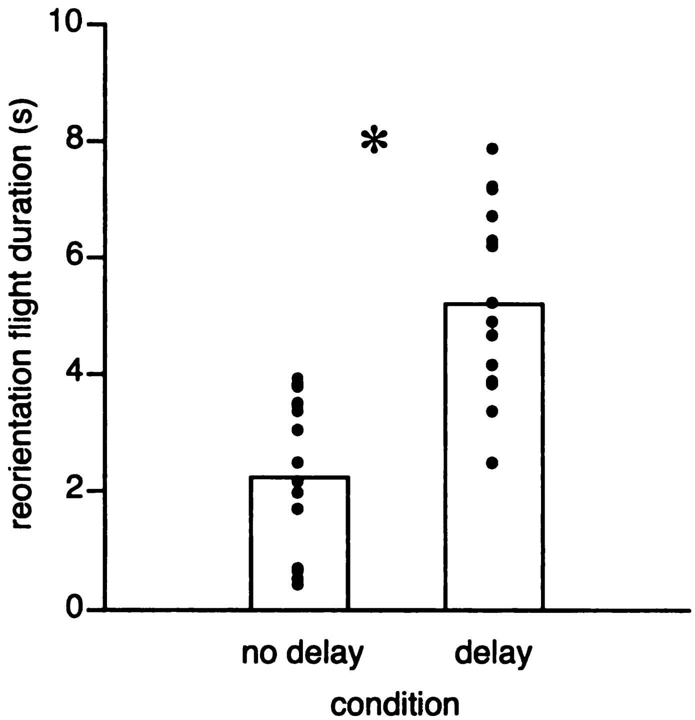


Figure 2.12. Effect of imposing a delay in availability of food on the duration on subsequent learning flights. Change in learning flight duration was calculated as the difference between the average of flight durations for the three visits prior to the 13th visit, where some bees experienced a delay, and the duration of the subsequent learning flight. In both conditions, bees departed from the opposite side of arrival starting on the 13th visit (condition similar to Turn condition in experiment 1). Changes in learning flight durations between the two conditions were significantly different (Wilcoxon: $Z = 4.01$, $p < 0.001$, $n = 31$). Means of the Delay and the No Delay group are significantly different from zero (Delay: $t= 11.99$, $p < 0.001$, $n = 14$. No Delay: signed-rank, $t = 76.5$, $p < 0.001$, $n =17$). Each point represents a single bee, and boxes indicate means.

effect of condition, but also no independent effect of reorientation flight duration when controlling for condition, and no interaction effect (Figure 2.13). After dropping the least significant term, which was the interaction term, we ran the model again, and still found no significant effects of condition ($F=0.19$, $p = 0.69$) or flight duration ($F= 2.73$, $p = 0.09$). Individual analyses for each group do not show significant correlations, although trends in both cases are in a positive direction (Delay: $R^2= 0.18$, $p = 0.18$, $n=12$; No Delay: $R^2= 0.08$, $p = 0.38$, $n=12$). Thus, we cannot conclude a causal relationship between reorientation flight duration and biases towards the departure side during the water tests because some facet of the delay itself may be influencing the subsequent biases during the water tests. One possibility is that bees in the Delay condition encoded information about the departure side during their extended search. However, it seems unlikely that a bee would learn information about a location prior to experiencing any reward there. Also, bees learn information about a new location upon departure, and information learned upon arrival is either not formed or not used during the first several visits of the 'TBL phase' (Lehrer 1993). Thus, it seems unlikely that tendency for bees in the Delay group to search the departure side is the result of learning that occurs during the delay rather than during the reorientation flights.

Patterns of foraging during water test

If the performance of reorientation flights influences a bee's bias for searching on the side of the table from which she departed, we might expect to see this pattern during the water test for the Delay group, but not the No Delay group. Furthermore, if the influence of reorientation flights were as potent as that of initial learning flights on

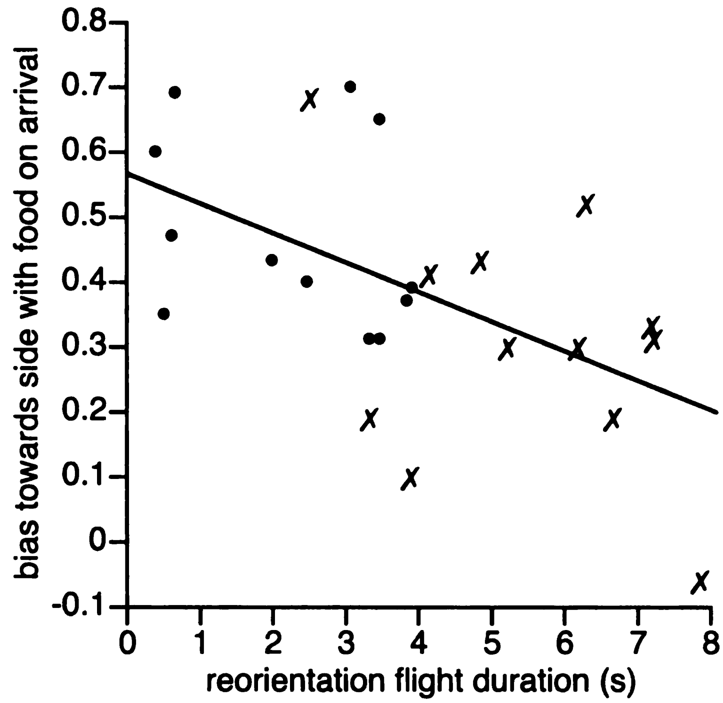


Figure 2.13. Relationship between change in reorientation flight duration and bias during water tests. Dots represent individual bees in the No Delay condition, and "x" represent bees in the delay group. Learning flight duration was measured for the 13th visit, which for bees in the Delay group followed an eight minute delay. The reorientation flight duration was measured as the change in learning flight duration measured relative to a baseline flight duration established by the bee prior to the delay. Bees in all conditions experienced a change in the position of the food on departure for all bees. Negative biases indicates biases toward the departure side. Combined data revealed a significant negative correlation between reorientation flight duration and biases during the water test (R^2= 0.30, $p < 0.01$, n=24). ANCOVA analysis, however, showed no significant effect of condition (F = 0.19, p = 0.67) or of reorientation flight duration (F = 0.19, p = 0.67) and no effect of an interaction (F= 0.15, p = 0.70).

subsequent search patterns, we would also expect to find no difference between biases of the Delay Group and the Initial group. In both groups, bees experienced six visits where they arrived and departed on opposite sides, but the bees in the Delay group also had prior experience at the table through the 12 visits of the *initial* phase where they arrived and departed from the same side. Bees in the Delay group did not have previous experience with the new departure side.

Comparison of water test biases between Delay and No Delay groups show that although the overall bias was towards the arrival side in both groups, bees in the Delay group had significantly different biases that tend more towards zero (Figure 2.14a). Thus, while bees in the Delay group favor searching in the arrival side of the table, they have a greater tendency to also search on the departure side than do bees in the No Delay group. This suggests that information about the departure side during the *post-change* phase is being learned during the reorientation flights and used when searching for food later on. As discussed in the previous section, it seems unlikely that differences in biases arose because bees in the Delay condition encoded information about the departure side during the delay period.

As expected, we also found a significant difference between the Delay and Initial groups (Figure 2.14b), which shows that bees in the Delay group had a bias towards search on the arrival side, while bees in the Initial group predominantly biased their search towards the departure side. These results suggest that the information gained through reorientation flights does not exert as much influence on foraging patterns as the information gathered during initial learning flights, and that past experience at a location exerts a strong influence on subsequent foraging patterns.

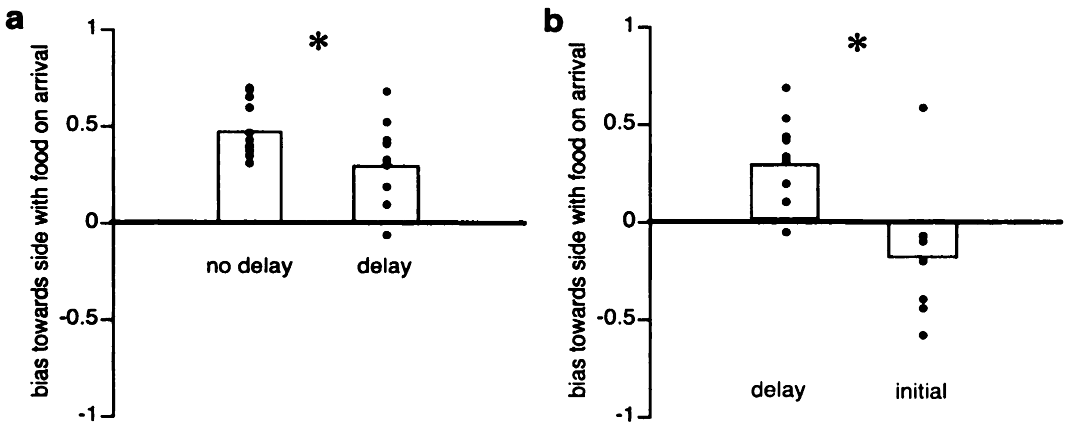


Figure 2.14. Comparison of biases towards side with food on arrival during water tests. Biases were calculated as the difference in the number of touches or landings made on the side where food was located on arrival during training and the number of stops made on the opposite side divided by the total. Positive biases indicate a bias towards the arrival side. Each data point represents a single bee, and boxes indicate means of each group. **a.** Water test biases of Delay and No Delay groups are significantly different (F= 5.43, p=0.03, n=24). Means of No Delay and Delay group are significantly different from zero (student's *t*-test: No Delay, $t = 5.49$, $p < 0.001$, n=12; Delay, $t= 11.10$, $p< 0.001$, n=12). **b.** In the Initial group, tests were performed on the sixth visit, while in the Delay group, tests were performed on the eighteen visit, six visits after the delay and change in food location. Biases of Initial and Delay groups are significantly different (F=13.80, $p < 0.01$, n = 19). Mean of the Initial group is not significantly different from zero (student's *t*-test: $t= -1.24$, p = 0.26, n=7).

Patterns of foraging across visits

As mentioned before, a bee's choice of where to first land is a good indicator of where she predicts the food is. Looking at this pattern across visits gives a sense of the shift in foraging patterns over time. In this analysis, scores were calculated in the same manner as they were for experiment 1 (Figure 2.10a). With scores from each visit, we averaged the first landing scores for visits 9-13 in the *initial* phase, which was a period in the phase in which bees were no longer performing learning flights. We then averaged the scores from visits 14-18 in the *post-change* phase and compared the two averages for each bee. We found in both the No Delay and Delay conditions, that bees were more likely to land on the departure side in the *initial* phase than in the *post-change* phase, when the departure side shifted to the opposite side of the table (Figure 2.15). Thus, it seems that reorientation flights do not cause bees to significantly shift their foraging patterns in terms of first landing choices. This result reinforces the conclusion that the information learned during reorientation flights does not replace the information the bees acquired during the *initial* training phase.

Patterns of foraging immediately following change and delay

As shown in previous studies (Lehrer and Collett 1994), and in experiment 1, bees shift their reliance on departure versus arrival information over time. Previously, we analyzed patterns during the water test or averages over several visits. Here, we examined foraging patterns on the visit immediately following reorientation flights: would bees rely on departure information newly gained through the reorientation flight? We compared biases in the trip immediately following the switch, which is also the trip

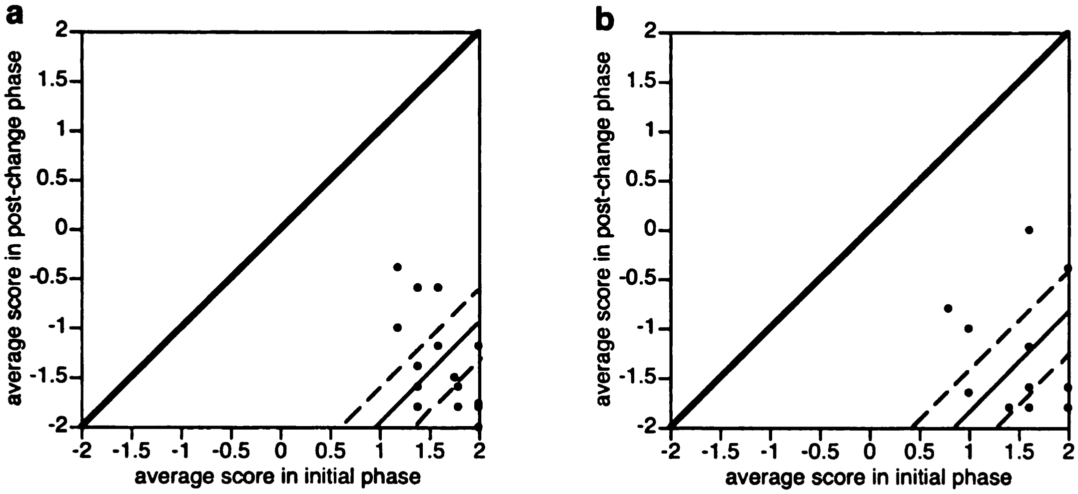


Figure 2.15. Comparison of average first landing scores in visits during *initial* phase and during the *post-change* phase. In the *initial* phase, bees arrived and departed from the same side; in the *post-change* phase, bees arrived on the same side as in the *initial* phase, but departed from the opposite side. Between the two phases, bees in the Delay group were subjected to an eight minute delay, which induced bees to perform reorientation flights (see Figure 2.12). Scores for first landings on each visit are calculated in the same way as in experiment 1 (Turns) (Figure 2.10a), and positive values are scored for landings on the departure side. An equal number of visits were used to calculate averages of the initial phase and the post-change phase for each bee. The exact number was determined by each bee, but was normally 5 visits. **a** and **b**.. In both the No Delay and Delay conditions, bees were more likely to land on the departure side in the initial phase than in the *post-delay* phase (No Delay: paired t-test: t= -17.14, $p < 0.001$, n = 16; Delay: paired t-test: t= -14.46, $p < 0.001$, n = 14). Each point represents a single bee. The *thick solid line* represents the point at which initial proportions and post-change proportions are equivalent. The *thin solid line* is displaced by an amount equal to the difference in the means of the two proportions, and the *dashed lines* indicate 95% confidence intervals.

after the delay for Delay group bees. Biases were calculated as previously described, and positive biases again indicate preferences for the arrival side. We found no significant differences in biases of the two groups, Delay and No Delay (Figure 2.15). In the No Delay group, 16/17 bees in the group had a bias of 1; of these 16, 12 found the food on their first landing, the others after two stops on the same side of the table. Similarly, in the Delay group, all 9 bees with a bias of 1 made one stop, landing directly on the feeder containing food. First, this shows that at this point, bees in both groups are very accurate in locating the feeder containing sucrose. These data also suggest that despite performing the reorientation flights, bees in the Delay group still predominantly choose to return first to the location of the food as learned during the *initial* phase. However, as seen in Figure 2.16, there is a tendency for bees in the Delay group to search on the departure side of the table compared to bees in the No Delay group. The difference is not significant, but any difference between the two groups could easily be masked by the dominance of the bees' preferences for first searching on the arrival side. If bees in the Delay group are more likely to forage on the departure side of the table, as these results (Figure 2.14) suggest, this implies that although reorientation flights do not strongly influence subsequent foraging patterns, bees do learn information about the departure side and will sometimes use it to guide their foraging choices.

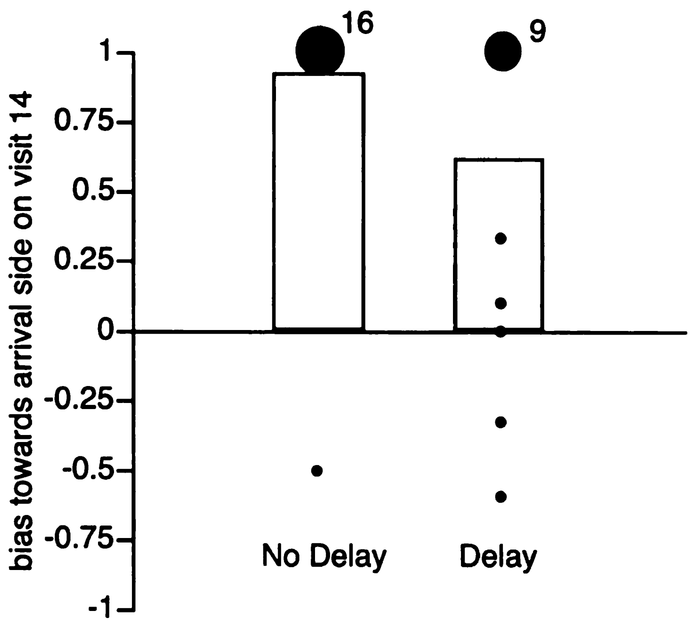


Figure 2.16. Comparison of biases on the 14th visit, one visit following the switch to a Turn condition. In both groups, food is still located on the same side as in the initial phase, but bees had departed from the opposite side on the thirteenth visit. Biases are calculated in the same manner as biases during the water test; the number of contacts on the arrival side minus the number of contacts on the departure side divided by the total. Positive biases here indicate a preference for the arrival side, and negative biases indicate preferences for the departure side. Boxes indicate means, and large black circles indicate values containing multiple data points; the numbers adjacent to the circles, which are scaled approximately to size, indicate the number of bees with biases of 1. We did not find a significant difference between the two groups (Wilcoxon: $Z= -1.93$, $p = 0.05$, $n = 14$ Delay, $n = 17$ No Delay).

DISCUSSION

In these experiments, we have found that memories formed during learning flights persist long after bees have switched to reliance on information learned upon arrival, and that this information can be used during subsequent searches for the food. We have also found that time invested in performing learning flights improves the likelihood that a bee will return directly to the location of the food source on her next visit, thereby increasing her foraging success and efficiency. Finally, we have shown how powerfully past experience impacts foraging choices. Although reorientation flights are thought to allow bees to update information about landmarks surrounding a food source, we found that bees still rely predominantly on information learned prior to a reorientation flight. However, our data also suggest that new information about landmarks is also encoded and used to relocate the food source. We discuss each experiment in turn.

Experiment 1- Effects of initial learning flights on foraging choices and patterns

Our results show that bees initially use information learned on departure to guide their return to the food, but over time, their preference for where they first land shifts away from the departure side, confirming Lehrer and Collett's (1994) finding. Our results expand on the 1994 study by Collett and Lehrer by showing that when bees learn differing cues on arrival and departure over a period of several visits, they retain and use both memories. Although their preferences for which cue they rely on shifts over time, the learning of retinal size cues does not simply replace memories of absolute distance cues learned on departure; the repeated learning of arrival information in the period following the ' TBL phase' (or the ' initial phase' in Wei et al. 2002) does not extinguish the information learned upon departure (Lehrer 1993). Rather, our results show that bees have spatial memories of both the arrival and departure side, and that they search both options when memories conflict.

This result seems to contradict one of Lehrer and Collett's (1994) results. In their study, bees were trained to a feeder and were allowed to view a cylinder, placed at a specific distance from the feeder, on both arrival and departure. Following training, bees were given a choice of two locations; in one location, the absolute distance between feeder and landmark was correct, but the retinal image size of the cylinder was not, and in the other location, the opposite was true. They found that following 22 rewarded visits, bees had a preference for the retinal image size, a cue that is learned on arrival rather than departure (choice frequency for retinal image size was 60%, while for distance, 40%). In our study, following 20 rewarded visits, we found that bees do not have a preference for either side, and thus no distinct bias for using retinal image size or absolute distance cues. However, this difference can likely be explained by the fact that we used a highly conservative measure of preference in our analyses. In the Turn condition of our study, we used ten individual bees, each tested once, whereas Lehrer and Collett tested four individual bees, each tested four times in a row. Also, in calculating preferences, we chose to use a proportion for each individual bee and assigned each bee a single value (n = 10), instead of pooling the total number of choices made by all bees in all tests. Thus, our analyses had lower power than those used in the Lehrer and Collett study. These differences in statistical methods may explain why we did not detect a preference for using information learned upon arrival following several rewarded visits. Additionally, slight differences in the number of total rewarded visits made by bees in each of the two studies (20 versus 22), or possible differences in distances between the food source and the hive may have also contributed to this difference in results. However, these factors seem unlikely to be significant. Thus, we do not consider that the seemingly contradictory results of these two studies are problematic.

Even if there is a preference for the arrival side that was not detected in this study because of low sample sizes, we observed bees visiting the departure side panel at a greater rate than the north and south panels. This suggests that visual memories learned from the departure side have not completely faded, and that they are still accessed and used. How and why bees access these memories and decide to use them, however, are still open questions. If the use of 2-D retinal size images is easier and sufficient to guide a bee to her goal, one might expect that the 3-D information about absolute distance might decay after the TBL phase, or initial phase since it is no longer necessary (Lehrer and Collett 1994). But if the information about absolute distances has been encoded into long term memory, which is established over multiple visits and can be formed in as little as two minutes (Menzel, 1999), then it is not surprising that bees are still able to utilize this information after twenty visits given that long term memories can persist for days, even months (Menzel, 1987).

From a foraging standpoint, the use of absolute distance information after the initial phase may be part of a strategy where bees shift to using other memories to guide their searches after the use of retinal size images proves to be unsuccessful. Such a strategy might ensure that bees have a sort of back-up system to resort to when the main information source fails. This is not unreasonable since absolute distance cues are more precise and less subject to the ambiguities of retinal size image. Thus, by using both sets of cues, bees may effectively increase their chances of finding the food.

While using departure information might be a good strategy, a simpler alternative explanation exists for why bees in the Turn condition visit the departure side during the water tests. Rather than being specifically guided by memories learned upon departure, it is also possible that in the course of searching around the table, bees recognize the departure location. This recognition then prompts them to investigate the area more closely and make contact with the feeders on the departure side. In our experiments, this is quite possible, as the arrival and departure locations are separated only by about a meter. Also, a bee's typical search pattern would allow for the possibility of coming into the vicinity of the departure location by chance; we have observed that bees begin by searching very close to the location where the food was in previous visits, and over time, their search widens to other nearby areas, but often returning to the original location. Future studies investigating this alternative explanation should be designed such that arrival and departure information lead bees to two different locations that are separated by a greater distance and made to be more distinct. This way, bees would not be able to easily stumble upon a familiar view of the departure information. Such an experiment, although logistically difficult, would help distinguish whether the use of absolute distance cues, at a time period when arrival cues are normally used, is guided by chance recognition or by directed search.

In the Turn condition of experiment 1, we verified that longer learning flights lead to better foraging accuracy; across the first few visits to a new location, we found a positive correlation between the summed duration of learning flights and the likelihood that a bee would first land on the departure panel. Although this correlation is not a strong one, this is not unexpected given the large degree of individual variation in foraging behavior (Thomson and Chittka, 2001). Thus, these results support our premise that by investing more energy in performing longer learning flights, bees are rewarded by gaining information that help them to be more accurate in locating a goal and thus be more efficient and successful foragers. However, further studies will be needed to understand the precise mechanisms by which extended learning flights allow bees to pinpoint a goal with greater accuracy.

Experiment 2- Effects of reorientation flights on foraging choices and patterns

We found that when reorientation flights were induced by delays in finding the food, the new information acquired by bees during reorientation flights did not replace the old memories and were not even used preferentially by bees in subsequent visits. Rather, bees continued to rely more heavily on previously learned memories to guide them to their goal. However, reorientation flights did allow bees to gain new information, which they used during the water test when their original memories failed. Furthermore, we found evidence that the duration of the reorientation flights is correlated with the likelihood of searching the departure side during the water test. Although bees still predominantly search the arrival side, bees that performed longer learning flights presumably learned more precise information about the departure side and were better able to recognize and search the departure side as a result.

The dominance of the arrival side during bees' searches is expected considering that they experienced rewards only when landing on the arrival side. However, in the *initial* condition, where bees fed and departed on opposite sides of the table for only 6 visits, bees searched predominantly on the departure side. Our data show that past experience exerts a greater influence on subsequent foraging choices than new information gained through reorientation flights, but the reason for this is still unclear. If reorientation flights serve to update landmark information, it is unclear why a bee would rely more on memories formed prior to the reorientation flight. One possibility, as mentioned in the introduction, is that bees continue to rely on retinal size cues because they may be easier to use for guidance. Only when these cues prove to be unreliable, do bees switch to using absolute distance cues learned through reorientation flight.

That information learned during reorientation flights does not replace the information the bees acquired during the *initial* training phase is not surprising in light of previous work by Robinson and Dyer (Robinson and Dyer, 1993). They showed that when bees swarm and relocate their hive to a new location, they reorient to the new site quickly, but are still able to remember the location of the old nest. Their results, along with ours, suggest that reorientation flights modify rather than replace existing spatial information, and also suggest that for bees, the use of spatial information is flexible. In both cases, the retention of old memories may provide an adaptive advantage should bees need to return to their old nest or foraging site because the new location proves to be unsuitable or unrewarding.

Although our work has demonstrated how information acquired through learning flights is utilized to improve a bee's foraging success, we still do not understand how bees decide how much effort to invest in learning and the timing with which this decision occurs. Our previous experiments have suggested that this decision is made before a bee departs because we found that duration is modulated by experiences that occur prior to take-off; we found that increasing sucrose concentration and longer delays were both correlated with longer reorientation flights (Wei et al. 2002). Results from experiment 2, however, suggest that reorientation flights may also be modulated during the flight itself. Our analysis comparing Delay and No Delay groups for reorientation flight durations in the trip immediately following the switch found that bees in the No Delay group still performed reorientation flights. Although these bees did not experience a delay, they still experienced a shift in location such that they were departing from a new side of the table. Thus, although their reorientation flights were not as long as those of bees in the Delay group, the fact that they performed even a relatively short reorientation flight suggests that the bees noticed some change in the visual scene.

Since the change took place during feeding, one could argue that the bee noticed the change in location as she was being displaced. This, however, is not a likely scenario given that previous work has established that bees do not respond to visual changes occurring while they are feeding (Menzel and Erber, 1978). Schone (1996) found that the critical factor in triggering learning flights in bees displaced from a familiar to a new site was a view of the scene immediately prior to release; bees that had a clear view of the new scene prior to departure did not perform reorientation flights, while bees that were denied a view did (Schone, 1996). In our study, bees may have detected their displacement in the moment before take-off. However, unlike bees in the Schone study, which were held in a container for 1-3 minutes while viewing the scene, bees in our study departed almost immediately after feeding. Thus, it is equally or more likely that our bees detected some change in the scene during departure and reoriented *en route*.

Future Directions

Given that reorientation flights are likely to be triggered by different factors, future studies should consider how information might be differentially coded or utilized depending upon the ecological situation necessitating the performance of reorientation flights. Here, we have investigated reorientation flights as modulated by uncertainty. However, we know from our previous studies (Wei et al. 2002) that reorientation flights can also be modulated in response to the value of the food reward. In this case, the spatial information guiding a bee to a location has not failed, but reorientation flights are still performed in response to an increase in sucrose concentration. To better understand why such reorientation flights are performed and how bees might utilize new information acquired through such flight, we will need to consider the differences between uncertainty-modulated and reward-modulated reorientation flights. In addition, by considering the ecological context in which these flights occur, we will gain a much clearer understanding of learning flights and their role in the foraging success of honeybees.

CHAPTER 3

THE ROLE OF LEARNING FLIGHTS IN RESPONSES OF HONEYBEES TO CHANGES IN SUGAR QUALITY AND DISTRIBUTION.

INTRODUCTION

In a competitive landscape where resources are unevenly distributed, foraging success depends upon the ability to find and exploit the most profitable areas. Nectar-feeding insects, especially bees, have provided an important model for empirical studies of such ideas in the optimal foraging literature. In bees, relatively simple decision rules guide them to concentrate searches for nectar in good areas and to move through poor ones (Kipp et al., 1989; Pyke, 1979; Schmid-Hempel, 1984; Schmid-Hempel, 1986). However, in a dynamic and patchy environment, the decision of where a bee begins her foraging bout should also impact her success. If bees are able to return to one of the more rewarding patches within a foraging area first, they would likely spend less time and energy collecting nectar than if they arrived at random locations within the area.

Previous studies have suggested that bees need to perform learning flights (Lehrer 1991,1993; Wei et al. 2002) to relocate a newly discovered, rich foraging area. During these specialized learning flights, a bee intensively examines the location and acquires information about the absolute distances between landmarks and the food source, which is then used to guide her return to the food source (Collett and Zeil, 1996; Lehrer, 1993b; Wei et al., 2002; Zeil et al., 1996). This landmark information, however, is costly to obtain. One cost is the energy expended in performing the learning flights. Another is the assumed cost of memory formation and storage. The decisions bees make about whether or not to perform learning flights should be determined by the benefits provided by the information learned relative to the costs of obtaining such information.

In most previous studies of learning flights, honeybees fed at artificial feeders, where they were able to fill their crops with highly concentrated sucrose solution at a single point source (Lehrer, 1991; Lehrer, 1993b; Schone, 1996; Wei et al., 2002). The payoffs for relocating these feeders were quite high, and not surprisingly, bees in our studies using artificial point source feeders almost always performed learning flights (Wei et al. 2002, Chapter 2). In natural conditions, however, bees rarely, if ever, fill their crops at a single flower blossom (Kearns and Inouye 1993). Rather, they collect nectar by visiting a series of blossoms and are often required to forage in several patches before completing a foraging bout (Schmid-Hempel, 1986; Waddington and Holden, 1979).

This raises the question of the function of learning flights in foraging landscapes where resources are widely distributed. Perhaps in areas where nectar is evenly distributed across a wide area, the navigational strategies that bring a bee into the general vicinity, such as use of celestial cues and large scale landmarks, are sufficient and learning flights are not necessary. In patchily distributed areas, however, learning flights may benefit bees by allowing them to shift their foraging patterns between visits in response to discoveries of discrete areas of richly rewarding nectar.

However, few studies have studied patterns of foraging made between visits or learning flights in natural contexts. Some early studies anecdotally noted whether bees entered and exited patches from a particular location and in some cases, noted the occurrence of learning flights (Ribbands, 1949). Thus, one of our main goals in this study was to consider the role of learning flights in a context where nectar is more naturally distributed and specifically to explore the role they play in shifting foraging patterns in response to resource changes.

A second goal of this study was to follow up one of our earlier findings that reorientation flights (recurrent learning flights at an already familiar location) can be modulated by sucrose concentration: when moved to a new, better food source[3], bees performed longer reorientation flights than when bees experienced little increase in the quality of the food (Wei et al. 2002). This finding was of particular interest because most earlier explanations for reorientation flights claimed that they function to update landmark information and are triggered by difficulty in locating a goal, which signals the need for an update (Iersel and Assem, 1964; Wehner, 1981; Zeil, 1993a). This claim was corroborated by our finding that reorientation flights are longer when bees were forced to search for the expected food reward for a longer period of time (Wei et al. 2002). Because sucrose concentration is a key factor in determining a bee's motivation to collect nectar from a particular source (von Frisch, 1967), reorientation flights, in addition to being modulated by uncertainty in locating food, are also modulated by motivational variables related to the quality of the food source. In our earlier study (Wei et al. 2002), we suggested that the reasons why reorientation flights are modulated by sucrose concentration can be understood best in an ecological context. When bees experience a substantial increase in sucrose concentration between visits, we suggested that the corresponding event is likely to be the landing on a different flower or in a different, more profitable patch, since nectar concentrations of most flowers stay relatively constant

[3] It is unclear whether a shift in location is necessary to trigger reorientation flights. In our study, after bees landed in familiar location, they were moved while feeding to an identical test apparatus only a few meters away. The visual scenes in each location were very similar.

despite fluctuations over the day due to evaporation (Barth, 1985). By performing learning flights in response to such discoveries, bees may be better able to exploit richly rewarding patches.

In this study, we combine observations in a field setting where bees freely forage on figwort flowers, *Scrophularia nodosa*, with more controlled, laboratory experiments using artificial feeders to test these ideas. Our goals were: 1) to characterize the occurrence of learning flights in response to the discovery of new foraging opportunities within a natural foraging patch, 2) to determine which attributes of floral nectar rewards play a role in inducing learning flights observed in a natural foraging context, and 3) to determine whether learning flights modulated by changes in nectar quantity and richness lead to changes in spatial patterns of foraging across visits.

METHODS AND MATERIALS

EXPERIMENT 1 (Figwort plot)

General methods

Study Site

An 8x10 m plot of the common figwort, *Scrophularia nodosa* L. (Scrophulariaceae) was kept in a field on the campus of Michigan State University, East Lansing, MI. The surrounding areas consisted of various agricultural fields. Aside from a similarly sized, nearby plot of *Scrophularia marilandica*, there were no other attractive nectar sources in the immediate vicinity. The flower blossoms of *S. nodosa* hold a relatively large volume of nectar and conveniently allowed for nectar collection at the field site. In the 2002 field season, the plot was composed primarily of *S. nodosa*, and was teeming with foragers including honeybees, bumblebees, wasps, and ants. In 2003, weed species were more dominant, so that the *S. nodosa* population was reduced to about 20% of its original density. Foragers in that year were much less numerous, especially with regard to honeybees. The peak figwort flowering season ran from approximately mid-June until the first week of July.

Bees

Three colonies of *Apis mellifera* were kept in Langstroth hives about 20 m from the *S. nodosa* plot, and were separated from the plot by a row of pine trees. Although honeybees at the figwort patch may have come from other hives, our observations indicate that most of the bees we observed were likely to have come from our hives.

Experimental methods

Observations of learning flights

To test whether learning flights might occur in response to the discovery of better foraging opportunities within a natural foraging area, we created a rich foraging patch within the *S. nodosa* plot. We set up two experimental conditions, covered and uncovered. The covered condition was intended to increase the nectar quality and /or quantity in comparison to the uncovered condition by preventing depletion of nectar by insects; the slower rate of depletion might increase the sugar concentration of nectar due to evaporation (Kearns and Inouye, 1993). For each observation session, two cages were erected in the plot. The cages were created from 3.5 cm diameter conduit tubing and 2.25 cm diameter wooden dowels connected by plumbing joints, and measured 1.2 m. x 1.2 m x 1.5 m. One cage was covered with white agricultural mesh, which prevented most foragers from visiting the plants within the cage. The other cage remained uncovered and accessible to foragers. Cages were set up at least 5-6 hours prior to each observation session, and the assignment of the covered cage was switched between observation session. In this way, the locations of the uncovered and covered patches varied between the two cage sites. This was done to control for any site biases because nectar production varies within and across plants (Kearns and Inouye, 1993). During each observation session, the mesh and cages were removed, except for the base of the cages, which formed 1.2 m x 1.5 m rectangular grids defining the boundaries of the patches.

At each patch, an observer recorded in 5 minute intervals, for a 30 minute period, the number of honeybees entering the patch and the behavior of each bee leaving the patch. The departure of each bee was scored as either 1) moving to another patch, 2) departing *S. nodosa* plot without a learning flight or 3) departing *S. nodosa* plot with a learning flight. The differences between these three behaviors were easily distinguished. Bees that moved to another patch within the plot typically kept low and moved short distances to adjacent patches, while bees that departed to the hive, or possibly another plot, flew high above the patch and at much greater speeds. Bees in the latter group either flew straight away or performed arcing, circling flight resembling learning flights. In categorizing learning flights, we described any flight that had any circling or arcing component as a learning flight. Due to the limitations of tracking multiple bees the intensive, face-forward phase of learning flights, described as the turn back and look (Lehrer 1991, 1993), was often obstructed from the observers view by other figwort plants. Although more loosely structured circling flights always follow turn back and look behavior, circling flights are not always preceded by the distinctive pivoting of turn back and look flights (von Frisch 1967, Schone 1996). In previous studies (Wei et al. 2002, Chapter 2), we have also included the circling portion of the learning flight in records of the durations because there is not a clear demarcation in the transition from pivoting to circling during flight.

Measurement of nectar quality and quantity

To determine whether the nectar quality and/or quantity differed in the covered and uncovered patches, we collected nectar samples from plants in each condition. Although the behavioral observations were made in 2002, we report data from samples collected during the 2003 season. Although there were likely some differences in the figwort plot between years, the most notable difference was the fewer number of foragers present in 2003. If this difference between years would bias the difference between the resources available in covered and uncovered patches, it would likely lessen the difference and thus underestimate the difference experienced by bees observed in 2002.

In 2003, a cage was set up around a patch of figwort that appeared similar in terms of plant density and height to those used in the experiments in 2002. The cage was alternately covered and uncovered on different days. This was done to minimize differences between plants, as nectar production varies within and across plants (Kearns and Inouye, 1993). As with the previous year, we left the cages covered for the same time period of at least 5 hours before collecting samples. Because nectar production rates vary over the course of the day (Kearns and Inouye, 1993), we collected samples at the same time of day, between 4 and 5pm. Twelve plants, of varying sizes and locations within the caged patch, were labeled with tape at their bases, and from those plants, we recorded the number of blossoms on each plant and collected nectar samples from every blossom that appeared to contain nectar. Visual inspection of the blossoms was sufficient to determine whether nectar volumes were large enough to be collected. Samples were collected in 5 µl or 10 µl microcapillary tubes and immediately sealed with Crit-O-Seal (Kearns and Inouye, 1993). Following collection of the nectar samples, the tubes were kept in a refrigerator until the samples were read at a later date. In all, we collected six sets of samples from these plants; three replicates each in the covered and uncovered conditions. The conditions were replicated in alternating sequence and spread out in time to account for variations due to weather, age of plants, or interspecific plant competition.

After measuring the volume of nectar in each sample tube, nectar concentrations were measured with a hand held refractometer (Atago N1). All measurements were made at room temperature. To increase the range of concentrations that could be read with our refractometer, nectar samples were mixed with an equivalent volume of distilled water. To reduce the influence of evaporation, samples were read as quickly as possible after the seals were broken on the tubes, and the mixing of the water and nectar was done swiftly and consistently.

EXPERIMENTS 2 (Concentration) and 3 (Volume and Concentration)

General methods

Bees

A colony of *Apis mellifera* was kept in an observation hive inside a large flight cage on the campus of Michigan State University, East Lansing, MI. The enclosed space measured 19.5 m (l) x 5.6 m (w) x 2.3 m (h) and was covered with a mesh fabric (designed to block 30% of incident light.) The honeybees' only source of food was sucrose solution provided in feeders, and pollen introduced directly into the hive. Preventing bees from flying to natural food sources guaranteed high levels of motivation for foraging in the experiments.

At a distance of approximately 7 m from the hive, we set up a stock feeder containing unscented sucrose solution at a concentration of 0.25 M. Unscented solutions were used in order to reduce the effect of odors on recruitment of bees to the test apparatus. We filled the feeder before experiments to establish a population of bees available for use in the experiments.

Scent-gland plugging

To reduce recruitment of other foraging honeybees to the feeders, we sealed the Nasanov glands, which produce recruitment pheromones, of all bees used in the experiments (Towne and Gould, 1988). Methods are described in Wei et al. (2002).

A second reason for plugging the scent glands was to reduce the influence of recruitment pheromones on the behavior of our test bees. Specifically, we wanted to ensure that the effects we observed on learning flights in our experiments were not attributable to odor cues left by subjects themselves. As a further precaution to reduce the influence of odors, we also replaced feeders that bees had fed on with fresh ones to eliminate odor marks bees may have deposited on the feeder. Additionally, we wiped each panel of the test apparatus where the feeders rested with a damp paper towel following each visit by a bee, and we rotated the position of the panels between visits, eliminating the ability of any odor cues to predict the location of the food. During each test, the panels used during the training phases were replaced with fresh panels.

Apparatus

In all experiments, bees were recruited to an octagonal shaped rotating table located 13.5 m from the hive. The table, with a 122 cm diameter, was mounted onto a platform, which was raised off the ground (Figure 3.1). The table was divided into 8 sectors, and a triangular foam panel, covered with matte textured white paper, was placed in each sector. A spare set of panels was kept out of sight for use during testing. Landmarks consisted of a single black cylinder, located in the center of the 8 panels, and two additional cylinders were placed on edges of the video camera stand, which stood behind the table. The landmarks were a blue and a yellow cylinder, whose locations were alternated to prevent any sort of bias for one color or the other. Each cylinder landmark was 31.6 cm tall and 9 cm in diameter. From the feeders, the center landmark subtended an angle of 29.9 degrees in height and 9.35 degrees in width. The camera stand supported a 2 m pole from which a video camcorder[4] was mounted and centered over the table.

On each panel, we placed a feeder centered approximately 5 cm from the edge of each panel base. The feeders were red candle holders (0.7 cm height, 0.8 cm diameter) that held 140 µl of liquid. Feeders filled to full volume contained ample sucrose solution to allow a bee to fill her crop, which has a maximum capacity of around 50 µl (Nunez, 1970), at a single feeder.

[4] Footage of arrivals, departures and search patterns during water tests were recorded; however, the data reported here were based on real time observations. We occasionally used video archives to reconfirm the accuracy of our field notes.

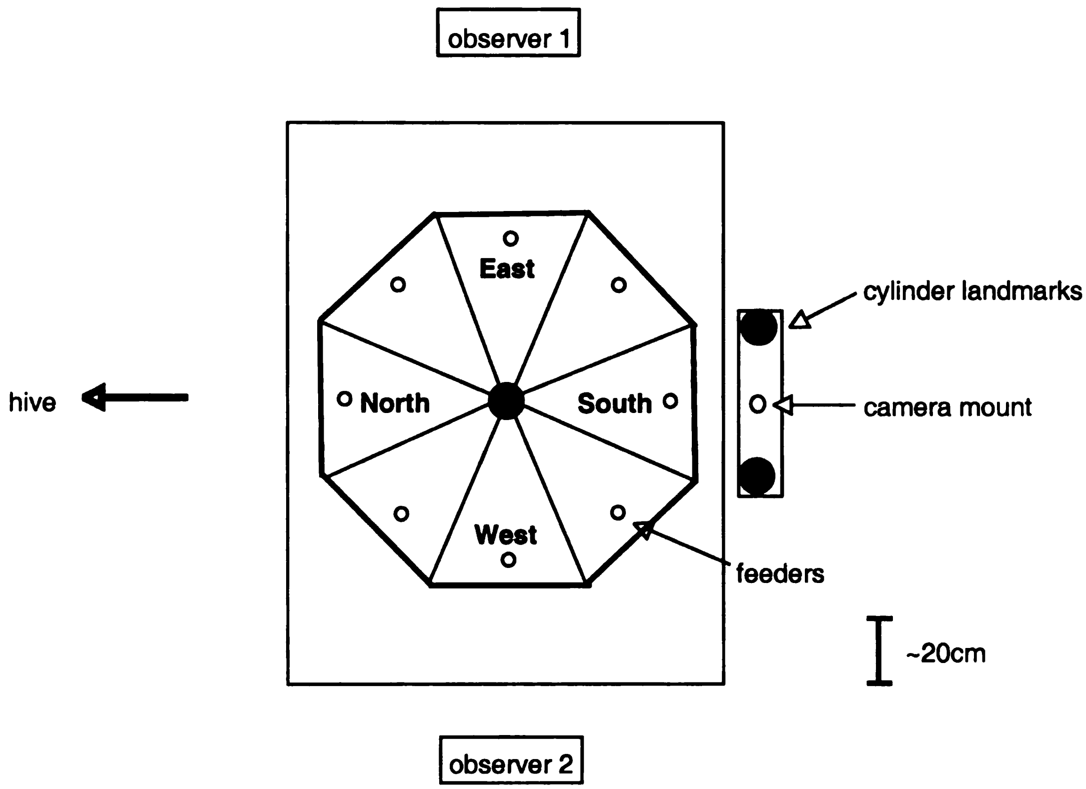


Figure 3.1. Diagram of experimental apparatus (drawn approximately to scale). Small circles indicate where feeders are located in each panel. Large, black circles represent cylinder landmarks. "East, West, North, and South" are labels for specific panel locations based on their compass orientation.

Recruitment to experimental apparatus

Honeybees are known to transfer to a new food source if the quality is higher than that of the food on which they were previously feeding (Lehrer, 1993b; Wei et al., 2002). In all of our experiments, individual bees feeding on low concentration sucrose (0.25 M) at the stock feeder were transferred to the test apparatus by using sucrose solutions of higher concentrations in the test feeders. We accomplished this by first introducing the concentrated solution to the bee via a pipette while she was feeding at the stock feeder. When the bee began feeding, she was placed onto the test feeder and carried by hand to the test apparatus. Some bees returned immediately to the new food source on subsequent trips, while others needed the recruitment process to be repeated from 1-5 times before returning to the test apparatus and finding the food without assistance.

Measurement of learning flights

Following each bee's visit to the experimental apparatus, the duration of learning flights was recorded using stopwatches. Two observers started their stopwatches at the moment the bee lifted off from the feeder and stopped timing when she either stopped flying in obvious arcing, circling patterns, or when she flew approximately one meter beyond the edge of the table. Because there was not a precise way to measure the exact duration of the learning flights, our measures include some variation. Although our measurements included a significant amount of circling in the vicinity of the food (see also Lehrer, 1993b), it also includes, more importantly, the intensive, face forward, examination time of the Turn Back and Look. We included this circling behavior because bees often turned away from the landmarks soon after departure, but well before they left the vicinity of the landmarks. This measure was validated using video in a previous study (Wei et al., 2002). When a bee departed from a feeder without a learning flight, the duration was recorded as "0". In these instances, the bee departed very quickly and headed directly towards the hive without any circling. Averages of the two hand recorded times were used in our analyses. The recorded times of each observer were consistently similar; in a sample of 373 recorded learning flight durations from the turn experiment, the mean difference between the two times was 0.09 s with a standard deviation of 0.47 s and a range of 0-3.64 s. Given that the sizes of the differences in learning flight durations were in the order of seconds (see Results), these measurement differences are relatively small.

These experiments required us to assess whether the duration of learning flights changed following an experimental manipulation. To do this, we established a baseline by averaging the departing flight durations of the three visits prior to the manipulation. At the point at which the manipulations were performed, these durations had stabilized such that learning flights did not show patterns of increasing or decreasing duration. Twelve visits were generally sufficient to reach this stable point. In most cases, the baseline was not zero. Often, the bees would perform one or two loops to orient themselves, but this behavior was distinctly different from the learning flights performed in initial visits, where several loops were made. The durations of reorientation flights were defined as the difference between the baseline value and the recorded flight duration following the experimental manipulation.

Measurement of spatial foraging patterns

In order to quantify the choices bees were making in terms of their spatial foraging patterns, we also recorded the following information about the foraging patterns of the bees on arrival: 1) the panel position of the first feeder where each bee landed and extended her proboscis onto the feeder (first landing), and 2) the sequence and distribution of contacts on the feeders (used to calculate search biases). The first landing data were presumed to reflect a bee's initial prediction about the location of food, and is a good indicator of the bee's accuracy in foraging. However, a bee's first prediction was sometimes incorrect, and her subsequent search patterns reflect additional predictions about where food might be. Thus, search biases were also measured as an indication of a bee's foraging accuracy. Finally, after the last rewarded visit was made (varies by experiment, see below), a water test was conducted. During this test, bees returned to find all feeders filled with water. As with each arrival, we kept track of each panel position where each bee landed and made contact with the feeder as she searched for the food. The water test ended when bees gave up searching at the table and returned either to the stock feeder or the hive. Without rewarding feeders to end a bee's search, the water test enabled us to observe the full extent of a bee's foraging decisions, and represents the most complete measure of foraging accuracy. The water test could be performed only once for each bee, and we were therefore limited to comparisons at a single time point.

Calculation of search biases

To measure search bias on a trip -by -trip basis, we calculated the difference in the number of stops made on either side of the table divided by the total number of stops. Further details are given in the results.

To measure search bias in the water tests, we calculated the difference in the number of stops made on either side of the table (A-B) divided by the total number of stops (A+B). For this calculation, we excluded the north and south panels. "A" equals the side where food was located upon arrival during the *post-change*, and "B" equals the opposite side.

During each water test, the total number of contacts varied because we tracked each bee until she left the table. In order to account for the effect of the total number of contacts on the variance of the bias calculation, we included only those bees that made a total number of 20 or more contacts. This conservative approach did not change the significance of our results. The criterion of 20 contacts was determined by choosing a sample size such that the variance of the bias was less than or equal to 5% (alpha = 0.05). In calculating these criteria, we assumed that the data followed a binomial distribution, where the probability of choosing either side is 50%.

We used two-tailed tests in all of our statistical analyses.

Experimental Methods

EXPERIMENT 2 (concentration)

In an earlier study, we found that sucrose concentration modulates the duration of reorientation flights (Wei et al., 2002) such that longer flights are observed when bees experience a substantial increase in sucrose concentration. We suggested that the ecological context for this finding is that sudden, large increases in the value of the food correspond to the discovery of new and superior food sources, which would be well worth remembering. In this experiment, we simulated a situation where bees foraging in an area with evenly distributed, low quality food suddenly encountered a localized, highly rewarding food source. Our goal was to quantify the bees' behavioral responses in terms of reorientation flights and changes in spatial foraging pattern following such a change in the distribution and quality of food in a relatively small area.

Individual bees were first recruited from the stock feeder to either the north or south panel of the table (Figure 3.1) with 0.5 M sucrose solution. At the table during the *initial* phase (first 12 visits) (Figure 3.2), every other panel contained a feeder filled to full volume (140 μl) with 0.5 M sucrose solution; the rest of the feeders were filled to full volume with water. This way, rewarding and unrewarding feeders were evenly distributed across the table. Unrewarding feeders were necessary to ensure that bees did not develop a bias towards the first feeder encountered. Between visits, we rotated the positions of the feeders by one panel so that the location of rewarding feeders varied (Figure 3.3a). After 12 visits, we changed the distribution and quality of the food at the table; following the switch, a single feeder, located either on the east or west panel (Figure 3.3a), contained a full volume of 2.25 M sucrose concentration, and the remaining feeders were filled with water. The position of the full volume and concentration feeder remained in the same location for the remaining 12 visits of the *post-change* phase, after which a water test was conducted. The control group of bees

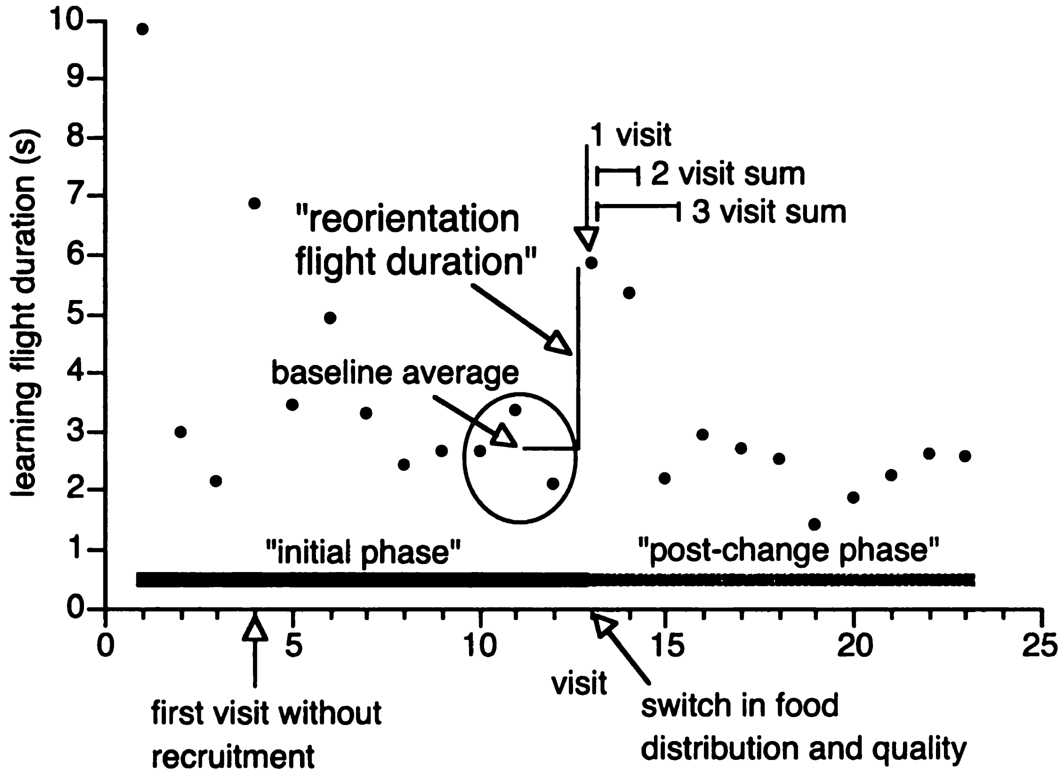


Figure 3.2. Change in learning flight duration over a series of visits by a single bee in experiment 2 (concentration). The *initial* phase, marked by the *black bar*, is the period during which learning flights are performed at a new location. The *post-change* phase, marked by the *gray bar*, is the period following the change in food quality and distribution. In experiment 2, the distribution and concentration of the food is changed from 0.5 M sucrose solution in every other panel (rotated between visits) to 2.25 M sucrose solution in a single panel on the east or west side. In experiment 3 (volume and concentration), the initial phase contains 8 µl of 1.0 M sucrose solution in all eight panels, and this is changed to a single feeder of full volume (140 µl) and concentration (2.25 M) on either the east or west side. "Reorientation flight duration" is calculated as the difference between the learning flight duration following the switch and the baseline average of the three previous visits. Sums of reorientation flight durations are made for one, two or three visits immediately following the switch (here, trip 13, 14 and 15), and sums of biases and first landings are made for the corresponding return visits (here, 14, 15, and 16).

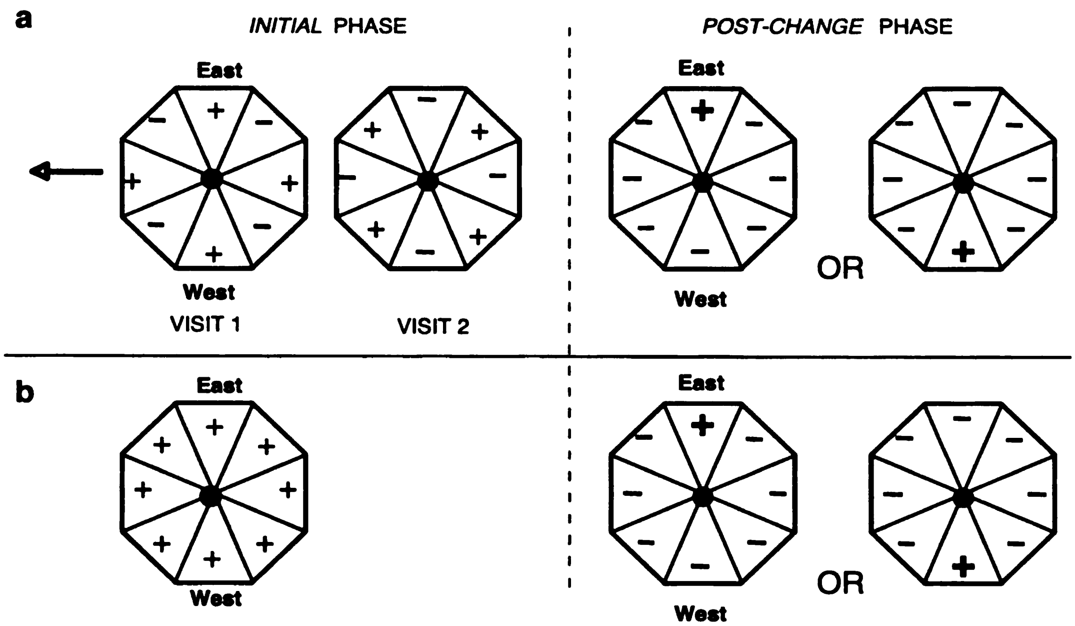


Figure 3.3. Diagram of experimental conditions. Octagons represent the experimental apparatus (Figure 3.1), which is divided into eight panels; "+" symbols in each panel show where food is located (bold font, larger "+" in *post-change* phase indicate higher sucrose concentrations, 2.25 M, and smaller "+" symbols in *initial* phase represent lower concentration sucrose solution, 0.5 M or 1.0 M), and "-" symbols represent feeders filled with water. **a**. *Initial* and *post-change* phases of experiment 2 (concentration); during the *initial* phase, positions of the feeders is rotated by one panel after every visit. Thus, feeders are in the same locations as visit 1 on all odd numbered visits, and visit 2 on all even numbered visits. In the *post-change* phase, food is located either on the east side or the west side; the location remains the same for every visit and for arrival and departure **b**. *Initial* and *post-change* phases of experiment 3 (volume and concentration); distribution of feeders is uniform through all visits in the *initial* condition. *Post-change* phase is the same as experiment 2.

experienced only the *initial* phase, where food was evenly distributed. We expected these bees to lack preference for either side of the table during the water tests, and thus provide a comparison for bees that had experienced the *post-change* phase.

EXPERIMENT 3 (Volume and concentration)

In experiment 2, we used feeders filled to full volume, which allowed bees to fill their crop at a single location. However, this is rarely the case in natural conditions because single flowers generally do not provide enough nectar to fill the crop, and we suspected that the manipulations performed in experiment 1 might have, in addition to influencing concentration, influenced nectar volumes. Changes in volume affect the flow rate, defined as the volume of nectar gathered over a period of time, experienced by a bee in a foraging bout; if individual blossoms contain greater volumes of nectar, bees can spend less time foraging and flow rate will increase. Since bees use flow rate, in addition to sucrose concentration, in assessing profitability, we wanted to assess a bees' responses to changes no only in distribution and quality of the food, but quantity as well.

As in experiment 2, bees were first recruited to either the north or south panels (Figure 3.1). Initial recruiting visits required the use of a fully filled feeder to ensure that the bee would not finish feeding and depart during transfer from the stock feeder to the table. To simulate a situation where food is evenly distributed and bees have to make multiple stops at feeders to fill their crops, all feeders at the table contained 8 μl of 1.0 M sucrose solution during the *initial* phase (12 visits after bee first returns to table without recruitment). For two bees, the initial conditions were slightly different: they experienced 5 μl of 0.5 M sucrose solution[5]. We still included the two 5 μl bees in our analyses because we felt that the slight change did not influence the original intent of the manipulation; they also experienced small volumes of lower concentration food evenly distributed throughout the table. Between visits, the feeders were replaced with a new set and refilled using a micropipetter. The table was also rotated at random so that bees would not rely on any odor cues left behind or cues particular to the table and panels. Following the *initial* phase, we changed the food distribution, quality, and quantity; feeders were switched so that only a single feeder contained 140 μl of highly rewarding 2.25 M sucrose concentration and the rest were filled with water. The position of the 2.25 M feeder was located either on the east or west panel (Figure 3.3b). Again, this position remained the same for the remaining 12 visits of the *post-change* phase, after which a water test was conducted. As in experiment 2, we had a control group of bees that experienced only the *initial* phase.

RESULTS

Experiment 1 (Figwort plot)

Effect of covering figwort patch

By covering cages with mesh, we intended to manipulate the profitability of the nectar available inside the cage. With the exception of a few ants, the mesh successfully blocked insects from foraging on the figwort blossoms inside the cage. Previous studies have shown that the exclusion of pollinators affects nectar production (Armstrong 1991

[5] These bees were the first two bees we trained, and we discovered that with 5 μl, the bees had not filled their crop after emptying all feeders, forcing us to refill feeders as the bees emptied them. This proved to be logistically challenging, so we adjusted the methods slightly for the remaining bees by increasing the volume in each feeder to 8 μl. We also increased the concentration slightly to 1.0 M for a greater contrast from the stock feeder.

as cited in Kearns and Inouye, 1993). Thus, we expected that we might find 1) an increase in the volume of nectar within individual blossoms in the absence of depletion by foragers and/ or 2) an increase in the concentration of nectar possibly due to the effects of evaporation on standing nectar. Although nectar concentrations of most flowers have been shown to remain relatively constant over the day (Barth, 1985), the exclusion of pollinators from the figworts in this experiment may allow for the normal daily fluctuations of nectar concentration due to evaporation to be exaggerated.

Comparing samples collected in each patch (see Methods), we found no difference in nectar volumes (Figure 3.4), but significantly higher concentrations in the covered patch (Figure 3.5). Volumes ranged from 0.23- 10.1 μl, with a range of 0.64 - 9.45 μl in the covered patches and 0.23 - 10.1 μl in the uncovered patches. Concentrations ranged from 5.2% to greater than 64%[6] sucrose or from 0.15 M to greater than 2.45 M (Kearns and Inouye, 1993) with a range of 14% to greater than 64% or 0.43 M to greater than 2.45 M for covered plots and 5.2 - 41.6% or 0.15 - 1.42 M for uncovered plots. These comparisons were based only on collected samples. However, the percentage of blossoms containing nectar were only a fraction of the total number of open blossoms, with ranges of 12.75% to 40.17% in the covered patch and 11.43% to 26.17% in the uncovered patch. Since a bee's judgment of profitability is averaged over an entire foraging bout, and not on a flower by flower basis (Waddington, 1980; Waddington, 1983; Wainselboim et al., 2003), we also wanted to get a better sense of the distribution of nectar across all the blossoms that a bee might potentially visit, which includes empty,

[6] The range that our refractometer could detect was from 0 - 64% sugar.

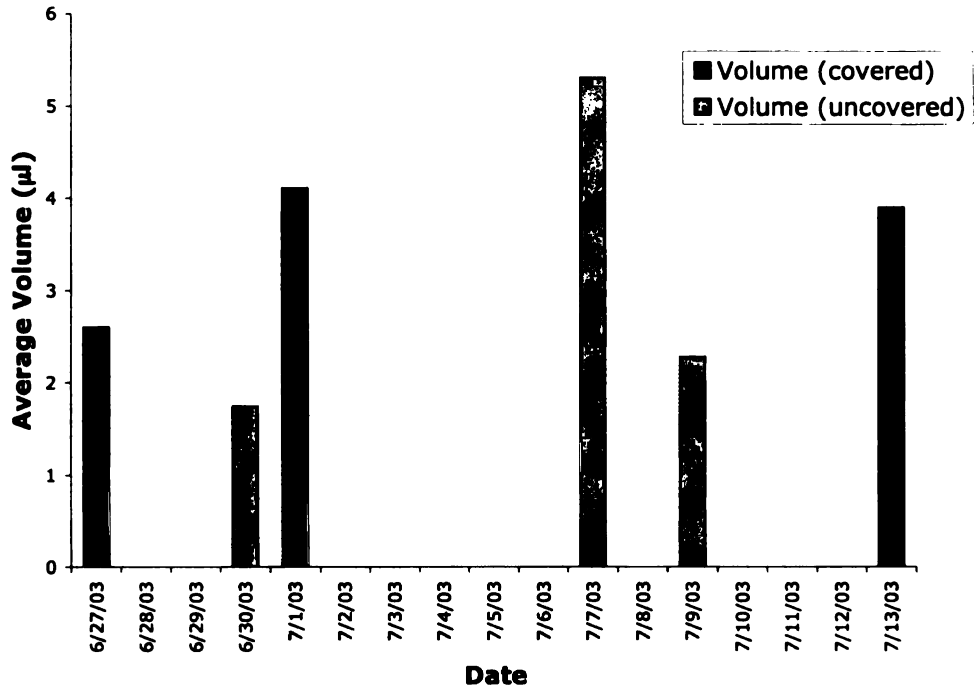


Figure 3.4. Average volume (µl) of individual nectar samples collected on a given day from each blossom containing nectar. There was no significant difference between nectar volumes of individual blossoms in covered versus uncovered patches (Wilcoxon: $Z=-1.07$, $p= 0.29$, covered $n=121$, uncovered $n=60$).

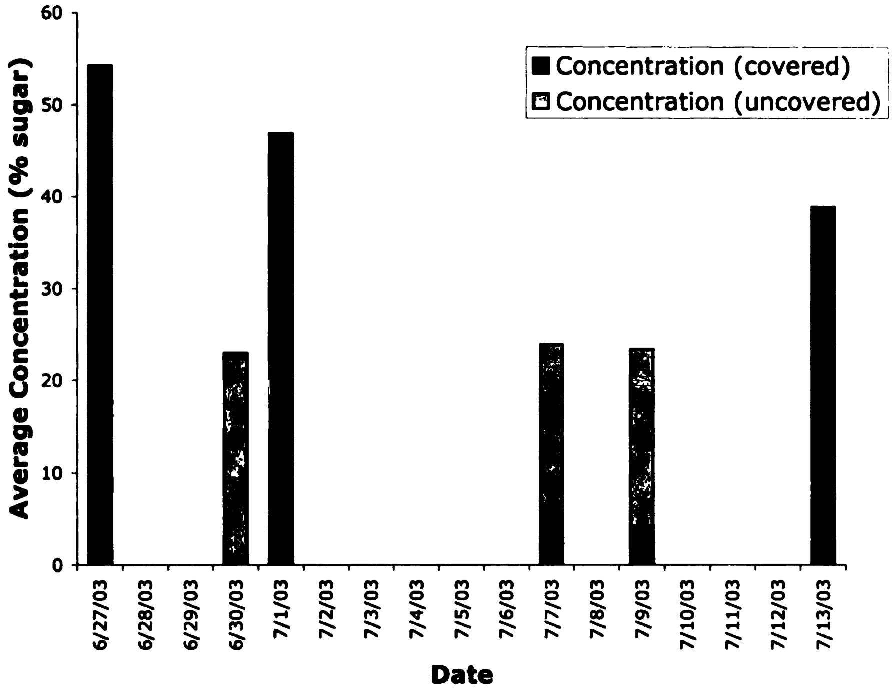


Figure 3.5. Average concentrations (% sugar) of individual nectar samples collected on a given day from each blossom containing nectar. Comparison of nectar concentrations of individual blossoms in covered versus uncovered patches shows a significant difference between the two treatment conditions (Wilcoxon: $Z= -8.92$, $p< 0.001$, covered $n=121$, uncovered $n=60$).

unrewarding blossoms. Thus, we also calculated the average mass of sugar for all open blossoms counted in each survey in each condition. We found that the average mass of sugar was greater for blossoms in the covered patch than the uncovered (Figure 3.6), which suggests that the covered patch was a richer, more rewarding area.

Observations of foraging honeybee population numbers over time

Another indicator that the nectar resources in the covered cage were more rewarding than in the uncovered cage was the number of bees that entered the patch. In each observation session, we gathered data from a covered patch and an uncovered patch, and paired analyses of these data found significant changes in the number of bees entering the patch over a 30 minute period (Figure 3.7); in the covered patch, more bees entered in the last 10 minute period than the first, while the opposite was true in the uncovered patch.

One concern might be that our data include some bees that have made repeated entries and exits to the observed patch. When possible, if we observed a particular bee moving outside the patch to forage and then returning to our observed patch, we counted her as if she had not left the patch. In these few instances, the bees made brief forays to immediately neighboring plants. We could not, however, follow longer forays outside of the patch, and returning bees may have been recounted (See Discussion).

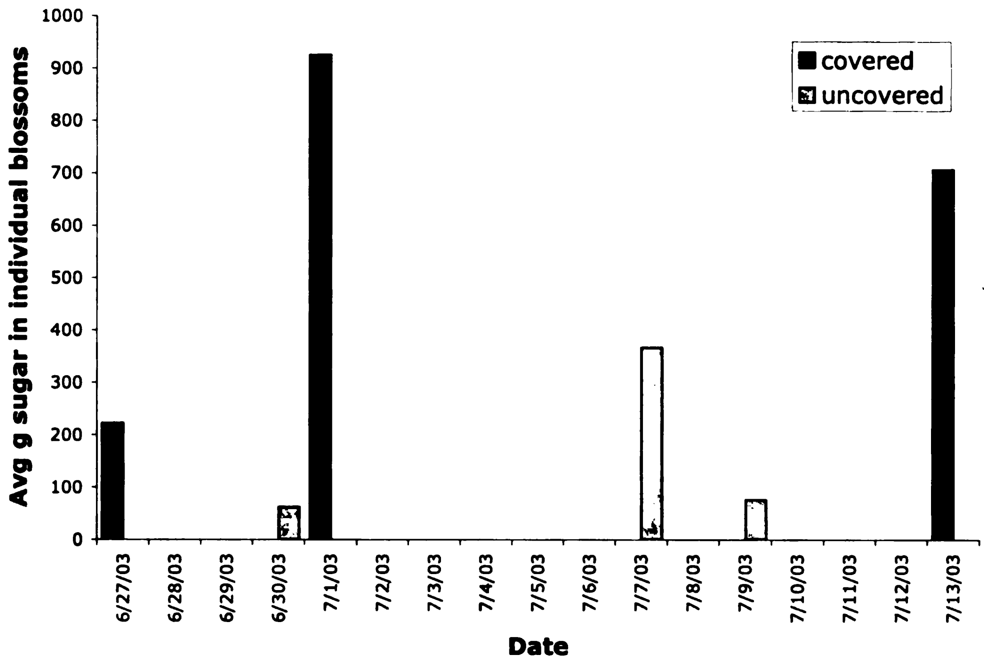


Figure 3.6. Average grams of sugar in individual blossoms in a given collection period. Averages include both blossoms from which nectar was sampled, as well as those that were empty. Comparison of the amount of sugar in individual blossoms in covered versus uncovered patches shows a significant difference between the two treatment conditions (Wilcoxon: $Z = -5.47$, $p < 0.001$, covered $n=405$, uncovered $n=366$).

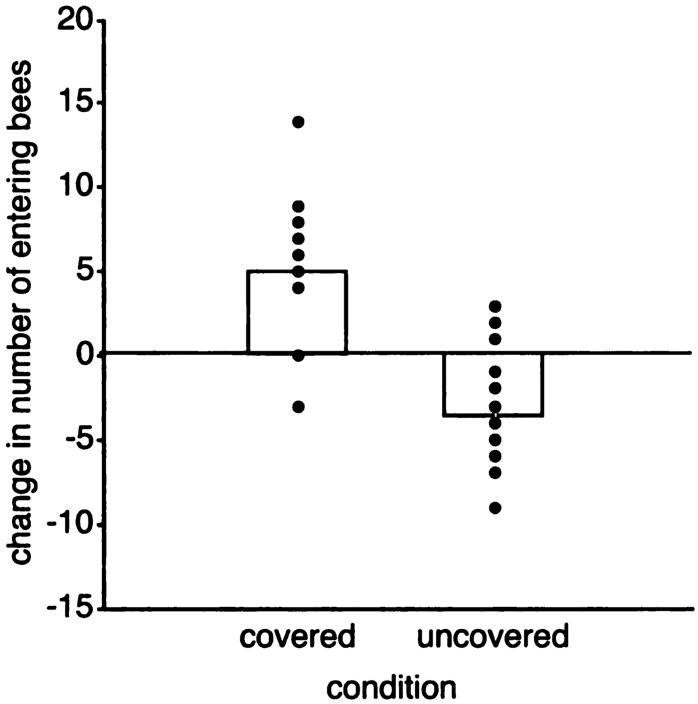


Figure 3.7. Comparison of the change in number of bees entering patches over the 30 minute observation period in covered versus uncovered patches. The change in number of entering bees was calculated as the difference in numbers of bees arriving in the first 10 minutes and the last 10 minutes of the 30 minute observation periods. Each data point represents the difference in number of entering bees in one observation period. Boxes represent means of these data points in each condition. During each of these observation periods, a covered patch and an uncovered patch were observed, and values from each session were paired for analysis (paired t-test: t=-5.71, p< 0.001, n= 13). Means of each condition are significantly different from zero (covered: t= 4.72, p< 0.001, df =12, uncovered: t= -3.20, p< 0.001, df =12).

Observations of departure behavior

Consistent with the expectation that learning flights would be observed when bees discovered the enhanced nectar resources within the covered cages, we observed a greater percentage of learning flights in the covered patch during each observation period than in the uncovered patch (except for one pair, where no learning flights were observed in either patch)(Figure 3.8). In fact, learning flights were observed almost exclusively from the covered patch.

Experiment 2 (concentration) and 3 (concentration and volume)

Foraging patterns following change in food distribution

When the foraging landscape changes from one of moderately rewarding, evenly distributed food sources to one with a very localized, richly rewarding area of food, do bees shift their foraging patterns upon return to the area? To examine this question, we first compared bees' biases at the end of the *post-change* phase using the water test. In each experiment, with one exception, every bee's biases in the water tests were on the same side as the location of the food (Figure 3.9). Comparisons with the control group, where bees experienced only the initial phase where food was evenly distributed, yielded only one significant comparison between the control group and bees trained to the east side in the *post-change* phase. Although the mean biases for the control groups are not significantly different from zero, they seem to suggest a possible bias towards the east side in experiment 2 and the west side in experiment 3. In spite of these tendencies, the differences in biases between the east and west groups still strongly suggest that by the

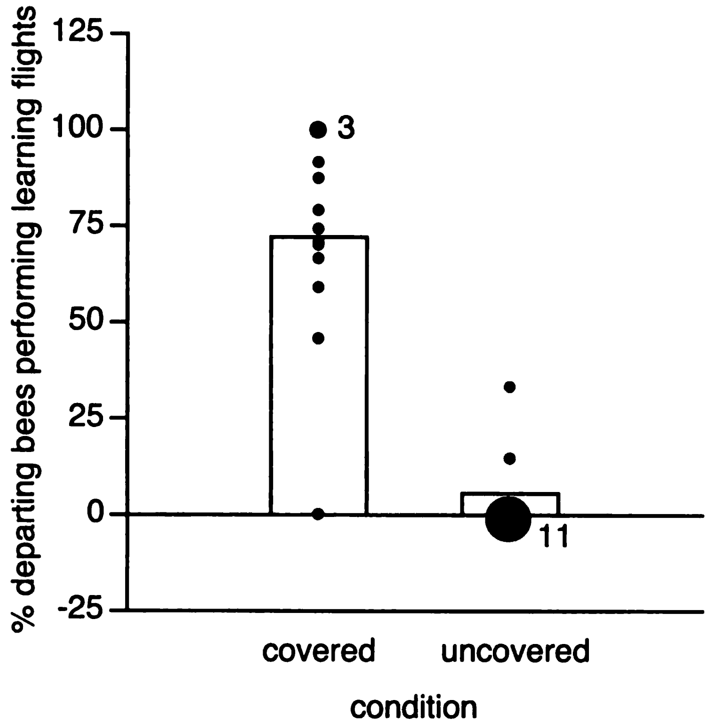
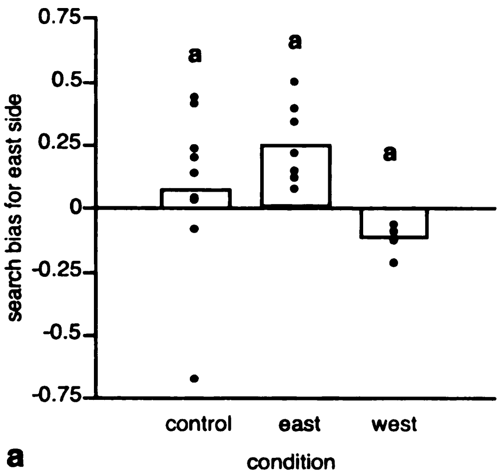


Figure 3.8. Comparison of the percentage of bees performing learning flights in covered versus uncovered patches (paired *t*-test: t= 8.72, p < 0.001, n= 13). The percentage was calculated from the total number of bees departing towards the hive; bees moving to adjacent patches were not included. Each point represents the percentage of bees performing learning flights in a given observation period; multiple values are indicated by larger dots, which are drawn approximately to scale. Boxes represent the mean percentage for each condition. During most observations of the uncovered patch, no learning flights were observed. Mean of the covered group is significantly different from zero (Wilcoxon sign rank: $t = 39.0$, $p < 0.001$, two-tailed *t*-test: $t = 9.48$, $p < 0.001$), while the mean of the uncovered group is not (Wilcoxon sign rank: $t = 1.5$, $p = 0.5$, two-tailed *t*-test: $t = 1.36$, $p = 0.20$).



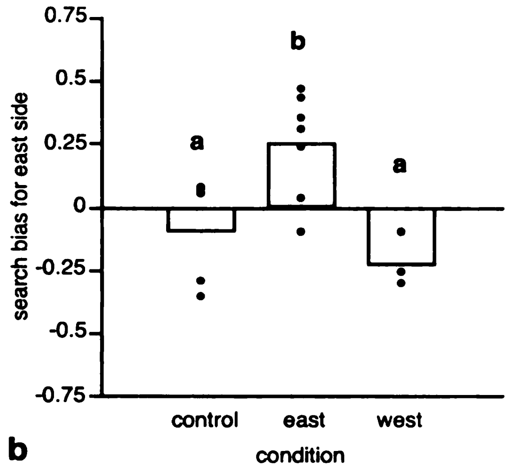


Figure 3.9. Comparison of search biases during water tests. Biases were calculated as the difference in the number of contacts with feeders on the east side and the number of contacts made on the west side, divided by the total. Positive biases indicate a greater number of stops on the east side. Conditions "east" and "west" indicate the side where bees departed and performed learning flights during the *post-change* phase. During this phase, arrival and departure locations were the same on every visit. Bees in the control group experienced the *initial* phase only and were tested following 12 visits. The East and West groups experienced the *initial* and *post-change* phase, and were tested after 24 visits. Each data point represents a single bee, and boxes indicate means of each group. **a.** Effects of increasing concentration and changing food distribution on search biases during water tests. Pairs sharing the same letter are not significantly different from each other according to Tukey-Kramer pairwise comparisons ($P<0.05$). Means of East and West groups are significantly different from zero, but the control group is not (student's *t*-test: East, $t = 5.08$, $p = 0.001$, n=8; West, $t= -4.04$, $p = 0.03$, n= 4; Control, $t= 0.74$, $p = 0.48$, n=9). **b.** Effects of increasing concentration, volume and changing the distribution of food on search biases during water tests. Pairs sharing the same letter are not significantly different from each other according to Nonparametric F-tests for pairwise comparisons ($P<0.05$). Means of East and West groups are significantly different from zero, but the control group is not (student's *t*-test: East, $t = 3.71$, $p = 0.01$, n=8; West, $t= -3.60$, $p = 0.07$, n= 3; signed-rank test: Control, $t= -1.5$, $p = 0.81$, n=5).

end of the *post-change* phase, bees have shifted their preferences towards the side where the highly rewarding food is located.

While search biases from the water tests describe the extent of a bee's preference for searching locations, we also wanted to know if a bee's choice of where to begin her investigation of the table shifted as well. If bees always began foraging bouts at random locations, we would expect the proportion of first landings on the departure side (as defined in the *post-change* phase) to be equal before and after the switch in food distribution. Instead, we found in both experiments that bees were more likely to choose the departure side for their first landing in the *post-change* phase, when food was located on the departure side, than in the *initial* phase, when food was evenly distributed (Figure 3.10). This suggests that bees shift their decisions of where to begin foraging in response to changes in the locations of food.

Role of reorientation flights in shifting foraging patterns

To determine whether the performance of reorientation flights influenced shifts in foraging patterns, we first calculated the changes in learning flight durations immediately following the switch in food distribution (see Methods). We found that in both experiments, departure durations increased significantly following the change in food distribution and quality (Figure 3.11). This suggests that reorientation flights were performed in response to the changes. Although the differences between the reorientation flight durations in experiments 2 and 3 are not significant (Student's *t*-test: $t = -1.47$, $p = 0.16$, $n = 24$), it appears that longer reorientation flights occur in experiment 3, where volume is also manipulated. This trend is influenced by the tendency of bees in

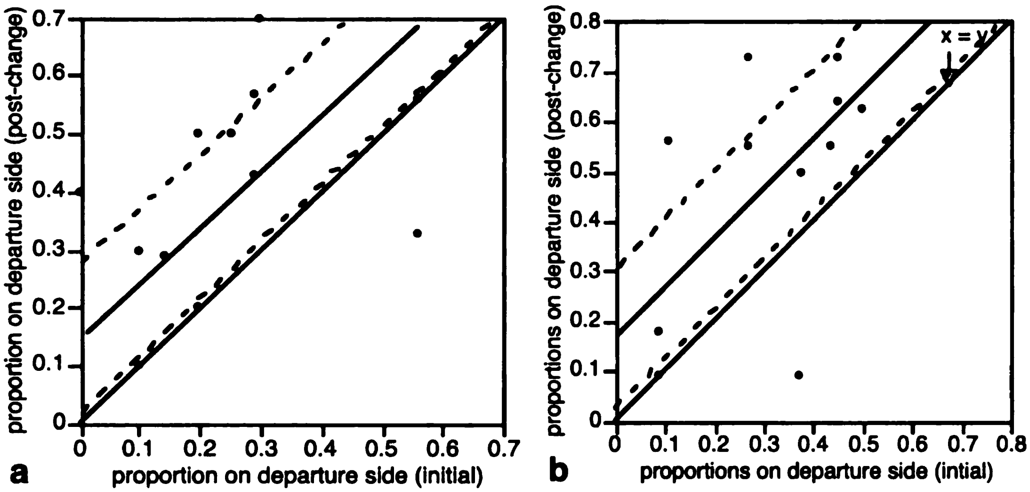


Figure 3.10. Comparisons of first landing choices for individual bees during the *initial* phase and the *post-change* phase, following the shift in food concentration, distribution, and volume (volume in experiment 3 only). **a.** Comparisons of choices made during phases of experiment 2 (concentration) (paired *t*-test: t= 2.83, p = 0.02, n = 13). **b.** Comparisons of choices made during phases of experiment 3 (volume and concentration) (paired *t*-test: t= 2.65, p = 0.02, n = 11). Proportions were calculated as the number of first landings made on the departure side, as defined during the *post-change* phase, divided by the number of visits in the phase. Thus, positive values indicate a greater proportion of bees making first landings on the side with food (same as the departure side) during return visits. Each point represents a single bee, and each proportion was calculated using the same number of visits in each phase (see methods). The *thick solid line* represents the point at which initial proportions and post-change proportions are equivalent. The *thin solid line* is displaced by an amount equal to the difference in the means of the two proportions, and the dashed lines indicate 95% confidence intervals.

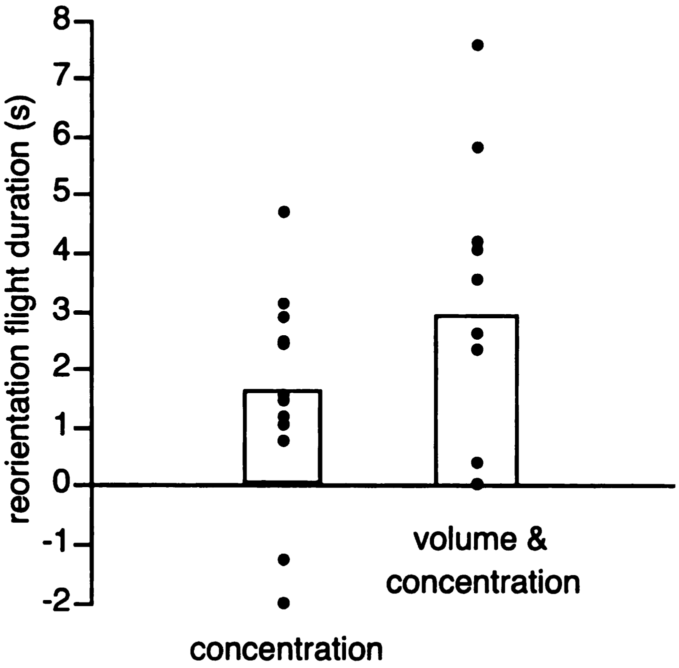


Figure 3.11. Diagram of reorientation flight duration following switch in food distribution, concentration, and volume (experiment 3 only). Reorientation flight duration was calculated for the first departure following the switch (visit 13). Each point represents a single bee. Boxes indicate mean values. Means were significantly different from zero for both experiment 2 (concentration) (student *t*-test: $t = 2.98$, $p = 0.002$, $n = 13$), and experiment 3 (volume) (student *t*-test: $t = 3.60$, $p = 0.005$, $n = 11$).

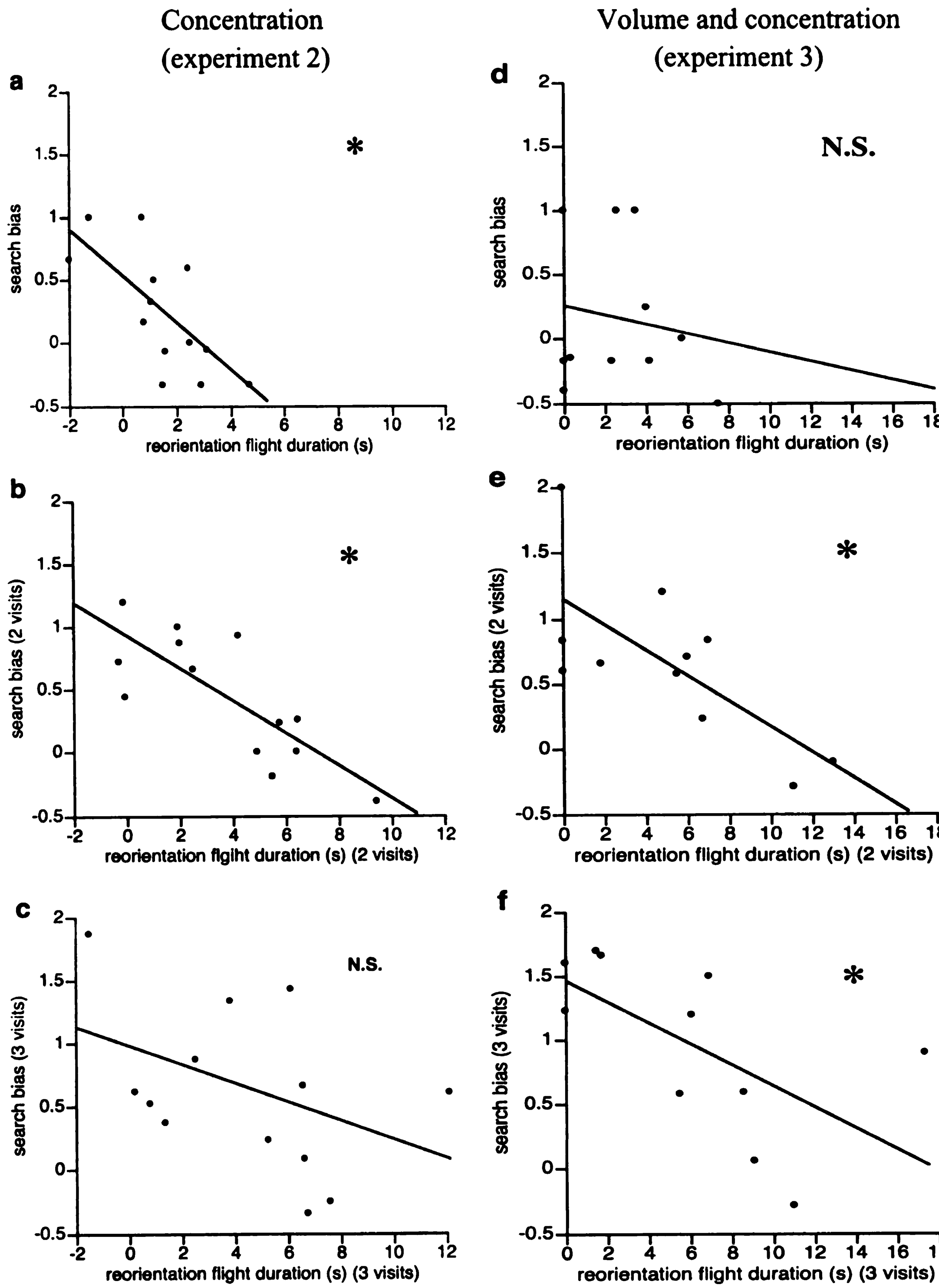
experiment 3 to not perform learning flights during the *initial* phase, which affects the baseline by which the changes in learning flight durations are calculated (see Methods). This tendency was very clear: when bees experienced the *initial* phase of experiment 3, where they fed at multiple feeders, each containing 8 µl of 1.0 M sucrose solution, they departed without a learning flight in 70% of the visits. However, when bees fed at feeders containing full volumes of 0.5M sucrose solutions during the *initial* phase of experiment 2, bees departed without a learning flight in only 11% of the visits.

In chapter 2, we found that the durations of learning flights performed at new locations were positively correlated with the probability that a bee would return to the location from which she departed on the previous visit. We also found that when reorientation flights were performed in response to a delay and shift in departure location, bees that performed longer reorientation flights were more likely to search the departure side during the water tests. Given this, we expected to find similar correlations with the duration of reorientation flights and subsequent foraging patterns in this study.

Instead, we found that bees performing longer reorientation flights were actually less biased towards searching on the departure side. Analyzing the relationship between reorientation flight durations and the search bias measure, which describes *all* the spatial choices bees make on each visit, we found negative correlations in both experiments (Figure 3.12)[7]. This surprising result suggests that reorientation flights may play

[7] As in chapter 2, we compared reorientation flight duration and biases during the water tests, but unlike the results in chapter 2, where reorientation flights were triggered by a delay, we found no significant relationships (for experiment 2: $R^2 = 0.0008$, $p = 0.93$, $n = 12$; for experiment 3: $R^2 = 0.03$, $p = 0.61$, $n = 11$). This is likely due to a ceiling effect; since the food was kept on the same side for arrival and departure throughout the *post-change* phase, bees that had performed short reorientation flights could still develop strong biases for the departure side during the water test using information learned on arrival.

Figure 3.12. Relationships between reorientation flight durations and biases for sides of the table on subsequent visits. **a, b, and c.** Relationships between the change in reorientation flight duration and subsequent biases in experiment 2 (concentration): for one visit (**a**) (R^2= 0.5, p = 0.007, n = 13), two visits (**b**) (R^2= 0.63, p = 0.001, n = 13), and three visits (**c**) (R^2= 0.18, p = 0.15, n = 13). **d, e, and f.** Relationships between reorientation flight duration and subsequent biases in experiment 3 (concentration and volume): for one visit (**d**) (R^2= 0.03, p = 0.62, n = 11), two visits (**e**) (R^2= 0.52, p = 0.01, n = 11), and three visits (**f**) (R^2= 0.37, p = 0.05, n = 11). Values are summed for both reorientation flight duration and biases in **b, c, e,** and **f.** (see Figure 3.8). Biases were calculated as the number of contacts made with feeders on the departure side, as defined in the *post-change* phase, and the opposite side, divided by the total. Positive biases indicate biases towards departure side, which is also the side where food is located in the *post-change* phase. Each point represents a single bee. "*" represents significant correlations, and "N.S." denotes nonsignificance.


Concentration
(experiment 2)
Volume and concentration
(experiment 3)
a
*
search bias
reorientation flight duration (s)
d
N.S.
search bias
reorientation flight duration (s)
b
*
search bias (2 visits)
reorientation flgiht duration (s) (2 visits)
e
*
search bias (2 visits)
reorientation flight duration (s) (2 visits)
c
N.S.
search bias (3 visits)
reorientation flight duration (s) (3 visits)
f
*
search bias (3 visits)
reorientation flight duration (s) (3 visits)

different roles when they occur in response to changes in the value of food compared to when they occur in response to uncertainty caused by delayed relocation of a goal.

DISCUSSION

In this study, we add to an already expansive literature on the foraging strategies of honeybees by considering decisions that bees make *across* visits about where to forage and about performing learning flights. Most previous studies on how bees maximize their foraging efficiency have focused on decisions made during the course of a single foraging bout (Banschbach, 1994; Giurfa, 1996; Greggers and Menzel, 1993; Hill et al., 2001; Keasar, 2000; Kipp et al., 1989; Nunez, 1982; Pyke, 1979; Ribbands, 1949; Schmid-Hempel, 1984; Schmid-Hempel, 1985; Schmid-Hempel, 1986; Waddington, 1985; Waddington, 2001). However, if nectar availability in natural foraging landscapes fluctuates and distribution is patchy[8], as is likely (Kearns and Inouye, 1993; Pleasants and Zimmerman, 1979), the choices bees make about where to begin their foraging bouts are also likely to impact their long term foraging success. These choices about where to forage are prefaced by decisions about the performance of learning flights: a bee must decide whether she will perform one and if so, for how long. Our results show that in a natural context, bees respond to the discovery of nectar-rich foraging areas by performing learning flights, and that in similar conditions in an artificial setting, learning flights influence subsequent foraging patterns, although in ways different than we predicted.

Our experiments in the figwort plot have demonstrated that bees respond to the discovery of a new, nectar rich foraging patch within the plot. In the covered patch,

[8] The level of patchiness in nectar distribution within natural populations is generally not well known, and contradictory evidence of nectar patchiness, or hot and cold spots, exists.

which was intended to simulate a nectar rich area, we observed an increase in the number of bees over a 30-minute period, while there was a decline in the number of bees in the uncovered patch. These population shifts might be explained by moment-to-moment foraging strategies of bees. Several researchers have documented the flight paths of bees foraging during a foraging bout and have discovered that bees tend to perform straight flights after feeding on low quality food, which moves them through poor quality area, and to perform tightly curved flights after feeding on high quality food, which slows their exit from rich areas (Pyke 1978; Heinrich 1979; Waddington and Heinrich 1981; Schmid-Hempel 1984, 1985). If bees foraging on the covered figwort patch were more likely to stay in the vicinity, it is possible that we recounted bees as they returned to the patch (see Methods). If so, the number of individual bees entering the patch may not have changed. Rather, bees in the covered patch may have been more likely to return and be recounted than bees in the uncovered patch. Alternatively, bees in the covered patch may have recruited more heavily to the area by releasing recruitment pheromones from their Nasanov glands, which bees are known to do at rich food sources (Seeley, 1995). If this were the case, then it would also be true that foragers who discover the covered patch are returning to the patch, since release of these volatile compounds occurs prior to feeding and is determined by a bee's expectations of reward (Fernandez and Farina, 2001). Thus, if the increase in the number of arriving bees were partially the result of the arrival of recruited bees, then it would also be true that the bees that discovered the covered patch initially are returning. Regardless of the explanation for this pattern, both suggest that bees responded to the discovery of a richer patch in a manner that is consistent with the idea that they were motivated to return to this location.

This motivation was likely driven by the increased nectar concentration and average sugar availability in the blossoms of the covered patch. The finding that nectar concentration in individual blossoms was always greater in the covered patch than the uncovered patch is most likely due to the effects of evaporation (Kearns and Inouye, 1993). The average amount of sugar per blossom was also significantly higher in the covered patch in part due to the higher concentration of nectar in the covered patch, but also in part due to the overall greater percentage of open blossoms with nectar when the patch was covered (overall, 29.9% in covered, 16.4% in uncovered). Although the differences in average amount of sugar per blossom were significant, blossoms in the patch which was covered on June 27, 2003, actually had on average a lower mass of sugar compared to July 7, 2003, when it was uncovered (Figure 3.6). Since these samples were not collected at the same point in time, this difference is likely explained by the fact that nectar production rates are affected by plant age, as well as seasonal and environmental factors (Kearns and Inouye, 1993).

Although we did not find a significant difference in average volumes of individual nectar samples between conditions, a difference may have existed between patches during the observation sessions. Due to practical considerations, we collected samples from the same plants on different days rather than from plants in a covered and uncovered patch simultaneously. Regardless, our finding that a greater proportion of blossoms contained nectar when the patch was covered (30% for covered, 16% for uncovered) suggests that the overall quantity of nectar in the covered patch was greater. If true, this would make the covered patch even more profitable: our finding that a greater percentage of blossoms contained nectar when the patch was covered than when uncovered suggests that the flow rate of sucrose, or the amount of sucrose intake per unit time, was also greater for foraging bees. Sucrose is a major component of nectar (reviewed in Kearns and Inouye, 1993), and the flow rate of sucrose determines food profitability (reviewed in Nunez and Giurfa, 1996). Thus, the profitability in the covered patch should be greater, as bees would spend less time collecting nectar loads and experience higher flow rates.

Our results also expand upon our previous findings, in experiments using artificial point source feeders, that learning flights are modulated by sucrose concentration (Wei et al. 2002) by demonstrating that bees foraging on real flowers in an open field also perform learning flights after feeding on a new, richer food source. Thus, at least in this particular set of circumstances, bees that foraged in an area that is less spatially defined than that of a point source artificial feeder, also performed learning flights in response to an increase in food profitability. In fact, a large percentage of bees departing the covered patch performed learning flights, while almost none of the bees departing from the uncovered patch did (Figure 3.8). However, some bees in the covered patch departed directly for the hive without performing learning flights. This observation might be explained by individual differences in past foraging experience (Wainselboim and Farina, 2003) or differences in colony state (Seeley, 1995).

Our experiments in the flight cage (experiments 2 and 3) were meant to mimic key aspects of a natural context, specifically circumstances where bees foraging in an area encounter a change in the quality, distribution and abundance of resources. These experiments provide further evidence that decisions to perform learning flights are informed by motivational states. During the *initial* phase of experiment 3, where 8 μl of sucrose solution was available in all feeders, bees departed without learning flights in 60% of all visits (9 bees, 75 visits). In this condition, bees fed on several feeders before collecting enough sucrose solution to complete their visit. In contrast, in the *initial* phase of experiment 2, where bees were able to fill their crops at a single feeder, departures without a learning flight occurred in only 8% of all visits (13 bees, 112 visits). Interestingly, in experiment 2, bees actually fed on a lower concentration food source (0.5 M) than bees in experiment 3 (1.0 M). However, food profitability is determined by flow rate (reviewed in Nunez and Giurfa, 1996), which is also influenced by food distribution in addition to sucrose concentration. Thus, in experiment 2, where feeders were filled to full volume, bees may have judged the food source to be profitable enough to warrant learning flights, while bees in experiment 3, where feeders contained only small volumes of sucrose solution, did not.

These results strongly suggest that bees maximize investments in learning by performing learning flights in response to the discovery of more profitable foraging patches. While previous studies have shown that foraging, recruiting, scenting behaviors, and even metabolic rate of foraging bees are modulated by changes in the quality and quantity of food rewards (De Marco and Farina, 2001b; Moffatt, 2000; Nunez, 1970; Nunez and Giurfa, 1996), our results provide an example of how motivational state can also influence active decisions to learn. By using motivational state to inform a decision to perform a learning flight, a bee may effectively be able to invest her energies in learning when the potential rewards for such learning are worthwhile.

Our hypothesis here is that by performing learning flights in response to the discovery of a profitable food source, a bee would use information gained during the flights to return to the profitable location more quickly and accurately. However, this hypothesis makes a few assumptions that, in retrospect, may not be entirely applicable to experiments 2 and 3. First, we assumed that bees are maximizing individual foraging success and that the best strategy for this is to return immediately to a rich food source. These assumptions were contradicted by our data showing that bees performing longer learning flights were less likely to return to the departure location in visits immediately following. These data raise a question: why are bees that performed longer learning flights *less* likely to search the side with the food than bees that performed none at all? If bees did not learn new information during reorientation flights, we might expect them to search the side with food with *equal* probability, not *less*. Rather, our results suggest that the performance of reorientation flights does impact subsequent foraging patterns, although not in the way we predicted.

In the context of this experiment, bees that are sufficiently motivated to perform a reorientation flight may also be motivated to explore the surrounding areas to determine the extent of the rich foraging area. Such exploratory behavior may make sense if we consider that bees need to maximize both individual foraging successes over the long-term and nectar intake of the entire colony. Furthermore, if their explorations fail to succeed in finding equally profitable food, they would still be able to return to the original location using information gained during reorientation flights. In this scenario then, such information can be used as a backup. In earlier studies (Chapter 2), we found evidence that bees use information gained through reorientation flights as backup when memories formed prior to the reorientation flight failed to guide them to the food. Further supporting the idea that information from reorientation flights may still be used in later visits is our finding that over repeated visits, bees shifted their foraging patterns in a manner that tracked the changes in resource distribution: when we simulated the discovery of a rich, locally concentrated food source by making highly concentrated sucrose solution available in only a single feeder, bees shifted their search patterns to concentrate in that area (Figures 3.9, 3.10). Unfortunately, we were unable to determine whether or not this shift in foraging pattern is influenced specifically by information gained during the reorientation flights, since the design of these experiments did not allow us to distinguish information learned on departure from that learned on arrival. This could easily be tested in the future using the Turn condition described in Chapter 2.

Another assumption we have made is that learning is adaptive and that information is always useful. But the value of information depends upon its potential to change behavior in a way that increases the fitness of the animal (Stephens, 1989). If learning entails costs associated with acquiring and storing information, and information is not always valuable, then learning may not always be adaptive. In experiments 2 and 3, one explanation for the results may be that the information gained during the reorientation flights is not useful in this particular context. Reorientation flights may be performed as a result of a general mechanism that triggers such flights when bees encounter richly rewarding nectar sources. For a behavior to be adaptive, the behavior must increase the fitness of the individual (or colony in this case). However, this does not require that the behavior is beneficial in all instances, as long as the costs are not greater than the overall benefits. For example, the honeybee waggle dance used to recruit nestmates to foraging sites increases the efficiency of a hive in collecting food (Dornhaus and Chittka, 2004; Sherman and Visscher, 2002; von Frisch, 1967) and thus presumably improves colony fitness. Recent studies have shown, however, that under some circumstances related to habitat and season, colonies with bees that dance are no more successful in collecting food than hives with bees that do not (Dornhaus and Chittka, 2004; Sherman and Visscher, 2002). Perhaps the same is true of learning flights; in some situations, such as when bees discover a new location for a food source, learning flights are adaptive, but in this particular scenario where bees are already familiar with the location, they may not be.

A second surprising aspect of our results in experiments 2 and 3 was the fact that bees performing shorter reorientation flights or none at all were able to find their way back to the departure point with relatively high accuracy (Figure 3.12). This seems perplexing because highly rewarding food was not found there previously. How are bees able to return to the departure location without information learned through reorientation flights? This can be explained if we consider that in experiments 2 and 3, bees were able to feed at all feeders around the table during the *initial* phase and since information about relative distances of landmarks can also be learned during arrival, bees likely had information about the departure location prior to discovering the highly rewarding food there. The discovery of a richly rewarding area may simply have activated the use of such information. Alternatively, bees may already have performed a few learning flights from that particular location during the *initial* phase and had information about the absolute distances between feeder and landmarks, which they could then recall when motivated to do so. This alternative, however, is unlikely given that learning flights in experiment 2 were performed in only 60% of all departures from any of the eight panels during the *initial* phase. This scenario is even less likely in experiment 3, where bees performed learning flights in only 8% of all departures during the *initial* phase.

In spite of the ambiguity in the results of experiments 2 and 3, this study has shown in the natural context of a figwort plot, where nectar is distributed among multiple blossoms, that bees perform learning flights in response to the discovery of nectar rich foraging patches. In a simulated natural context, with sucrose solution distributed among multiple feeders, we also showed that bees perform learning flights in response to the unexpected discovery of richly rewarding food. We found that over time, the bees also shift their within-visit foraging patterns to concentrate search in the vicinity of the food (Figure 3.9), and between-visit patterns to search first at or near the panel with the rich food (Figure 3.10). Unexpectedly, we also discovered that immediately following reorientation flights, bees that have spent more time learning are *less* likely to search near the location of the food. This result raises critical questions about the function of learning flights triggered by changes in nectar quality. Although these issues are not resolved with our data, our approach in exploring learning flights in more natural contexts has allowed us to uncover these intriguing results that would not have been possible using standard methods with point source feeders. By considering the natural context in which bees forage, we have formed a bridge between studies of learning flights and studies of foraging strategies, and have paved the way for a richer understanding of the function of learning flights in an ecological context.

CHAPTER 4

DIRECTIONS FOR FUTURE STUDIES

INTRODUCTION

Learning, the process of gaining knowledge, is generally believed to be beneficial. When animals face decisions of how much to invest in learning, they must negotiate a trade-off between the costs of obtaining information and the value of the information gained, which depends upon its potential to change behavior in a way that benefits the animal (Stephens 1989). This is not an easy task, as both are influenced by a host of complex, often intertwined, influences that include intrinsic physiological and psychological factors, and extrinsic ecological ones. Studying the decisions animals make about how much effort and resource to spend in learning is also difficult because it is often hard to judge when animals are learning.

The learning flights of bees and wasps offer the possibility of studying the benefits and costs of learning in a more direct way than is usually possible. One reason for this is that the function of learning flights is fairly well defined, in that they provide insects with rather specific kinds of visual information about landmarks. This makes it possible to manipulate the information available, and thereby to have a fairly clear idea about what information is potentially available to bees. A second aspect of learning flights that makes them useful as a model system for studying the benefits and costs of learning is that they are the result of an active decision on the part of the insect. If we make the assumption that insects perform learning flights only in circumstances when it would be useful, then we can infer the benefits of the behavior from the circumstances in which it is performed.

Taking advantage of these convenient features of learning flights, I have explored the decisions made by honeybees in allocating energetic and cognitive resources to the performance of learning flights. First, I have documented the precise ways in which various factors influence the modulation of learning flight duration in situations where bees learn the location of a point food source relative to an array of artificial landmarks (Wei et al. 2002). I have also explored the value of the information gained during learning flights in terms of its use in improving a bee's ability to relocate a food source (Chapters 2 and 3). My results have given me insight into the process by which honeybees make active decisions to learn in response to their changing needs for information and to changes in the foraging landscape. My work has also raised additional questions about this decision-making process in honeybees, and has found intriguing results that suggest new avenues of research. I discuss these directions for further study in this chapter.

Follow-up questions

Why do longer flights lead to better foraging?

In chapter 2, I found that learning flight duration is positively correlated with the accuracy of a bee in relocating a food source. The mechanism governing this relationship, however, is still not understood. What feature of an extended learning flight allows bees to be more accurate in pinpointing a goal? To answer this question, we must first consider the structure of learning flights and how the pattern of widening arcs and circles enables bees and wasps to extract landmark information. Zeil and colleagues (Zeil, 1993b; Zeil, 1997; Zeil et al., 1996) have shown that wasps pivot around a goal while fixating the goal with the lateral part of their retina, which allows them to fixate the scene at the end of the arcs. This feature may allow wasps, and bees, to learn some visual representation at the end of these arcs, presumably in the form of "snapshots" (Zeil et. al. 1996, Zeil 1997). These snapshots may then be used by the wasp upon return in an image matching process that guides them to the food: by matching their snapshot memory to the viewed scene, wasps are guided to minimize the degree of mismatch until the location is pinpointed. Because longer learning flights are correlated with a greater number of arcs and circles (C. Wei, personal observation), bees have more opportunities to form snapshots of the scene, and may thus be able to pinpoint a food source more accurately.

However, Lehrer and Bianco (2000) have suggested that "the relatively small number of fixation points during the TBL would not suffice to accomplish image matching unless the insect, on its subsequent arrival, keeps returning to those fixation points." From my observations, and those of Lehrer and Bianco (2000), we know that the return flights of bees do not follow the precise trajectories of the learning flights, which suggests that bees are not exposed to the exact same images as seen on departure. This does not preclude the possibility that bees may have other ways of assessing their proximity to the food even though they do not view the scene from the same vantage points (Cartwright and Collett 1982; Lehrer and Bianco 2000). Thus, the feature of extended learning flights that allows for greater accuracy on returns may be a feature other than the increased opportunity to form static snapshots. One such feature might be the pivoting motion of the flights. The particular pivoting structure of learning flights is thought to allow absolute distance information to be extracted from motion cues that are scaled relative to the wasp's distance from the goal (Zeil 1993;Zeil, 1997). Although the features of learning flights in wasps and bees are not exactly the same, they share enough similarities that these results may apply to bees as well. As the arc radius of the learning flight expands, bees have the opportunity to gauge distances from multiple viewpoints, which may increase the precision with which bees are able to localize the goal upon return.

The decaying nature of learning flights

Another important question about the modulated nature of learning flights is the question of why they are performed over a series of visits. Often, a single, long learning flight is sufficient to allow bees or wasps to return to their goal (Becker, 1958; Capaldi and Dyer, 1999; Wei et al., 2002). However, bees continue to perform learning flights for several visits after the initial visit, and the durations of these flights slowly decay. In mammals, exploratory behavior follows a similar pattern of decay over time, and novelty has been shown to govern exploratory behavior such that intensity of exploration is highest when an animal is least familiar with an environment and reoccurs whenever the environment changes (Berlyne, 1966; Thinus-Blanc and Gaunet, 1999). Thus, at least in mammals, the extent of exploratory behaviors is determined by the animal's state of knowledge or familiarity with an environment, which in turn increases with exploration. This feedback loop may explain the steady decline in exploratory behavior.

Both the exploratory behaviors in mammals and learning flights in insects follow the pattern of decreasing response as a result of repeated exposure to a stimulus known as habituation (reviewed in Shettleworth, 1998; Thinus-Blanc and Gaunet, 1999). Both behaviors also reappear when animals encounter a novel stimulus, a phenomenon known as dishabituation. Although habituation is often described in terms of stimulus- reflex responses, as in the classic example of gill withdrawal in *Aplysia*, habituation also occurs with complex behaviors, such as the well-known example of orientation responses of human babies to novel visual and auditory stimuli (reviewed in Shettleworth, 1998). Several models have been proposed to explain habituation. The simplest one, Sherrington's Reflex Model (reviewed in Shettleworth, 1998), can explain habituation in simple reflex pathways, such as in the gill withdrawal response of *Aplysia*, but it cannot explain more complicated behaviors, such as exploratory behavior. However, more complex models, such as Sokolov's Neuronal Model or Wagner's SOP Model (Standard Operating Procedure of memory), both of which incorporate the role of learning (reviewed in Shettleworth, 1998), may be able to explain the mechanistic reasons for the decaying nature of exploratory behavior in honeybees.

With a better understanding of the modulated nature of learning flights in reference to general learning theory, we may be poised to better understand the functional reasons for why learning flights are performed over a series of visits instead of in a single, extended flight. One possible advantage for such a pattern of learning flights may be that bees are able to reinforce learning that occurs on the first visit, and thus allow for longer lasting memories to be formed.

Circling flights versus the Turn Back and Look

During the observations of the bees in the figwort patch in Chapter 3, I observed that the structure of learning flights were different from those observed in my experiments in the flight cage. The flights in general consisted of wider arcs and circles, and seemed to be more akin to what has been called circling flights (von Frisch 1967). I noticed that some of these flights were prefaced by the more intensive, narrowly arced patterns described as the Turn Back and Look behavior (TBL) (Lehrer 1991, 1993), although the frequency of occurrence was difficult to determine. These two patterns have been described as two separate phases of learning flights (Schone 1996, Lehrer 1993), with the TBL acquiring information about nearby landmarks and the circling flight possibly acquiring information about celestial cues, as well as information on the spatial relationships between the feeding site and distant landmarks (reviewed in Schone 1996). In my studies inside the flight cage, I did not make this distinction between the TBL and the circling flight because in our experiments, it was not easy to tell when one pattern (TBL) transitioned into the other (circling).

This ill-defined transition between the TBL phase of learning flights and the circling phase calls to question the usefulness of this distinction. The difference between learning flight patterns in the flight cage and the figwort patch suggest that the visual environment determines learning flight structure (see Chapter 1). In most studies of learning flights, the landmarks and cues used were visually salient (e.g. Lehrer 1991, 1993; Wei et al. 2002; Zeil 1993). In my studies, I observed TBLs most clearly in my experiments using the box apparatus, which featured a prominent landmark at the location of the food. Landmarks and cues used in a natural context, however, may not be so obvious, and may be more spatially distributed. In the figwort patch, the presence of the observers seated a meter or so away were the closest landmarks, and I observed learning flights that spanned greater radii and were more loosely structured than the flights performed at the boxes. The arcing radius may be the defining difference between TBLs and circling flights and may be determined by the proximity of useful landmarks near the food source. However, what constitutes a useful landmark in a natural context is unclear, and individual landmarks may not even be necessary to guide an insect back to a location is also not known. In principle, panoramic images can be used in view-based homing (Zeil et al., 2003). Further studies describing the structure of learning flights in response to varying visual parameters will bring us closer to understanding what controls the structure of learning flights. Only with this information can we determine whether TBL behavior and circling flights serve two separate functions, one to learn nearby landmark cues and the other to learn celestial cues and large-scale landmarks, or if they simply reflect differences in visual environments.

Why do bees depart a rich patch without a learning flight?

In chapter 3, I found that bees perform learning flights in response to the discovery of rich foraging patches. I also noted, however, that some bees did not. While individual variation in behavior is expected, it is worth taking a moment to ask what circumstances might influence a bee not to perform learning flights when departing the richer, "covered" patch in our figwort studies. In my discussion, I suggested that differences in behavior of bees departing the covered patch might be explained by individual differences in past foraging experience (Wainselboim and Farina, 2003) or differences in colony state (Seeley, 1995). Both of these factors might influence how individual bees might judge the value of the food in the covered patch differently.

In my experiments, I suspect that past foraging experience may have influenced judgments of profitability that in turn influence decisions to perform learning flights. Since bees evaluate food source profitability over the entire foraging bout, bees that foraged for greater periods of time on less rewarding areas adjacent to the covered patch before discovering the rich area would have judged the overall area to be less profitable than those bees that discovered the covered patch earlier in their foraging trip (Waddington, 1980; Waddington, 1983; Wainselboim et al., 2003). Thus, these bees may have departed the covered patch without performing learning flights. This possibility remains to be tested.

Strategies of exploration versus exploitation

In Chapter 3, I also noted the unexpected finding that bees that performed longer learning flights were actually *less* likely to search the side with the food than bees that performed none at all. My results suggested that the performance of reorientation flights does impact subsequent foraging patterns, although not in the way we predicted.
I suggested that one possible explanation in this context is that when bees are sufficiently motivated to perform a reorientation flight, they are also motivated to explore the surrounding areas. This raises the question of why bees might choose a strategy of exploration instead of one of exploitation.

In previous studies of learning flights in honeybees, bees have fed at unusually profitable feeders where sugar solutions were available *ad libitum* and at high concentrations (Lehrer, 1991; Lehrer, 1993b; Wei et al., 2002). In this context, bees almost always chose a strategy of exploitation, and this led to the assertion that bees always return to a location following the performance of a learning flight (Lehrer 1993). However, in foraging areas with naturally distributed nectar, exploitation might not always be the best strategy, and bees may not always return to the location from which they departed. I suggested in Chapter 3 that exploration might be a better strategy for maximizing individual foraging over the lifetime of an individual. By initially exploring adjacent resources while allowing recently exploited flowers to replenish, perhaps bees are better able to track fluctuating resources over time. Another possibility is that exploration helps bees to determine the extent of a rich area and to recruit foragers to a wider area, possibly by scenting, and thereby maximizing the nectar intake of the entire colony. However, whether nectar resources are distributed in patches is not well known, and evidence exists for and against the presence of nectar "hot and cold spots" (Kearns and Inouye, 1993). Thus, the merit of these speculations first requires the spatial distribution of nectar in bee flowers to be better quantified. Second, a field study is needed to see if my results from our flight cage experiments also hold in a more natural situation where bees are feeding on real flowers and viewing natural scenes during learning flights. This would require the development of new methods that would allow for the tracking of individual honeybees in an open field. Although only touched upon here, studies in more natural contexts are the most promising avenue to a better understanding of the function of memory in natural foraging.

Additional Avenues of Inquiry

During the course of our experiments, I found a couple of unexpected results and interesting patterns. These findings raise questions about other decision-making processes in honeybees. I discuss each of these results in turn.

Dancing in the absence of food

During the delay experiments of Chapter 1, I noticed that many bees subjected to the longer delays disappeared from the area around the arena after they had searched for several minutes. I suspected that these bees had returned to the hive. In a follow-up study, using a glass-walled observation hive, I confirmed that many bees did return to the hive after several minutes of searching at the arena, and that, not surprisingly, the cumulative proportion of bees returning to the hive increased as the delay increased. More interesting is the finding that approximately a third of the bees that returned to the hive performed a dance despite their failure to obtain a nectar load (Figure 4.1). These dances looked like normal recruitment dances, but in general were shorter and less vigorous than dances performed by the same bee following rewarded visits. Also, several bees, including one of the dancers, performed shaking signals or dorso- ventral abdominal vibrations (DVAV), which are thought to signal to the nectar-receiving bees in the hive the need to reallocate their focus to a different task, which in this case is foraging (Nieh, 1998; Seeley, 1995). Both dances and DVAVs are generally thought to signal the presence of a profitable food source (reviewed in Seeley, 1995), and enhance recruitment to food. Both signals are also thought to be modulated in their expression according to both the quality of the food and the needs of the colony (Nieh, 1998; Seeley, 1994).

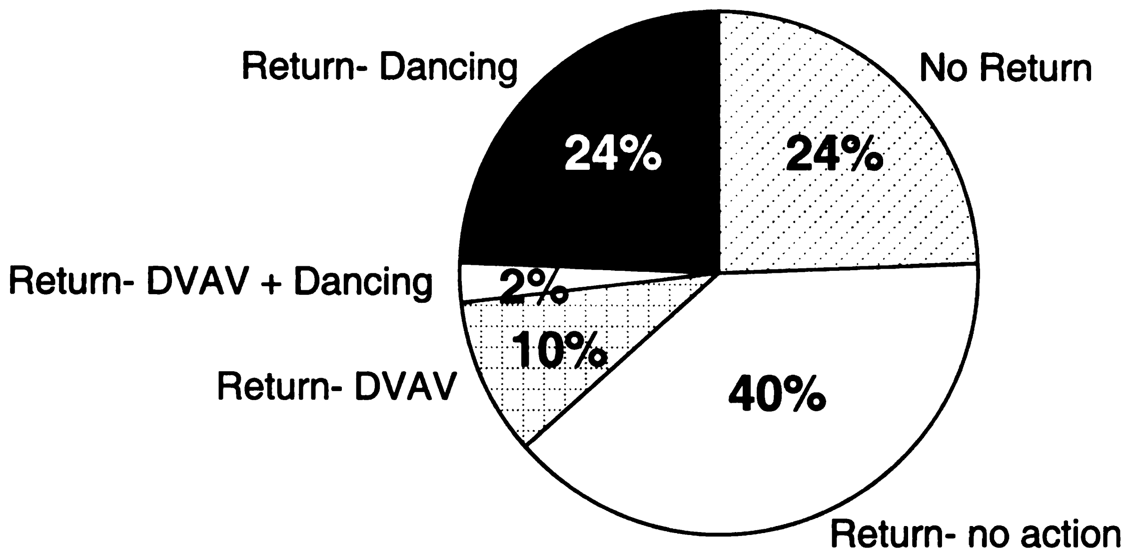


Figure 4.1. Behavior of 41 bees during a 10 minute delay at the arena in which food was unavailable. Overall, 10 bees (24%) searched continuously at the arena, while 31 bees (76%) returned to the hive after searching at the arena. Of those that returned, 4 performed a dorso-ventral abdominal vibration (DVAV), 10 performed dances, and one did both.

Investigations of how bees judge profitability of a food source have assumed that assessments are made on a trip-by-trip basis (Schmid-Hempel, 1985; Seeley, 1994). In this context, it is unexpected that some bees should dance or perform DVAV shaking signals without having experienced the cues associated with a successful foraging trip, and it raises questions about the mechanisms governing a bee's decision to dance. My observation that bees performed such signals to a source that they had just found to be unrewarding may suggest that their assessment of the food's profitability was not based on the most recent experience alone. Rather, their behavior must have been influenced by their experience in previous rewarded visits. The value of such a strategy may be that it minimizes the impact of temporary depletion of flower patches due to competition. By continuing to dance even without a successful load, foragers may assure that there will be bees available to collect nectar when the source is replenished.

Persistence in searching for food: how does a bee decide when to give up?

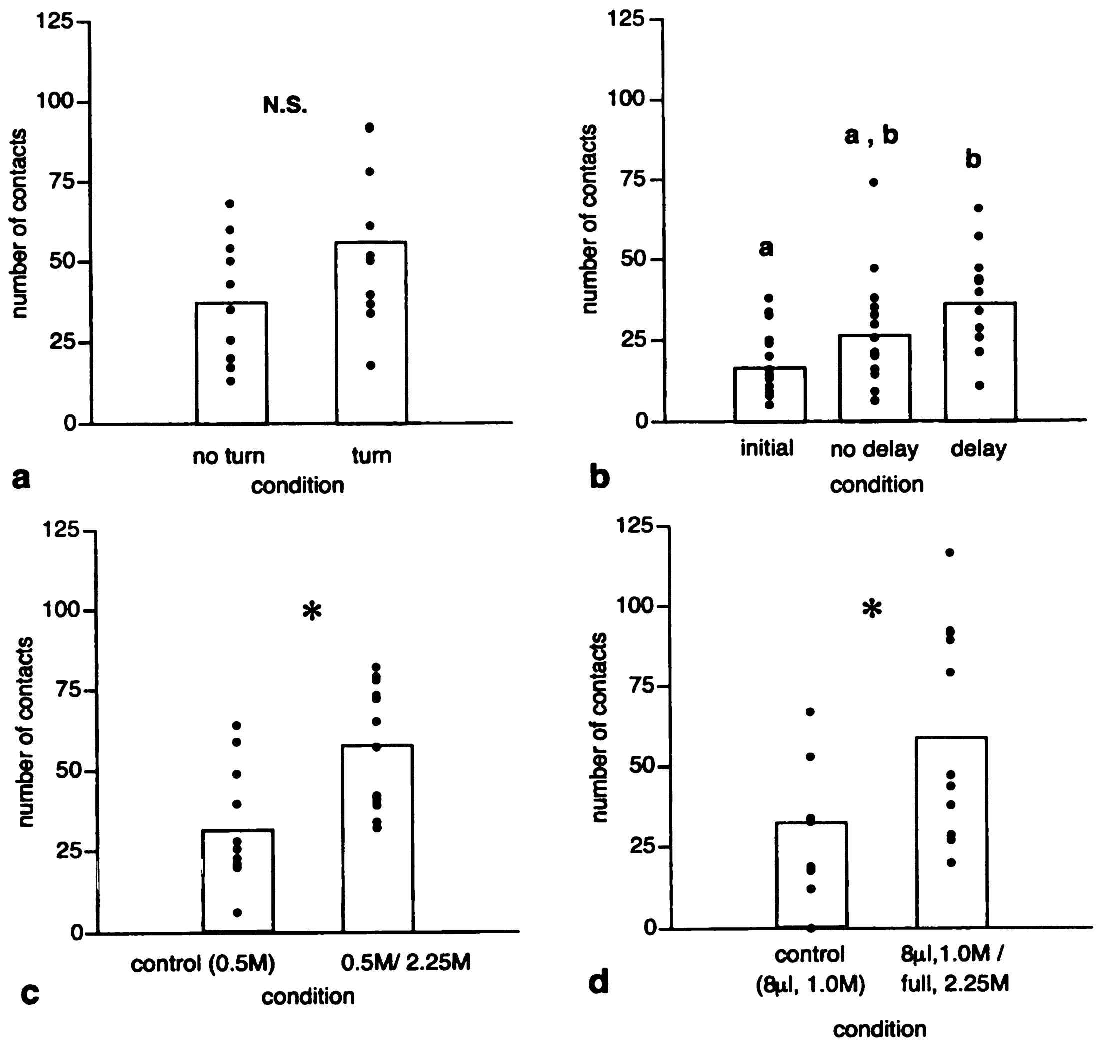
During this experiment where I observed the dances without rewards, I also noticed that the amount of time before a bee would end her search at the arena and return home varied greatly between bees. These observations suggest interesting questions about the decisions bees make about how much time and energy to invest in searches. In other words, how does a bee decide when to give up?

Results from chapters 2 and 3 are pertinent to this question. In several experiments, bees were subjected to another situation where we removed the food source at a familiar location, forcing bees to search for an extended period of time upon return. This occurred during the water tests, where all feeders were filled with unrewarding water[9], which required bees to inspect the feeders before determining if they were rewarding or not. For each water test, bees arrived at the table and frequently contacted feeders while searching for food. The point when a bee would give up this search was often distinctive, with a bee leaving quickly and abruptly (although this was not always the case in the arena). Sometimes, bees would return to their previous feeding site, the stock feeder, while others headed directly back to the hive. As in the arena, the point at which this giving up moment occurred varied greatly between individuals. However, there was also a difference between *conditions* in search persistence. Comparing the number of contacts bees made with feeders during the water test, I found: 1) In experiment 1 of Chapter 2, bees in the Turn and No Turn conditions did not differ significantly in the number of contacts they made, but the data suggest that bees in the Turn condition may have been more persistent (Figure 4.2a). 2) In experiment 2 of Chapter 2, bees in the Delay group were more persistent than bees in the Initial group, while bees in the No Delay group made an intermediate number of stops (Figure 4.2b). 3) In experiments 2 and 3 of Chapter 3, bees that experienced a *post-change* phase, where 140 ml of 2.25 M solution was available in a single location, were more persistent than bees that had only experienced the *initial* phase, where low concentration food was evenly distributed (Figure 4.2c,d).

These data do not reveal which factors contribute to the persistence of a bee in searching for a known food source, but they do suggest some interesting possibilities. The last result reported above (Figure 4.2 c,d) suggests that differences in motivational level can explain why bees in the non-control group are more persistent in their searches:

[9] Bees do collect water, but in these cases, our subject bees were foraging for nectar. None of our bees stopped to feed when the feeders contained water.

Figure 4.2. Comparisons of the number of contacts with feeders made during water tests. Each point represents a single bee, and boxes indicate means. **a**. Experiment 1 (Chapter 2). In both groups, water tests were performed after 20 visits ($F_{1, 19} = 3.71$, $p = 0.07$, n=21). **b**. Experiment 2 (Chapter 2). In all conditions, during the six visits prior to the water test, bees departed from the opposite side of where they found food upon arrival (turn condition). Bees in the *Delay* and *No Delay* groups also experienced twelve visits where they arrived and departed from the same side (no turn condition) prior to the change in food location and switch to the turn condition. Pairs sharing the same letter are not significantly different from each other according to Tukey-Kramer pairwise comparisons ($P < 0.05$). **c**. Experiment 2 (Chapter 3). Bees in the control group were tested after twelve visits experiencing low concentration (0.5 M) food, which was evenly distributed across the table (see Chapter 3 methods). Bees in the "0.5 M/2.25 M" group experienced an additional twelve visits, where high concentration food was located at a single panel, before the water test. ($F_{1, 12} = 8.90$, $p = 0.007$, n=22). **d**. Experiment 3 (Chapter 3). Bees in the control group experienced twelve visits where 8 ml of 1.0 M sucrose concentration was available in all feeders. Bees in the "8 μl/1.0 M- full/2.25 M" group experienced an additional twelve visits, where 140 ml of high concentration (2.25 M) food was located at a single panel, before the water test ($F_{1, 18} = 5.07$, $p = 0.03$, n=20).



125
100
75
50
25
0
number of contacts
N.S.
no turn
turn
condition
a
a , b
b
a
initial
no delay
delay
condition
b
*
control (0.5M)
0.5M/ 2.25M
condition
c
*
control
(8µl, 1.0M)
8µl,1.0M /
full, 2.25M
condition
d

bees in the non-control group had been feeding on food that was far superior to what the control bees had experienced. This alone is not surprising. However, the data in Figure 4.2b show differences in persistence across groups where all bees were fed with 140 ml of richly rewarding, 2.25M sucrose solution. Thus, motivation alone, as influenced by food profitability, cannot explain this difference. Rather, this result, and possibly the previous one as well, may be explained by the difference in experience between groups of bees. In all three cases (Figure 4.2 b,c,d), the group where bees were more persistent experienced an additional six (Figure 4.2b) or twelve (Figure 4.2c,d) visits to the octagonal rotating table before the water test. With a greater number of visits to a location, bees have more opportunity to gain information about the foraging area, and may therefore be more certain of where the expected location of the food is, which in turn increases their persistence in searching.

An alternative explanation for our results is that bees that make more contacts with feeders do so because they have expectations of the food in more locations than bees that are less persistent. In all groups where bees were more persistent in their overall search during the water tests, bees were also likely to have expectations of finding reward at a greater number of locations on the table: 1) In Chapter 3, the Control groups found food at all locations, and were thus unlikely to have learned any particular location. The Experimental groups, however, experienced 12 additional visits where they found food located in a single panel. Thus, these bees should have developed an expectation of reward at a single location on the table (Figure 4.2 c,d). 2) In Chapter 2, bees in the Initial condition were shown to prefer search on the departure side. Bees in the Delay and No Delay groups both had expectations of the food on the arrival side, but in the

Delay group, bees performed longer reorientation flights and showed a greater tendency to also search the departure side. Thus, bees in the Delay group were likely guided by expectations to find the reward in two locations compared to the Initial and No Delay groups, where bees likely had expectations of the food in only one location (Figure 4.2b). 3) Our results in Chapter 2 showed that bees in the Turn condition had memories for both sides of the table, while bees in the No Turn condition, where they always arrived and departed from the same side, had only learned one side (Figure 4.2a). Perhaps bees with expectations of finding food at a greater number of locations are equally persistent in searching any given location, but invest more energy in searching overall because they have more places to investigate.

Whether a bee's investment in searching is regulated by the strength of her expectation of finding food at a particular location and/or by the number of locations where she expects to find food will require further studies to distinguish. To test the multiple locations hypothesis, a relatively easy experiment could be set up where bees would be trained to a varying number of locations (2 or 3), each with distinctive visual cues. During training, bees would alternately experience the various sites for a long enough period of time that they would be very familiar with each site. If the multiple locations hypothesis is true, one would predict that bees should make a greater total number of contacts when they have learned 3 rewarding locations compared to 2. Alternatively, assuming that bees are more persistent in their searches when they have stronger expectations of finding food at a particular location, a result where bees trained to 3 locations were *less* persistent in their searches might suggest that with greater numbers of locations, bees have lesser expectations of finding food at any one location, and thus search less.

To test the possibility that the strength of a bee's expectation for finding food at a particular location influences a bee's decision of how long to search, we must first consider that expectations are influenced by factors including the probability of finding reward at a particular location and a bee's certainty that she is in the correct location. Disentangling these two factors from each other is not an easy task. One possible approach, similar to one taken in chapter 1, might be to compare relatively certain conditions, where bees are given salient, reliable cues that accurately predict the location of food, and more uncertain conditions, where cues are less salient and reliable. By manipulating features of the feeders and landmarks near the feeder, as well as the number of predictive cues, variation in the predictive power of various cue combinations can easily be created. Since much is known about the cues used by bees to pinpoint a goal, such as landmarks (Collett and Zeil, 1997) or features of the flower itself (reviewed in Menzel, 1985), a combination of salient cues that would accurately predict the location of a food goal could easily be created.

Beyond honeybees

The results presented in this dissertation and the incidental observations described above have not only raised interesting questions about the learning flights of honeybees, but they have also highlighted a fascinating behavior that is a special example of exploratory behaviors found in taxa across the animal kingdom (Boal et al., 2000; Joubert and Vauclair, 1986; Nicholson et al., 1999; Thinus-Blanc and Gaunet, 1999; Zeil et al., 1996). In vertebrates, exploratory behaviors have been described in the context of exploration of novel objects and environments for the purpose of developing or updating spatial representations (reviewed in Thinus-Blanc 1999). These behaviors decrease over time, consistent with the phenomenon of habituation. When novelty is encountered, dishabituation and re-exploration is observed. Similarly, exploratory behaviors in invertebrates, such as learning flights in bees and wasps, and learning walks in ants (Nicholson et al., 1999), also exhibit this pattern of decreasing intensity of behavior over time and reoccurrence in novel environments. Unlike exploratory behaviors described in other species and the orientation flights of bees and wasps, learning flights and learning walks occur *after* the discovery of a rewarding food source. As an example of backwards conditioning, the existence of these behaviors in insects raise the question of whether such examples exist in other taxa or if such behavior is restricted to insect species.

Anecdotally, hummingbirds have been observed performing behaviors similar to the "turn back and look" behavior in honeybees. If these reports are true, comparisons of such behaviors between vertebrate hummingbirds and invertebrate honeybees could potentially yield insight into the fundamental nature of active learning behaviors. However, we would not need to venture across the vertebrate divide to find fruitful comparative study. Within wasps and bees, many interesting comparisons can be made. For example, we might ask if there are differences in the occurrence and features of learning flights in solitary versus social bees. We might also ask why ground nesting bees continue to use absolute depth cues over the long term (Brunnert et al., 1994), while honeybees use them transiently (Collett and Lehrer 1994; Chapter 2). As I demonstrated in Chapter 3, considering learning flights in various foraging contexts allowed us to broaden our view of learning flights and deepen our understanding of its functional role. Likewise, by considering learning flights in a broader evolutionary context, we can gain not only a deeper understanding of learning flights in honeybees, but of active learning behaviors and exploratory behaviors as a whole.

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