THE ROLE OF ORIENTATION FLIGHTS ON HOMING PERFORMANCE IN HONEYBEES

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Summary

Honeybees have long served as a model organism for investigating insect navigation. Bees, like many other nesting animals, primarily use learned visual features of the environment to guide their movement between the nest and foraging sites. Although much is known about the spatial information encoded in memory by experienced bees, the development of large-scale spatial memory in naive bees is not clearly understood. Past studies suggest that learning occurs during orientation flights taken before the start of foraging. We investigated what honeybees learn during their initial experience in a new landscape by examining the homing of bees displaced after a single orientation flight lasting only 5–10 min. Homing ability was assessed using vanishing bearings and homing speed. At release sites with a view of the landmarks immediately surrounding the hive, 'first-flight' bees, tested after their very first orientation flight, had faster homing rates than 'reorienting foragers', which had previous experience in a

different site prior to their orientation flight in the test landscape. First-flight bees also had faster homing rates from these sites than did 'resident' bees with full experience of the terrain. At distant sites, resident bees returned to the hive more rapidly than reorienting or first-flight bees; however, in some cases, the reorienting bees were as successful as the resident bees. Vanishing bearings indicated that all three types of bees were oriented homewards when in the vicinity of landmarks near the hive. When bees were released out of sight of these landmarks, hence forcing them to rely on a route memory, the 'first-flight' bees were confused, the 'reorienting' bees chose the homeward direction except at the most distant site and the 'resident' bees were consistently oriented homewards.

Key words: honeybee, *Apis mellifera*, homing, orientation, navigation, learning, spatial memory.

Introduction

Like other animals that forage from a central place, honeybees face the task of finding their way on foraging trips that may take them up to 10 km from home. Over such distances, only visual information such as landmarks and celestial orientation cues can provide a basis for rapid and accurate homing. These references are useful only if the animal can learn the spatial relationships defined by these visual features. Most studies of large-scale spatial learning in insects have focused on the contents of spatial memory in highly experienced bees (for reviews, see Dyer, 1994; Wehner, 1981). Far less work has been done on how spatial memory develops in naive bees as they begin to gain experience in a new landscape (Opfinger, 1938; Becker, 1958; Vollbehr, 1975). We addressed this problem by studying the homing ability of honeybees that have made only a single orientation flight in a particular landscape.

We observed the homing behavior of honeybees displaced from the nest after they had completed a single orientation flight. Path integration was not a possible mechanism of homeward orientation for the bees in our experiments because we denied them the experience of tracking their position during

the outward trip (Müller and Wehner, 1988; Wehner et al., 1996). This task is a direct test of landmark learning during the orientation flight; only if bees were familiar with landmarks should homing occur efficiently.

Becker (1958) was the first to establish a role for orientation flights in learning the large-scale landscape features that bees use for homing. These flights are a conspicuous behavior used by a variety of hymenopteran insects before departing from a place to which they will later return (Wehner, 1981; Lehrer, 1996; Jander, 1997). An orientation flight at the nest entrance begins as a departing bee turns and hovers back and forth, turning in short arcs, apparently looking at the hive entrance. Then, the bee increases the size of the arcs until, after a few seconds, she flies in circles while ascending to heights of 5–10 m above the ground. This spiraling flight takes the bee out of sight of human observers. She returns a few minutes later, always without nectar or pollen.

Naturalists recognized this behavior long ago, but Becker (1958) was one of the first to examine its role in long-distance homing. She caught bees when they returned from their first

flight, marked them and then displaced them to various sites in the landscape. From a range of distances within 700 m of the nest, many bees returned home, whereas bees displaced without orientation flight experience did not. Thus, the bees that made orientation flights must have learned something about the landscape that allowed them to find home. The homing success was poorer from greater distances, which presumably reflects limits to the spatial scale that the orientation flight covers (Fig. 1). Becker (1958) also found that the ability to return home after a single orientation flight generally improves with increasing age. Intriguingly, bees with previous flight experience in a different landscape were more successful at homing from all distances. This suggests that something about their prior experience allowed them to acquire more information during the orientation flight than did bees whose orientation flight was their first foray outside the nest. A further observation was that intervening features such as forests or hills did not appear to hinder homing after an orientation flight (Becker, 1958), suggesting the possibility that the orientation flight allows bees to learn the relationships between visually isolated parts of the landscape.

Becker's work, although highly intriguing, left some very important questions unresolved. First, the data of Becker (1958) make it hard to assess the level of performance achievable after a single orientation flight, because she made no studies of bees that had had extensive flight experience in the landscape in which she worked. Second, it remains unclear whether the first orientation flight in a new terrain enables bees to learn only the visual features of the hive and its immediate surround or also allows them to learn landmarks located far from the hive. The latter possibility would be extremely interesting, because it would suggest that, during their first orientation flight, bees begin to piece together knowledge of the routes connecting visually isolated parts of the terrain. Becker's observation that bees reach home from great distances does not prove that bees have used landmarks at these distances; they might use a random or systematic search strategy to reach locations from which they could see the next location or landmarks near it. Third, the difference in homing ability between bees with different levels of visual experience is not adequately explained. The higher homing success of bees with prior experience in a different landscape may be a result of differences in the spatial scale of the orientation flight. Another explanation for the homing differences may reflect differences in the sorts of information that the bees are capable of learning (e.g. locations in the vicinity of the hive compared with routes connecting different parts of the terrain).

To examine these issues, we sought to replicate Becker's (1958) basic technique of testing the behavior of bees after a single flight, but using substantially refined methods. Whereas Becker (1958) released groups of bees at the same time and recorded only the fraction that returned home, we released bees singly so that we could measure the initial orientation of individual bees. This method is of crucial importance, because it allowed us to assess whether bees have learned the homeward direction associated with the landmarks at different release positions. Furthermore, we used the flight time between the release point and the hive to develop an improved assay of homing success. Finally, we used bees with foraging experience in the test landscape ('resident bees') as a comparison by which to judge the performance of the two categories of experimental bees: first-flight bees and forager bees whose previous flight experience was in a different locale.

We used six release positions, all far enough away to deprive the bees of a view of the nest, and otherwise differing in distance, direction and whether bees at the release position could see landmarks in the vicinity of the nest. From some release points, bees could probably see large landmarks immediately surrounding the nest, but from none of the release positions could bees see the hive itself. From the other release sites, landmarks immediately surrounding the hive were not visible; homing from these sites would be dependent on the bees' memory of the relationship between the visible landmarks and the nest itself. An ability of bees to fly efficiently homewards from these locations would imply that they had developed a simple route map for linking the release site to the hive (Cartwright and Collett, 1983; Dyer, 1996).

From each release position, the bees' initial orientation and eventual homing success should provide clues as to the nature of their learning of landmarks during the orientation flight. If only features of the hive itself are learned during the orientation flight, then we expected random orientation from all release positions (because none was close enough to provide a direct view of the hive). If the orientation flight is used for learning features of the local panorama around the hive, then we expected homeward orientation from sites with a view of such landmarks immediately surrounding the hive, and random orientation from sites without a direct line of sight to the nest environs. [Prior experiments in our landscape (Dyer, 1991) showed that bees do not fly high enough upon release to see over obstacles such as woodlots or hills.] Finally, if route
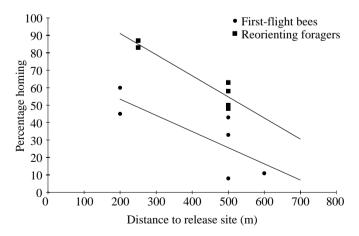

Fig. 1. Percentage of displaced bees reaching the hive from release points in a landscape in which they had made only a single orientation flight. The data were taken from Table 7 in Becker (1958). The equations for the regression lines are as follows: first-flight bees, $y=-0.093x+71.91$, $r^2=0.61$; reorienting foragers, $y=-0.121x+115.25$, $r^2=0.89$.

memories are formed during the orientation flight, then directed orientation from all sites would be expected, regardless of the location of the sites with reference to the hive; we anticipated, however, that there would be a ceiling effect on homing related to the distance of the release site. We examined these possibilities in honeybees with different types of prior flight experience to assess what particular factors account for differences in homing performance.

Materials and methods

Honeybees

We used honeybees from the Zoology Department research apiaries at Michigan State University. The bees were a mixture of European subspecies, predominantly hybrids of Italian-derived *Apis mellifera ligustica*.

Study sites

The experiments took place during the months of May–September of 1993–1996. We conducted the experiments near the Michigan State University Research Farms. Farmland, woodland and residential developments surrounded this abandoned pasture, named 'Greiner' after the owners of the property. Release locations A–F were established to cover a range of factors that might affect orientation ability. The sites all differed in the distance or the direction from the hive, or both (Fig. 2). All sites also differ in the panoramic scene visible from the release point. From sites A, B and F, bees could see landmarks directly adjacent to the hive, whereas sites C, D and E offered no view of landmarks near the hive. In addition, all sites except A and B differed in their distance from and direction towards the hive, and were in different directions; sites A and B were the same distance but differed in their direction from the hive.

Treatment categories

Building on Becker's (1958) studies of the orientation flight, we tested the homing ability of bees with various degrees of experience in a given terrain. We studied bees in three categories of experience. The details of handling the bees before their use in an experiment varied with treatment and will be discussed more fully below.

'Reorienting foragers' were worker bees that had foraging experience in a different landscape prior to the experiment. These bees came from small, queenright colonies that contained between 4000–12 000 bees and were maintained in a small forest clearing 8 km east of the study landscape. At this location, these bees were free to forage in the surrounding environment as members of healthy colonies. When introduced to the testing site, they performed an orientation flight on their first departure from the nest. Becker (1958) described these bees as foragers; in our experiments, they are called reorienting foragers to identify them as bees without previous flight experience in the Greiner landscape, although they did have ample flight experience in their home location. In Becker's study, such bees were called 'flight bees'.

'First-flight bees' had never been outside the nest before the experiment and, hence, were visually naive. Visually naive colonies were created using worker bees that emerged from their pupal cells in an incubator and were then installed into an observation hive. Two Langstroth frames of comb containing capped brood, pollen and nectar served as the foundation for each colony. Except for a mated queen, the only adult bees in these hives were bees reared in the incubator. Each 'naive' colony was kept inside the laboratory and fed 30 % sucrose solution when not employed in an experiment. The study site was the only landscape that these naive workers had the opportunity to explore, and their only flight experience in this terrain occurred during the orientation flight just before their test. These bees were tested starting 5–7 days after they emerged as adults and were sufficiently mature to begin flying. Becker (1958) called this category of bees 'beginners'.
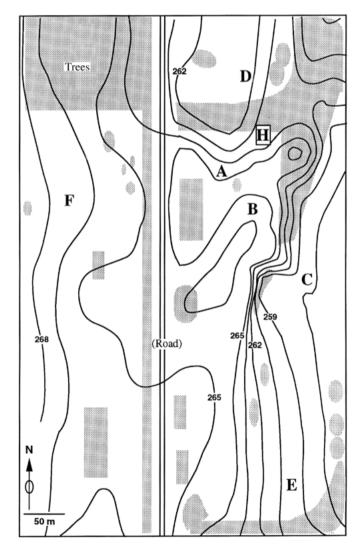

Fig. 2. Map of the Greiner study site showing the position of the hive (H), the six release sites (A–F) and the approximate topography, as indicated by the contour lines, showing approximate elevation in meters. The stippled areas indicate landscape features, such as trees, woodlots or buildings.

Finally, 'resident bees' were bees from a large queenright colony that was located in the test location. A two-storey commercial colony was placed in the testing landscape (site H in Fig. 2) and allowed to forage normally for at least 2 weeks. Foragers from this colony had ample opportunity to learn the terrain of the Greiner location; they were likely to have performed orientation flights well before we used them in the study. We tested their homing ability after capturing the bees on their return from a foraging flight. Becker (1958) did not test any bees in this category of experience.

General protocols

On the night before an experiment with reorienting foragers, we closed one of the hives after injecting smoke into the entrance, and then placed it in the back of a truck and drove it to the laboratory. The next morning, we moved the hive to the testing landscape. Some aspect of this experience induces the foragers bees to perform reorientation flights on departure from the nest on the subsequent morning (see Robinson and Dyer, 1993). Preliminary trials using this procedure indicated that at least 95 % of the bees that fly out of these colonies during experimental manipulations have at least some previous flight experience on the basis of trials in which all bees with experience in the training site were identified using a self-marking system (Boylan-Pett et al., 1991).

For trials with first-flight bees, we carried the colony from the laboratory to the field site on the morning of the experiment. Workers in these hives were sufficiently motivated to perform orientation flights without the addition of smoke.

Reorienting and first-flight bees were allowed to complete one orientation flight at Greiner by moving a closed beehive into the pasture and placing it at the location marked H in Fig. 2. After a 20 min settling period, we opened the colony and allowed 60–100 bees to depart. We then closed the colony and removed any bees that remained on the colony entrance. Bees that departed typically conducted an orientation flight that took them away from the hive for several minutes. When they returned, we captured each bee singly in a 20 ml glass vial and recorded the duration of its time away from the nest during the orientation flight measured from the time when we opened the hive. The duration of the orientation flight is an approximate measure of the exact time the bee was away from the nest.

Resident bees were experienced foragers that we captured as they landed on a screen that had been placed loosely on the entrance of a hive that had stood at H for several weeks. This screen slowed the bees' entrance to the hive and allowed us to capture them in vials. To ensure that the bees we selected were foragers and not simply bees returning from an orientation flight, we preferentially selected bees carrying pollen or bees that were obviously engorged with nectar. The screen was removed after we had captured 40 bees. The captured bees were subsequently handled in the same manner as the bees in the other treatment conditions.

The vials containing the bees were placed in an ice-filled cooler for 2–10 min to chill the bees and immobilize them for marking. After chilling, each bee was labeled individually with a plastic numbered tag, placed in a clean vial and fed with an unscented 25 % sucrose solution. While there is some evidence that localized cooling of small parts of the bee brain affects the formation of long-term memories (Erber et al., 1980; for a review, see Menzel, 1990), there is no evidence that cooling the whole bee affects memory (Menzel et al., 1974) or learning in the context of our experiments.

We conducted a short experiment to determine whether unknown features of the Greiner landscape might have biased the orientation of bees in ways unrelated to learning during the orientation flight. In particular, we wanted to know whether there was any tendency for bees that had not completed an orientation flight, but were motivated for homing, to head towards the location where the test hive was normally placed. For this control, we trained bees from a colony at the Entomology Teaching and Research Site (Entomology Site) to find a feeder located approximately 3 km south of their colony. We marked the bees with plastic numbered tags while they drank sugar water from the feeder. After at least 3 days of foraging experience, the tagged bees were captured from the feeder in vials just as they were about to head home. The Entomology Site is located approximately 2.5 km from the Greiner landscape. We released these bees singly at Greiner site A while a large test beehive stood at H. We expected that the bees would not be oriented towards H and that they would not actually reach the hive at H. If anything, bees might be expected to fly north, the direction of the 3 km homeward flight from the feeder at the Entomology Site. Alternatively, the bees might fly to the west, the actual direction of the Entomology Site from Greiner. If bees headed towards H, then we would conclude that some bias existed that tended to draw bees towards that location. This result would indicate that the orientation flight does not provide information that was not already available to bees flying in the landscape for the first time.

Data collection and analysis

Except where noted, we tried to test bees only on warm and sunny days to remove any confounding influences of weather. One hive and one release site were used for one experiment on each test day. We displaced one group of approximately 40 bees and released them one by one. Two or three observers tracked the bees visually. Each bee's vanishing bearing, or the compass bearing at the moment when the bee vanished from human sight, was measured with a compass (Dyer, 1991). Bees usually flew in a few wide circles just after release, no more than 10 m off the ground, then chose a flight direction leading away from the release site. Most bees could be followed by sight for more than 40 m. Bees lost from view within 10 m, or those still moving on a circular flight path when they vanished, were not measured. This measurement is commonly used in studies of insect orientation (Gould, 1986; Dyer, 1991, 1993; Dyer et al., 1993). Some bees needed additional food in order to fly; we fed these lethargic bees sugar solution from a pipette and allowed them to rest for at least 3 min before releasing them.

We conducted multiple trials in each treatment category until we had compiled at least 40 vanishing bearings per release site

for each treatment. This required many more than 40 releases per site because departing bees were often lost from view before a reliable vanishing bearing could be obtained. Across all treatment groups and all release sites, we recorded vanishing bearings for 45 % of all bees released (*N*=3055). There was no reason to suspect that this resulted in a biased sample.

We made every attempt to remove possible effects of observer bias in recording the vanishing bearings. These studies were conducted over a 4 year period, however, and a number of field assistants participated in the collection of the observations. Because of the nature of the experimental procedure, it was not possible to conduct blind experiments. We are confident that the data represent a conservative measure of the bees' homing ability because of the strict criteria that we established for measuring the flight direction.

The vanishing bearings were plotted on polar histograms to illustrate the directions flown by the bees. Statistical analyses of the circular distributions follow Batschelet (1981). The mean angle of a distribution of vanishing bearings is the 'center of mass' of the data on a polar histogram. The mean vector length, **r**, is a measure of the concentration of the data points, or the degree of clustering (Batschelet, 1981). For perfectly aligned data, **r**=1 while for randomly distributed data, **r**=0. The Rayleigh test was used to determine whether the circular distributions were non-randomly distributed. The mean angle is considered significantly different from a predicted angle if the prediction lies outside the 95 % confidence interval of the mean, as determined from Fig. 5.2.1 of Batschelet (1981). The length of the mean vector, **r**, and the sample size (Batschelet, 1981, p. 86) influence the angle of deviation.

To record homing times, an observer stationed at the hive during the releases captured marked individuals and noted the time of their return. From synchronized stopwatches at the release point and at the hive, we could determine the flight duration to the nearest minute. Bees that did not return within 45 min were not included in the analysis because we observed the hive for only 45 min after the release of the last bee. This procedure placed an upper limit on the homing time, which resulted in a right-censored distribution of homing times. The data on homing speed of the bees were treated with survival analysis. This statistical method employs nonparametric procedures to examine data for which there is a defined point event, conventionally called 'failure', that occurs after a given length of time (Cox and Oakes, 1984; Muenchow, 1986). In this case, the homing time, or the time elapsed from a bee's release until its arrival at the hive, is equivalent to its 'failure time,' even though by failure to stay in the field it is actually succeeding in its goal to reach home.

The analysis compares the distributions of homing times from different treatment groups or from different release sites. Its great advantage is that it can deal with the presence of non-normally distributed data that are 'censored' because of the termination of the experiment or the attrition of subjects from the sample (SAS Institute Inc., 1988; Fox, 1993). Using the univariate SAS procedure LIFETEST in a computer program written by Fox (1993), we completed estimates of the survival distributions and computed rank tests for association of the

response variable, homing time, with the variables of treatment and release site within a single factor. Wilcoxon tests tested the homogeneity of the survival curves, which allowed us to determine whether the distributions differ between the three treatments. The Wilcoxon rank statistics and covariance matrices were used to conduct pairwise comparisons among the treatments and among the release sites (Fox, 1993, 1996).

Other linear statistical methods follow Zar (1996).

Results
Duration of the orientation flight

For the experiments at Greiner, we used a general linear model procedure to test for the effects of the two treatments, reorienting and first-flight, on the duration of the orientation flight. We found a highly significant effect of treatment (*F*=13.99, d.f.=1, *P*<0.002) on the duration of the orientation flights of bees in each treatment group. Bonferroni *t*-tests revealed that the reorienting bees had a longer mean return time (9.6±0.18 min, mean ± S.D., *N*=1243) than the first-flight bees (8.4±0.17 min, *N*=1001).

Homing at Greiner
Control experiment for orientation bias at Greiner

The vanishing bearing for bees displaced to Greiner site A from the Entomology feeder are plotted in Fig. 3. As illustrated by the polar histogram, the bees are randomly distributed (Rayleigh test, *z*=0.9633, *N*=57, not significant). Additionally, of 91 bees released across 3 days, only one arrived near the hive placed at H, the normal location of hives during our
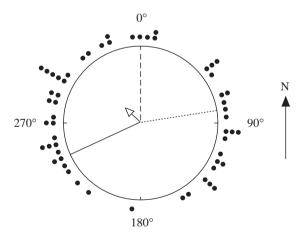
Fig. 3. Polar histogram showing the distributions of vanishing bearings (*N*=57) for bees taken from a feeder at the Entomology Teaching and Research site on Collins Road and released at Greiner site A. The polar histogram illustrates the mean bearing (322 °) with an arrow, and the length of the mean vector (the length of the arrow) (**r**=0.13) represents a measure of dispersion. The solid line marks the true direction of the Entomology Site from Greiner. The dashed line indicates the direction from the feeder at the Entomology Site to the hive. The dotted line shows the direction from site A to the hive at Greiner. These data indicate that there is no bias in the landscape (Rayleigh test: *z*=0.9633, not significant).

experiments. It is unlikely, then, that bees in the experiments would find the hive using some feature intrinsic to the landscape but not related to the orientation flight.

Vanishing bearings

At all release locations, resident bees were non-randomly oriented and headed in the predicted homeward direction (Fig. 4). Table 1 summarizes the conclusions from the vanishing bearing data which suggest that randomly selected resident foragers were well acquainted with the landmarks around the release sites.

Sites A and B were both located in the same part of the landscape as the hive and should have offered bees a view of landmarks directly associated with the hive (Fig. 2). The sites
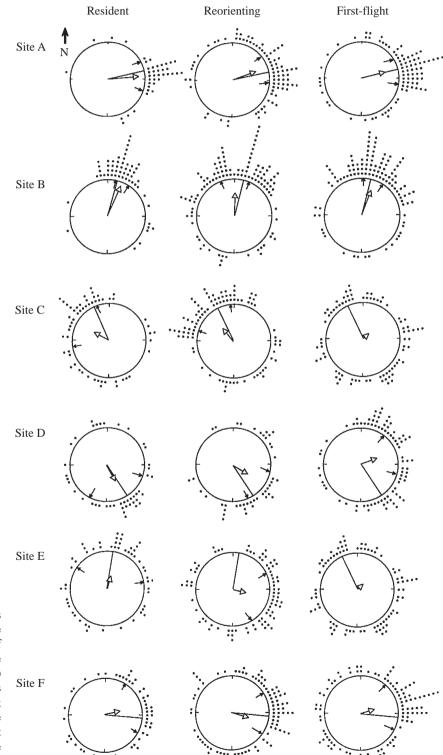
Fig. 4. Polar histograms showing the distributions of vanishing bearings for bees released in the Greiner landscape. The open arrow in the center of each polar histogram shows the mean bearing; the length of the mean vector (the length of the arrow) is a measure of dispersion. The radius line marks the true homeward direction, and the small black arrows indicate the 99% confidence interval of the mean. A mean bearing is considered not significantly different from a predicted angle if the prediction falls within the confidence interval.

Table 1. *Descriptions of release sites and summary of conclusions based on polar histograms of vanishing bearings*

| | Distance to hive (m) | Homeward direction (degrees) | Treatment group | Vanishing bearing | | | | |
				Mean (degrees)	N	r value	Rayleigh test, z	Significance
Site A	100	79	Resident	85	40	0.81	26.24	*P<0.001*
Site A			Reorienting	72	82	0.54	23.91	*P<0.001*
Site A			First-flight	79	84	0.62	32.29	*P<0.001*
Site B	100	14	Resident	21	57	0.8	36.48	*P<0.001*
Site B			Reorienting	1	105	0.5	26.25	*P<0.001*
Site B			First-flight	18	106	0.63	42.07	*P<0.001*
Site C	165	335	Resident	299	56	0.37	7.67	*P<0.001*
Site C			Reorienting	321	88	0.36	11.40	*P<0.001*
Site C			First-flight	62	79	0.10	0.79	*P<0.5*
Site D	70	151	Resident	154	44	0.36	5.70	*P<0.005*
Site D			Reorienting	130	43	0.43	7.95	*P<0.005*
Site D			First-flight	74	83	0.39	12.62	*P<0.001*
Site E	375	8	Resident	15	53	0.28	4.16	*P<0.02*
Site E			Reorienting	106	79	0.31	7.59	*P<0.001*
Site E			First-flight	74	75	0.22	3.63	*P<0.05*
Site F	500	94	Resident	78	48	0.36	6.22	*P<0.002*
Site F			Reorienting	92	88	0.36	11.40	*P<0.001*
Site F			First-flight	77	96	0.30	8.64	*P<0.001*

were located in different compass directions from the hive, however. Both reorienting foragers and first-flight bees were non-randomly oriented and headed in the homeward direction upon release at both site A and site B (Fig. 4).

Site F is similar to sites A and B in that it also offers a view of the landscape around the hive, although it is located quite distant from the hive itself (Fig. 2). Again, reorienting foragers and first-flight bees were significantly oriented in the homeward direction from this site (Fig. 4). From this site, located 500 m from the hive, human observers could see the hive in the Greiner landscape. It is unlikely that bees could see the hive from this location, but it is possible that bees could see other visual orientation cues (landscape scale features such as woodlots or treelines) to allow them to depart successfully in the homeward direction. Other possible explanations for the clustered vanishing bearings here could be wind coming predominantly from the west or the position of this site on the high end of a hill. Additionally, if bees oriented their departures towards the azimuth of the sun (which was in the east during these experiments) (see von Frisch, 1967; Vollbehr, 1975), then it may not be possible to attribute homeward orientation of either group to landmarks. Thus, it is possible that neither group of bees new to this site learned the route connecting it with the hive.

Site C was visually separated from the visual panorama around the hive by a woodlot and a small hill, but it is located relatively close to the hive (165 m). At this site, reorienting foragers were non-randomly oriented and headed in the predicted direction, while first-flight bees vanished in random directions (Fig. 4; Table 1). That bees with previous flight experience are clustered towards home suggests that they had learned something during the orientation flight about the route connecting this release position with the hive, while naive bees had not.

Site E was both visually and spatially separated from the hive and the home landscape (Fig. 2); hence, homing from this site would require a route memory. While the mean vanishing bearing indicates non-randomness, reorienting bees were not headed in the homeward direction (Fig. 4). The vanishing bearings of first-flight bees released here appeared to be clustered, but within very wide confidence intervals, giving less weight to the histogram peak (Fig. 4). Bees in both categories of visual experience generally departed in an easterly direction and did not appear to be oriented homewards. At this site, bees often circled high into the air before flying in a given direction, which may be an indication of their uncertainty (Wolf, 1926; Menzel et al., 1990; Schöne et al., 1995).

Like sites C and E, site D also does not offer a direct view of the hive or of landmarks associated with it. This site, however, is located only 70 m behind the hive (Fig. 2) and it offers a very different visual scene from other sites; it is surrounded by a series of woodlots and a few dead trees. Reorienting foragers were oriented and headed homeward at this site (Fig. 4; Table 1), but first-flight bees, while significantly oriented, did not consistently choose the homeward direction (Fig. 4). Like the bees in these treatment categories at site E, the bees tended to depart towards the east. Upon release from the vials, the bees often circled extensively and flew high overhead before disappearing. The results resembled those at site C, where reorienting and resident bees, but not first-flight bees, behaved as if relying on knowledge of the route between the release site and the hive.

To summarize the results from measurements of vanishing bearings, we found that all three treatment groups oriented homewards when in the vicinity of landmarks near the hive (sites A and B). When bees were released out of sight of these

landmarks, hence forcing them to rely on a route memory, the first-flight bees were confused, the reorienting bees did head in the homeward direction except at the most distant site, and the resident bees were consistently oriented homewards. Certain of the polar distributions show random or highly skewed orientations that contain clusters of vanishing bearings headed towards home. It is possible that some fraction of the bees in such samples had learned the homeward direction at the release site. Still, we can safely assume from these distributions that the bees are on average more confused.

Homing speed

We used survival analyses to examine the flight times between release point and the hive. The cumulative percentage homing time and its standard error were calculated using the SAS procedure LIFETEST, which uses the χ^2 approximation for Wilcoxon tests plotted against time. Note that this technique compares groups not only on the the basis of eventual homing success but also on the basis of the shapes of the cumulative homing curves.

The homing curves of resident, reorienting and first-flight bees at Greiner are shown in Fig. 5. Generally, for resident bees, the homing curves for bees released from all sites are similar in shape and in the total numbers of bees returning to the hive; there is rapid initial accumulation over the first 10 min, followed by a gradual reduction in the rate of arrival at the hive. However, the differences between the sites imply that the bees at each position may differ from one another. The Wilcoxon test supports the implication that the treatment groups differ (χ^2=21.7, d.f.=5, P<0.0006). This statistic means that homing from the six sites differs, but does not reveal which sites differ from one another. We used the covariance matrix for the Wilcoxon statistic generated by the SAS procedure to calculate z statistics for each pairwise comparison and used a Bonferroni adjustment to stabilize the experiment-wise error rate, as suggested by Fox (1993). Table 2 contains the results of these multiple comparisons; in only three cases do these tests indicate statistical differences between two curves. Each of these significantly different comparisons involves site F, a distant release point, with other sites close to the hive. The cumulative percentage homing from all sites is roughly equivalent for all sites at the termination of the experiment, which supports the idea suggested by the vanishing bearing data that there are not many differences between the homing abilities of resident bees across sites. The existing differences imply that homing is faster from closer sites.

For reorienting foragers, the Wilcoxon test of homogeneity of the homing curves supports the inference that the release sites differ (χ^2=75.63, d.f.=5, P<0.0001). Bees released at site D, the closest release location, have the highest percentage of return. The two most distant sites, E and F, have curves with the slowest rate of return and after 29 min, begin to diverge. The curve for site A resembles the curve for site B until the 15 min mark; after this time, the accumulation rate of site A bees at the hive resembles that of bees released from site C. The multiple-comparison testing reveals statistical support for
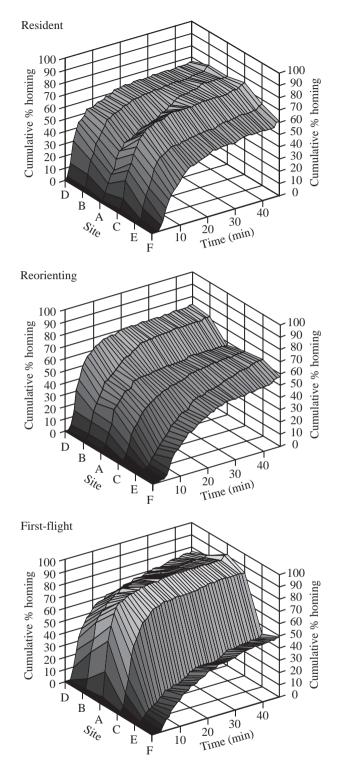

Fig. 5. Cumulative percentage homing for bees released at Greiner. Each wire-frame plot illustrates the cumulative frequency of bees homing over time from the six release sites. The results from each of the three treatment groups are plotted. Resident bees had the most uniform homing rate across all sites, while first-flight bees were more variable. The surface created by the curves for first-flight bees suggests that homing is dependent upon an interaction between the distance and the visual surround. In all treatment groups, the effect of distance upon homing rate is clearly illustrated. Tables 2–4 show the results of pairwise comparisons.

Table 2. *Comparisons of resident bees homing at Greiner*

Comparison	z score	Probability	Conclusion
A and B	1.478	0.069	Same
A and C	2.048	0.02	Same
A and D	1.97	0.024	Same
A and E	0.783	0.217	Same
A and F	1.998	0.023	Same
B and C	0.378	0.353	Same
B and D	0.356	0.361	Same
B and E	1.903	0.028	Same
B and F	3.037	0.001	Different
C and D	0.012	0.50	Same
C and E	2.41	0.01	Same
C and F	3.671	0.00001	Different
D and E	2.331	0.01	Same
D and F	3.552	0.0002	Different
E and F	0.944	0.173	Same

The comparisons are based on Wilcoxon rank statistics and involve a Bonferroni adjustment to keep the experiment-wise error rate to $P<0.05$.

The z scores listed correspond to their single-comparison probabilities. For multiple comparisons, the corrected cutoff point for significance depends on an adjusted $z>2.932$.

These statistics correspond to Fig. 5.

Table 3. *Comparisons of reorienting bees homing at Greiner*

Comparison	z score	Probability	Conclusion
A and B	2.323	0.01	Same
A and C	1.404	0.08	Same
A and D	3.579	0.0002	Different
A and E	3.193	0.0007	Different
A and F	3.262	0.0006	Different
B and C	3.353	0.0004	Different
B and D	0.891	0.192	Same
B and E	5.078	1.912×10^{-7}	Different
B and F	5.156	1.264×10^{-7}	Different
C and D	4.595	2.168×10^{-6}	Different
C and E	1.523	0.064	Same
C and F	1.574	0.058	Same
D and E	6.589	2.212×10^{-11}	Different
D and F	6.684	1.161×10^{-11}	Different
F and E	0.042	0.483	Same

The comparisons are based on Wilcoxon rank statistics and involve a Bonferroni adjustment to keep the experiment-wise error rate to $P<0.05$.

The z scores listed correspond to their single-comparison probabilities. For multiple comparisons, the corrected cutoff point for significance depends on an adjusted $z>2.932$.

These statistics correspond to Fig. 5.

the patterns illustrated by Fig. 5; the plots of homing frequency that resemble one another (for example, sites F and E) are not statistically different from one another. In general, curves that appear visually distinct from one another are statistically different. The majority of the pairwise comparisons indicate statistical differences between the curves (Table 3). Homing is generally faster from the nearer sites.

In the figure illustrating the homing frequencies of first-flight bees (Fig. 5), there is a dramatic difference between the shapes of the curves for bees released at the most distant sites and those at the closer sites. Sites E and F have a similar form to one another, but are quite different from sites A, B or C. Site D, which does not share a similar visual surround with the other sites, is the closest release location. The cumulative homing curve at this site is intermediate to the other curves. The populations of first-flight bees differ ($\chi^2=269.26$, d.f.=5, $P<0.0001$) as given by the Wilcoxon test of homogeneity across the release sites. Pairwise multiple comparisons between the release locations indicate statistical differences between most sites (Table 4). Only three sites, A and B, B and C, and C and D, are not different from one another. Once again, there is a distance effect. Of the three homing surfaces illustrated in Fig. 5, the surface for the first-flight bees appears less consistent across sites than in the other treatment categories.

Flight behavior of displaced bees on release

We observed the bees carefully as they departed from the vials and, although it is difficult to describe their flight behavior quantitatively, we noticed a behavior that warrants further discussion. During a typical release, the bees circled around the release position before departure. Occasionally, after the bee had disappeared from the area, we noticed a marked bee flying around the site as if lost. This behavior occurred only with first-flight and reorienting bees, never with resident bees. We caught these bees to prevent them from interfering with our observations of bees in subsequent

Table 4. *Comparisons of first-flight bees homing at Greiner*

Comparison	z score	Probability	Conclusion
A and B	2.459	0.0069	Same
A and C	4.501	3.381×10^{-6}	Different
A and D	6.985	1.429×10^{-12}	Different
A and E	12.0	0.000001	Different
A and F	13.204	0.000001	Different
B and C	1.768	0.039	Same
B and D	4.057	0.00003	Different
B and E	8.342	0.000001	Different
B and F	9.961	0.000001	Different
C and D	2.386	0.009	Same
C and E	6.667	1.304×10^{-11}	Different
C and F	8.612	0.000001	Different
D and E	4.016	0.00003	Different
D and F	6.332	1.21×10^{-10}	Different
E and F	3.125	0.0009	Different

The comparisons are based on Wilcoxon rank statistics and involve a Bonferroni adjustment to keep the experiment-wise error rate to $P<0.05$.

The z scores listed correspond to their single-comparison probabilities. For multiple comparisons, the corrected cutoff point for significance depends on an adjusted $z>2.932$.

These statistics correspond to Fig. 5.

releases. It became clear that the tendency of bees to return to the release site was greater with first-flight bees than with reorienting bees and was greater at release sites not offering a view of the hive environs. This pattern provides a further indication of the interplay between previous flight experience and current visual conditions in influencing homing performance in bees with experience limited to a single orientation flight.

An interesting situation involving this flight behavior occurred during an experiment when the weather did not cooperate with our plans. On 3 August 1994, we initiated an experiment with a naive colony. Normally, we conducted experiments only when celestial cues were available to the bees, but on this day, the first-flight bees completed their orientation flight under a completely overcast sky. Between the time of the orientation flight and the release time at site E, the sky had cleared and the sun appeared. Of the 37 bees that we released, only three returned to the hive. Most of the bees (81 %) returned to the release site and were captured there. Four bees remained unaccounted for at the end of the experiment. On no other occasion did so many bees return to the release site.

One explanation for this result might be the change in the sky conditions between the time of the orientation flight and the time of the displacement. It is possible that the novelty of the celestial conditions induced confusion in the bees; the first-flight bees were not given the opportunity to learn anything about the sun or the sun-linked patterns of polarization in the sky during their orientation flight. The appearance of solar information may have interfered with the bees' ability to use learned landmark information to choose the homeward direction. Another possibility is that the shape of the orientation flight is different under an overcast sky, as would be suggested by the results of Vollbehr (1975), who reported that orientation flights are directed towards the sun's azimuth.

Discussion

The orientation flight is a dramatic turning point in the life of a young bee. Having worked for 2–3 weeks in the confines of the hive, the bee performs a specific behavior that allows her to begin to learn features of the landscape that she will use during the rest of her life as a forager. Our research confirms that extremely rapid learning occurs during the orientation flight and that the acquired information can be used immediately for homing.

Our research has replicated the pioneering findings of Becker (1958) and provided new insights into the acquisition of spatial information that occurs during the orientation flight. Our results indicate that the ability to find home when displaced into the landscape after a single orientation flight is partially dependent on the distance of displacement and on previous visual experience. This distance effect implies, not surprisingly, that the quality of learned information is highest for locations near the nest.

The vanishing bearings show that, from distances of 100–200 m, bees are guided not by a random or systematic search strategy, but can select the home direction upon initial departure from a site experienced during a single flight in the landscape. From none of the release sites should bees have been able to see the hive directly, so homeward orientation implies that bees learn more than merely the appearance of the hive during the orientation flight. Specifically, at least in the case of reorienting bees, the orientation flight must have allowed the bees to form a memory of the route connecting visually isolated parts of the terrain. Homing was better from sites that offered a view of the landmarks in the vicinity of the nest than from sites visually isolated from the nest environs, even when the distance was roughly the same. For example, bees in the same treatment category homed more directly from sites A and B than they did from sites C and D, even though the former sites were not very much closer to the nest; indeed, site D was the closest of all. In addition, homing was better from site F than from site E, even though they were both located quite far from the hive.

Another important contribution of our research is the insight it provides into the effects of prior experience on homing performance. The vanishing bearing data from sites C and D indicate that reorienting foragers can learn landmarks that function as simple routes on the basis of a single flight in the landscape, but newly flying bees (first-flight bees) cannot. In the normal context of behavioral development, bees may conduct more than one orientation flight prior to the initiation of foraging in order to develop fully their knowledge of the landscape (von Frisch, 1967; Wehner, 1981). One possible explanation for this may be found in the shorter duration of orientation flights in first-flight bees than in reorienting bees: perhaps they simply did not cover as much territory. However, although the difference in orientation flight duration is significant, it does not seem large enough to explain the apparent inability of bees to learn the routes home from sites C and D, which were not much further away from the hive than sites A and B. Of course, these first-flight bees might have structured their orientation flights differently, staying out almost as long as reorienting bees but not venturing far from the hive.

Another intriguing possible explanation for the difference between first-flight bees and reorienting bees is that their prior experiences equip them differently for the task of learning new landmarks during their orientation flights. First-flight bees must learn and acquire landmark information, while reorienting bees are challenged with 're-learning' visual features of a new landscape. In addition, first-flight bees have never had the opportunity to learn the sun's course: perhaps this knowledge is necessary for efficient learning or homing. Re-learning might require longer orientation flights because of constraints in the way that 'old' and 'new' memories are processed, separated or stored. It could possibly enable the reorienting bees to learn spatial relationships that the first-flight bees cannot, especially routes connecting visually isolated parts of the terrain.

Although first-flight bees were generally rather poor in their

homing performance from distant release sites or from sites requiring the use of route memory, they did quite well at other sites. The vanishing bearing data from sites A, B and F are particularly striking. These three sites correspond to locations that have a direct view of landmarks in the immediate vicinity of the nest. That naive bees appear as well oriented as bees in the other two treatment categories is impressive given their brief flight experience overall and their limited exposure to this terrain.

The vanishing bearings for reorienting and first-flight bees at site E and for first-flight bees at site D are significantly oriented on the basis of the results of the Rayleigh test, although the bees are not consistently headed in the homeward direction. In all three of these cases, the distribution of bearings is spread out mainly in the easterly direction. The bees fly away towards the east, towards the area of the sky where the sun is located when these experiments were conducted. Vollbehr (1975) demonstrated that the first orientation flights completed by bees are aimed at the sun's azimuth and that they probably approach the hive after the orientation flight from the direction of the sun. It is possible that the sun affects the behavior of the bees when they cannot orient using non-celestial visual landmarks. The vanishing bearings collected at site F across all treatments are also headed in the easterly direction, which corresponds to the direction homewards from this site. That there is a bias at sites E and D may mean that the patterns at site F are also the result of a bias. Replicating these experiments at a time of day when the sun was in the western sky, or using release locations with different compass directions relative to the hive, would help to elucidate the effect of the sun on homing after an orientation flight.

One somewhat surprising pattern is that differences in homing success measured by vanishing bearings are not always congruent with differences measured through survival analysis. Given that the vanishing bearing data for bees at sites A and B in the three treatments appear equally well oriented, the results of the survival analyses are somewhat perplexing. At sites A and B, the first-flight bees accumulate at the hive more rapidly than bees in the other treatments. Their rate of return is faster than that of reorienting bees or resident bees, and this difference is highly significant.

In addition, at site C, where the vanishing bearings seem to indicate that the first-flight bees are disoriented, the corresponding plot of cumulative percentage homing indicates that the first-flight bees do not differ from the resident bees. Or consider the data from site D: from the homing rate analysis, it appears that there are no differences between the treatments, but from the vanishing bearings, first-flight bees are not headed in the homeward direction. They are statistically non-random and appear to be distributed towards the east.

The discrepancies between the vanishing bearing and homing success data sets are perhaps not surprising if we consider that vanishing bearings indicate the bees' initial choice of the homeward direction only and not the flight behavior of the bees after their disappearance from the area. We had no way of knowing what they do (or what they see

and use to guide their flight) until they arrive at the hive. Any distractions, including the choice of an incorrect landmark, may steer them off course. Alternatively, bees that are randomly oriented (as in the case of first-flight bees at site C) may encounter a familiar scene immediately after disappearing from our view and quickly discover the route towards the nest.

The orientation flight briefly exposes honeybees to a new terrain, and yet this short experience in a novel area allows bees to recognize the homeward direction after displacement from the nest. This ability raises the question of how the orientation flight allows bees to encode visual information in memory so rapidly. Particularly intriguing is the ability, seen in reorienting bees, to learn simple routes during the orientation flight. Learning routes need not imply that bees learn the sequence *per se* of a series of scenes between a release site and the hive. An ability to learn the homeward direction relative to landmarks in each of a series of locations visually isolated from the nest environs would allow bees to follow routes efficiently even if they had not learned the sequence explicitly.

Understanding the spatial pattern traced out by bees during the orientation flight would provide invaluable insights into orientation behavior and learning. Consider, for example, the implications of two contrasting flight patterns. First, bees might trace out a floral-like pattern, heading away from the nest in one direction then, guided by a path-integration process, heading back homewards, then looping out in another direction. A pattern such as this is seen in ants searching for their nest when lost (Wehner and Srinivasan, 1981). This pattern would seemingly simplify the task of learning the homeward heading relative to landmarks in locations out of view of the nest, because the bees could simply learn the visual images seen while being guided homewards by their path-integration systems. Alternatively, suppose that bees move away from the nest in a concentric spiral, then return home after a specified amount of time. This strategy would have at least two disadvantages compared with the 'flower-petal' strategy. First, the path-integration system would need to keep track of position for the entire orientation flight, instead of restarting on each loop back to the nest. Furthermore, bees would have to learn homeward headings relative to landmarks that they have seen while flying not radially but tangentially relative to the homeward direction. In other words, the bees' view of landmarks during learning would be different from the view they would have when aimed homewards, which would seem to require some sort of internal transformation not needed in the example of the flower-petal flight path. The actual paths of orientation flights by honeybees can now be followed using new radar tracking technology (Riley et al., 1996), so it may be possible to elucidate this problem.

The research reported here provides a firmer basis for the further study of learning processes in honeybees. First, our experimental approach has proved to be a reliable and robust method for studying performance after known degrees of experience with spatial relationships in a natural environment. Furthermore, we have documented a variety of specific factors that influence the performance of bees in using learned

information about the terrain. These include the distance of the release site, the visual isolation of the release site from the nest and the level of the bee's previous experience in the terrain. Preliminary evidence suggests that the presence of celestial cues is another influence either on the acquisition of spatial information about landmarks or on the use of such information. Further studies of these factors and how they interact should lead to a deeper understanding of the learning processes underlying a naturally occurring behavior.

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