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Bees travel novel homeward routes by integrating separately acquired vector memories

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Abstract. The question of whether bees can take novel short cuts between familiar sites has been central to the discussion about the existence of cognitive maps in these insects. The failure of bees to show this capacity in the majority of previous studies may be a result of the training procedure, because extensive training to one feeding site may have eliminated or weakened memories for other sites that were previously trained. Here we present a novel approach to this problem, by rewarding honey bees, *Apis mellifera carnica*, at two feeding sites, one (S_m, 630 m southeast from the hive) at which they could feed in the morning, and the other (S_a, 790 m northeast) at which they could feed in the afternoon. We then displaced bees to S_a in the morning and to S_m in the afternoon either from the other feeding site or from the hive. Bees were also displaced to two novel sites, one at a completely unfamiliar location (S_4) and another that was located halfway between the two feeding sites (S_3). Bees displaced from either of the feeding sites never took novel short cuts; instead, they used the homeward directions that would have been correct had they not been displaced. Bees caught at the hive entrance, however, chose the correct homeward direction not only when displaced to both feeding sites, but also when displaced to S_3, although not from S_4. Control bees that had been trained to only one of the feeding sites were not able to travel directly home from S_3 excluding the possibility that bees used landmarks close to the hive. This is the first evidence that bees take a novel short cut by activating two vector memories simultaneously. The potential mechanisms of integrating the two memories are discussed. Since bees took novel short cuts in only one direction (to the hive) and only when displaced from the hive (not the feeders), we conclude that inference of a cognitive map in bees would be premature.

Navigation in honey bees, *Apis mellifera*, between the nest and feeding sites is governed by several orientation strategies and forms of memory (Wehner & Menzel 1990; Wehner 1992). Path integration provides the bee with a home-directed vector at any given time during its twisting search flight. This integration requires only a short-term, or working, memory, in which the calculation of home direction is updated from one moment to the next. The contents of this working memory are short-lived, that is, its contents from a minute ago are no longer accessible and have been replaced with the present estimate of home direction. Familiar sites in a bee's flight range can also be stored in long-term memory, so that the information can be retrieved after several hours or days. Several locations can be stored in the long-term memory at any one time. Such sites are specified in the bee's memory by means of goal-directed vectors (Wehner 1992; Chittka et al. 1995a). The vectors, both in path integration and orientation along familiar routes, are computed by relating the directional components to celestial cues (sun, polarized light pattern: Wehner & Wehner 1990; Wehner et al. 1996) and landmarks (Chittka & Geiger 1995a; Chittka et al. 1995b). The distance components are calculated using visual distance measuring routines (Esch & Burns 1996; Srinivasan et al. 1996) and, again,

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landmarks (Chittka et al. 1995a; Chittka & Geiger 1995b). Snap-shot memories of the visual scenes at the goals allow for precise location of the nest entrance and the flower (Cartwright & Collett 1983; Chittka et al. 1992; Collett 1992, 1996). The structure of the spatial memory arising from the interaction of separate long-term memories has been a question of debate over the last 10 years since Gould's (1986) claim that bees might employ a cognitive map sensu Tolman (1948) and O'Keefe & Nadel (1978), which would allow them to take novel short cuts between two familiar sites. Although various examples of novel short cuts have been described (Menzel 1989; Dyer 1991; Dyer et al. 1993; Capaldi & Dyer 1995; Geiger et al. 1995) none of them has indicated unambiguously a cognitive map, but instead could be explained as movements towards familiar landmarks seen from a new angle (Wehner & Menzel 1990; Dyer 1991; Menzel et al. 1996). Does this mean that insect navigation relies solely on stacks of independent memories about vectors, sites and routes, and does not use the potential of an integrated geocentric spatial memory structure (Thinus-Blanc 1987), in which vectors are associated with the visual scenes en route and at the goals?

It has long been known that bees retrieve a vector memory when compass cues are not available by referring to the landmarks associated with the vector, and can even use it as a reference for dance communication (von Frisch 1965; Dyer & Gould 1981). They also attach home vectors to the particular visual scene at the feeding site (Wehner et al. 1990; Menzel et al. 1996), and they indicate the correct vector towards the feeding site in their dance performance when they are stimulated inside the hive with the particular odour of the feeding site, even at night (Lindauer 1954, cited in von Frisch 1965, page 354f). Thus, long-term memories of goal vectors are indeed connected to visual scenes, and cues of the feeding site provide the opportunity to integrate local memories into geostable vector memories.

Here we ask whether novel navigational behaviours emerge from the combination of visual scene and goal vector memories. We show that novel routes are chosen on return to the hive from a release site that resembles features of two known feeding sites and discuss whether this novel behaviour can be explained (1) as a kind of path-integration process on a large scale, (2) as a compromise between conflicting memory-retrieval processes triggered by the visual scene, or (3) as an indication of a computational process integrating two simultaneously activated goal vector memories.

GENERAL METHODS

Study Area and Experimental Set-up

Honey bees, *A. m. carnica*, were trained and tested in a flat agricultural area close to the town of Amöneburg (North Hesse, near Marburg, Germany). The landscape is dominated by a cone-shaped mountain which is surrounded by a wide flat valley. We selected this area because the mountain provided a dominant distant landmark which could be seen from everywhere around it. Thus, release sites could be arranged such that learnt compass directions conflicted with the present position of the dominant distant landmark.

We trained bees to forage at two feeding sites, one (S_m) in the morning (0900 to 1200 hours) and the other (S_a) in the afternoon (1400 to 1700 hours). S_m was located 630 m southeast (115°) of the hive and S_a 790 m northeast (40°) of the hive (Fig. 1). Sugar solution (0.5–2 M sucrose solution depending on flight activity) was provided at each site at the particular times of the experiments. We chose this training procedure to establish two sets of vector memories in each bee. Regular foraging by a group of 20–40 bees was established within 2 days, and experiments started after the third day. S_m was located among harvested agricultural fields without local landmarks within a radius of 150 m. S_a was marked by a low bush standing about 60 m to the side. The mountain peak appeared at a visual subtended angle of 5° in height at S_m and of 8° at S_a. Thus, S_m and S_a differed with respect to the local cues and the appearance of the dominant distant cue.

The hive stood at the edge of a cornfield behind a small, low barn. Judging from the spatial resolution of the bee eye (1° angular visual resolution, Wehner 1981), the barn was not visible from distances greater than 120 m. Bees flying towards S_m from the hive crossed over uniform grassland and harvested fields without any obvious landmarks; those on the way towards S_a flew over a small country road in the first third of their flight and then continued to fly over harvested fields.

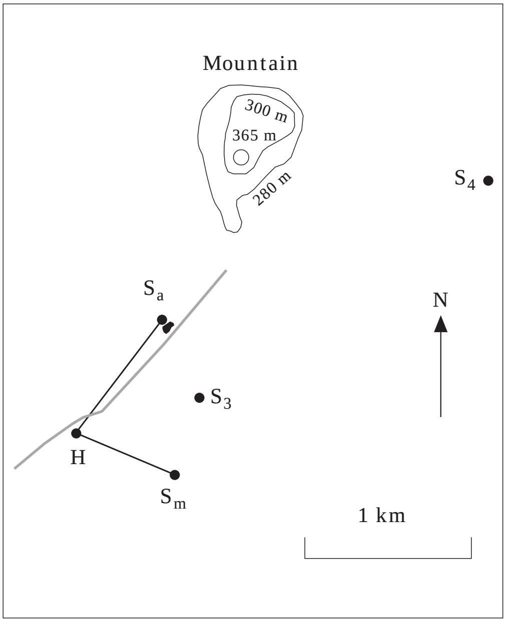

Figure 1. The feeding sites, S_a and S_m, and release sites, S_3 and S_4, used in the experiment. H: hive. The positions of a local landmark, a bush, near S_a, and of a road passing near both the hive and S_a are shown.

The mountain appeared to the left (under 90°) for bees heading towards S_m, and straight ahead for those flying towards S_a.

The release site S_3 was located in a large and uniform grassland area 720 m to the east of the hive (80°). A few scattered trees and a small clump of trees stood at distances greater than 200 m and, thus, should have been barely visible to the bees. The mountain top appeared at an elevational visual angle of 6° from S_3. The local cues at S_m and S_a were not visible to the bees from S_3. S_4 was located in a pasture 3.5 km northeast (60°) of the hive and east of the mountain peak (Fig. 1). Local landmarks were rather different from those at S_m, S_a and S_3; a row of bushes following a creek and a few scattered high trees further away.

Experimental Procedure

We performed the experiments late in 1991, 1992 and 1993 (August, September) when natural food sources were scarce. We introduced a two-frame colony in an observation hive into the study area immediately before training the bees to the feeding sites. There were no natural food sources in the sector (seen from the hive) between 0° (north) and 180° (south), and flowers that could have been visited were more than 500 m away to the west between 220° and 280°. Most importantly, there were no natural food sources around and between the experimental sites. It is most unlikely that bees visited natural food sources before being recruited to the feeding sites because of their distance. Therefore, the area was unfamiliar to the bees, at least with respect to information collected on foraging flights, and the bees visited only the experimental feeding sites. We marked a large proportion of the bees in the hive with number tags, and kept a full record of each individual bee visiting the feeding sites at any time over the whole experimental period. Each bee used in the translocation experiments visited the feeding sites for at least 2 days. The study period was limited to 2 weeks in each of the 3 years.

For a translocation test, we captured individual bees in 20 ml glass vials either at the hive entrance when they were just arriving at the hive or when they were leaving it (hive arriving and hive departing bees), or at the feeding station when they prepared to leave the feeder (after feeding to satiation) or at the moment they landed at the feeder but had not yet started feeding (feeder departing and feeder arriving bees). Up to eight bees were transported within 10 min to the release site and they were released within the next 10 min (see below for site details). Thus, bees were kept in the container for less than 20 min. Bees do not appear to learn anything about the landscape or the compass directions when they have an open view during transport (Geiger et al. 1994; Schöne 1995, 1996), but since illumination might influence their motivational state, we kept the bees in the individual containers in the dark for the whole time between capture and release. We carried out all of the releases in sunny or only partially overcast weather, so that either the sun could serve directly for compass orientation, or patches of the blue sky were available for bees to reconstruct the position of the sun by evaluating the plane of polarized light. Temperatures ranged from 15 to 29°C. No releases were performed if wind speed exceeded 12 km/h. We released bees individually to exclude social facilitation, and because we could determine the vanishing bearing of only one bee at a time. A new bee was released only when the last one had vanished from our visual range.

Using a compass, we measured the vanishing bearing of each bee (the angle being measured clockwise from the north) as it departed from the release site. As described elsewhere (Gould 1986; Menzel et al. 1990; Wehner et al. 1990; Dyer 1991) bees circled for a few seconds and then continued in a rather straight path in a certain direction. Three observers recorded the path of the bee by looking from below up towards the bee, which was clearly seen as a dark spot against the sky, and pointing towards the bee with outstretched arms. The last observer to lose the bee (well beyond 20 m in most cases) noted the vanishing bearing from the release site with an accuracy of 5°. Bees landing within sight or that were lost within a circle of less than 15 m were not recorded.

A bee's motivational state changes four times during a foraging bout, and these states may determine the kind and strength of navigational memories. Hive departing and feeder departing bees (either at S_m or at S_a) head towards a goal (feeder, hive), and have activated the respective goal vector memories. Hive arriving and feeder arriving bees (either at S_m or S_a) have just applied the respective goal vector memory. We may expect, therefore, that goal vector memories differ between bees with different motivational states. To test this possibility, we displaced bees from both feeders and the hive. These two categories were further broken down into bees that had just arrived (at the respective feeders or the hive) and bees that were about to depart from any of these locations.

Statistical Analysis

We employed standard circular goodness-of-fit tests to determine whether vanishing bearing distributions were significantly different from random distributions. In the case of unimodal distributions, the test employed was the *V*-test, which tests H_0 (the population directions are randomly distributed) versus H_A (the population directions are not randomly distributed around the circle, but instead are concentrated around a hypothetical direction; Batschelet 1981; Zar 1996). Wherever samples appeared not to have been drawn from von-Mises populations, we employed a modified Hodges–Ajne test for uniformity versus a specified angle (Zar 1996), which tests non-parametrically for uniformity against an alternative that specifies an angle. A condition of these tests is that the hypothetical direction of H_A is determined before the experiment is performed. In our case, we were interested in seeing whether the distributions were random (H_0) or centred on the hive-ward or feeder-ward directions (H_A), as described below. This condition is thus fully satisfied. For comparing two circular distributions, we employed the Watson U^2-test (Zar 1996). This non-parametric test procedure is recommended in place of the parametric Watson–Williams two-sample test when at least one of the sampled populations is not unimodal or when there are other considerable departures from the assumptions of the latter test procedure. As we could not ascertain whether the distributions' parent distributions followed a von-Mises distribution and whether the two distributions' population dispersions were the same, we employed the non-parametric test procedure. When we had tied data, we used the Watson U^2-test modified for ties.

Where bimodal distributions were clearly recognized, we employed the broken axis approach (Holmquist & Sandberg 1991), which tests H_0 (the population directions are randomly distributed) versus H_A (the population directions are not randomly distributed). This test also returns the mean angles for the two modes of bimodal circular distribution. However, there is no test yet available that tests whether either mode is centred on a particular direction.

In several cases, it was not possible to test whether our sample's apparent distributions were drawn from von-Mises distributions, and thus we did not estimate the confidence intervals of the distributions' mean angles. Since, in several instances, the samples were clearly bimodal, we also did not determine the median angles' confidence intervals. Instead, we relied on the tests mentioned above.

EXPERIMENT I: BEES DISPLACED ON ARRIVAL AT HIVE

Methods

Bees were caught on arrival at the hive after their return from one of the feeders. Bees returning from S_m in the morning were displaced to S_a and S_3, and bees returning from S_a in the afternoon were displaced to S_m, S_3 and S_4. The bees were motivated to get back to the hive to

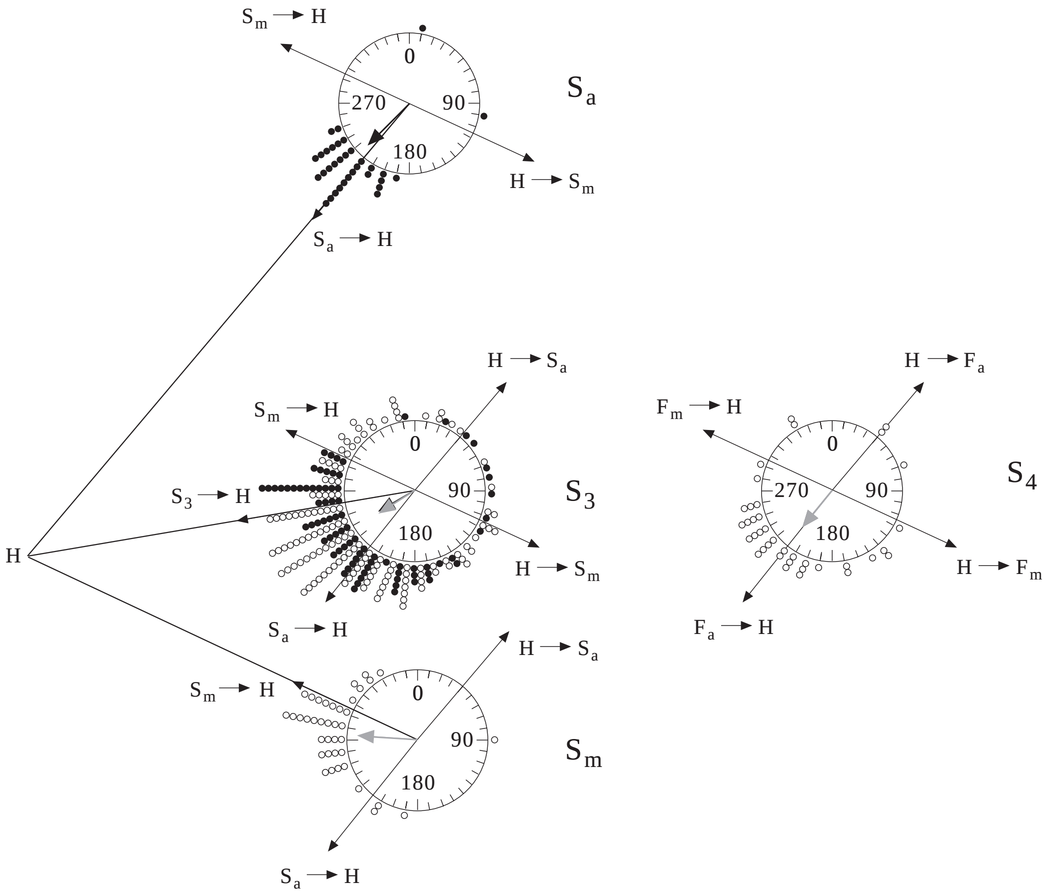

Figure 2. Distributions of vanishing bearings of bees caught on arrival at the hive from feeding sites S_m in the morning (●) and S_a in the afternoon (○) and subsequently displaced to S_m, S_a, S_3 or S_4. F_a and F_m indicate the feeding sites the bees have just come from. The thick arrow within the circle gives the mean angle of distribution of vanishing bearings as the centre of mass of the data on a polar histogram (indicated in the diagram with a black arrow for closed circles and a grey arrow for open circles). The two arrows for S_3 are very close. The number of bees released at each place (N) and the mean vector length (r) (Batschelet 1981) are: S_a: $N=33$, $r=0.86$; S_3: ●, $N=81$, $r=0.58$; ○, $N=130$, $r=0.57$; S_m: $N=39$, $r=0.83$; S_4: $N=37$, $r=0.61$. The long arrows in the diagrams indicate the directions the bees might have taken (see Fig. 1).

discharge their foraging load. Thus, we asked if these bees would take the correct direction to the hive.

As pointed out in the General Methods, it is unlikely that any landmarks near the hive were visible from S_3. Nevertheless, we performed a control experiment to exclude the possibility that those bees that chose the correct compass direction from S_3 used a simple piloting strategy. We used as control bees those that had visited only the afternoon site S_a. Again, these bees were caught on arrival at the hive from a foraging trip to S_a and displaced to S_3. If landmarks close to the hive were visible from S_3, these bees should choose the correct path towards the hive.

Results and Discussion

The bees oriented towards the hive at S_a, S_m and S_3; at S_4 bees chose the compass direction that would have brought them to the hive if they flew the same route once more (from S_a to the hive in the afternoon) (Fig. 2; S_m: Hodges–Ajne test, $P<0.05$; S_a: V-test, $P<0.05$; S_3: Hodges–Ajne test,

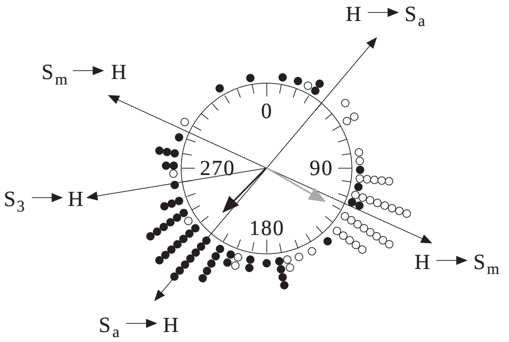

Figure 3. Distributions of vanishing bearings of bees released at S_3. These control bees had visited only one feeding site. Two groups of bees were tested: hive departing bees in the morning which foraged only at S_m (○, grey arrow), and hive arriving bees in the afternoon which foraged only at S_a (●, black arrow). The number of bees released (N) and the mean vector lengths (r) are: ○: $N=40$, $r=0.71$; ●: $N=55$, $r=0.574$.

$P<0.05$; S_4: Hodges–Ajne test, $P<0.05$). The distributions of bees released at S_a and S_m are significantly different from the distribution of bees released at S_3 at the respective times (Watson U^2-test corrected for ties in both cases, $P<0.01$). Control bees did not take the correct route towards the hive but picked the compass direction from S_a to the hive (Fig. 3; Hodges–Ajne test: $P<0.05$).

Our results show the following.

(1) Bees displaced to a feeding site that was visited only at another time of day managed to identify the release site correctly by its surrounding landmarks, and derived the appropriate home vector from these landmarks. This means that the bees can indeed retrieve memories specifying both entrained routes at any time of day.

(2) Bees displaced to an unfamiliar site located between the familiar feeders (S_3) took a direct course towards the hive, even though they had not previously visited this site. This is evidence that they can take novel short cuts. Provided they were not simply guided by landmarks at the hive visible from S_3, these bees must have integrated memories from both routes to steer a novel course.

(3) Bees displaced to the remote unfamiliar site, S_4, flew the compass direction that would have been correct if they had been displaced to the feeding site they had just come from (F_a). There is no statistically significant difference between distributions at S_a and S_4 (Watson U^2). This result indicates that bees do not simply calculate an average of familiar flight vectors whenever they find themselves at a novel site.

The finding that control bees picked the compass direction from S_a to the hive provides clear evidence that experience with both S_a and S_m is necessary to take a novel short cut from S_3.

EXPERIMENT II: BEES DISPLACED ON DEPARTURE FROM HIVE

Methods

Bees departing from the hive are motivated to get to one of the two feeding sites, depending on the time of day. We displaced departing bees to the novel site, S_3, or to one of the feeding sites at the 'wrong' time of day to see if they could take novel short cuts towards the feeder to which they were about to fly. We performed the same controls as in experiment 1, displacing hive departing bees to the remote unfamiliar site S_4 in the morning. To ensure that the bees that flew back home directly from S_3 did not simply use landmarks near the hive as cues we displaced to S_3 hive departing bees that had only visited S_m, to see if they would manage to fly directly to the hive.

Results and Discussion

Instead of flying towards S_m which was their original heading, bees displaced to S_a in the morning headed back towards the hive (V-test: $P<0.001$; Fig. 4). As in experiment 1, this indicates that memories normally used only at another time of day are accessible, but only to travel to the hive.

The distribution of vanishing bearings of bees displaced to S_m in the afternoon was bimodal (broken axis approach: $P<0.001$; Fig. 4). Neither of the two peaks of the distribution pointed to S_a, which was the bees' destination prior to displacement. Instead, roughly half of the bees vanished towards the hive, as did the bees released at S_a in the morning. The other half of the bees chose the compass direction from the hive to S_a, as if they had not been displaced. This result is in agreement with earlier findings, which showed that displaced hive departing bees often ignore even familiar landmarks and rely on compass cues exclusively

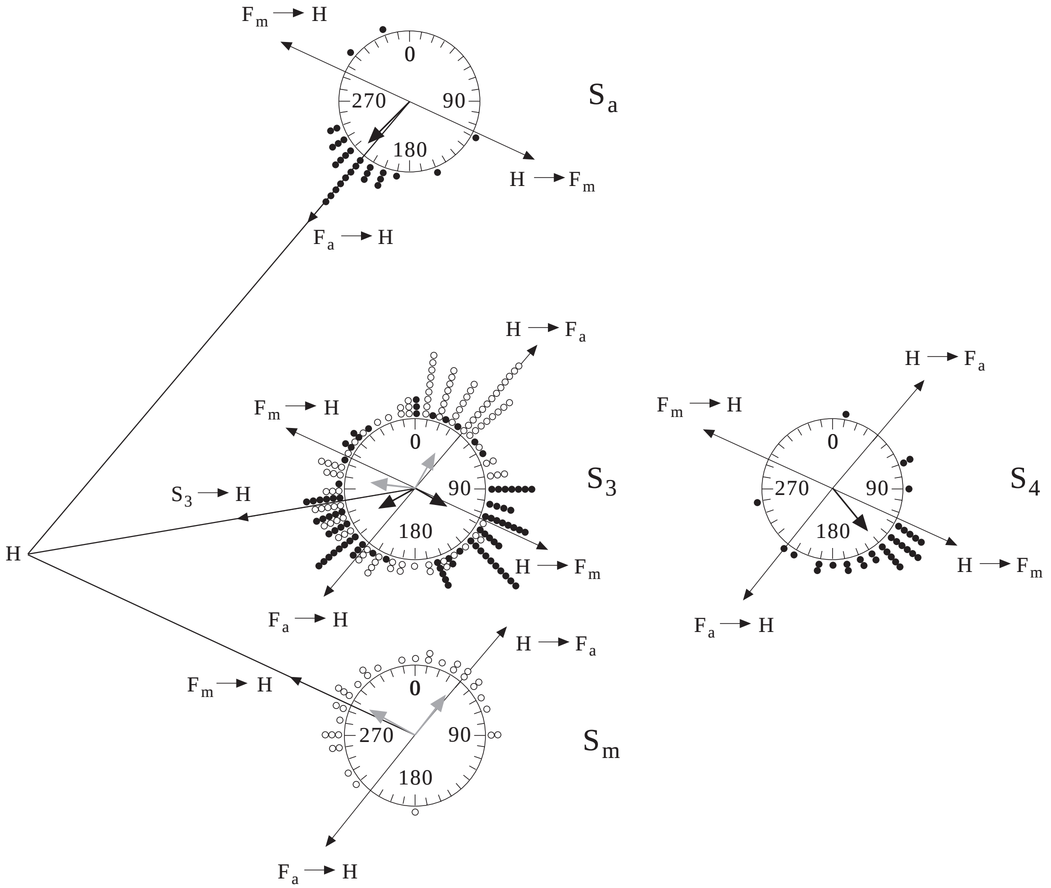

Figure 4. Distributions of vanishing bearings of bees caught at departure from the hive towards S_m in the morning (●, black arrows) and S_a in the afternoon (○, grey arrow), and subsequently displaced to S_m, S_a, S_3 and S_4. F_a and F_m indicate the feeding sites the bees have just come from. Two arrows indicating the centre of mass of data are given for those cases where the broken axis approach (see Methods) proves two independent distributions. The number of bees released (N) and the mean vector lengths (r) are: S_a: $N=28$, $r=0.82$; S_3: ●, $N=85$, $r=0.45$; ○, $N=107$, $r=0.50$; S_m: $N=33$, $r=0.69$; S_4: $N=32$, $r=0.75$.

(Menzel et al. 1990). The distribution of bees released at S_a is significantly different from that at S_m (Watson U^2-test corrected for ties: $P<0.01$).

The difference in behaviour between bees displaced to S_a and S_m can be explained only by differences in the landmarks that surround these sites. We suspect that the tendency of hive departing bees to use sun compass cues is depressed when landmarks are prominent enough to indicate to the bees that such a choice would be wrong. S_a was not only marked by a nearby bush, but was also much closer to the mountain peak than S_m, which may be why bees never simply chose the compass direction from the hive to S_m when displaced to S_a. These differences show that the behaviour of bees at any one release site was dependent not only on motivation, but also on the local features that identified each site.

In experiment 1 we found that hive arriving bees displaced to S_3 flew a novel course directly towards the hive. Were hive departing bees able to fly from S_3 directly towards the feeder to which they had set out to fly? The answer is no. Both groups of bees tested (those displaced in the morning and in the afternoon) had bimodal distributions of vanishing bearings (broken axis approach: both $P<0.001$; Fig. 4), but none of the peaks corresponded to the directions to the two feeders. Instead, both groups of bees contained a fraction that vanished towards the hive. These bees had apparently given up their original motivation to fly towards one of the feeders, and chose instead to fly home. This result confirms that novel short cuts can be taken towards the hive, but not towards a feeder. The second peak in each group of hive departing bees corresponds to the feeder direction that would have been correct from the hive at each given time. These bees apparently ignored the landmarks specifying S_3 and flew according to the sun compass direction that would have been correct from the hive.

Control bees displaced to S_4 behaved as if they had not been displaced and continued to take the compass direction that would have been appropriate from the hive to S_m (Hodges–Ajne test: $P<0.05$; Fig. 4). Bees displaced to S_3, after visiting only S_m, used the compass direction from the hive to S_a (Hodges–Ajne test: $P<0.05$; Fig. 3). This shows that landmarks close to the hive were not used; in addition, it means that experience with both S_m and S_a was necessary to ‘calculate’ a middle course from S_3.

EXPERIMENT III: BEES DISPLACED FROM FEEDING SITES

Methods

We have already established that the ability to take novel short cuts is motivation dependent, but not always in the sense that the short cut taken actually follows logically from the motivation. For example, hive departing bees never chose a short cut towards one of the feeders to which they headed out when caught. Instead, only about half of the bees took a novel short cut, but they flew back towards the hive instead of the feeder locations. In this experiment, we explored the effects of motivation further, by catching (and subsequently displacing) bees on arrival at the feeders or at the moment of departure from one of the feeders. We expected that feeder arriving bees should be motivated to feed, and thus to fly back towards the feeder after displacement, if their navigation capacities had allowed them to do so. Similarly, feeder departing bees should be motivated to travel to the hive, and thus to take a short cut to its location when displaced. We considered this particularly likely since we found in experiments I and II that bees could take short cuts towards the hive even when not motivated to fly towards it (hive departing bees).

Results and Discussion

None of our expectations were met by the bees displaced from S_m. All of these bees, irrespectively of whether they were displaced to S_a or S_3 and independently of whether they were feeder arriving or feeder departing bees, chose only one direction (Fig. 5). The distributions shown in Fig. 5 are statistically indistinguishable from the compass direction that would have guided them back from S_m to the hive (V-test: in both cases $P<0.05$).

Not even the feeder departing bees, which had set out to fly back to the hive, chose its correct direction. This result is all the more surprising since these bees should have been more motivated to fly back to the hive than hive departing bees, and at least as motivated as hive arriving bees. Nevertheless, although hive arriving bees and a significant proportion of hive departing bees would fly directly towards the hive, feeder departing bees displaced from S_m never chose this direction. Apparently, the compass flight vector instruction that is uploaded into the working memory on arrival at and on departure from a feeder is so strong that it overrides all input from local landmarks.

We were interested not only in how the chosen direction depended on motivation, but also on site-specific cues. Bees displaced from S_a should be in the same motivational state as those displaced from S_m, but the distribution of their vanishing bearings is bimodal (broken axis approach: $P<0.01$; Fig. 5). Some bees behaved like those displaced from S_m: they chose the compass direction to the hive that would have been correct in the absence of a displacement. However, a second sub-population of these bees vanished in the actual hive direction from the novel location. These bees must have correctly ‘assessed’ their novel location to retrieve the true direction of the hive. Note that this is not the same as taking a novel short cut: all these bees had experience with both S_m and S_a and thus were not choosing any route they had not previously travelled. Because of this difference between bees displaced to S_m and S_a from the respective opposite location, we conclude that the choice of direction depends not only on motivation, but also on local cues at the release site.

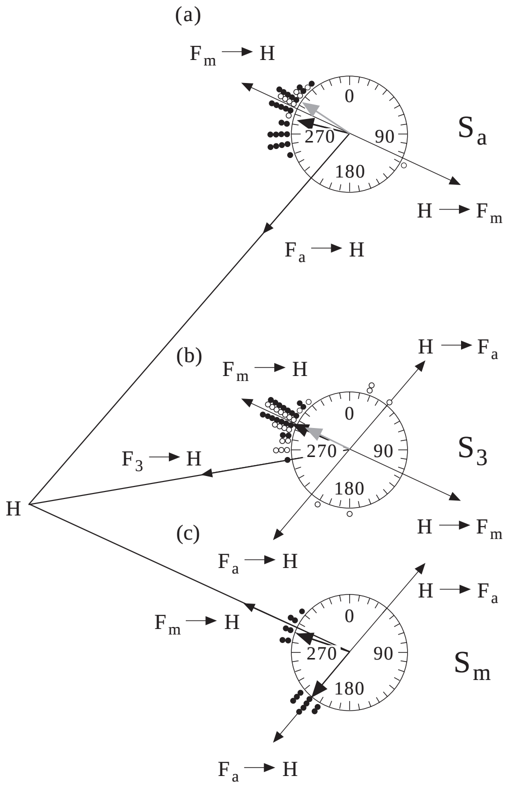

Figure 5. (a,b) Distributions of vanishing bearings of bees caught at departure (○) from, or on arrival at, S_m (●), and subsequently displaced to S_a and S_3. (c) Distribution of vanishing bearings of bees caught at departure from S_a, and displaced to S_m. F_a and F_m indicate the feeding sites the bees have just come from. Two arrows indicating the centre of mass of data are given for those cases where the broken axis approach (see Methods) proves two independent distributions. The number of bees released at each place (N) and the mean vector lengths (r) are: S_a: ●, $N=24$, $r=0.95$; ○, $N=9$, $r=0.77$; S_3: $N=19$, $r=0.98$; S_m: $N=16$, $r_1=0.99$, $r_2=0.98$.

GENERAL DISCUSSION

Navigation in honey bees arises from a rich repertoire of behavioural routines which are intimately connected by the motivational state during foraging, incidental and associative learning, and selective memory retrieval. This study provides evidence for: (1) the association of home vector memories with specific locations of feeding sites; (2) the dependence of memory retrieval on motivation; and (3) the selection of a novel flight direction towards the hive, if two home vector memories are simultaneously activated. We discuss these three aspects in turn.

Site-specific Retrieval of Home Vector Memory

A basic property in bee navigation is dead reckoning, or path integration, a process that guides the bee along direct lines between its goals, for example nest and feeding site (Wehner 1992). These straightened flight paths are related to celestial cues in a time compensated fashion, and, thus, are geostable compass directions (von Frisch 1965; Wehner 1992). Many observations and experiments provide compelling evidence that bees store the respective compass directions in a long-term reference memory and apply these memories when communicating about feeding places using the dance behaviour (von Frisch 1965), when leaving the nest or feeding site, and when they are displaced to an unfamiliar release site (Wehner & Menzel 1990; Collett 1992, 1993; Dyer 1994, 1996). The visual scene experienced during flight is also associated with the compass direction, and it enables the bee to refer to the compass direction at times when the celestial cues are not visible (von Frisch 1965; Dyer & Gould 1981; Chittka & Geiger 1995b). Our data corroborate Wehner et al.'s (1990) finding, in that the visual scene at the feeding site is also associated with the compass direction allowing the bee to choose the home vector specific for the feeding site. Furthermore, our results indicate that visual scenes with few landmarks at the feeding site (S_m in our case) induce home flights at a lower probability and lower accuracy than more enriched visual scenes, as at S_a.

It is not yet known whether all components of the visual scene (local cues and distant ones, e.g. the skyline pattern as it appears from the feeding site), and whether cues other than visual (olfactory or magneto-sensory, the latter as indicated by findings of Collett & Baron 1994) are associated with the home direction. Furthermore, nothing is known about the learning process involved in this association. Is it a kind of incidental learning similar to that known from bees and wasps which, after feeding, fly in circles around the feeding site, then turn around and look at it (Opfinger 1931; Lehrer & Collett 1994)? Or is it a forward associative learning process in which the appetitive stimulus (sucrose) becomes the reinforcing stimulus (Menzel 1990)? In the latter case an angle of 180° would have to be added to the compass direction of the just finished approach flight for storing the compass angle of the home flight. Incidental learning is in fact more likely, because bees released at an unfamiliar place return to the release site within 10–20 min if they do not find the hive (unpublished data). There is no external reinforcing stimulus such as a food reward under these conditions, and still bees manage to relocate precisely the place from where they had started after a search flight of certainly more than 1–2 km as judged from their flight speed (5–15 km/h).

Motivation-dependent Retrieval of Vector Memories

Bees go through four motivational states during a foraging bout. We presume that each state is characterized by its own set of active memories (those that reside in the working memory). For example, when a bee sets out to fly from the hive to a known feeder, we assume that the respective flight vector is retrieved from a long-term memory, and loaded into a more active form of memory, or working memory, which contains only the information about which vector is used next. Feeder departing bees retrieve the ‘home vector’ in a similar fashion. Hive arriving and feeder arriving bees have just applied the respective vector memories, and thus these memories may be set to zero or into a less active form (Wehner 1992). At the same time, we presume that all of this information stored in long-term memory is retrieved from there, depending on the motivational state and visual cues at a given site. Although displacement experiments have been carried out with bees in different motivational states (e.g. hive departing bees: Gould 1986; Menzel et al. 1990; Dyer 1991; Dyer et al. 1993; hive arriving bees: Wehner et al. 1990; feeder departing bees: Menzel 1989; Wehner & Menzel 1990), a thorough analysis of the effect of motivation on navigation has been lacking. Our experiments provide such data. Bees in all four motivational states were tested at release site S_a, three motivational states at S_m and at S_3 (in the morning), and two motivational states at S_3 (in the afternoon) and at S_4. We found that indeed bees in different motivational states behaved very differently.

Bees displaced from the feeders applied the home vector memory of the feeding site at which they were collected, and not (with the exception of a sub-population released at S_m) the home vector associated with the release site (Fig. 5). Bees displaced from the hive were much more ready to replace their active vector memory with one retrieved by the visual scene at the release site. As a result, three of the four groups displaced from the hive (Figs 2 and 4) vanished in the direction of the hive, and one group (hive departing bees at S_m) split into two sub-groups, one returning to the hive and one continuing according to the compass direction in its active memory form. Hive arriving bees appeared to be more prepared to use a site-specific home vector memory, judging from the fact that some hive departing, but no hive arriving, bees resisted the site-specific retrieval process (Figs 2 and 4). This latter result may indicate that the zeroing process induced by arriving at the hive may have emptied the active vector memory. As a consequence, hive arriving bees should be disoriented when released at an unfamiliar site, because they do not have a vector available in their working memory, nor do they encounter familiar landmarks to guide them. Random distributions of vanishing bearings were indeed found in earlier experiments under these conditions (Wehner & Wehner 1990; Chittka et al. 1992; R.M., unpublished data). In our study, however, the vanishing bearings of hive arriving bees displaced to the unfamiliar site S_4 were not randomly distributed (Figs 2 and 4). The bees applied the vector memory they had just used: they continued to fly in the direction they had flown before displacement. The reason for the discrepancy between our results and those cited earlier is unclear. One possibility lies in the difference of local features that surrounded the unfamiliar sites in these studies. In the earlier studies (Chittka et al. 1992; R.M., unpublished data), bees foraged in (and were displaced into) highly structured landscapes that were very different from one another. Here, bees were displaced into a mostly featureless landscape whose only familiar cue was the dominant mountain. The latter was not appropriately used, as evidenced by the observation that bees did not fly directly home. However, the open landscape at S_4 may have caused the bees to assess that they were still en route from a feeder to the hive, which may have helped them to reactivate the weakened home vector memory.

We conclude that the memories of vectors both for the outbound and inbound flights exist in a retrievable long-term memory, and that the accessibility differs in different motivational states. In our experiments, each bee stored two outbound and two inbound goal vectors, and the context (time of day, visual scene, motivation) determined which was brought into an active form of working memory.

When are Novel Routes Taken?

Bees released at the familiar sites either flew straight back to the hive or continued to fly in the compass direction they would have taken at the capture site. No bee flew from any release site directly to a feeding site. This result confirms those of many authors (Menzel 1989; Menzel et al. 1990; Wehner et al. 1990; Wehner & Wehner 1990; Dyer et al. 1993; Geiger et al. 1995; but see Gould 1986) all of which indicate that bees do not take novel short cuts towards a goal, unless familiar landmarks at the goal are recognized from the release site. However, in the present study, bees displaced from the hive did take novel short cuts, but in only one direction, towards the hive. The controls unambiguously prove that these short cuts are taken in the absence of landmarks that might mark the target.

All bees released at S_3 experienced the location for the first time. They never flew from S_m to S_a, or vice versa, either during training or during testing. The flight direction from S_3 to the hive was indeed novel to them, both with respect to the compass direction and the ground over which they passed. This novel route was taken only by bees that visited both feeding sites (comparison of Figs 2 and 4 with Fig. 3) indicating that both landmark piloting and guidelines on the ground can be excluded, and that information about both feeding sites, for example their home vectors and/or their visual scenes, is necessary. Landmark piloting can also be excluded on the basis of the low spatial resolution of the bee eye. Distant cues behind the hive as seen from S_3 were not available. Guideline orientation with reference to the only feature of the landscape, the country road, which might have guided the bees, can also be excluded, because bees that visited only S_a should have followed this guideline, but did not (Figs 2 and 4). Tests at an additional release site (S_4) excluded the possibility that bees simply activate all home vector memories as an emergency programme when they find themselves at an unfamiliar release site (Fig. 2), and, thus, fly a compromise direction irrespective of where they are. Rather, the novel route requires features that resemble at least partially those of both feeding sites.

Two functions of these features are conceivable.

(1) One possibility is that the similarity encountered at S_3 with both familiar sites S_a and S_m is used directly to calculate the novel flight route. In that case, the bee's attempt to match the visual scene at S_3 with both scene memories of S_m and S_a might lead to a compromise home direction similar to a behaviour found by Collett & Baron (1995) for flight manoeuvres along small and very close visual cues. Since local landmarks resembling those of S_m or S_a did not exist at S_3, only distant landmarks or the skyline come into question in our experiments. We consider this rather unlikely, because such features did not guide the bees at the fully unfamiliar site. As shown in Fig. 4, bees flew according to their compass memory at the unfamiliar site S_4 and did not refer to the distant landmark (the mountain) which appeared to be rotated by 100–110° compared with the feeding sites.

(2) The guidance towards the hive might result from an integration of the two simultaneously activated home vector memories. The two memories may be retrieved concurrently because of the partial resemblance of the visual scene at S_3 with those of S_m and S_a. A vector addition, even in its simplest form (vector addition without weighting vector length before adding) can be performed only if the content of at least two vector memories reside in working memory. The 'uploading' of these two vectors from long-term memory must be triggered by landmark cues that have some resemblance to both familiar feeding sites. The bee must compare the panorama at the release site with the two remembered panoramas at the feeding sites, and 'infer' that it has been displaced to an intermediate location.

Why are Novel Routes Taken only under Some Conditions?

The ability of some groups of bees to find short cuts to the hive contrasts sharply with the finding that no bees ever took short cuts between the feeding sites, or from S_3 to one of the feeders. Local cues visible from the other feeding site were not available in our experiments. However, distant cues could have been used for piloting. For example, hive departing bees in the afternoon heading out for S_a (which is located straight towards the mountain) could have simply continued to fly towards the mountain when released at S_m and would have followed a short-cut route. They did not choose this direction. Thus, while other studies indicate that bees use local landmarks for piloting (e.g. Chittka et al. 1995b; Geiger et al. 1995), our results show that they do not use the skyline pattern for this purpose. This makes sense: such cues would be misleading because visual resolution is low in bees and therefore only rather close landscape features can be used. These features are, however, sensitive to motion parallax for the distances travelled and would not allow reliable navigation in most cases. Bees should also not have been 'confused' by the fact that they would have had to fly over differently structured ground between S_m and S_a, because (1) the agricultural area was rather similar between the hive and the feeding sites S_m and S_a, and between the two feeding sites, and (2) there were no guidelines along the flight paths other than the country road, which they had to cross on their way from the hive to S_a. Bees would have crossed the road in a rather similar way if they flew from S_m or S_3 to S_a.

The most likely explanation why bees did not fly from S_m to S_a or vice versa is that at these fully familiar release sites, the information of the home vector associated with these sites is shifted from long-term memory to working memory, and this dominates their behaviour. As a consequence, motivation changes and bees return to the hive rather than continue flying to the feeder. Similarly, bees released at S_3 did not fly to one or the other feeding site according to their motivation because the two home-directed vectors retrieved from long-term store dominate their navigation. It is, however, difficult to understand why feeder departing bees did not manage to take a short cut from S_3 to the hive, even though they were trained the same as, and likely to be more motivated to get to the hive than, hive departing bees. A more profound understanding of the dynamics of spatial memory retrieval and its dependence on motivation is necessary to unravel this enigma.

Are Bees Guided by a Vector Map in Homing?

The capacity of an animal to take a novel short cut has been taken as indication of a map-like organization of spatial memory frequently referred to as a 'cognitive map' (Tolman 1948; O'Keefe & Nadel 1978; Thinus-Blanc 1987; Bennett 1996). There is no evidence yet that bees store spatial arrangements of landmarks and scenes in such a way (Wehner & Menzel 1990; Wehner 1992; Collett 1996; Dyer 1996; Menzel et al. 1996) because simpler navigation mechanisms (route orientation or landmark piloting; Bennett 1996) either cannot be excluded (Gould 1986), or were found to explain novel short cuts (Menzel 1989; Dyer 1991; Geiger et al. 1995). Two questions arise from these observations and the present ones. First, what may explain the difference between the many negative results cited above, and ours? Second, do our results demonstrate the existence of a cognitive map in bees?

In all the previous studies, bees were extensively trained along one route or along two routes in succession, released at a site that they had not visited before (Menzel 1989; Wehner & Wehner 1990; Dyer et al. 1993), or that had been a feeding place some time before (Menzel et al. 1990; Wehner et al. 1990; Dyer 1991). Extensive training along one route could lead to an elimination of information about the spatial arrangement of landmarks around another. If so, the negative results cited above are not surprising. The information that was thought to be accessible to bees, because they had been trained to two sites in succession, or across a whole area (as in Menzel et al. 1990), may simply not have been available any longer. Because of the prolonged training along the ultimate route, their memory of the landscape may have collapsed to a linear, one-dimensional arrangement of marks along the route travelled last. With the training schedule used here, we circumvented this danger by establishing memories for two routes simultaneously. Under these circumstances, we find that bees will indeed take novel short cuts, without the help of landmarks used for piloting.

In this sense, our results meet the basic requirement to prove a cognitive map (Tolman 1948; O'Keefe & Nadel 1978; Thinus-Blanc 1987; Bennett 1996). However, we caution against making this inference too rapidly. We support the notion that what our bees do is an instance of basic cognition, that is, an activation of separately acquired memories and their application in a novel, adaptive sense (Markl 1985). Retrieval of more than one memory by the associated cues, generalization between the cues, and context-dependent learning have been frequently observed in associative appetitive learning of bees (Menzel 1990). Spatial learning may follow similar rules. However, whether such a memory organization is classified as a cognitive map depends on how rich the map is. If it allows bees to navigate in only one direction (home), and in only some motivational states (hive arriving, and to a degree, hive departing bees but not feeder-related motivation), we think that the inference of a map would be premature. If, however, multiple vectors between landmarks, feeding sites and the hive are stored together with the particular sensory properties of these sites, we might conclude that a map has been established. Our data, however, do not allow us to infer that the representation of the bee's foraging range is that complex. More data are needed, particularly from experiments that avoid the limitations possibly caused by the extensive training to too few feeding sites, compared with the number of such sites linked by natural foraging bouts on flowers. Such experiments might also include more structured landscapes than the one selected here. A more structured landscape may support more local features, and a stronger association between them and compass vectors. There is no reason to assume that the memory of bees may not be rich enough to contain several, if not many, of these associations simultaneously, if the elimination of these local associations by focusing on one site and one flight route is avoided. Finally, we advocate analysing the learning processes involved in navigation more thoroughly.

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