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Original article

Vector integration and novel shortcutting in honeybee navigation

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Abstract – Honeybees that had been trained to visit two feeders simultaneously were released at five sites located further away from the training area. Harmonic radar tracking was used to record the complete homing flights. The bees performed multiple straight flight components (SFCs) between curved search flights. SFCs reflect vector directions between the two feeding sites and the respective vectors between the feeding sites and the hive. Direct flights back to the hive were also observed. The latter belong to a homing strategy that requires the bee to identify its location relative to the hive. We interpret these two navigation strategies as reflecting the application of a directional component of novel shortcut flights. Taken together, our findings indicate that bees apply several different directional components of vectors which are either experienced directly during flight or derived from long-distance vector integration or mapping.

navigation / vector integration / shortcut flights / cognitive map

1. INTRODUCTION

Early studies on navigation in honeybees (Wehner and Menzel 1990; Dyer 1991) relied on the observation of vanishing directions after animals had been trained to a feeder, were collected at the feeder or at the hive entrance and then transported to a release site further away from the route between hive and feeder (catch-and-release experiments). In all these cases, the bees performed rather straight flights after some circling in the immediate surroundings and flew into a direction they would have taken if they had not been displaced. These observations were interpreted as indicating an egocentric navigation strategy which does not take any allocentric components into account. The only study using the same procedure which deviated from these results was reported by Gould (1986) who observed bees vanishing into a direction that would have brought them to their intended goal (feeding site) in a geocentric relation to the spatial layout of the goal and the release site. Although Gould's experiments were repeated several times they could not be confirmed. Therefore, the debate settled on the conclusion that bees do indeed lack a geocentric form of navigation based on allocentric spatial references.

However, bees arrived back at the hive even if they were not trained to a distant feeder, and it did not take them much longer than bees that were trained to the release site (Menzel et al. 2000). Under these circumstances bees circled longer at the release site searching for the feeder they were used to finding in the immediate

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vicinity of the hive, but then took off in the direction of the hive. It was, therefore, suggested that bees refer to two forms of navigation memory, a vector-based memory and an allocentric landmark memory that stores some spatial relations between locations. The solution to the question of what kind flight paths bees perform when homing from a remote release site was supplied by the harmonic radar for tracking the full flight path of bees between release and arrival at the hive (Menzel et al. 2005). Indeed, most bees were found to first apply the vector memory that would have brought them back to the hive if they had not been transported to the unexpected site and released, but they subsequently steered towards the hive either directly or via the feeder. This latter homing strategy was independent of any beacon at the goal (hive or feeder) and did not require any far distant landmark structures, e.g. for sequential image matching to the panorama. These results posed the question about the structure of such an allocentric memory.

The Menzel et al. (2005) studies showed that bees apply not only the highly salient-learned vector of the route flight but also a novel shortcut flight which can be formally considered as a derived vector flight resulting from the subtraction of the route flight vector and a vector which would have brought the bees straight back to the hive from the current location. This latter flight vector was used by two thirds of the bees in the Menzel et al. (2005) study, whereas one third of the bees performed according to the derived vector leading them first to the feeder and then to the hive. The assumed integration process can be conceptualised as a triangulation process that includes the identification of the current location relative to the hive as based on an associated home directed vector to local landmarks, a vector retrieved from memory (to the vector of the route between hive and feeder in both its outbound and inbound components) and the calculated vector from the current location to the feeder. Another possibility can be excluded which assumes a learned vector from a landmark to the feeder because bees never flew such a route. Notice that such a triangulation process requires also a change of motivation from inbound to outbound. In the case of the Menzel et al. (2005) experiments, one third of the bees would have changed their motivation and two thirds would have kept their home directed motivation. If bees would apply multiple triangulation processes based on more than just one learned route vector, retrieval of several to many hive and feeder-related vectors and changes between motivational states a network of triangulated spatial relations would result, possibly leading to a rich network of interconnected learned and retrieved vectors.

Honeybees trained to a stable and spot-like feeding place fly along a stereotypical straight route and learn the compass direction and distance as well as the landmarks along this route. The vector components (direction and distance) of the outbound flight are encoded in the waggle dance and communicated to colony members inside the hive (von Frisch 1965). Extended landmarks allow the bee to retrieve the compass direction even if the sun or spots of polarised light in the sky are not available (von Frisch and Lindauer 1954; Dyer and Gould 1981). These observations support the interpretation that there is a major component in bee navigation that relies on flight vectors learned from stereotypical route flights. Landmarks are considered to both act as a back-up for retrieving the directional component when the sun compass cannot be used and to indicate directions towards the feeding place and the hive (Towne and Moscrip 2008). Recently, a neural net has been implemented in an attempt to simulate navigational strategies in ants and bees based on large-scale vector integration (Cruse and Wehner 2011). The basic components reflect the same assumptions as made in the Menzel et al. (2005) analysis namely different vector memories for outbound and inbound travels, identification of loci with respect to their home-directed vectors, and motivational change for outbound and inbound travels. The computational rules are considered as decentralised arguing against an integrated form of spatial memory as conceptualised in a cognitive map (Tolman 1948). However, multiple memory vectors that are respectively associated to landmark structures will lead to a network of retrieved and derived connections that is formally equivalent to a map-like memory structure (Menzel et al. 2005). The question is how complex such a network of connections is and how flexible it can be used in homing strategies.

In an attempt to elucidate the complexity of vector memories, we challenge the bees by asking whether they are able to integrate two feeder locations and perform shortcut flights between three locations, the hive, and the two feeders. Do they fly along a vector that would bring them from one feeder to the other although they had not previously been trained to do so? Do they choose to fly to one of the feeders or the hive after being released at an unexpected location? Do they perform such vector flights in sequence and relate them to landmark structures?

We recorded the flight paths of single test bees using harmonic radar (Riley et al. 1996) which allows us to track bees over a distance of more than 1-km radius (Menzel et al. 2005; Menzel et al. 2011). These flight paths show that the bees do in fact learn two feeder locations, recruit their vector memories of the route flights between the hive and the two feeders, and perform novel shortcuts, both between the two feeders and between various locations within the test area and the hive, and change their motivation for the outbound and inbound vectors even within the same homing flight. We conclude that bees homing after being trained to two feeders refer to a dense network of vectors both learned and derived from vector integration. The dominance of vector guidance under these training and test conditions may override other forms of navigation less dependent on intense training along routes.

2. MATERIALS AND METHODS

Honeybees (*Apis mellifera carnica*) were kept in regular-sized colonies and trained to two feeding stations with sucrose solution at concentrations adjusted to the needs of the experiment. The test area was an extensive flat pasture (approx. 1.5×1.5 km, close to Klein Lüben, Brandenburg, geographic coordinates: N 52.97555, E 11.83677) without natural landmarks apart from the structure of the ground and a few coloured tents (3.5 m high) (see Menzel et al. 2005). None of the three locations (hive H, the two feeders FC and FD) nor the horizon provided any navigation cues. Figure 1 gives the layout of the experimental arrangement. Bees were trained to the two feeders (FC and FD) by providing food at only one of them for 20–30 min and then changing to the other for 20–30 min. The distance between H and FC and H and FD was 165 and 170 m, respectively, and the angle between these two routes was close to 50° (distance between FC and FD, 160 m). A group of bees was marked individually with coloured number tags, and a full protocol of their foraging behaviour was established. Only those bees that visited the two feeding stations roughly equally frequently were included in the translocation and tracking experiments. Each test bee was used only once for a translocation and tracking experiment. A total of 33 bees were radar tracked after being released separately at one of the release sites (see Table I).

The respective test bee was collected at one of the two feeders either immediately after arrival with very little feeding or after completion of feeding. It was then transported to one of the release sites (R8, R9, R10, R11, and R12), and a radar transponder was fixed to the number tag on its thorax. Transport and preparation for radar tracking lasted less than 10 min. During the flight of the test bee, both feeding sites were devoid of any landmarks visible from a distance (all utensils on the ground were covered with a green sheet, and all staff removed themselves from the flight area or hid behind the hive). All other bees trained to the feeders were caged during the flight of the test bee. This was accomplished by collecting the arriving bees before the particular test bee was selected. When the test bee arrived at the hive, it was caught, the transponder was removed, all caged bees were released, and training continued. Scent was not used at any stage of training or testing. Weather conditions during the test flights were fine or fair with wind speeds of <5 m/s. Wind direction was

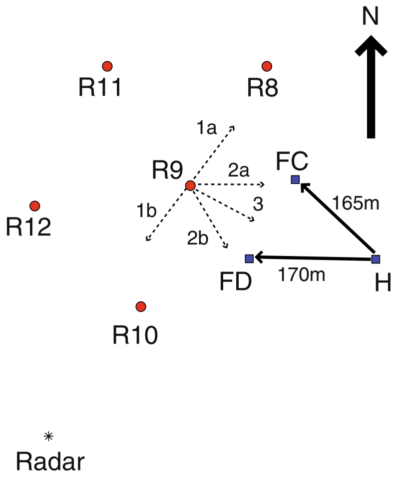

Figure 1. Training and test layout. A group of bees were trained from the hive to FC and FD. They only flew along the *arrows* pointing from the hive to FC and to FD. *R8–R12*, release site locations. Most of the releases took place at R9. *Dotted arrows at R9*, flight direction categories resembling the directional components of the vectors the bees may apply: from FD to FC (flight direction category no. 1a), from FC to FD (flight direction category no. 1b), from one of the feeding sites back to the hive (flight direction category no. 2a: FD to hive; flight direction category no. 2b: FC to hive), and the vector from the release site R9 (or any other location) to the hive (flight direction category no. 3). The respective flights may be correct for the respective location or incorrect for the respective location. For example, flight direction category no. 3 performed close to the R9 release site as shown here will be location correct, but flight direction category no. 1a performed at R9 will be location incorrect. Notice that flights along flight direction category no. 2a and 2b may resemble the directional component of the vector flight from one of the two feeders to the hive or a novel shortcut flight from the release site to the intended goal (one of the two feeders, FC or FD). These two interpretations cannot be distinguished because of the geometric layout of the feeder and the release sites, e.g. a flight along flight direction category no. 2a can be interpreted either as a location-incorrect vector flight according to the home vector from FD to H, or as a location-correct goal-directed flight from R9 to FC. Bees released at a release site may first perform circling search flights which may bring them at some distance from the release site. Therefore the initiation of the first straight flight may not happen at the release site. Furthermore, straight flights may be interrupted by circling search flights leading to multiple secondary straight flights.

monitored continuously and found to be dominated by a westerly directions thus blowing from the training and release sites towards the hive. No correlation of flight behaviour with wind conditions was found.

The operation of the harmonic radar device has been described previously by Menzel et al. (2005). In short, we used a system with a 9.4-GHz radar transceiver (Raytheon Marine GmbH, Kiel, NSC 2525/7 XU) combined with a parabolic antenna providing approx. 44 dBi. The transponder fixed to the thorax of the bee consisted of a dipole antenna with a Low Barrier Schottky Diode HSCH-5340 of centred inductivity. The second harmonic component of the signal (18.8 GHz) was the target for the radar. The receiving unit consisted of parabolic antenna tuned to 18.8 GHz, connected to a low-noise pre-amplifier directly coupled to a mixer (18.8 GHz oscillator), and a downstream amplifier with a 90-MHz ZF Filter. A 60-MHz ZF signal was used for signal recognition. The transponder had a weight of 10.5 mg and a length of 12 mm. We used silver or gold wires with a diameter of 0.3 mm and a loop inductance of 1.3 nH.

The flights of the test bees consisted of curved components and stretched components. We analysed these flights by determining the origin and end of straight flight components (SFCs) using a custom written MathLab program based on the following criteria: the angle between three consecutive radar measurements (radar paints) needed to be smaller than 30°, and the flight distance between these three paints more than 50 m. The location of the origin of each SFC and its compass direction as well as its length were computed and plotted on a map of the test area. Since one test flight may contain more than one straight component, we put them in order of sequence.

Statistics Circular data were analysed according to Batschelet (1981) and Fisher (1996) using MatLab (Mathworks Inc.) and the toolbox for circular statistics (Berens 2009). We tested of nonuniformity using three different tests, Rayleigh's test, Hodges–Ajne test, and Rao's spacing test for nonuniformity. For data partitioning, we used a *k*-mean cluster algorithm for circular data. An incremental approach was applied to determine the most appropriate

Table I. Data sheet of all test animals with the number of the animal, the feeding site where it was collected (FC or FD), the release site, the total number of straight flight components (SFC) of that particular test animal, and the direction relative to north (angle) of the first, second, and third SFC.

Date	no. bee	Caught at	Released at	Number of SFCs	Angle SFC 1	Angle SFC 2	Angle SFC 3
8/26/2008	10	FC	R9	4	79	69	75
8/26/2008	11	FD	R8	1	112	–	–
8/26/2008	12	FD	R9	4	86	21	147
8/26/2008	13	FC	R9	3	147	88	84
8/26/2008	14	FD	R8	1	355	–	–
8/26/2008	15	FD	R7	1	55	–	–
8/27/2008	16	FC	R9	6	213	32	19
8/27/2008	17	FD	R9	4	75	118	322
8/27/2008	18	FD	R8	2	175	251	–
8/27/2008	19	FC	R10	3	109	352	46
9/20/2008	22	FD	R9	3	153	142	161
9/20/2008	23	FC	R9	5	220	180	90
9/20/2008	24	FD	R9	4	162	256	93
9/29/2008	25	FC	R9	2	139	123	
9/21/2008	28	FD	R9	3	79	141	194
9/21/2008	29	FD	R9	1	119	–	–
9/21/2008	30	FC	R9	1	150	–	–
9/26/2008	31	FC	R11	2	130	132	
9/25/2008	32	FD	R12	5	44	106	119
9/25/2008	33	FD	R12	2	77	122	
9/25/2008	34	FC	R11	3	193	187	120
9/25/2008	35	FD	R12	2	12	106	
9/25/2008	36	FC	R11	4	236	135	124
9/26/2008	37	FD	R12	7	78	171	206
9/26/2008	38	FD	R12	1	88	–	–
9/17/2008	39	FD	R9	1	96	–	–
9/17/2008	40	FD	R9	1	119	–	–
9/18/2008	41	FC	R9	3	99	96	208
9/19/2008	42	FC	R10	4	220	145	108
9/25/2008	43	FD	R12	1	90	–	–
9/24/2008	44	FD	R12	1	115	–	–
9/21/2008	45	FD	R9	1	108	–	–
9/25/2008	46	FC	R12	1	79	–	–

number of clusters. Starting with a number of two clusters and proceeded up to ten, we checked for conditions in which all clusters contained at least three individual SFCs. Optimal conditions were found for five (Figure 4a) or six (Figure 4b) clusters. In any other cluster number, we had either a very poor separation or found clusters with only one SFC. For the different cluster groups in Figure 4a, b, we applied a nonparametric test for circular data (analogue to the Kruskal–Wallis test). Since in both cases

(Figure 4a, b) we found significant differences ($p<.05$), we used a nonparametric test (analogue to Wilcoxon rank sum test) for two circular medians for pair-wise comparisons. We used the Lilliefors test for testing of normality in the linear binned data of Figure 5 (binning size, 10 m).

3. RESULTS

3.1. Flight tracks after catch and release

Test animals visited two feeding sites (FC and FD) about equally frequently over at least 1 day, and they never flew between FC and FD (or FD and FC) during training. The latter condition was controlled by a complete list of all visits to the feeders on the level of the individual animal, and a near-complete observation of flight trajectories of leaving bees. The experiment started by catching one of these bees at either FC or FD. With the exception of three control bees, all bees were caught immediately after landing. The three control bees were allowed to fill their crop completely and were caught when preparing for the return flight. All test bees (Table I) returned to the hive within less than 30 min although the furthest release site (R11 and R12) was 450 m from the hive and there was no beacon at the hive or panoramic structure which would allow the animal to head towards the hive over distances beyond the visual catchment area estimated to be 30 m radius. Figure 2a–d shows four representative examples of full flight tracks. Curved and straight flight components are apparent. In the following, we shall focus on the as indicated by the red arrows in Figure 2a–d. As described in section 2, SFCs were detected automatically by a custom written program and plotted as vectors in the map of the test area. SFCs performed as the first flight component after release are given in bold arrows in Figure 2a–d, those performed later in dotted arrows. SFC were categorised in two respects, according the kind of flight direction as shown in Figure 1 (flight direction categories no. 1a, 1b, 2a, 2b, and 3) and with respect to whether they were correct for the location or incorrect. Assigning SFCs to the respective flight direction category that were correct for the location were straight forward because these SFCs were very accurate. For location-incorrect flight-direction categories, we assigned the respective SFC to that category that was closest in its directional component to one of the categories. An exception is category no. 3 (SFC towards the hive) because it could be initiated at different locations and thus had different directional components. However, this assignment did not cause any problems because nearly all of these SFCs ended up close to the hive.

Some animals headed straight back to the hive from the release site (e.g. SFC 1 and 2 in Figure 2a and see also Figure 1 line 3) or performed first flights that would have brought them from the feeding site where they were collected to the other feeding site (Figures 2b (SFC 1), d (SFC 1) 1 (lines 1a and 1b)). Note that the bees were not trained to fly between FC and FD, and we never saw an animal flying from one feeding site to the other during training. Since the open feeder was always attended by an experimenter, uncontrolled foraging flights can be excluded but animals may have been exploring the two feeder sites without their flights being monitored and such exploration flights may have included direct flights between the two feeders. However, such flights were not rewarded.

Test animals released at one of the release sites also applied one of the two inbound vectors from FC or FD to the hive H indicating that bees change their motivation from outbound to inbound although they were collected at the feeder immediately at arrival. Such a vector flight is seen in Figure 2c where the first SFC marked 1 closely followed the inbound vector from FD to H. Notice that flight directions of SFCs from close to the release site R9 to one of the two feeder sites cannot be distinguished from flight direction components no. 2a and 2b, the inbound vectors from the respective feeders. We, therefore, released animals also at other release sites (R10–R12,

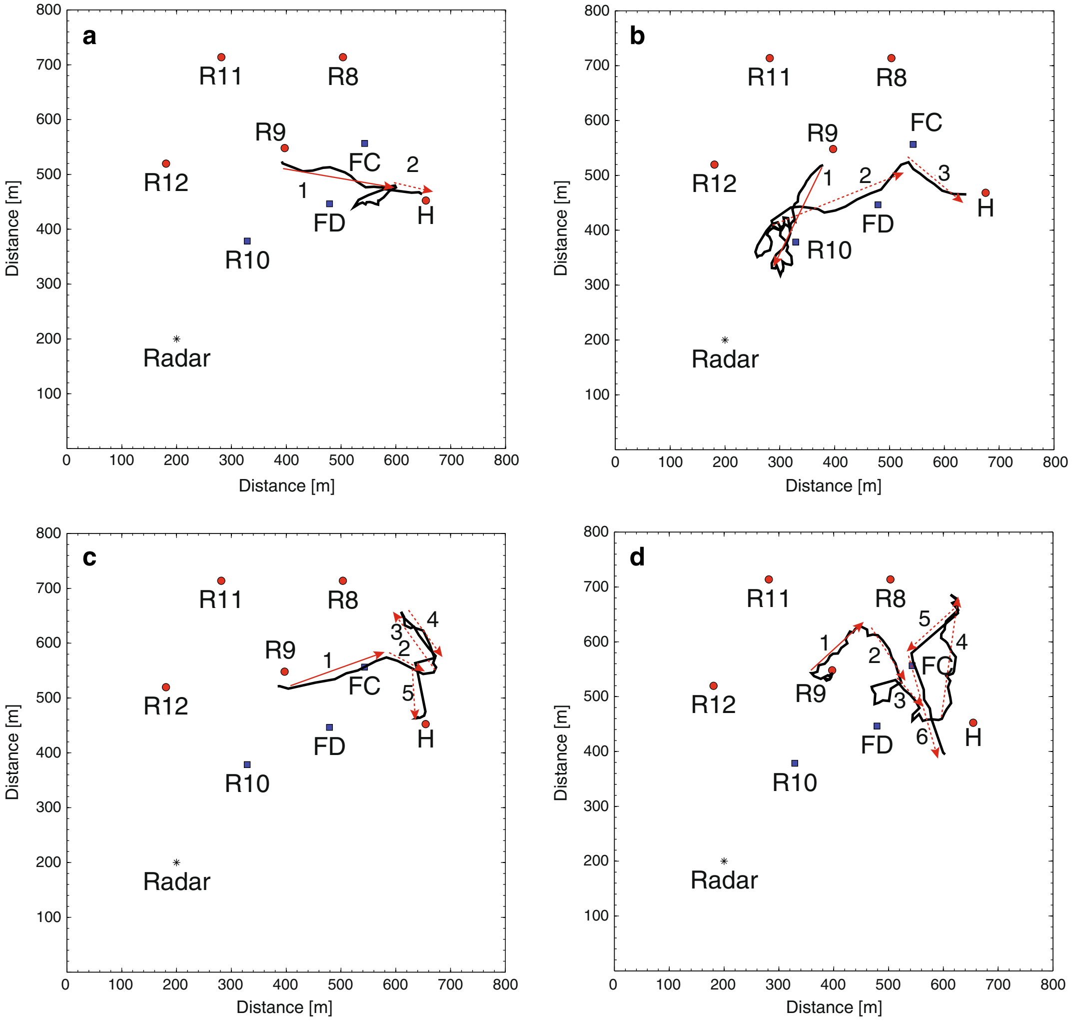

Figure 2. Four examples of complete flight tracks and assignment of first straight flight components (SFC) (*bold red arrow*) and secondary SFCs (*dotted red arrows*). The respective test animals were released at R9 after they were collected at FC (**a**, **b**, **d**) or at FD (**c**). The animals in (**a**) and (**c**) were collected after they filled their suck, those in (**b**) and (**c**) were collected immediately after touchdown. The consecutive SFCs are numbered. **a** This bee flew straight back to the hive (first and short secondary SFC) following flight direction category no. 3 (see Figure 1). **b** This bee performed first a SFC that would have brought it from FC to FD (flight direction category no. 1b) if it had not been transported to R9. Thus, it followed a location-incorrect flight direction category no. 1. Then it applied a short SFC of category 2a (*dotted line*, *2*), then a outbound component of category 2b (*dotted line*, *3*), immediately reversed the direction (*dotted line*, *4*, inbound component of 2b), and finally flew back to the hive (*solid line*, *5*, category no. 3). **c** This bee first followed a flight path close to the direction from FD to FC (flight direction category no. 1a). Since the bee did not terminate its first SFC at FC, this flight is categorised as location incorrect, then it took a direction of inbound category 2a (*dotted line*, *2*) arriving close to feeder FC, and finally flew back to the hive following category inbound 2 b (*dotted line*, *3*). **d** This performed first a location-incorrect SFC according to flight direction category no. 1a, then a category 2b inbound flight (*dotted lines*, *2* and *3*), then reversed the direction by applying an outbound category 2b flight (*dotted line*, *4*), then took a SFC according to category 1b (*dotted line*, *5*) which is characterised by an outbound motivation, and finally flew back to the hive following inbound category 2b flight (*dotted line*, *6*).

Figure 1), but we say in no case a first SFC towards one of the feeders sites.

Secondary SFCs resembled a series of SFCs which finally brought the animal back to the hive relatively quickly (dotted red lines in Figure 2a–d). As mentioned previously, the hive could not be seen at distances of >30 m, and thus any flights towards the hive from distances greater than this potential visual catchment distance were not guided by a beacon at the hive. The sequence of consecutive SFCs also indicated that flight vectors may be interrupted and new vectors performed. Sometimes the animal continued in the same direction after an interruption with a search flight component (Figure 2a (SFC 1 and 2)), or the next SFC had a different direction (Figure 2b–d). In some cases, two consecutive SFCs were performed after a 180° turn (Figure 2c (middle SFCs)) giving the impression that the animal corrected the SFC it had just applied by switching from an inbound to an outbound motivation or vice versa. Since most homing flights contained up to three SFCs, we analysed a maximum of three SFCs. The sequential application of more than one SFC in a homing flight may indicate that several vector memories exist side by side and are activated sequentially allowing the animal to switch between both motivation (inbound–outbound) and vectors memories.

The layout of the three sites H, FC, and FD as shown in Figure 1 allows us to derive five flight direction categories that may be applied as first or secondary SFCs either with a location-correct or location-incorrect directional component of the respective SFC. For example, in Figure 2a both SFCs 1 and 2 belong to flight direction category no. 3 and both are location correct since both are directed towards the hive from the respective initiation of the SFC. In Figure 2b, the first SFC belongs to flight direction category no. 1b which is location incorrect. The second SFC in Figure 2b appears to belong to flight direction category no. 2a and is location correct because it is initiated close to the correct location (FD), and the third SFC appears to belong to flight direction category no. 2b and is location correct since it is initiated at the correct location (FC) resembling the inbound component of the flight from FC to H.

3.2. First straight-flight components

Representative examples of first SFCs are shown in Figure 3a–c for test bees released at R9. Three categories can be distinguished, flights according to the vector components between the two feeders (Figure 3a, flight direction category no. 1a and 1b), flights resembling homing flight vectors from one or the other feeder (Figure 3b, flight direction category no. 2a and 2b), and flights directed to the hive (Figure 3c, flight direction category no. 3). In the first case, bees may performed this first SFC immediately after release (Figure 3a, bees 16, 22, 23, 24, and 42) or after extended circling search flights that brought them to regions further away from the release site (Figure 3a, bee 15). Such extended searching was also observed in bees that performed straight homing flights (Figure 3c, flight direction category no. 3), but since bees usually return to the release site after initial searching the first SFC towards the hive was usually initiated close to the release site. First SFCs according to flight direction category no. 2a or 2b (Figure 3b) were not preceded by different search flights after release indicating that the animals may have switched motivation from outbound to inbound and activated a home-directed vector memory when released. Animals performing flight direction category no. 1a or 1b tended to behave according to a vector that would have brought them from the feeding site where they were collected to the other feeder. Bees 15, 22, and 24 in Figure 3a were collected immediately on arrival at FD and applied a flight vector that would have brought them to FC if they had not been transported to R9. Animals 16, 23, and 42 in Figure 3a were collected at FC and applied a flight vector that would have brought them to FD if they had not been transported to R9. Only animal no. 23 in Figure 3a did not behave according to this rule. It is obvious that bees following a flight vector according to the bee line between the two

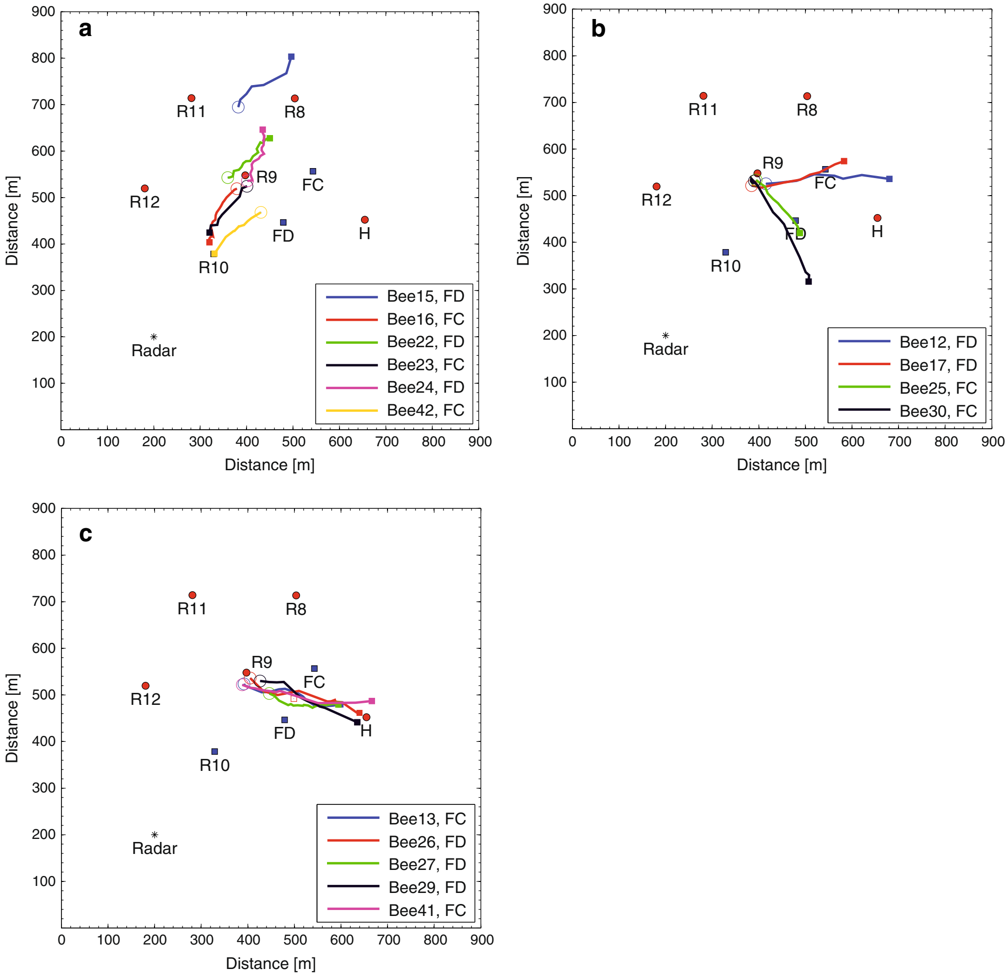

Figure 3. Representative examples of first SFCs of test bees released at R9. The number of the test bee and the feeder from which it was collected is given in the respective inset. The *circle* gives the start point of the respective SFC and the *filled square* its end point. The landmarks characterizing the feeding places and the release site are rather inconspicuous, thus the animals may not be able to notice at the release site that they have been moved to an unexpected location. **a** Animals followed the vector components of the bee line between the two feeders (flight direction category no. 1a or 1b, all flights belonging to the category location incorrect). Two of the animals (bees 15 and 42) performed first extended search flights before the first SFC shown here. Besides one animal (bee 24) the animals behaved as if they did not recognise the transport to R9, kept their motivation (outbound) and chose the direction which would have brought them to the respective other feeder. **b** Animals flew along a path resembling homing flight vectors from that feeder from which they were collected. Thus the animals switched motivation from outbound to inbound (flight direction category no. 2a or 2b). Bees 12 and 30 continued their SFCs beyond the feeder location they cross over making it very likely that they applied location-incorrect vector memories for flight direction category no. 2a (bee 12) or flight direction category no. 2b (bee 30). Bees 17 and 25 terminated their SFC close to a feeder location and thus may have applied a location-correct flight towards the respective feeder, but this possibility cannot be distinguished from the application of their respective home vectors. **c** Animals performed novel shortcut flights back to the hive (flight direction category no. 3). Bees 29 and 41 flew the whole distance to the hive and thus applied a location-correct flight direction category. Bees 13 and 27 terminated their flight direction category earlier and thus may have applied a location-incorrect flight direction category.

feeders would have ended up at the other feeder had they not been transported. They did not compensate for the displacement and appeared to apply a vector memory that is incorrect for the displacement conditions. Accordingly these flights were incorrect for the location. The flights following flight direction category no. 3 in Figure 3c, however, were location correct.

The four representative examples of first SFCs shown in Figure 3b comprise flight paths that resemble inbound vectors between the feeding sites and the hive. Animals no. 12 and 17 were transported from FD to R9 and animals no. 25 and 30 from FC to R9. These animals applied the respective home vector at the release site that would have brought them back to the hive if they had not been displaced, thus they belonged to the category location incorrect. The flight paths crossed over the respective other feeder or terminated there allowing for the possibility that the animals might have performed novel shortcut flights from the release site to the locations of the feeders. In that case, these flights would have belonged to category location correct. Notice that these locations were not marked by any features signalling the feeder location (see section 2). The geometric layout of the locations (hive, FC, FD, and R9), however, did not allow us to distinguish between feeder-directed flights from R9 and flight directions according to flight direction category no. 2a or 2b (vector from either FC or FD to the hive), and as mentioned above, we did not record any first SFC directed towards the feeder locations from other release sites.

Figure 3c shows five representative examples of direct flights back to the hive. These flights can be classified as novel shortcut flights because the animals had not performed this type of flight during training nor, most likely, at any other time during foraging as there were no natural food sources in the R9 region. Thus these flights were assigned to the location-correct category. One animal (no. 26) made a few sharp narrow turns at the bee line between FC and FD and then continued along the same direction until it reached the hive. Animals 13 and 41 came from FC, the other three from FD. Bee no. 13 had filled its crop at FC, whereas the other animals were collected immediately on arrival without any feeding. Half of the first SFCs at R9 (n=20) were directed toward the hive (flight direction category no. 3, n=10), whereas none of the first SFCs from animals released at R7, R8, R10 and R12 belonged to flight direction category no. 3 (n=7). Animals released at R9 flew roughly equally frequently towards the hive when transported from FC or FD (FC, n=4; FD, n=6). The other flight directions were about equally frequent in animals released at R9 (flight direction categories no. 1a, n=2; 1b, n=3; 2a, n=2; and 2b, n=3). From the six animals transported from FD to the release sites R8, R10, and R12 five performed flight direction category no. 1b flights which would have brought them from FD to FC had they not been displaced. Both animals transported from FC to these release sites performed flight direction category no. 1a flights which would have brought them to FD. Thus animals performing flight direction category no. 1a or 1b flights were likely to behave as if they had selected the flight directions which would have brought them from the feeder at which they were collected to the other feeder but now performed as location-incorrect flights. Note that the animals had never before flown this shortcut.

3.3. Angular distribution of all SFCs

Figure 4a shows the distribution of first SFCs together with the flight direction categories according to Figure 1. As a consequence of the experimental layout flight direction categories no. 1a, 1b, and 3 were rather different, whereas flight direction categories no. 2a and 2b were rather similar to that of 3. Using the methods of cluster analysis described in section 2, we found five clearly separated clusters (p<.02). The clusters given with pink and black, possibly also green dots in Figure 4a corresponded well to flight direction category no. 1a, 1b, the other two (or three) clusters (red and blue and possibly green dots in Figure 4a) included flight direction category no. 2a, 2b, and 3. These latter directions were rather similar (Figure 1). It is not surprising that the distributions of directions of secondary SFCs fell less well into different clusters

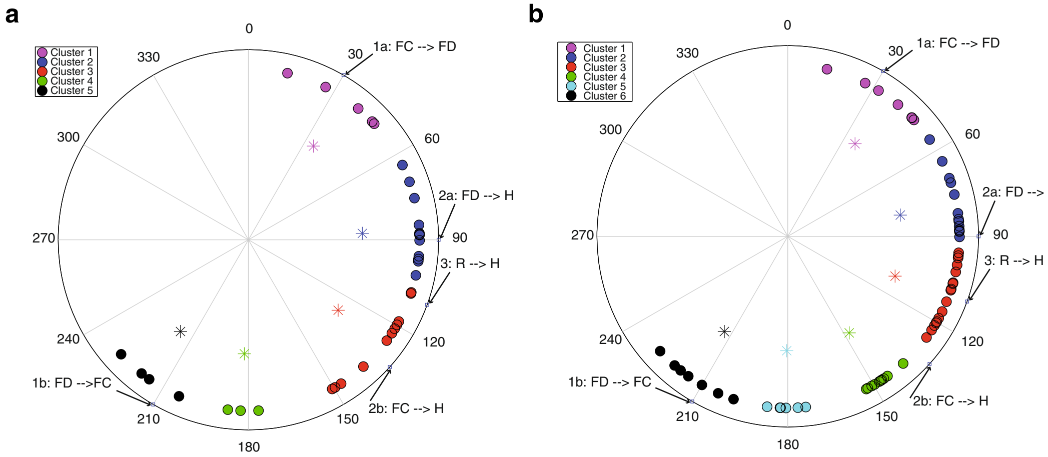

Figure 4. Cluster analysis of the first (**a**) or of all (**b**) SFCs. First SFCs fall into five clusters. The *arrows* give the respective flight direction categories as defined in Figure 1. The *asterisks* mark the respective average vector. We tested of nonuniformity using three different tests, Rayleigh's test, Hodges–Ajne test, and Rao's spacing test for nonuniformity. The results of all three tests were $p<.05$ for both distributions in (**a**) and (**b**). For data partitioning, we used a *k*-mean cluster algorithm for circular data. For the different cluster groups, we applied a nonparametric test for circular data (analogue to the Kruskal–Wallis test). Since in both cases (**a** and **b**) we found significant differences ($p<.05$), we used a nonparametric test (analogue to Wilcoxon rank sum test) for two circular medians for pair-wise comparisons and found $p<.05$ for all pairs.

(Figure 4b). Our analyses lead to six close clusters (separated with $p<.05$). Secondary SFCs were usually initiated closer to the hive and possibly guided by local landmarks guiding the animal back to the hive from different directions. It is thus not surprising that the distributions of secondary SFCs were broader than expected if only flight directions that were independent of the location were chosen. For example, the last secondary SFC shown in Figure 2c (dotted line 5) may be either a location-incorrect flight belonging to flight direction category no. 2b or a location-correct flight towards the hive (no. 3). Such location-correct flights towards the hive would be shortcuts originating at different locations thus leading to a broader distribution of directions.

We also analyzed the lengths of all SFCs (Figure 5) and found an inhomogeneous distribution but the three peaks are not significantly different due to the small number of measurements. The first peak around 140 m may correspond to the three sides of the triangle H-FC-FD-H, and the other peaks to SFCs stretch-

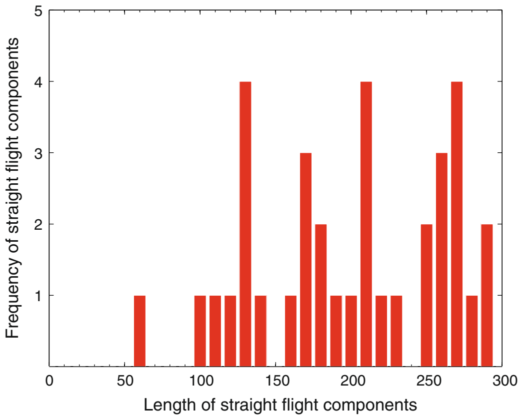

Figure 5. Frequency of the length of all SFCs. The distribution does not follow a normal distribution (Lilliefors test for linearly binned data; bin size, 10 m), but no significant differences are found between the various peaks due to the limited number of data. The first peaks correspond to the distance of the two trained routes and that between the two feedings sites (FC and FD). Release sites had distances to the hive of >280 m (see Figure 1).

ing over longer distances, e.g. in direct homing flights from the respective release site. However, when we examined the distribution of different flight direction categories we did not find shorter SFCs for no. 1a, 1b, 2a, 2b, and longer SFCs for no. 3. We conclude from these findings that the length of SFC is less well controlled than their respective directions.

4. DISCUSSION

So far navigation in bees has been tested in catch-and-release experiments after training to one feeding site establishing one route-based vector memory. Here we trained bees to two feeders and analyzed their SFCs during homing behaviour. We found multiple SFCs which can be interpreted as applications of multiple vector memories. These multiple vector memories may already appear in the first SFC after release or later in secondary SFCs. The vector memories belong to two forms, the experienced flight vectors reflecting the routes between hive and each feeder (flight direction category and flight direction category no. 2a and 2b), and vectors derived from vector integration (flight direction category no. 1a, 1b, and 3). These latter vectors have not been experienced by the bee but must have been derived from the spatial relations of the three important locations, the hive and the two feeders (FC and FD). Flight direction category no. 1a and 1b connect the two feeders; flight direction category no. 3 connects the release site (here mostly R9) with the hive. The respective SFCs can be either correct for the location where they are initiated or incorrect, besides flight direction category no. 3 which is always location correct. Consider for example flight direction category no. 1a or 1b, the short cuts between the two feeders. If these directions are chosen outside the connection between the two feeders FC and FD then they are location incorrect (Figure 3a), if chosen anywhere along the line between FC and FD then they are location correct. It is interesting to note that all SFCs belonging to location-incorrect flight direction category of no. 1a or 1b were chosen further away from the hive than the bee line between FC and FD possibly indicating that the animals related their choices to the overall spatial relations of the three sites H, FC, and FD.

SFCs of flight direction category no. 1a or 1b are always derived flight directions irrespective of whether they are location incorrect or location correct. An experimental bee finding itself at an unexpected site after arriving at, e.g. FC from the hive might recall from its memory the other outbound flight vector (e.g. H to FD), keep its outbound flight motivation, and perform vector subtraction between these two vectors (the vector already applied and the activated vector). Such vector integration would lead to flight direction category no. 1a or 1b along the connecting line between FC and FD. Indeed most of the animals performing no. 1a and 1b flights behaved in a way which would have brought them from the feeder where they were collected to the other feeder.

The choice to fly according to flight direction category no. 2a or 2b (the directional component of the home flights from the two feeders) requires a change in motivation from outbound flights to inbound flights. It is therefore not surprising that SFCs along no. 2a or 2b were rare as first SFCs and frequent as secondary SFCs. These flights may be initiated at a greater distance from the two feeder locations or between the feeders and the hive. In the first case these directions frequently coincided with the shortcuts from the current location to either of the two feeders (e.g. following flight direction category no. 2a from close to R9 would lead to FC and following no. 2b to FD). The layout of the three locations H, FC and FD did not allow us to distinguish between vector flights along flight direction category no. 2a and 2b and the respective location-correct shortcuts towards the feeders. It is quite possible that some no. 2a or 2b SFCs may in fact be location-correct shortcuts to one of the feeders because they were frequently terminated at these locations although all materials marking these locations were removed during tests. Location-incorrect flight direction category of no. 2a and 2b may bring the bee closer to the hive or further away (e.g. in the case where no. 2a is applied closer to FC and no. 2b closer to FD).

Such location-incorrect flights of no. 2a or 2b were often longer than the corresponding vector from one of the feeders to the hive. It is thus not surprising that the analysis of the SFC lengths indicated much longer flights than the learned vectors (Figure 5). If flight direction category no. 2a or 2b were initiated close to a feeder site (no. 2a close to FD and no. 2b close to FC) then these flights were always location-correct indicating that the local landmarks reminded the animal about the correct homing flight.

SFCs of flight direction category no. 3 require a change of motivation similar to flight direction category no. 2a and 2b. First SFCs belonging to flight direction category no. 3 were rather frequent, clearly documenting novel shortcuts towards the hive. We observed that half of the first SFCs at R9 ($n=20$) were directed toward the hive (flight direction category no. 3, $n=10$), whereas none of the first SFCs from animals released at R7, R8, R10, and R12 belonged to flight direction category no. 3 ($n=7$) possibly indicating that the area around R9 may resembled more closely the landmarks characterizing FC and FD, and thus would favour the activation of both home vectors from the two feeders leading to either a compromise flight vector or to vector integration of both vectors.

An alternative interpretation assumes that the geometric relations between R9 and hive favoured the novel shortcut towards the hive, a behaviour that would require knowledge of the geometric relations between the respective locations. As in the study of Menzel et al. (1998), these data do not allow to distinguish between these two interpretations. It has been argued multiple times that the process of retrieving a vector memory on the basis of landmarks, switching motivation between outbound and inbound, and vector subtraction conceptualises are more parsimonious neural procedure than a geometric representation in spatial memory (Collett and Collett 2000, 2002; Merkle and Wehner 2008; Sommer et al. 2008; Wehner et al. 2006). Indeed, a rather simple model of memory retrieval, motivational switch and path integration (Cruse and Wehner 2011) formally meets the requirements to predict navigational performance of bees as tested in the study presented here and in the Menzel et al. (2005) study. Whether the model captures a more parsimonious neural implementation is a different question and must be kept open as long as we do not have any data on the neural processes in the insect brain allowing the animal to navigate over long distances in a highly flexible way and to communicate about locations using the same spatial reference frame (Menzel et al. 2011).

Any further interpretation of the data presented her needs to incorporate the following conditions: (1) The test area did not provide any cues visible from the three sites (FC, FD, and R9) thus beacon orientation and panorama matching is excluded. (2) The local conditions at the releases sites did not differ in any obvious way. It thus can well be that the animals at a release site retrieved inbound vector memories from both feeding sites and flew a compromise angle steering towards the hive (first SFCs of category no. 3.) This interpretation is supported by the finding that flights along direction category no. 3 were often longer than the learned vectors. One might assume that an analysis of hive-directed flights from different locations might allow us to distinguish between the vector integration hypothesis and the geocentric map hypothesis. Unfortunately, this is not possible with our experimental layout because SFCs along flight direction category no. 3 from other locations than close to R9 were rather close to either flight direction category no. 2a or 2b.

Taken together, the data clearly document the application of several forms of vector memories both retrieved and derived from vector integration. Furthermore, bees switch between inbound and outbound motivation and between different vector memories in sequential straight flight sectors. Any interpretation needs to consider the intense training to two feeders and the rather inconspicuous landscape in which we performed these experiments. Both factors may have favoured reference to vector memories rather than to those forms of memory that are established during exploratory orientation flights early in the life history of each forager bee. We argued in earlier studies (Menzel et al. 2005, 2011) that spatial relations of landmarks may guide bees in their search behaviour when they are relieved from the initial dominance of vector memories. Further experiments are required to elucidate the relation between navigation mechanisms controlled by spatial memory acquired during exploratory and route flights. Furthermore, it needs to be tested further whether the resulting network of multiple vector components learned during multiple successive route flights may establish a map-like representation allowing the bee to make shortcuts according to its actual state of motivation.

5. CONCLUSIONS

First and secondary straight flight components in homing behaviour of captured, transported and released bees reflect multiple vector memories belonging to two classes of directional components, novel shortcuts and trained-route flight directions. Novel shortcuts (flight direction category no. 1a, 1b, and 3) appear when animals take the direct connection between the two feeders (in both directions), and when they fly straight back to the hive. If flight direction category no. 1a and 1b were expressed as first SFCs than they were incorrect for the location, and therefore did not bring the animal to the intended goal. In such a case, the animal must have applied vector integration between the vector components of the route memories from the hive to the two feeders. The same flight direction category no. 1a or 1b could also be performed at the locations of the two feeders. In that case, the animals applied the vector-integrated direction correct for the location. In the case of flight direction category no. 3 straight flight components brought the animal closer to the hive. This behaviour resulted from location-correct application of vector integration of routes between hive and the two feeders and is bound to a change of motivation from outbound to inbound. Flight directions according to flight direction category no. 2a and 2b, the inbound components of the trained routes, brought the animals close to one of the feeders or further away from a feeder depending on which of the two directions were chosen. Flights towards a feeder location may contain a component of location-correct novel shortcutting towards the respective feeder location. However, the spatial arrangement of the hive and the two feeders in our experiment (along an equal sided triangle) did not allow us to separate such potential shortcuts from location-incorrect vector flights. Irrespective of these limitations we conclude that both vector memories of the learned routes and novel shortcuts towards the hive characterise the navigation strategy of honeybees after intensive route training. Location-correct shortcuts may be explained by the integration of the trained vectors (combined with a motivational change from outbound to inbound), but an allocentric map-like organization of the navigational memory cannot be excluded.

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Intégration vectorielle et réduction des distances de vol dans la navigation des abeilles.

Navigation / intégration vectorielle / raccourcissement du vol / carte cognitive

Vektor Integration und kürzeste Flugstrecken bei der Navigation von Honigbienen

Navigation / Vektorintegration / kürzeste Flugroute / Kognitive Karte

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