

RESEARCH ARTICLE

Variations on a theme: bumblebee learning flights from the nest and from flowers

Théo Robert^{1,*}, Elisa Frasnelli^{1,2}, Natalie Hempel de Ibarra¹ and Thomas S. Collett³**ABSTRACT**

On leaving a significant place to which they will return, bees and wasps perform learning flights to acquire visual information to guide them back. The flights are set in different contexts, such as from their nest or a flower, which are functionally and visually different. The permanent and inconspicuous nest hole of a bumblebee worker is locatable primarily through nearby visual features, whereas a more transient flower advertises itself by its colour and shape. We compared the learning flights of bumblebees leaving their nest or a flower in an experimental situation in which the nest hole, flower and their surroundings were visually similar. Consequently, differences in learning flights could be attributed to the bee's internal state when leaving the nest or flower rather than to the visual scene. Flights at the flower were a quarter as long as those at the nest and more focused on the flower than its surroundings. Flights at the nest covered a larger area with the bees surveying a wider range of directions. For the initial third of the learning flight, bees kept within about 5 cm of the flower and nest hole, and tended to face and fixate the nest, flower and nearby visual features. The pattern of these fixations varied between nest and flower, and these differences were reflected in the bees' return flights to the nest and flower. Together, these findings suggest that learning flights are tuned to the bees' inherent expectations of the visual and functional properties of nests and flowers.

KEY WORDS: Insect navigation, Place learning, Route learning**INTRODUCTION**

Bees and wasps perform what are known as learning flights when they leave a location to which they will return. Such sites may be their nest (e.g. Becker, 1958; Hempel de Ibarra et al., 2009; Tinbergen, 1932; Wagner, 1907; Zeil, 1993a) or a feeding site (e.g. Lehrer, 1993; Opfinger, 1931), or, in the case of parasitic wasps, the location of a host's nest (Rosenheim, 1987). Learning flights are structured so that the insects can learn enough about the surrounding landscape on a single flight to guide a successful return. This basic function of the flight is similar across species and the different goals to which the insect returns. It is thus not surprising that there are similarities between, for instance, the learning flight of the sand wasp *Cerceris* when leaving its nest (Zeil, 1993a) and the wasp *Vespula* when leaving a feeder (Collett and Lehrer, 1993). By contrast, nests and flowers have very different functions and properties. Nest holes can be inconspicuous, whereas flowers usually advertise themselves to be

visually striking. An insect normally has only one nest to which it is essential that it returns. In contrast, flowers of one species are dispersed, often in patches of the same colour, thereby attracting pollinators from a distance (Benitez-Vieyra et al., 2007; Gumbert and Kunze, 2001; Johnson et al., 2003). With many flowers close together, there is less need to learn a single plant's precise location; moreover, individual flowers are often short-lived. We investigate here in what ways a bumblebee's learning flights at the nest and at a flower are tailored to such differences.

The specific questions with which we approached this issue were first whether learning flights are longer when a bumblebee leaves its nest than when leaving a flower. Differences in duration could allow bees to obtain more precise information for learning the location of an inconspicuous nest, either through repetition of particular manoeuvres or through the performance of a greater range of manoeuvres. Typically, individual bees and wasps perform learning manoeuvres on several departures from the nest or a newly discovered flower. Because a conspicuous flower provides an obvious target for a bee's approach, fewer learning flights may be needed for a rapid and successful return to a flower than are needed to reach the nest. Does the duration of learning flights in fact decay faster over a sequence of departures from a flower than from the nest?


A second related question is whether the bees' learning manoeuvres might be more focused on a conspicuous flower than on the surroundings and more distributed in space when learning the location of an inconspicuous nest that can often only be located through its relation to surrounding visual features. Concentrating a learning flight on a flower might occur not only because it is an obvious target for guiding a future return, but also because, by learning its appearance better, bees would be more able to recognise the same flower elsewhere.

To answer questions of this kind, we made the visual scene at the nest and the flower as similar as possible (Robert et al., 2017). Differences in learning flights would then indicate intrinsic differences related to the nature of the goal and the bees' internal state, rather than a consequence of the surroundings in which the flights occur. *Bombus terrestris* is particularly appropriate for such a study as this bee both nests in the ground and will also forage at low lying flowers such as clover. We could thus mark the nest hole with a coloured ring on the ground and use an identical ring as a flower, with black cylinders nearby to provide additional locational cues (Robert et al., 2017). We could then examine the learning flights of the same bee as it left its nest and a flower. To investigate how bees looked at their surroundings during learning flights, we compared the ways in which the bees faced the array of cylinders during learning flights at the nest and the artificial flower.

Return flights were also analysed to see whether differences in learning flights at the nest and flower are reflected in later return flights to those places. Lastly, we performed tests with the nest hole covered and the sucrose dispenser removed to compare how the bees search for their nest or the flower in the presence or absence of the ring.

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MATERIALS AND METHODS

Experimental procedures

Experiments were conducted in a greenhouse (8×12 m floor area) at the Streatham campus of the University of Exeter, UK. Bumblebees, *Bombus terrestris audax* (Linnaeus 1758), from commercially reared colonies (Koppert, Haverhill, UK), were marked individually with coloured number tags. The colony was placed under a table, the ‘nest table’, and we recorded the flights of worker bees as we allowed them to leave their nest, one at a time (see ‘Training’ below), through a hole in the centre of the table. The nest hole was surrounded by a matte purple plastic ring (5 cm outer diameter) placed flat on the table top. In addition, three black cylinders (17×5 cm) were placed in a 120 deg arc around the nest hole with their centres 24.5 cm from the hole. The artificial flower, an identical purple ring with a sucrose dispenser (50% w/w) in its centre, marked by the same arrangement of cylinders, was placed on the top of another table, the ‘flower table’, 5 m away.

Both tables (1.5×1.8 m) were covered with white gravel that was frequently raked. Both the flower and nest ring were frequently cleaned. The behaviour of bees leaving the nest and the flower was recorded continuously during the experiments at 50 frames s⁻¹ with video cameras (Panasonic HC-V720, HD 1080p) that were hung 1.35 m above each table and captured an area of about 70×90 cm in an image of 1920×1080 pixels.

Training

Bees were naive to the experimental surroundings and at the start of training had not previously left the nest. To avoid bees interacting with each other, gates were operated so that bees left the nest one at a time. After a bee performed a learning flight close to the nest, it flew around the greenhouse and, after it had landed or slowed down, it was caught in a butterfly net and transferred into a tube. A few minutes later, the bee was introduced to the artificial flower by placing the tube over a similar flower on a third table, the ‘training table’, which, like the flower and nest tables, had a purple ring, but did not have an array of cylinders. Once the bee started to drink, the tube was slowly removed. Bees on the flower generally continued drinking and, when done, typically after 30–50 s, performed a learning flight on leaving the flower. The bees were then left to fly and return to the nest table, where they entered the nest through the centre of the ring. On the bee’s second departure from the nest, the training table was covered and the bee was left to find the flower on the flower table.

Most bees, after their pre-training, found and fed from the flower and we worked with the 19 bees that did so. One of the 19 started to fly erratically partway through the experiment and its later flights were not analysed. Bees had six departures from the nest and made five visits to the flower before tests were given.

In order to check whether the duration or pattern of flight at the flower table might be affected by having the same arrangement of cylinders on the two tables, we performed the following control experiment. The nest table had neither cylinders nor a purple ring and the flower table had the purple ring and the usual array of three cylinders. A second change made to speed up the experiment was to dispense with the training table. After bees had left the nest, they were caught and placed directly on the flower. We recorded and analysed the bees’ learning flights on their departure from the flower.

Tests

Each bee received four different tests: two at the nest table and two at the flower table. Before each test, either the nest was covered by a sheet of plastic topped with gravel, or the sucrose dispenser on the

flower table removed. The cylinder array was translated to a new position and the gravel raked. In one set of tests, the purple ring was absent. In a second set, the ring was placed in its normal position with respect to the cylinder array. Different purple rings were used for tests and training. The tests were given during four foraging circuits after training was complete. One test was given on each circuit, alternating between tests at the nest and the flower. The bee’s flight on its approach to the tested table was recorded until it stopped approaching the general location of the nest or flower. The usual nest or flower arrangement was then restored and the bee returned home or fed from the flower.

Data analysis

The positions and body orientations of the bees were extracted from the video recordings using custom-written codes in MATLAB (Philippides et al., 2013). The duration of a flight was defined by the time it took a bee to cross a circle of 24 cm radius centred on the nest entrance or the flower. Cumulative distance indicates the distance that a bee travels before it first crosses a circle of a given radius.

In some figures, flight parameters are plotted against normalised time. Each individual bee’s flight was then divided into ten equal durations and the relevant value of the flight parameter computed for each of the ten divisions. This procedure allowed changes in the parameter to be examined across flights of different durations. In some analyses, the flight was divided into two portions, with the first third taken as the ‘initial phase’ and the remainder as the ‘later phase’. Fig. S1 illustrates the usefulness of this procedure.

Fixations relative to a particular position (e.g. nest or flower or cylinder) are defined as times during the flight when the image of, say, the nest was stationary on the retina (within ±3 deg) for at least 80 ms (see Robert et al., 2017 for a detailed explanation of the algorithm for extracting fixations). Fixations of the nest itself (or flower) is the subset of fixations in which the image of the centre of the nest or flower lies within ±20 deg of the bee’s longitudinal axis (see also Fig. S2). Because real and virtual cylinders are relatively close, cylinder fixations are restricted to when the centre of the cylinder lies within ±10 deg of the bee’s longitudinal axis. Fixation rate is the ratio of the number of fixations to the total number of frames within a specified time interval.

Although views memorised at the nest and flower are likely to be insulated from each other by the differing contexts (Fauria et al., 2002), we checked for interference by training a separate cohort of bees with no cylinders or ring on the nest table and with the usual cylinder array and purple ring on the flower table. A single learning flight was recorded at each table. The results of this control experiment (Fig. S3) show that, despite some differences, the basic structure of the learning flights at the flower table is similar.

Tests were analysed using Adobe CS6 Premiere Pro to measure durations and Photoshop to collect the coordinates of the bees’ landings. Landings were recorded as one of three types: (1) landings following an approach to the goal from the edge of the table; (2) repeated landings that occurred after landings of type 1 (data not shown); and (3) landings near the bottom of cylinders, with the bee usually facing a cylinder.

When bees first arrive at the nest or flower table, they often do so at speed and remain in view very briefly before disappearing from the video frame. In later approaches to the table, they reduce their flight speed and often land. Even bees that do not land tend to slow down at particular points on the test table and appear to be searching. These ‘slowdowns’ give another measure of where the bees are when they locate the nest and flower. We extract slowdowns from each bee on the first approach to a test table in which the bee

reduced its speed. The position of a slowdown is taken as the point where flight speed is at a minimum. The first three of such 'slowdown' troughs are extracted automatically from each bees' first approach and their positions noted.

Statistical analyses were conducted in R (3.3.0; <https://www.r-project.org/>). The package 'circular' was used to compute circular means and the mean resultant length (ρ). Wilcoxon tests were used for comparisons between flights; for example, the first and fourth departures from or returns to the nest or flower. A few departures ($N=23$ out of 152) in which the bees flew abnormally and crash-landed or bounced on the gravel were not analysed. Return flights immediately following an abnormal learning flight were also excluded. Four additional return flights were not recorded because they occurred while the camera's memory card was being changed. Because of these complications, the number of flights included in each statistical test varied. Sample sizes are given in the figure legends.

RESULTS

Durations of and areas covered by learning flights at nest and flower

Differences between the durations of learning flights at the nest and the flower and the areas that the bees cover during these flights are great enough to be obvious when scrutinising the first and fourth flights of a single individual. In the example of Fig. 1, the two flights at the nest (LN1 and LN4) are more than twice as long as they are at the flower (LF1 and LF4), with the bee covering a larger area on its flights from the nest. In its first flight, the bee looks back to face the nest and the flower; by the fourth flight, the bee continues to look back to face the nest, but it moves directly away from the flower with scarcely a backward glance (Fig. 1). These obvious differences hold generally across the sample of learning flights (Fig. 2), as shown by cumulative plots of the durations and distances of the flights plotted against distance from the nest or flower. In fact, the bees' very first learning flights from the flower are at the training table when it

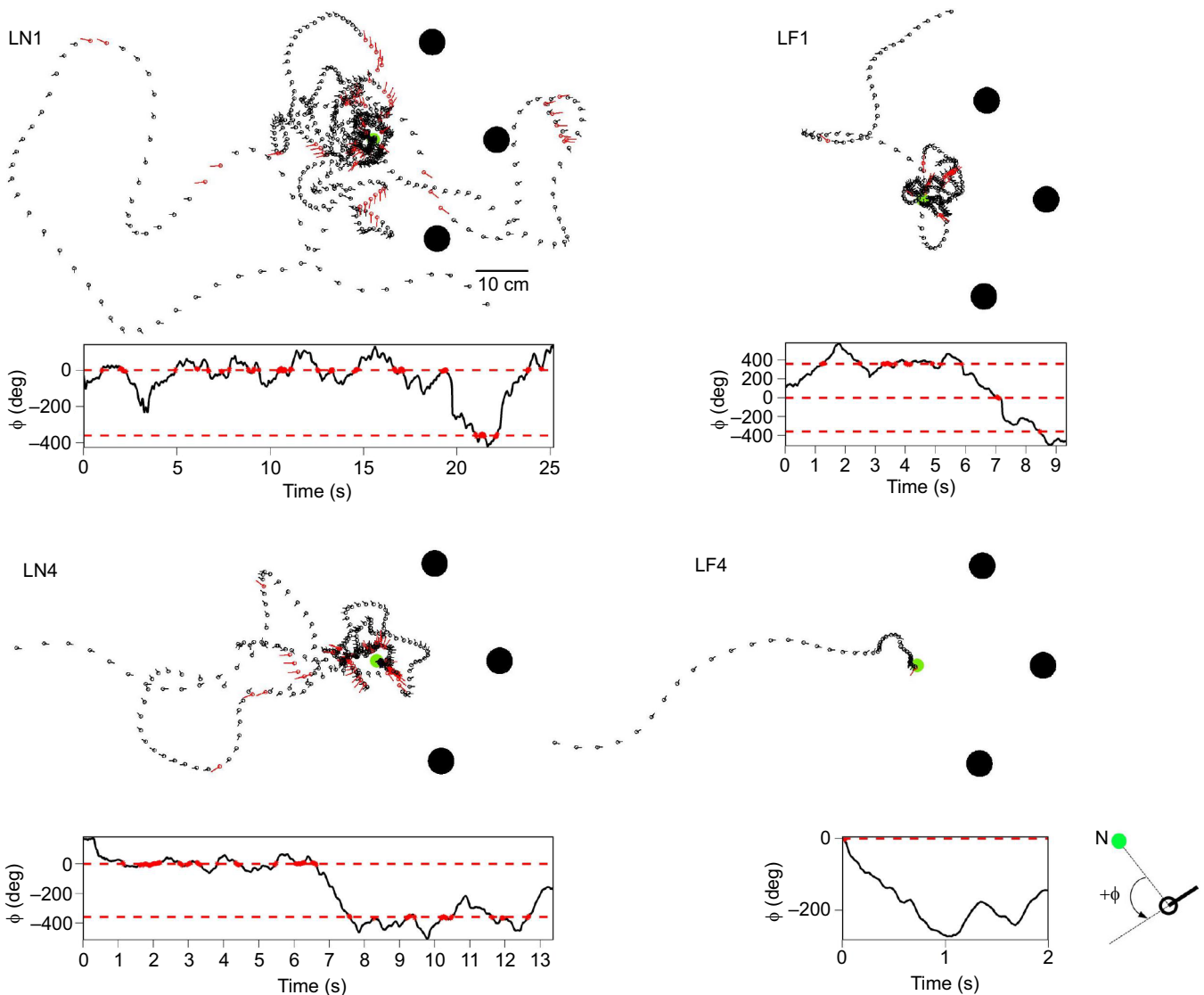


Fig. 1. Top views of the first and fourth learning flights of a single bee at the nest (LN1, LN4) and flower (LF1, LF4). The small white circles show the positions of the bee plotted every 40 ms during its trajectories and the tails show its body orientation. Red circles and extended tails show when the bee faces the nest or flower (± 10 deg). The large black circles represent the positions of the cylinders and the green circle the nest or flower. Plotted below each trajectory is the orientation of the bee relative to the nest or flower (ϕ , deg). Dashed red lines show the orientation in which bees faced the nest or flower. Inset on the bottom right shows ϕ with the arrow pointing in the positive direction.

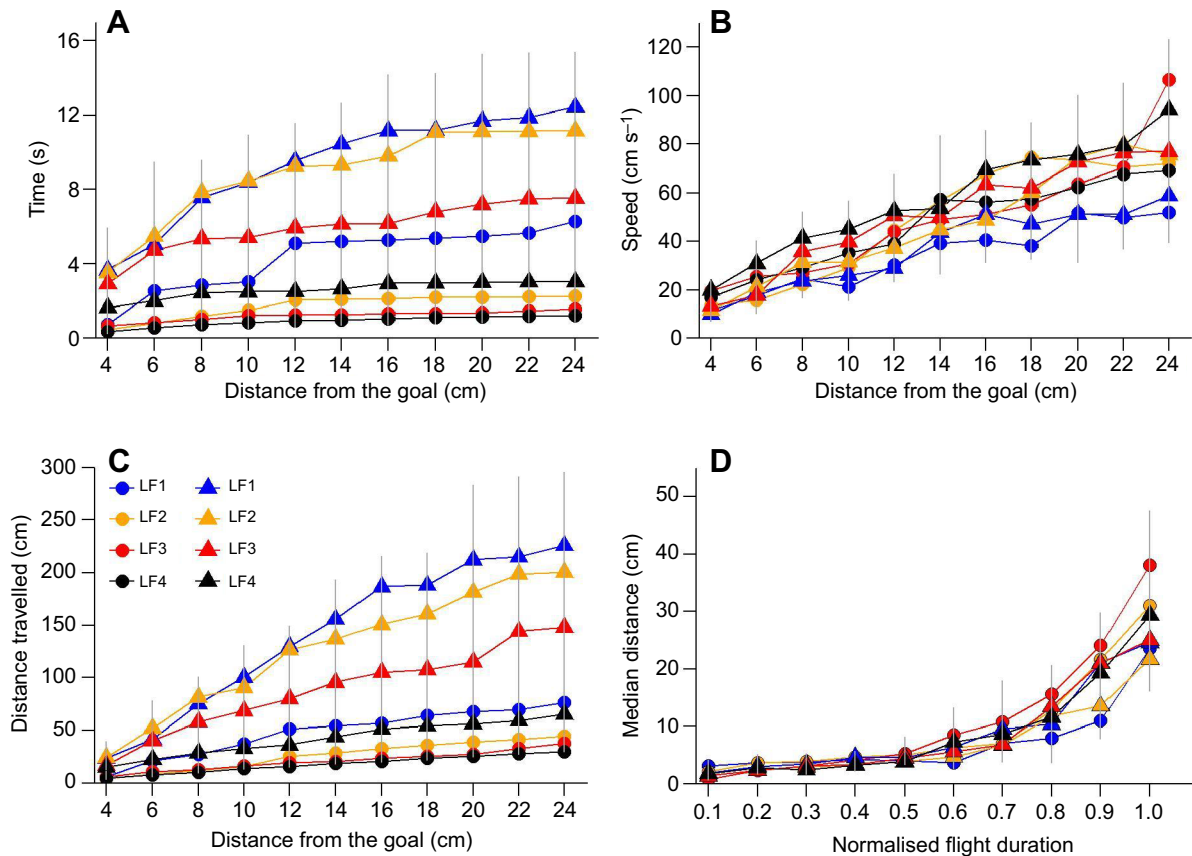


Fig. 2. Some parameters of the first four learning flights at the nest (LN1–LN4) and flower (LF1–LF4). (A) Median times [and interquartile ranges (IQRs)] taken by bees to cross for the first time a specified radial distance from the nest or flower to a maximum radial distance of 24 cm (LN1/LN2/LN3/LN4: $N=19/18/18/18$ bees; LF1/LF2/LF3/LF4: $N=15/12/13/13$ bees). (B) Similar plot but showing speed in relation to distance from the goal, with each bees' speed computed within a ± 40 ms interval around the crossing time. (C) Similar plot of trajectory length. (D) Bees' median distance from nest or flower plotted against normalised time (see Materials and methods). The median duration of LN1 (12.44 s, 5.1 IQR) was longer than LF1 (6.26 s, 4.05 IQR; Wilcoxon, $N=15$, $W=4$, $Z=-3.18$, $P=0.001$). Similarly, LN4s (3.04 s, 2.95 IQR) were significantly longer than LF4s (1.20 s, 0.68 IQR; Wilcoxon, $N=13$, $W=3$, $Z=-2.97$, $P=0.003$). Both LN and LF became significantly shorter over successive visits (LN1 versus LN4: $N=18$, $W=161$, $Z=-3.29$, $P=0.001$; Wilcoxon, LF1 versus LF4: $N=12$, $W=78$, $Z=-3.06$, $P=0.002$).

becomes familiar with the flower with no cylinders present (see Materials and methods). The median duration of these flights is 6.63 s [5.82 interquartile range (IQR), $N=22$], which is no longer than that of LF1 (median duration 6.26 s; 4.05 IQR, $N=15$) (Wilcoxon test, $N=15$, $W=73.5$, $Z=-0.77$, $P=0.44$). In the distance plot (Fig. 2C), the steeper the slope, the longer the distance the bee covers within a specified annulus before leaving it. Thus, for the first learning flights at the nest, the slope is steepest until the bee has travelled approximately 16 cm from the nest and remains relatively flat thereafter. Note that the slope is roughly constant at the start, even though the area increases with the distance of each annulus from the centre, indicating that the bees spend a preponderance of the flight close to the nest.

The slope for the first learning flight from the flower is shallower than from the nest at the start of the flight (Fig. 2C). After the bees are approximately 12 cm from the flower, the slope becomes almost flat, indicating that at this point the bees fly directly away. The plots of the cumulative durations of the flight reflect this change (Fig. 2A), and the slopes of the plots also show corresponding drops at 16 cm and 12 cm. Because flight speed increases during each flight (Fig. 2B), these points of inflection are more pronounced in the plots of duration.

By plotting the bees' median distances from the nest or flower against normalised flight duration (Fig. 2D, Fig. S1), we see for all flights that, over the first 30–40% of the flight, bees remain within a

few centimetres of the goal. After this initial phase, the bees gradually travel further from the nest or flower. Because of the striking difference between the initial and the later phases of the flights, we consider them separately in some of the following analyses.

Further evidence that bees cover larger areas during their learning flights at the nest than at the flower is seen in heat maps that show the bees' positions accumulated across the first learning flights of all bees (nest, Fig. 3A; flower, Fig. 3B). The same data are re-plotted in two ways. The first (Fig. 3C) shows the normalised number of data points in successive 5 cm annuli centred on the nest or flower. This number declines with distance from the nest and flower, but more rapidly in flights at the flower than at the nest. The second plot (Fig. 3D) is of the normalised number of data points in 45 deg radial segments centred on the nest or flower. The bees spend more time in the segments opposite to the cylinders at both nest and flower, but this radial asymmetry is greater for flights at the flower.

Looking patterns during learning flights at nest and flower

Two sets of visual cues in the immediate vicinity of the nest and the flower could supply the bees with information to guide their returns to the nest and flower. The first is the ring that acts as a flower or surrounds the nest hole, and the second the three cylinders 24.5 cm away from the nest and flower. With what spatial and temporal patterns do bees view these cues during their learning flights and

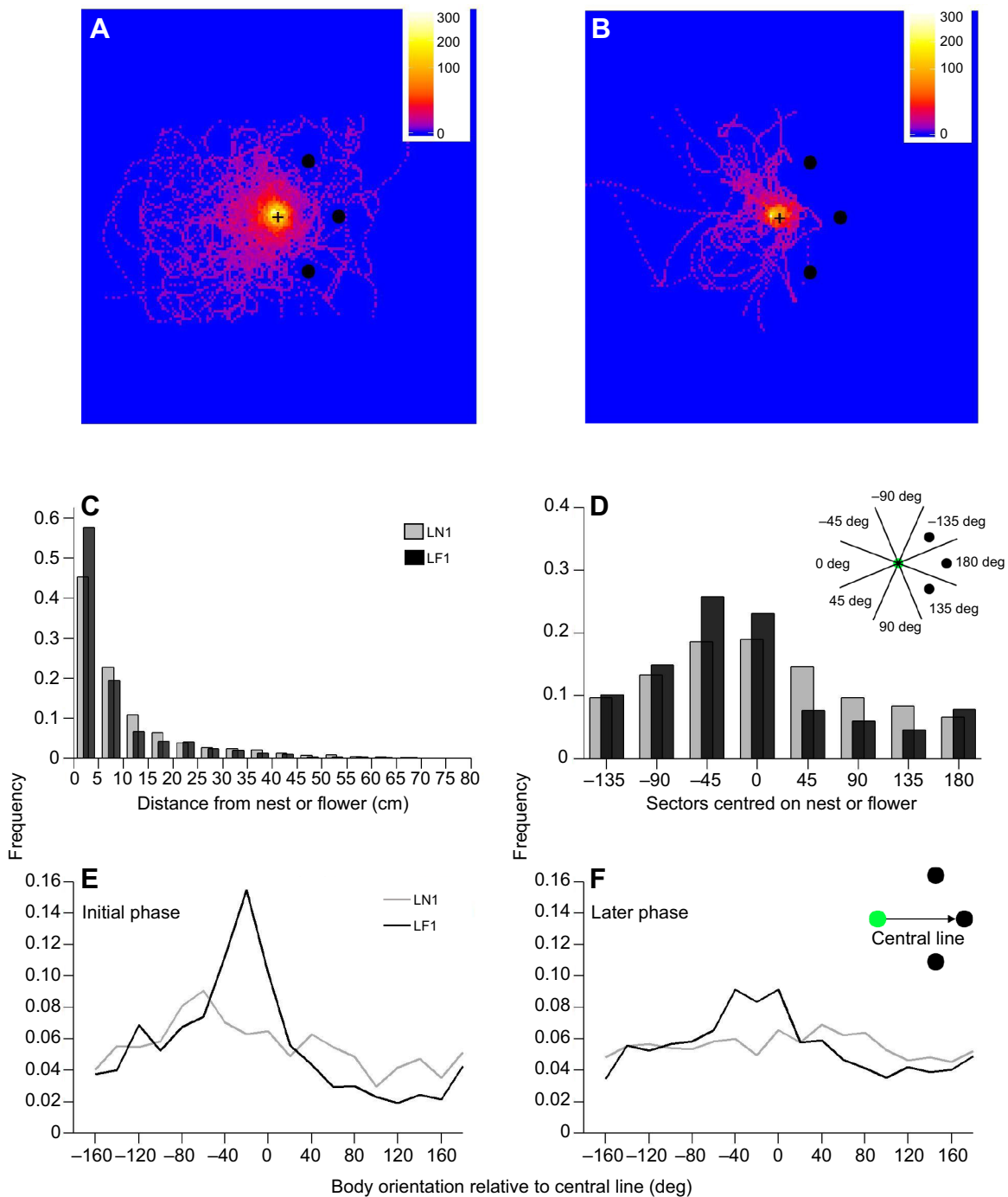


Fig. 3. Some spatial and directional parameters of LN1 and LF1. (A,B) Heat maps of the positions of the bees during LN1 (A) and LF1 (B). The black + marks nest hole or flower. Black circles mark the cylinders. Scale shows the number of frames in which a given area (1×1 cm) was visited. LN1: $N=19$ bees, LF1: $N=16$ bees. (C) Frequency of all bee positions in A and B grouped by distance from flower or nest. The overall median distances from nest and flower do not differ ($N=16$, $W=90$, $z=-1.34$, $P=0.2$). (D) Frequency of bee positions in A and B grouped by the radial sector centred on flower or nest in which they occur. Mean angular position of LN1 is -17.7 deg [vector amplitude (ρ)=0.25], and of LF1 is -42.3 deg ($\rho=0.40$). (E) Frequency distribution of the bees' body orientation relative to the central line of the array (see inset on the right) for the initial phase of LN1 and LF1. Bin width is ± 10 deg. Mean orientation of LN1 is -49.98 deg ($\rho=0.16$) and of LF1 is -40.46 deg ($\rho=0.38$). (F) As in E, but for the later phase of LN1 and LF1. Mean orientation of LN1 is 5.04 deg ($\rho=0.06$) and of LF1 is -33.38 deg ($\rho=0.19$).

how do viewing patterns vary between learning flights at the nest and flower?

In the initial portions of learning flights, bees tend to face in the direction of the cylinder array. In flights at the flower, bees focus strongly on the centre of the array and in flights at the nest they face

more broadly with a peak towards the bottom cylinder of the array (Fig. 3E). In the later portion of the flights, the focus is reduced in flower flights and absent in nest flights (Fig. 3F). In the following two sections, we dissect in more detail the ways in which bees fixate the nest, the flower and the cylinders.

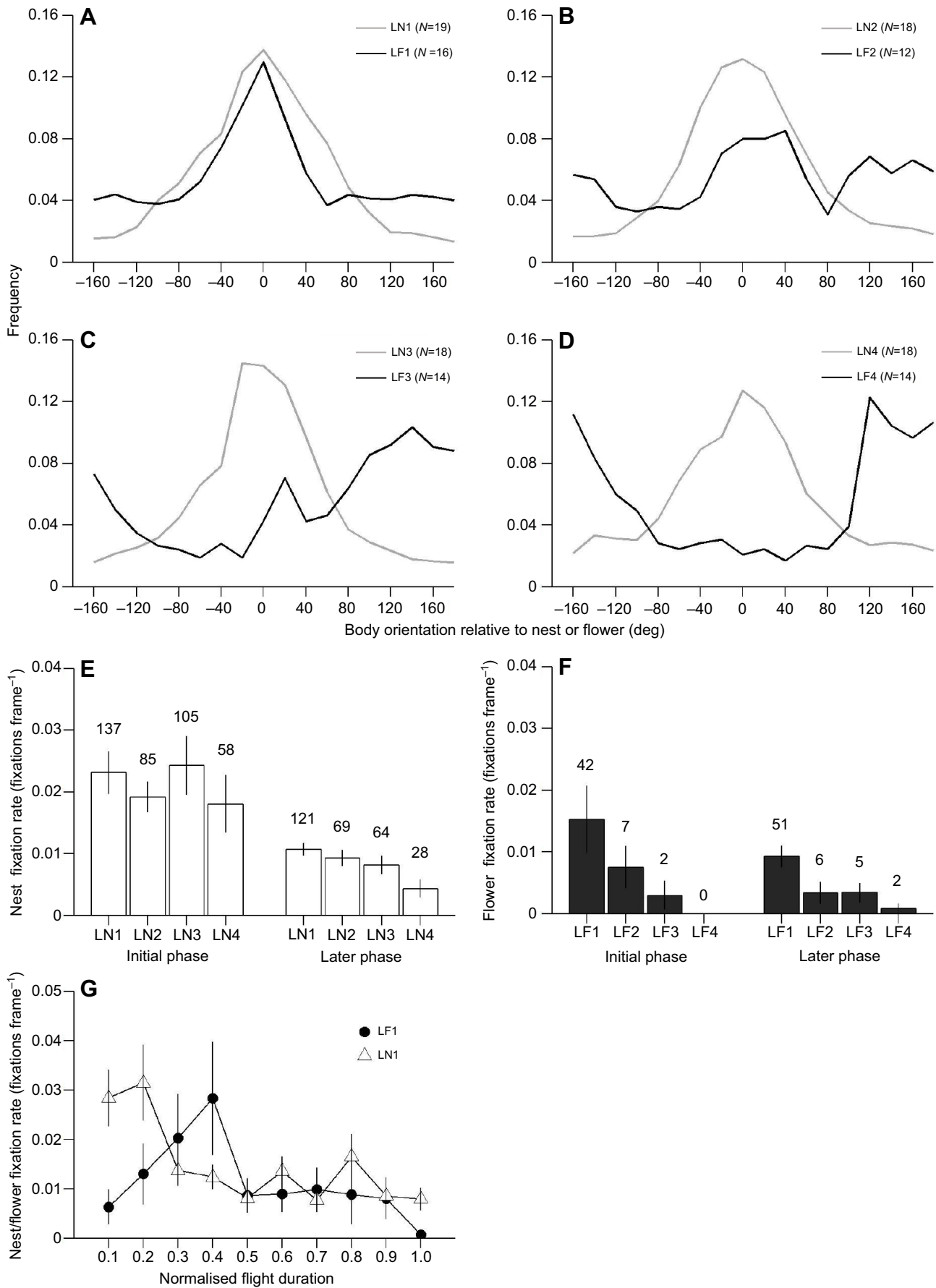


Fig. 4. See next page for legend.

Fig. 4. Fixating nest and flower. (A–D) Bees' body orientation relative to the nest or flower during LN1–LN4 and LF1–LF4. Data are pooled across the complete flights of all recorded bees; bins ± 10 deg. (E,F) Mean nest and flower fixation rates during the initial and later phases of each nest (E) and flower (F) learning flight. Numbers above the bars give the number of fixations. Rates of fixation of nest are higher in the initial phases (LN1 initial versus LN1 later, $N=19$, Wilcoxon test, $W=171$, $Z=-3.05$, $P=0.002$; LN4 initial versus LN4 later, $N=18$, Wilcoxon test, $W=45$, $Z=-2.67$, $P=0.008$). This is not the case for the rates of flower fixations. (G) Time course of mean nest and flower fixation rates of LN1 and LF1 calculated over the flight's normalised time. Here, and for subsequent similar figures, bins go from 0 to 0.1, >0.1 to 0.2, etc. and are labelled with their end values. Number of bees in E–G as in A–D.

Nest and flower

In their first learning flights, bees look preferentially at the nest and the flower (Fig. 4A; Fig. S2). The peaks of the distribution of body orientations relative to the nest or flower pooled over all frames from all bees occur when the bee is aligned with the nest or flower. Alignment in the nest direction is sustained across all four flights at the nest. Although the peak in the direction of the flower is strong in the first flight, it diminishes in later flights (Fig. 4B–D). By flight four, the bee flies directly away from the flower so that the peak of the distribution is in the opposite direction. The tendency is the same if we examine fixations (see Materials and methods) of the nest or the flower.

To analyse the density of looking over successive time intervals, we adopted a measure that we term fixation rate (see Materials and methods). The nest fixation rates vary around 0.02 across the initial phase of all four flights (Fig. 4E) and are significantly lower in the later phase. Flower fixation rates decay more rapidly over successive flights than do nest rates in both the initial and later phases of the flights (Fig. 4F). Nest fixation rates (Fig. 4E) do not differ significantly from flower fixation rates during LN1 and LF1 (Fig. 4F) in either the initial or later phases of the flights. The rates differ later in the sequence of flights, beginning with LN2 and LF2 (initial phase LN2 versus LF2: $N=12$, Wilcoxon test, $W=65$, $Z=-2.03$, $P=0.04$; later phase LN2 versus LF2: $N=12$, Wilcoxon test, $W=74$, $Z=-2.75$, $P=0.01$) and continuing to LN4 and LF4 (with the exception of the later phase of LN3 and LF3: $N=14$, Wilcoxon test, $W=67$, $Z=-1.50$, $P=0.13$). On a finer time scale, with fixation rates during LN1 and LF1 plotted against the normalised duration of the flight, the nest and flower rates have a similar maximum of about 0.03 towards the start of the flight when the bee is close to the nest or flower (Fig. 4G).

Cylinders

It is a little more difficult to determine whether bees face the cylinders more often than they face in other directions. To do so, we compare the number of fixations towards the real cylinders and towards nine virtual cylinders (Fig. 5A,B). Virtual cylinders are placed to fill the gaps between real ones to complete a circle of cylinders centred on the nest or flower placed 30 deg apart at 24.5 cm from the flower or nest. To test whether there were more real cylinder fixations than virtual ones, we count for each flight of each bee the mean number of fixations towards the three real cylinders placed at -60 , 0 and 60 deg on the circle, and the mean number of fixations towards the four virtual cylinders flanking the real ones at -90 , -30 , 30 and 90 deg (Fig. 5A,B).

There are significantly more fixations towards the real cylinders (4.44 ± 0.41) than towards the virtual ones (3.63 ± 0.49) in the first learning flights at the nest (Wilcoxon test, $N=19$, $W=200$, $Z=-2.25$, $P=0.02$) and at the flower (mean number of fixations of real: 2.08 ± 0.52 , and virtual: 1.03 ± 0.33 cylinders; Wilcoxon test, $N=16$, $W=100$, $Z=-3.31$, $P=9 \times 10^{-4}$). The darker colours on the bar graph

indicate fixations in which, from the bee's vantage point, both nest and cylinder are aligned. These joint fixations are relatively rare in nest flights, but commoner in flower flights. During later learning flights at both the nest and the flower, there are fewer cylinder fixations and real cylinders did not attract significantly more fixations than the flanking virtual ones (data not shown).

Like fixations of the nest and flower, the majority of fixations of the cylinders occur close to the bee's departure point (Fig. 5C,D). In nest flights, more distant fixations are mostly on the side of the nest away from the cylinders and, like the directions of cylinder fixations, are distributed broadly. In flower flights, the distributions of directions and positions of cylinder fixations are more focused (cf. Fig. 3). It seems unlikely that these differences in viewing directions at the nest and flower tables can be attributed to differences in the more distant panorama within the greenhouse as seen from the vantage point of the nest or flower. The panorama as viewed from those positions look similar (Fig. S4).

The cylinder fixation rates during the first learning flights from the nest and the flower peak at different normalised times (Fig. 5E,F); however, note that overall flower flights are considerably shorter. On flights from the nest, the fixation rate of the bottom cylinder peaks at the start of the flight's normalised duration followed soon by the middle cylinder at a slightly lower fixation rate and still later the top cylinder at the lowest rate of the three. One tentative interpretation of this pattern is that bees tend to look from the bottom to the top of the cylinder array. On flights from the flower only the central cylinder attracts appreciable fixations with a broad peak in the fixation rate about a third of the way through the normalised duration of the flight. These patterns suggest that the bees' viewing strategy differs spatially across nest and flower flights.

Return flights

Bumblebees returning to the flower and the nest (Fig. 6A,B) fly in a characteristic zigzag path, tending to face the nest or flower at the extrema of the zigzags (Philippides et al., 2013). As happens during learning flights, but in the reverse direction, the approach to the flower seems more direct than that to the nest. This difference is also apparent in cumulative plots of the durations and distances of all the recorded bees when plotted against the bees' distance from the nest and the flower (Fig. 6C,D). Returns to the nest take slightly longer and the trajectories are less direct than those to the flower on both the first and later flights.

By contrast, approaches to the flower and the nest are similar in the way that the bee slows down, often hesitating, circling or hovering close to the nest or flower just before landing. Surprisingly, there are no striking differences between the first and fourth returns. Indeed, the bee's first approach to the flower on the training table (RF0), before it has performed any learning flights at the flower, is about as fast and as long as its fourth return (Fig. 6C,D).

On their returns, bees tend to approach the nest and flower from a direction in which the array of cylinders lies beyond the goal (Fig. 6A,B), matching their learning flights (Fig. 3D) and enabling the bees to view the array through much of the approach (cf. Zeil, 1993b). The bees' body orientation relative to the central line suggests that bees look closely at the area containing the flower and central cylinder and survey the scene more broadly when approaching the nest (Fig. 3E,F). But statistical tests using each flight to the flower or nest as a data point do not show these differences to be significant (Fig. 7A,B). Such differences between flower and nest are more marked in plots of the bees' body orientation relative to the flower or the nest: bees returning to the flower are more focused on the flower than are bees returning to the

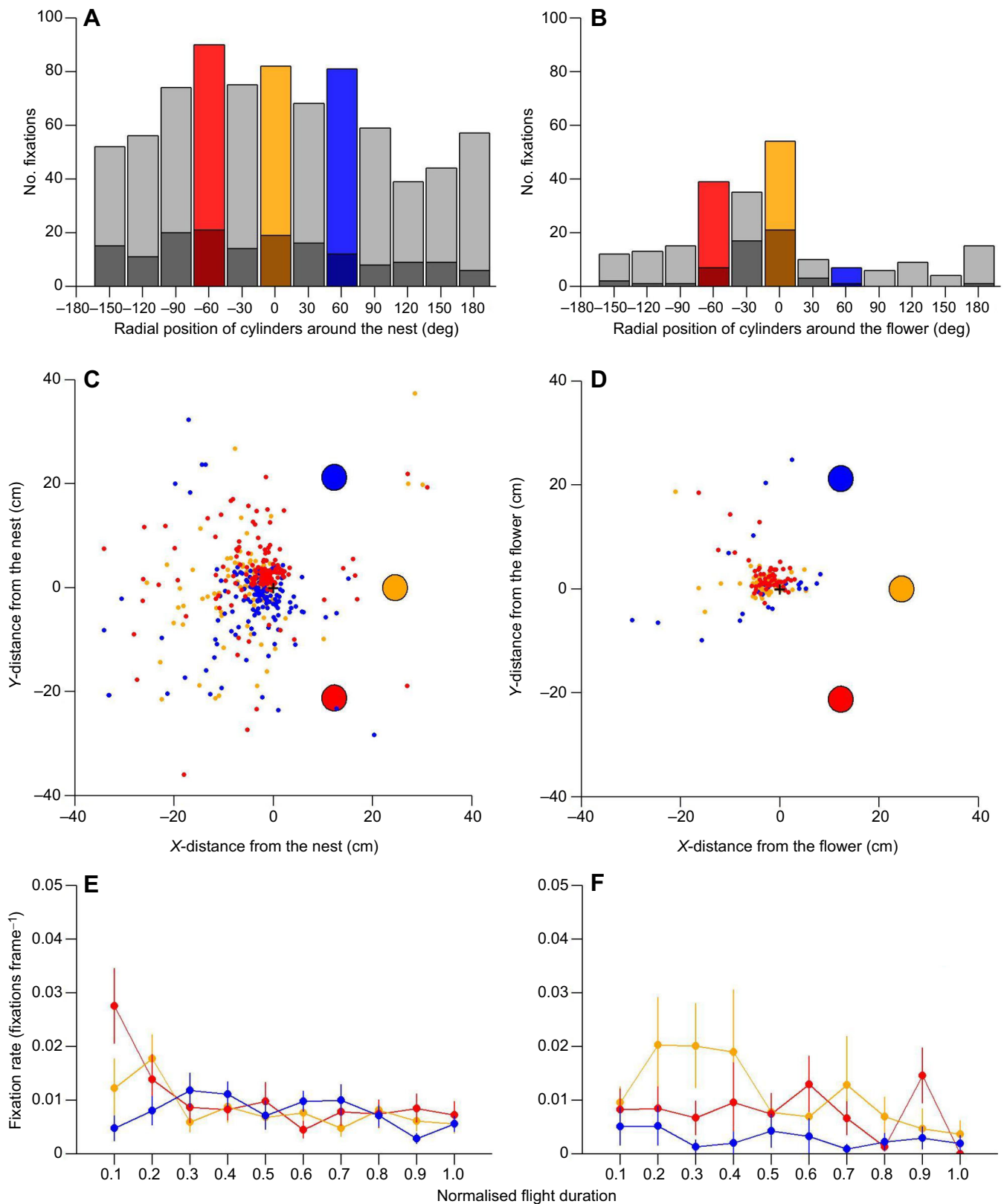


Fig. 5. Cylinder fixations. (A,B) Number of cylinder fixations towards real (coloured) and virtual (grey) cylinders during LN1 (A) and LF1 (B) (see Results for details; LN1/LN2/LN3/LN4: $N=19/18/18/18$ bees; LF1/LF2/LF3/LF4: $N=16/12/14/14$ bees). The darker colour bars show the number of fixations simultaneously toward the cylinder and the nest or flower. (C,D) Position of bees relative to the cylinders upon each cylinder fixation during LN1 (C) and LF1 (D). The cross shows the nest or flower. (E,F) Time course of mean fixation rates over the normalised durations of LN1 and LF1. Red, orange and blue refer to -60 , 0 and 60 deg radial positions of cylinders around the nest or flower.

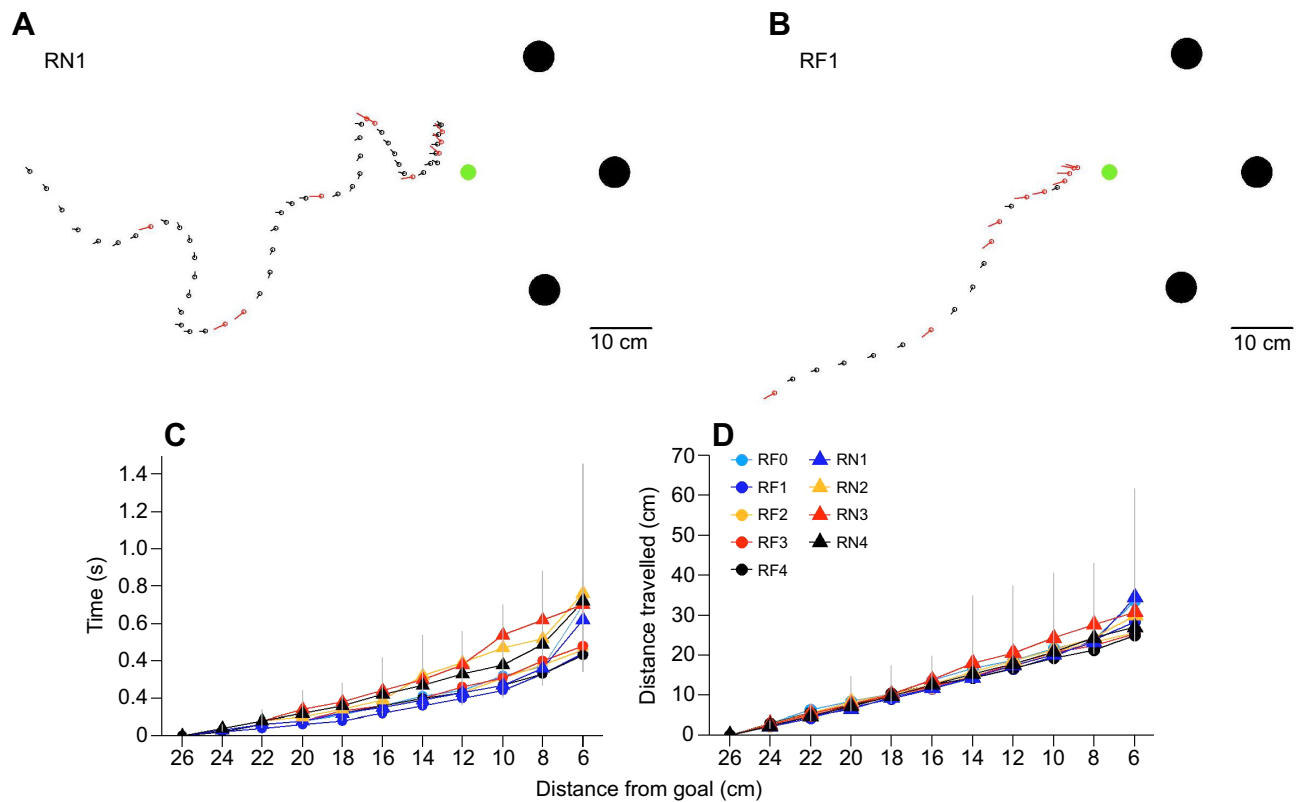


Fig. 6. Some properties of return flights. (A,B) The first return flight of one bee to the nest (RN1) and flower (RF1). The small white circles show the positions of the bee plotted every 40 ms during its trajectories and the tails show its body orientation. Red circles and extended tails show when the bee faces the nest or flower (± 10 deg). The large black circles represent the positions of the cylinders and the green circle the nest or flower. (C) Median times and IQRs taken by bees to cross a specified radial distance on their returns to the nest or flower in 2 cm steps from 26 to 6 cm (RN1/RN2/RN3/RN4: $N=18/16/17/18$ bees; RF0/RF1/RF2/RF3/RF4: $N=14/16/12/14/14$ bees). The bees' behaviour just before landing, when they were very close to the nest or flower, was very variable and we did not analyse this part of the return flights. (D) Similar plots of trajectory length. The median trajectory length of the first return to the nest was 34.41 cm (36.86 IQR), compared with 28.34 cm (6.56 IQR) for that of the flower (Wilcoxon test, $N=15$, $W=20$, $Z=-1.99$, $P=0.05$). Median lengths of the fourth returns to the nest (27.07 cm, 9.92 IQR) and flower (24.91 cm, 3.06 IQR) differed significantly (Wilcoxon test, $N=14$, $W=10$, $Z=-2.48$, $P=0.01$).

nest focused on the nest (Fig. 7C,D). The frequency of fixations with respect to the nest or flower (Fig. 7E,F) makes the same point. Later returns are similar (data not shown). Thus, return flights match learning flights in that bees view the nest within a broad spatial context and focus more narrowly on flowers.

A further parallel between return and learning flights concerns the bees' fixations of the cylinder array. During the first learning flight at the nest, bees first and most often fixated the top cylinder (Fig. 5A, E), whereas, at the flower, they focus on the central cylinder more often (Fig. 5B,F). The same difference between flights at the nest and the flower occur on the first return flight, but not on later returns. Thus, on their first return flight to the nest, bees mostly fixate the bottom cylinder (Fig. 8A) and, on their return to the flower, they fixate the central cylinder (Fig. 8B). The darker areas on the histograms of Fig. 8A and B illustrate that returning bees tend to align themselves so that they look across the nest when fixating the cylinder.

Tests of localisation

The results so far indicate significant differences in flights at the nest and the flower. Are these differences reflected in the results of tests designed to examine the precision of the bees' ability to localise the nest and flower?

In all tests, the array of cylinders is shifted from its normal position. In the first two tests at the nest and flower, the ring is removed so bees have to rely on the cylinders during their search for the nest or flower. In two further tests, the ring is present and is

shifted with the array of cylinders. We obtain two measures of where bees suppose the nest or flower to be. The first and most direct is where they landed. Landings often happen late during a search. They are preceded by moments in which the bees' flight speed slows down greatly. The positions of the troughs of these 'slowdowns' (see Materials and methods) is a second useful measure.

Despite longer and more elaborate learning flights at the nest than at the flower, in tests without a ring bees are no more accurate in their landing positions on the nest table than on the flower table. In both cases the landings cover an appreciable area (Fig. 9A). The distributions of distances from the virtual nest (8.15 ± 0.50 cm) are similar to the distances from the virtual flower (7.16 ± 1.23 cm; Fig. 9C,D). When the rings are there, bees landed precisely on them, whether the rings represent the nest or the flower (Fig. 9B). In these conditions, landings are close to the centre of the ring whether it marks the flower (1.43 ± 0.09 cm) or the nest (1.84 ± 0.19 cm).

The positions in which bees slow down in tests with no ring are nearly as accurate as the landing positions (Fig. 10A,C), with little difference in the bees' distance from the virtual nest (11.8 ± 1.1 cm) or flower (10.5 ± 1.49 cm; Fig. 10E,F). However, the spatial organisation of these searches did differ between nest and flower tests. Slowdowns around the virtual nest are distributed along the vertical axis, parallel to the cylinder array, and at the flower they spread along the central line of the flower and array. In tests with rings, the bees mostly slow down close to the ring, but there are also

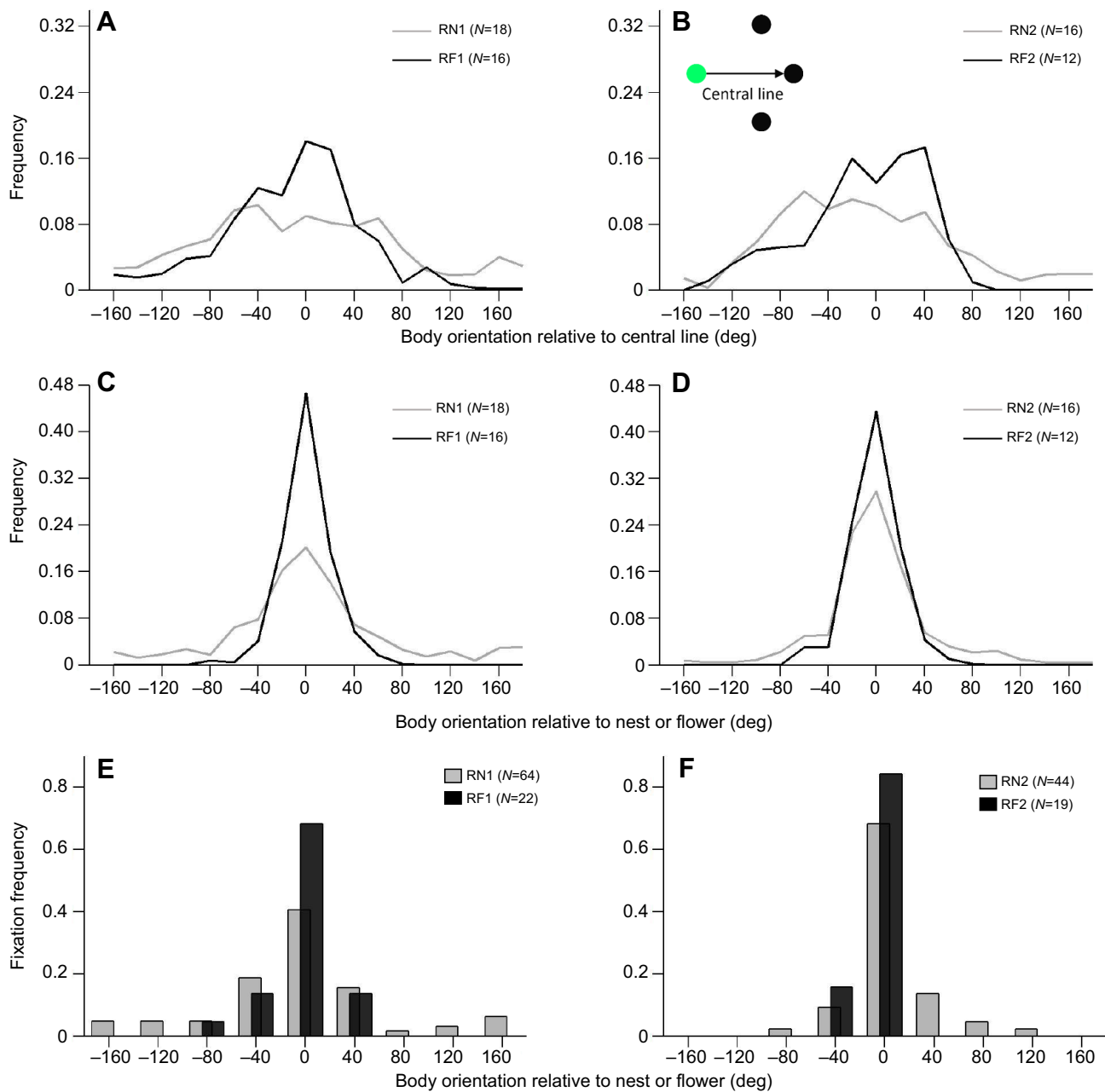


Fig. 7. Looking at visual cues during returns. (A,B) Body orientation relative to the central line of the cylinder array (see inset in B) during RN1 (circular mean: -14.52 deg, ρ : 0.33), RN2 (circular mean: -20.11 deg, ρ : 0.45), RF1 (circular mean: -9.01 deg, ρ : 0.64) and RF2 (circular mean: -5.81 deg, ρ : 0.69). The ρ values of the RF1 and RN1 distributions or the RF2 and RN2 distributions do not differ significantly (RN1 versus RF1: $N=15$, Wilcoxon test, $W=81$, $Z=-1.19$, $P=0.233$; RN2 versus RF1: $N=10$, Wilcoxon test, $W=37$, $Z=-0.97$, $P=0.33$). (C,D) Body orientation relative to nest or flower during RN1 (circular mean: 2.82 deg, ρ : 0.54), RN2 (circular mean: 0.77 deg, ρ : 0.77), RF1 (circular mean: -0.53 deg, ρ : 0.94) and RF2 (circular mean: 1.51 deg, ρ : 0.94). The ρ values of the RF1 and RN1 distributions or the RF2 and RN2 distributions differ significantly: RN1 versus RF1: $N=15$, Wilcoxon test, $W=113$, $Z=-3.01$, $P=0.003$; RN2 versus RF1: $N=10$, Wilcoxon test, $W=47$, $Z=-1.99$, $P=0.047$. (E,F) Frequency of fixations relative to nest or flower during RN1, RN2, RF1 and RF2; bins ± 20 deg.

outlying points at some distance from the ring (Fig. 10B,D). Mean distances are thus similar between nest (10.8 ± 2.15 cm) and flower (11.2 ± 3.51 cm), although the precise cluster on the flower table is not replicated on the nest table.

The slowdowns with the ring on the flower and nest tables are particularly useful since we can see where the bees look when they are relatively close to the virtual goal. We examine the bees' body orientations relative to the cylinder array when the ring is present and the bee was close to it (Fig. 10G). Bees above the virtual nest are oriented towards the nest and the bottom part of the cylinder array [circular

mean: -75 deg, vector amplitude (ρ): 0.48], as they are during learning and return flights. Bees above the virtual flower are more oriented towards the centre of the cylinder (circular mean: -14.1 deg, ρ : 0.58), as in learning and return flights. However, it should be noted that these differences are not significant (Watson–Wheeler test, $W=2$, $P=0.3$).

Are the bees more persistent in their search for the nest than the flower?

To analyse the bees' persistence, we score the frequency of landings after an approach. When the ring is absent, the rate of landings is

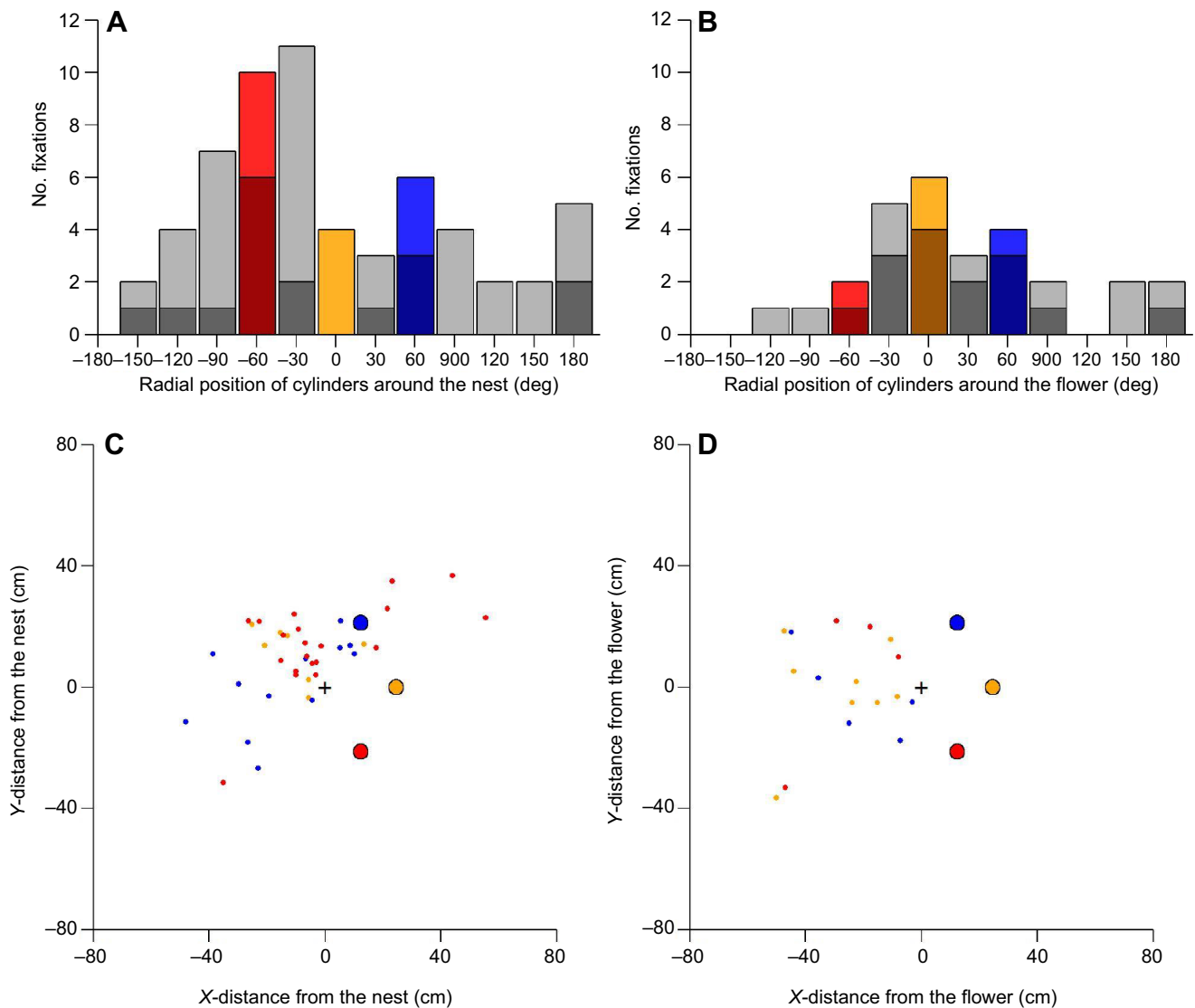


Fig. 8. Fixations relative to real and virtual cylinders during RN1 and RF1. (A,B) Number of cylinder fixations towards real (coloured) and virtual (grey) cylinders during RN1 (A) and RF1 (B). Darker colour bars show the number of fixations simultaneously toward the cylinder and the nest or flower. (C,D) Positions of cylinder fixations during RN1 and RF1. The cross shows the nest or flower. Red, orange and blue circles refer to -60 , 0 and 60 deg radial positions of cylinders around the nest or flower.

higher at the nest (0.040 ± 0.005 landings s^{-1}) than at the flower (0.015 ± 0.004 landings s^{-1} ; Wilcoxon test, $N=17$, $W=20$, $Z=-2.57$, $P=0.01$). However, when the ring is present the rate of landings is unchanged at the nest (0.039 ± 0.004 landings s^{-1}) but increases during flower searches (0.089 ± 0.010 landings s^{-1}) to become higher than at the nest (Wilcoxon test, $N=16$, $W=100$, $Z=-3.41$, $P<0.001$).

We also score the mean duration of search bouts. In the absence of the ring, nest searches are longer (17.7 ± 1.84 s) than flower searches (6.60 ± 1.06 s) (Wilcoxon test, $N=17$, $W=4$, $Z=-3.43$, $P<0.001$). When the ring is present, nest searches continue to be longer (46.40 ± 9.36 s) than flower searches (8.06 ± 0.62 s) (Wilcoxon test, $N=16$, $W=0$, $Z=-3.52$, $P<0.001$). Nest searches are significantly longer with the ring than when the ring is absent (Wilcoxon test, $N=16$, $W=5$, $Z=-3.26$, $P=0.001$). However, the presence or absence of the ring has no significant effect on the length of flower searches (Wilcoxon test, $N=16$, $W=30$, $Z=-1.86$, $P=0.06$).

Taken together, these tests of persistence indicate that bees searched longer for the nest than for the flower and that the presence of the ring sometimes makes bees search for longer.

DISCUSSION

Structural differences between learning flights at flower and nest

The major differences that we have identified are that flights at the nest are much longer than those at a flower. Learning flights at both locations tend to become shorter with each departure, with a faster decay at the flower than at the nest. During their longer trajectories at the nest, bees survey the immediate nest surroundings over a larger area than they do the flower and fixate different features. The rate and number of fixations is higher in flights from the nest than from the flower and fixations also occur over a longer range of distances from the goal.

Wei and co-workers (Wei et al., 2002; Wei and Dyer, 2009) measured the durations of honeybee learning flights when leaving a feeder and showed that they were longer when sucrose concentration was higher. This change indicates that bees may take more care to learn a location when the value of the reward grows. It is hard to compute the relative values of a nest and flower, but intuitively the same may apply – a nest is forever, but a flower

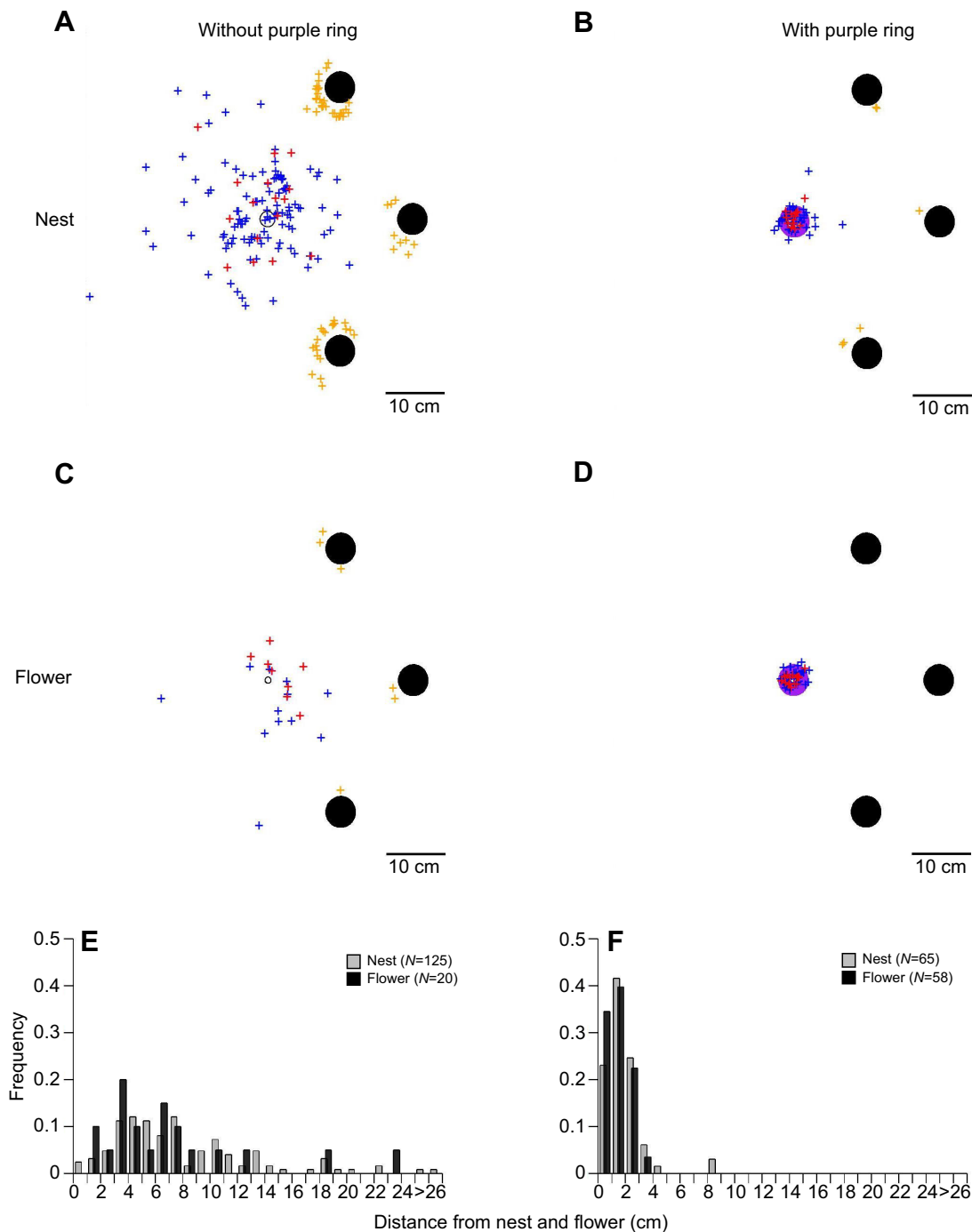


Fig. 9. Positions and orientations of bees during landings with and without ring. (A,B) Positions of the landings during the test at the nest with (B) and without (A) the ring. The virtual position of the goals relative to the cylinders are marked by white circles in A and purple circles in B. Black discs mark cylinder positions. The position of landings after an approach are shown by blue crosses, with the first of the landings (i.e. one per bee) shown in red. The orange crosses show landings at the base of the cylinders, which were not included in the analysis. (C,D) As in A and B but for landings at the flower. (E,F) Frequency of landings at different distances from the nest or the flower without (E) and with (F) the ring.

may only be a one day's stand. But, equally, learning enough to locate what is usually an inconspicuous nest hole may need more effort than learning to locate a flower and the lengths of learning flights may be adapted to these requirements.

Indeed, these differences occur when the visual surroundings of the nest and flower are made to be as similar as possible, indicating that the observed differences are the result of internal modulations of the learning flights set by the function (nest or flower) of the bee's

current location. The modulations could perhaps be triggered at the nest by external factors, such as a sudden absence of nest odour or the sudden presence of daylight, and at the flower by the availability of nectar. Arguing against such triggering is that similar differences are found on return flights to the nest and flower when these particular triggering factors are not present. It seems more likely that the bees' differing internal states at the nest and the flower are caused by neuromodulators subtly adjusting neural circuits, with the level of

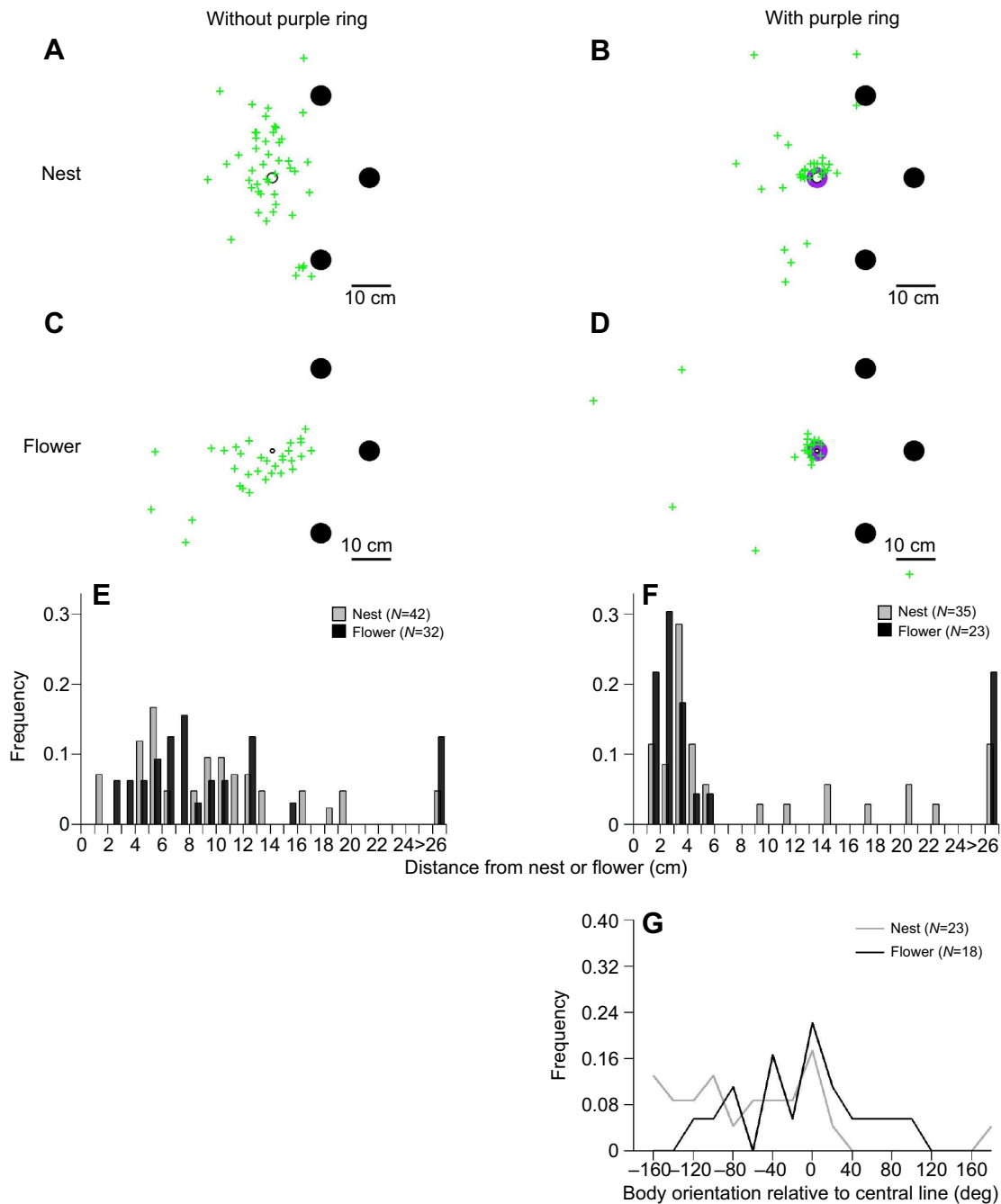


Fig. 10. Positions and orientations of bees during slowdowns with and without ring. (A,B) Positions of the slowdown points (green crosses) during the early search bouts at the nest with (B) and without (A) the ring. The virtual position of the goals relative to the cylinders are marked by white circles in A and purple circles in B. Black discs mark cylinder positions. (C,D) As in A and B but for slowdowns at the flower. (E,F) Frequency of slowdowns at different distances from the nest or the flower without (E) and with (F) the ring. (G) Body orientation relative to central line of the array during slowdowns with the ring when bees were within 6 cm of the centre of the ring.

modulation controlled by a variety of factors (reviewed by Griffith, 2012). Interestingly, the internal state in which a memory is formed may also be required for its expression (Krashes et al., 2009).

Accuracy and persistence of search

Although the differences between the flights might suggest that bees learn more about the surroundings of its nest than a flower, the tests revealed no difference in the bees' ability to localise the nest or flower. One possibility is that the surroundings in which we tested the bees may have been insufficiently challenging to reveal any

substantial differences in their ability to accurately pinpoint the location of the flower or the nest. We should also remember that the bumblebees in the present experiments could learn their surroundings on arrival and departure, so that their behaviour during tests could in part have been the result of information acquired on both outward and return journeys (Lehrer and Collett, 1994).

Nonetheless, bees persisted for longer when searching for their nest than when looking for a flower. Why should that be? There are at least two kinds of answers. The first is functional: persistence of

search may just be part of the different sets of characteristics that are intrinsically associated with a permanent home and a transient flower. The second type of answer is mechanistic: stronger synaptic connections associated with stronger nest memories and the resulting prolonged activity somewhere in the circuitry could cause bees to search for longer.

Lastly, why under some conditions do the bees persist in searching for the flower and not give up quickly when the ring is absent. In contrast to a situation in which landmarks indicate the position of a learned inconspicuous food source (Lehrer and Collett, 1994), the flower here was clearly visible. One might suppose that searching for a flower which has disappeared from its former location is less worthwhile than searching for a nest that lacks one of several cues marking its location. Perhaps, in the particular set-up of these experiments, the central cylinder was often fixated with the flower (Fig. 5) and became part of the flower so that its continued presence means for the bee that the flower is still there. Another possibility, which is supported by the bees' natural foraging behaviour, is that the location itself becomes valuable. In these experiments, the bees at the flower could drink concentrated sucrose solution to capacity and consequently both the flower and the place may be rewarding. In normal foraging, when bees have obtained reliably good rewards from a particular species within a patch of flowers, they continue to visit the same patch after the particular flower species that they are harvesting stops flowering and explore it for other species (Heinrich, 1979; Ogilvie and Thomson, 2016).

What is learnt when?

Bumblebee learning flights divide naturally into two portions: an initial phase of about a third of the flight in which the bee keeps very close to the nest or flower followed by a later portion in which the bee gradually increases its distance from the nest or flower (Fig. 2D). It is striking that most of the fixations of the nest, flower and cylinders occur during the initial phase, suggesting that views of the goal surroundings are stored during this phase, when the bee is very close to the nest or flower location, and may learn the distance of visual cues from the goal. Supporting evidence that visual information is stored during the initial phase comes from finding that the cylinders selected for fixation during learning flights differ between nest and flower and that the same preferences are seen in the cylinders that bees fixate during the first return flights to the nest or flower and during tests at these places. The fact that, on return flights, bees tend to fixate cylinders from positions in which they are also fixating the nest (Fig. 8A,B) reinforces earlier conclusions (Philippides et al., 2013) that bumblebees are likely to learn the compass bearing of nearby visual features relative to the nest during the initial phase of their learning flights.

It is interesting that bees only find it necessary to fixate cylinders during their first learning and return flights at the nest and the flower. Thereafter it seems that they can be guided by the cylinder in its retinal position relative to the nest without having to look at the cylinder directly. The orientation of the bees' body when they reduce speed during tests when very close to the nest or the flower (Fig. 10G) suggests that bees may revert to looking directly at the same cylinders when checking their location.

So far, we have supposed that what bees look at during the initial phase of their first learning flights depends on their visual surroundings on departure and whether they leave their nest or a flower. The pattern of looking in the control experiment (Fig. S3) suggests that how bees inspect their surroundings may also be influenced by what they had seen on their previous approach to the

flower or nest. Bees in the control experiment were placed directly on the flower and so had no opportunity to learn anything about the flower's surroundings until their learning flight on departure. The distribution of facing directions in the initial phase of this learning flight (Fig. S3C) was similar to that of bees in the main experiment when they left the nest (Fig. 3E). Bees, when first leaving the nest, also have no prior knowledge of the nest's surroundings. Might the broader scan seen in both the learning flights from the nest and from the flower in the control experiment be a consequence of the bees' unfamiliarity with their surroundings? Could the more focused facing direction when leaving the flower in the main experiment (Fig. 3E) be a consequence of what bees learnt while approaching the flower for the first time? Testing this possibility will involve repeating the main experiment but varying what bees see on their first approach to the flower.

In the later phases of learning flights at the nest, bees tend to fly away from the nest and then back towards it in a sequence of loops of increasing size (Philippides et al., 2013). It seems likely that during these loops bees acquire views while flying towards the nest that can later guide the bees' homeward returns (cf. Stürzl et al., 2016). This possibility is supported by correlations between learning and return flights. During return flights, the compass directions in which bees face the nest are aligned with the preferred direction of nest facing during learning flights (Hempel de Ibarra et al., 2009). This later phase of learning flights may thus be adapted to learning short routes, perhaps only centimetres long, that lead back home.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.H.d.I., T.S.C.; Methodology: T.R., N.H.d.I., T.S.C.; Software: T.R.; Validation: T.R., N.H.d.I., T.S.C.; Formal analysis: T.R., N.H.d.I., T.S.C.; Investigation: T.R., E.F.; Resources: N.H.d.I.; Data curation: T.R., E.F., T.S.C.; Writing - original draft: T.R., T.S.C.; Writing - review & editing: T.R., E.F., N.H.d.I., T.S.C.; Supervision: N.H.d.I., T.S.C.; Project administration: N.H.d.I.; Funding acquisition: N.H.d.I., T.S.C.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.172601.supplemental>

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