



Bumble bees strategically use ground level linear features in navigation

Joanna S. Brebner^{a,*}, James C. Makinson^{a,1}, Olivia K. Bates^{a,2}, Natacha Rossi^a,
Ka S. Lim^b, Thibault Dubois^{c,3}, Tamara Gómez-Moracho^c, Mathieu Lihoreau^c,
Lars Chittka^a, Joseph L. Woodgate^a

^a Department of Biological and Experimental Psychology, School of Biological and Chemical Sciences, Queen Mary University of London, London, U.K.

^b Department of Agroecology, Rothamsted Research, Harpenden, U.K.

^c Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS, University Paul Sabatier – Toulouse III, Toulouse, France

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Extended ground level structures like roads or field edges can be important cues for navigating animals, seen for example in road-following pigeons. In a landscape devoid of skyline cues but with a rectangular grid of pathways and roads, we used harmonic radar to track free-flying bumble bees, *Bombus terrestris*. Individual bees consistently used ground level linear features for navigation in a wide range of behavioural contexts. Bee exploration flights, search behaviour and foraging routes were shaped by linear features, with bees frequently flying along and parallel to pathways and roads. Comparisons of flight trajectories across these behavioural contexts show that individuals modulated their use of linear features strategically with respect to their individual goals and experience. Bees searching for a feeder used linear features to target their search, while foragers often followed pathways to return to their hive without overshooting. These findings on a major pollinator have important implications for the placements of bee colonies for agriculture and floral resources for conservation.

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Ground level elongated linear structures, such as rivers, forest edges, paths and roads provide unique visual information which can affect animal dispersal and navigation (Billington, 2003; Gesicki, Cech, & Bingman, 2019; Schiffner & Wiltschko, 2014). For instance, pigeons, *Columba livia*, follow roads (Guilford, Roberts, Biro, & Rezek, 2004; Lipp et al., 2004), bats fly along hedgerows and treelines (Billington, 2003) and turtles follow coastlines (Luschi, Papi, Liew, Chan, & Bonadonna, 1996). Such features may be particularly relevant for pollinators that exploit agricultural terrains which often present sparse visual environments with prominent man-made linear features.

There is tentative evidence that bees use such linear structures for navigation. For example, von Frisch and Lindauer (1954) trained honey bees, *Apis mellifera*, to fly along continuous forest edges, lake edges and roads and observed that they would prioritize this information over cues gained from their sun compass (von Frisch, 1967). Menzel et al. (2019) observed that some experimentally displaced honey bees tended to use gravel roads, field edges, hedgerows and irrigation channels to return to their hive. Collett and Graham (2015), reviewing other radar studies (Degen et al., 2016; Osborne et al., 2013; Wolf et al., 2014), further observed that displaced honey bee foragers seemed to follow a pathway then a hedgerow back to their hive, while honey bees performing their first exploration flights appeared in some cases to follow field edges.

These observations suggest that bees use ground level linear features for some navigational tasks. However, none of the studies were designed to examine this question in a quantitative and experimental manner and cannot address the likelihood or degree of use of these features, or the variety of contexts in which they are used. Interpreting these observations is made more difficult

* Corresponding author.

E-mail address: j.s.brebner@qmul.ac.uk (J. S. Brebner).

¹ Present address: Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW Australia.

² Present address: Department of Ecology and Evolution, Biophore, UNIL-Sorge, University of Lausanne, Lausanne, Switzerland.

³ Present address: Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia.

because the landscapes in which the bees were tracked contained potential landmarks such as trees and buildings that bees could have used for navigation. Thus, important questions remain about the likelihood of bees using ground level linear features, the contexts in which they are used and whether their use depends on a bee's experience with the local landscape. If bees consistently use ground level linear features for navigation, this may affect the areas they are most likely to discover, pollinate and fly through.

Here we examined how and to what extent bees learn to use linear features during navigation by using a harmonic radar to track free-flying bumble bees, *Bombus terrestris*, in a unique, visually sparse landscape, characterized by a ground level rectangular grid of roads, pathways and field borders. While compass cues remained, the horizon provided no skyline cues that bumble bees could exploit to indicate location (Fig. 1a; for more detail see Appendix, Field site panorama). In the absence of skyline information, any influence of ground level cues can be isolated, allowing us to quantify bumble bees' use of linear features in exploration flights, searching flights, foraging flights and over route development.

METHODS

Bees and Field Site

Field work was carried out on a rice farm (Finca Casudis, La Puebla del Río; latitude 37.13562305; longitude: -6.080421855), south of Seville, Spain, between 20 April and 15 May 2018. The field

site was composed of a series of unflooded rice paddies on flat terrain, with few to no above ground level features (Fig. 1a; Appendix, Field site panorama). The rice paddies were all 420 m by 200 m, set out in a grid and separated by raised pathways of compacted earth (approximately 0.5 m higher than the fields). All pathways running WSW–ENE comprised two single-lane roads separated by a narrow irrigation channel. Every second pathway running WNW–ESE was a single-lane road, while the others were narrow footpaths, approximately 1 m wide. A small packed-earth runway ran along one of the WSW–ENE roads, from the main farm outward (thicker pale line, Fig. 1b). To the north of this road was a footpath along which the hives were placed (Hive Location 1) for experiments 1–4, while to the south, the hives were placed in a field (Hive Location 2) for an additional test (see Appendix, Experiment A3). All pathways (including roads, footpaths, field edges and the runway) are referred to as this environment's linear features.

Eight commercially bred *B. terrestris* colonies (with 150–200 individuals on average; Koppert Biological Systems, Spain) were used. Each colony was transferred to a custom-made wooden hive box (30 × 21 cm and 16 cm high), with a Perspex tunnel (26 × 4 cm and 4 cm high) ending in a small Perspex platform (4 × ca. 6 cm), through which the bees could access the outside world. Small sliding barriers could be used to block the tunnel, allowing us to control which bees could go in and out. Two hives (placed side by side) were used at a time to maximize the number of active foragers. Bees were allowed to feed freely from experimental feeders

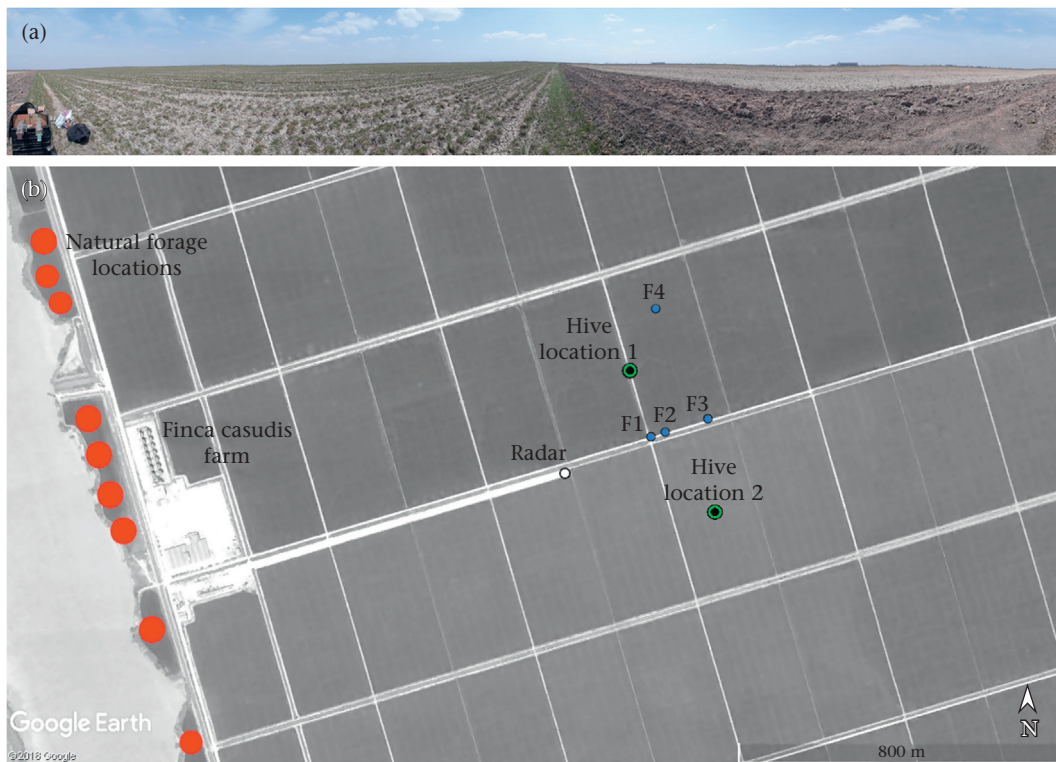


Figure 1. Panorama and satellite view of the field site. (a) 360° panorama of the field site from the hive position. The hive, with a black tarp covering, is visible on the left of the image. To the right are the grain silos at the farm, barely visible as a small dark grey building above the horizon. The landscape lacks skyline cues (see Appendix, Field site panorama). (b) Satellite image with relevant locations. Bumble bees were tracked from two hive locations (Hive Location 1 and Hive Location 2, represented by green circles). Hives were located at Hive Location 1 for experiments 1–4 and at Hive Location 2 for experiment A3. Bees in our experiments foraged at experimental feeders (F1, F2, F3 and F4, represented by light blue dots). F1 and F4 were both 168 m from the hive, with F1 along the footpath directly connected to the hive and F4 northeast from the hive in the centre of a field. F2 and F3 were along a dirt road perpendicular to the footpath where the hive was placed and directly connected to F1, 172 m and 238 m from the hive, respectively. F1 and F2 were 35 m apart, while feeders F1 and F3 were 168 m apart. Different feeder treatments were used for experiments as follows: F1 and F2 from 20 to 23 April, F1 and F3 from 26 to 30 April, F1, F3 and F4 on 4 May, and F1 and F4 from 5 to 8 May. Flight paths were tracked using harmonic radar (represented by a white dot), located at the end of a packed earth runway which ran east from the farm. The farm Finca Casudis is located to the west, next to the banks of the river Guadalquivir, where some natural forage sources (represented by red ellipses) could be found. The satellite image used is from Google Earth, ©2018 Google. Note that all rice paddies were dry (no vegetation, brown) at the time of the experiments.

in a flight tent (hexagonal, 3.9 m at widest point, 1.95 m per side, 2.5 m high) for 2–4 days before the hive was transported to the field site. The flight tent was located more than 15 km from the field site, in a different visual environment (either situated in a walled garden or next to a wall on one side and a dense grove of orange trees on all others. The horizon was not visible from either location). Bees were only able to fly within the flight tent, so that their visual experience prior to the field site was restricted to a small space walled with fine white mesh, while the fields and paths in our field site were generally uniformly coloured (mostly brown) during data collection (Fig. 1a). Individual bees were thus familiar with the feeder design but naïve to the field site and to their location within it at the beginning of experiments. Feeders were made of a raised blue platform (20 × 20 cm), approximately 1 m above ground level, identical to those used in Woodgate, Makinson, Lim, Reynolds, and Chittka (2017) supporting a gravity feeder (described in chapter 2, section 5 in von Frisch, 1967). A 1 m tall feeder could have been visible to bees from a maximum range of 25 m (calculated from the smallest measured bee spatial resolution of 2.3°; Dyer, Spaethe, & Prack, 2008). This is a generous estimate, since the feeders were thin and located on the lower-level field beside the footpath so that they appeared less than 1 m above the horizon. We are therefore confident that feeders could not be used as landmarks for bee orientation outside this 25 m range.

Natural foraging sources were present along the river Guadalquivir (west of the farm, over 1200 m from the hive site), but were largely absent within the rice paddies themselves. Experimental feeders providing ad libitum 30% (by weight) unscented sucrose solution were placed 160–250 m from the hives, either along the linear features (Feeders F1, F2 and F3) or in the middle of a rice paddy (Feeder F4; Fig. 1b).

All tracked bees were given unique colour and letter identification using custom-made metal tags (Qualitech, March, U.K.), attached to their thorax using superglue (Loctite Power Flex Gel, Henkel Ltd., Hemel Hempstead, U.K.). Bees were primarily tagged within the flight tent before the hive was transported to the field site, but this tagging process continued after experiments started so that newly emerged bees could also be tracked. What was known about individual experience was carefully noted for reference.

Harmonic Radar

Individual bees were tracked using a 32 mm harmonic radar described in previous studies (Riley et al., 1996), located approximately 310 m from the hive (Fig. 1b). To allow radar tracking, a lightweight transponder (16 mm long, approximately 20 mg) was attached to the metal tag of a focal bee as it left the hive, using a small magnet at the base of the transponders. This allowed us to attach and remove it from the metal tags very quickly without capturing or handling the bee, thus minimizing stress due to manipulation.

The radar rotated constantly, scanning through 360° once every 3 s. When a bee with a transponder was within a line of sight of the radar up to approximately 1 km, the radar returned its distance and azimuth from the radar once every rotation. Flight path tracks were converted to GPS coordinates by triangulating the radar signals against two locations whose GPS coordinates were known, using a custom-written Matlab script (Mathworks Inc., Natick, MA, U.S.A.). These tracks were visualized by plotting them on top of a satellite image of the field site. We tracked a total of 177 flights from 83 individuals (Appendix Table A1).

Ethical Note

Bees were kept outside (first in a distant location within a flight tent and then in the field site), covered with tarp to protect them

from rain and sun. The hive boxes kept the bee colonies in a dark environment, comparable to their natural underground hives. Bumble bees store food (nectar and pollen) in their hive, and workers have intrinsic motivation to leave and forage for the colony. Supplementary pollen was provided every 2 days. Colonies were checked regularly for food stores and were provided with sugar solution if their stores were low.

Individual foragers were tagged with metal tags, involving one short capture per individual, where tags were attached to the thorax using super glue. Metal tags allowed us to apply and remove our magnetized radar transponders without capturing them, involving only brief containment within the tunnel leading to their hive. This minimized stress and reduced handling time to only a few seconds. Radar transponders were small enough to allow for normal bumble bee flight (Woodgate et al., 2017).

Once the colonies were no longer being used for experiments, they were humanely killed via freezing, to prevent commercially bred bees from affecting local wild bumble bee populations.

The research described here aligns with the ASAB/ABS Guidelines for the use of animals in Research. No licences or permits were required for these experiments.

Experiments

Details of dates, bumble bee colonies, number of tracks and individuals for each experiment can be found in Appendix Table A1. Tracks where individual identity was unknown were excluded from our data set and analyses. In all cases described below, the colonies were placed at the first hive location 'Hive Location 1' (Fig. 1b). Colonies were placed at location 'Hive Location 2' (Fig. 1b) for an additional test (see Appendix, Experiment A3).

Experiment 1

Do bees use ground level features during exploration flights? The first flights of bees in a new environment focus on exploring the surrounding landscape and, in the case of bumble bees, also the food sources within it (Osborne et al., 2013; Woodgate, Makinson, Lim, Reynolds, & Chittka, 2016). These 'exploration flights' usually consist of several loops in all directions around the hive, starting small and progressively widening (Osborne et al., 2013). Individuals with no previous flight experience in this environment were tracked on their first exploration flights in the field site. This was done using sliding barriers in the hive tunnel to allow access to the outside for only the focal bee and no others. These first flights were primarily recorded on 24 April, when there were no artificial feeders present in the field site. Three more first flights were recorded on 4 May, when several feeders were present in the field.

We also recorded flights displaying typical looping behaviour around the hive, characteristic of exploration flights (Osborne et al., 2013; Woodgate et al., 2016), between 30 April and 8 May, from bees in colonies whose tunnels were kept open to allow free access to the field site. We could not be certain whether these bees had previously flown, so we called these tracks 'apparent exploration flights'. Feeders were present during these recordings (Appendix Table A1).

Experiment 2

Do bees use linear features during repeated back and forth flights to forage locations? Once bees had completed their exploration flights, we opportunistically tracked foraging bumble bees while all individuals had free access to the field site. Two main feeder treatments were set up during this time. All colonies were given a day of exposure to the field before feeders were introduced, and different colonies were used for each treatment.

In the first treatment, we placed feeders in positions F1 and F2 (35 m apart) along linear features (Fig. 1b). F1 was located next to a crossroads, connected to both the hive and F2 by linear features. The hive and F2 were not placed on the same linear feature, so that the straightest path from the hive to F2 did not involve flight along a linear feature. The intention was to compare flights to F1 (where the most direct flight coincided with a flight over a linear feature) and to F2 (where the most direct flight crossed over open field). Since most of the foraging flights tracked in this condition (60%) involved individual bees visiting both F1 and F2 in the same foraging trip, we set up a second treatment.

In the second treatment, we placed feeders in positions F1 and F3 (168 m apart) along the same linear features. F1 remained next to the crossroads, connected to both the hive and F3, while F3 was located on the same path as F2 had been, but further from F1 to increase the cost of joint visits to F1 and F3. As with the previous condition, we aimed to compare tracks to F1, along a linear feature, with tracks to F3, where a flight following linear features would take the bee on a longer flight via the crossroads, and the most efficient flight would be across the field.

Some of the bees we tracked did not visit our experimental feeders but flew instead to distant locations, primarily in the direction of the river. Many of these bees returned with pollen. We therefore categorized nonlooping flights to distant locations as natural forage flights (Appendix, Experiment A2). Unlike the flights to feeders, the final goals of these natural forage flights were out of range of the radar. This means that the flight we tracked was the portions of outbound and inbound flight within range of the radar, up to 1 km from the hive location. Outbound flight portions therefore stopped before bees reached their destination, while inbound flights began when the bees passed into the radar's range.

Experiment 3

Does bees' reliance on linear features change with experience? One bee was repeatedly tracked while habitually foraging at feeder F3 (Fig. 1b). Here, the most direct route from the hive to this feeder would involve no flight along linear features (238 m), while an alternative route continuously following linear features would be 41% longer (336 m). The inbound and outbound portions of flight were manually categorized as follows: if the flight approaching the goal (feeder for outbound and hive for inbound portions of flight) followed the associated linear feature for at least the final 30 m, then it was labelled as 'using linear features' to reach the goal, while if the bee did not follow the associated linear feature on its way to the goal, it was labelled 'not using linear features'. Tracks in which the bee followed linear features after or not immediately before reaching the desired goal were classified as 'not using linear features'. We used 30 m as a cutoff point since it is larger than our generous calculated maximum range (25 m) at which bees might be able to discern a feeder and use it as a beacon. We assessed the frequency of each of these flight strategies as the bees gained experience.

Experiment 4

Do bees use linear features to search for a removed feeder? When a bee does not find a goal (e.g. hive or feeder) in its usual position, it searches using a distinctive flight pattern, performing looping flight in all directions around the goal's last known location (Reynolds, Smith, Reynolds, Carreck, & Osborne, 2007). We identified focal bees from individuals that regularly visited a single feeder, either F1 or F3 (Fig. 1b). When the focal bee returned from a flight to its preferred feeder, we used the tunnel dividers to confine all bees to the hive. All feeders were then removed from the field. The focal bee was released from the hive and then tracked until it returned to the hive. The focal bees tested searched for either F1 ($N = 3$ bees) or F3 ($N = 3$ bees).

Track Analyses

Proximity score

To quantify use of ground level linear features by bees, we analysed all tracks for the bees' proximity to linear features. All tracks were reduced to nonstationary data by excluding points that showed less than 1 m of motion since the previously recorded bee position.

We used a satellite image of the field site, acquired from Google Earth Pro (Google, Mountain View, CA, U.S.A.), which we converted to a black and white image (1116 x 632 px) showing the positions of all linear features. The pixel coordinates for all linear features in the image were extracted using custom-written Matlab script (Mathworks Inc.). The rest of the calculations were done using custom-written C# code (C# version 2.10.0).

We translated all radar track coordinates into the same pixel coordinate system as the image, using the relative positions of the hive and feeder F1 for reference. We calculated the Euclidean distance between each bee track coordinate and the closest linear feature and rescaled it to real-world distance (m). This allowed us to assess the minimum distance to any linear feature for every point in the bees' flight path. From this we calculated a 'proximity score', the proportion of points in the track (excluding stationary points) that were less than 5 m from any linear feature.

Since flights were centred on the hive, which was positioned next to a linear feature for experiments 1–4, and since the field site was a regular grid of linear features, it is to be expected that bees would often approach or cross linear features even if they do not use them in any way for navigation. To ask whether bees spent a greater proportion of their time close to linear features than expected by chance, we calculated a track-specific 'control score'. This control represented the average expected proximity score from the same track rotated multiple times around the hive. Specifically, we rotated every track around the hive position in 10° increments and calculated a proximity score, as described above, for each rotated track. The control score is the mean of all proximity scores from the 35 rotations and estimates the level of proximity we would expect by chance if the bee's flight path was independent of landscape features, while still centred on the same hive position.

We tested whether bees followed linear features more than expected by chance during exploration flights using a series of paired-samples *t* tests (after testing each data set for normality using a Kolmogorov–Smirnov test; a Wilcoxon matched-pair signed-rank test was used as a nonparametric alternative if the data were not normally distributed), comparing proximity scores to their paired control scores. The null hypothesis was that there would be no significant difference between proximity and control scores, suggesting that bees spent no more time close to linear features than expected by chance. The dependent variables for each test were the proximity and control scores from flights categorized as first flights or apparent exploration flights (experiment 1). Our method of generating control scores assumes that flights have no specific destination, so is not suitable for assessing flights to feeders. We report proximity scores for foraging and searching flights, but do not test them against a control.

In all cases, a mean was calculated for all repeated measures from an individual before statistical testing so that all tests were performed on data representing one data point per individual.

Flight bearings

Flying along linear features is not the only way in which bees might use linear features in navigation. Bees might also fly parallel to linear features, or their internal representation of space may be influenced by the grid of features around them even when out of

sight of linear features. We therefore calculated the bearings flown by bees in our experiments to determine whether they showed any biases matching the orientations of linear features.

Flight bearings were calculated as degrees anticlockwise from east between every two consecutive points of nonstationary track. For each track, these angles were sorted into 18° bins, and the proportion of data points falling into each bin was calculated. This allowed tracks to be assessed together (with every track equally weighted) to explore group level effects. These data were plotted in circular bar charts, and the raw data were tested for uniformity for first and apparent exploration flights (experiment 1) to assess initial biases in the flight path and for searching flights to removed feeders (experiment 4), to assess biases during targeted searching. We tested whether the flight bearings in each experiment were uniformly distributed using Kuiper's test of uniformity, which is sensitive to multinomial distributions (Birch, 2018; Landler, Ruxton, & Malkemper, 2018), since flights parallel to linear features would be predicted to have four modes at 90° intervals. Kuiper's tests were performed using R (RStudio Team, 2016).

RESULTS

Experiment 1

We tracked 14 bumble bees during their first flights and 13 on apparent exploration flights (see Appendix Table A1). Exploration flights showed a visible bias for linear features, where bees frequently flew along and parallel to surrounding roads, field borders and pathways (see Fig. 2a–d). Significantly higher proportions of bees' flights were in proximity to linear features than expected by chance during first flights (paired-samples *t* test: $t_{13} = 3.438$, $P = 0.004$; Fig. 2e) and apparent exploration flights (paired-samples *t* test: $t_{12} = 3.648$, $P = 0.003$; Fig. 2f). The distribution of flight bearings was significantly nonuniform (Kuiper's test of uniformity: $V = 3.2932$, $N = 32$, $P < 0.01$), showing more flight in the directions of linear features aligned with that associated with the hive than in any other direction (Fig. 2g). This corresponded not only to bees flying along the footpath associated with the hive, but also to bees flying parallel to that linear feature and along other identically oriented linear features nearby (Fig. 2). One bee showed extensive flight along a field border parallel to the footpath along which the hive was located, suggesting an aliasing error (where the bee mistook one linear feature for another, similar one; Fig. 2a). These flights are distinctly different to previously published examples of bumble bee exploration flights, which showed quasicircular loops in all directions (see Osborne et al., 2013; Woodgate et al., 2016).

A bias towards following linear features during exploration flights can predict where individual foragers discover food sources and so may lead to colony level biases in exploitation of resources. Some support for this is provided by a further experiment in which a feeder located along a linear feature received far more visits than an identical one in the middle of a field (see Appendix, Experiment A1).

Experiment 2

In the first treatment, feeders F1 and F2 were presented together (Fig. 3). Here, 12 of 15 bees were observed flying to both feeders, for a total of 17 of 28 flights to both feeders rather than only to F1 (five flights from five bees) or only to F2 (six flights from five bees). In the second treatment, feeder F1 remained in the same place, while F2 was removed and replaced by F3, 168 m from F1 along the same road as F2 had been. Only one bee visited both feeders in a single

flight, compared to 28 flights of three bees to F3 only. No bee was recorded flying to F1 only during this time.

In both treatments, bees flying to feeders consistently spent a high proportion of flight within 5 m of linear features (flights to F1: mean \pm SD = 0.88 ± 0.18 , $N = 10$ bees; flights to F1 and F2: mean \pm SD = 0.80 ± 0.11 , $N = 8$ bees; flights to F2: mean \pm SD = 0.71 ± 0.09 , $N = 5$ bees; flights to F3: mean \pm SD = 0.59 ± 0.11 , $N = 3$ bees). When bees flew to feeders other than F1, where the shortest potential route would not follow linear features, they still frequently flew over linear features more than necessary (Fig. 3a–d), particularly during their final approach to the feeder or hive. Some bees did not visit feeders and instead flew beyond the range of the radar, presumably to natural forage. The return portions of these flights almost always followed the hive's footpath on the final approach to the hive (see Appendix, Experiment A2). Nevertheless, even bees flying back and forth to feeders F2 and F3 never followed linear features for the entire route, instead cutting across the corner of the field, demonstrating that they were not entirely dependent on linear features. The use of linear features in the last approach then likely indicates a strategy to locate the exact hive position.

In a further experiment, where neither hive nor feeders were located on linear features, we still observed extensive flight along footpaths, field borders and roads, and more flight close to linear features than expected by chance (Appendix, Experiment A3). Linear features were therefore salient in these experiments not only because the hive and feeders were located on them.

Experiment 3

One bee was recorded on 21 foraging trips over 4 days to feeder F3. The most direct route from the hive to this feeder would involve no flight along linear features while an alternative route continuously following linear features would be 41% longer. As the bee gained experience, we observed a switch in flight strategy (Fig. 4). In the first 10 foraging trips, 60% of outbound and inbound flights went directly over linear features for at least the last 30 m approaching the goal (the feeder for outbound flights; the hive for inbound flights; Fig. 4a–h). By contrast, in the last 11 foraging trips, only 14% of outbound and inbound flights went directly over linear features during the final approach to the goal (Fig. 4i–p). This suggests that linear features initially played an important role in helping the bee precisely locate the goal, but that with experience the bee was able to form a more efficient route and approach the goal without this guide.

Experiment 4

Searching flights (seven flights from six bees) showed consistently high proportions of flight within 5 m of linear features (mean \pm SD = 0.70 ± 0.17 ; Fig. 5). These numbers are comparable to those from flights to feeders, despite the lack of looping behaviour in these cases, suggesting that searching bees largely restricted their search to linear features.

During their search, bees not only flew repeatedly over the stretch of road along which the feeder was usually found, but also along other linear features (Fig. 5). Initially, bees returned to familiar locations such as the crossroads or even the hive itself before repeating their route to the feeder location. Later in their search, bees often scanned other parallel and perpendicular linear features (Fig. 5a–d). This suggests that bees either widened their search space for the lost feeder or switched strategy and searched for novel feeders, while relying on their learnt association between the feeder and road to guide them.

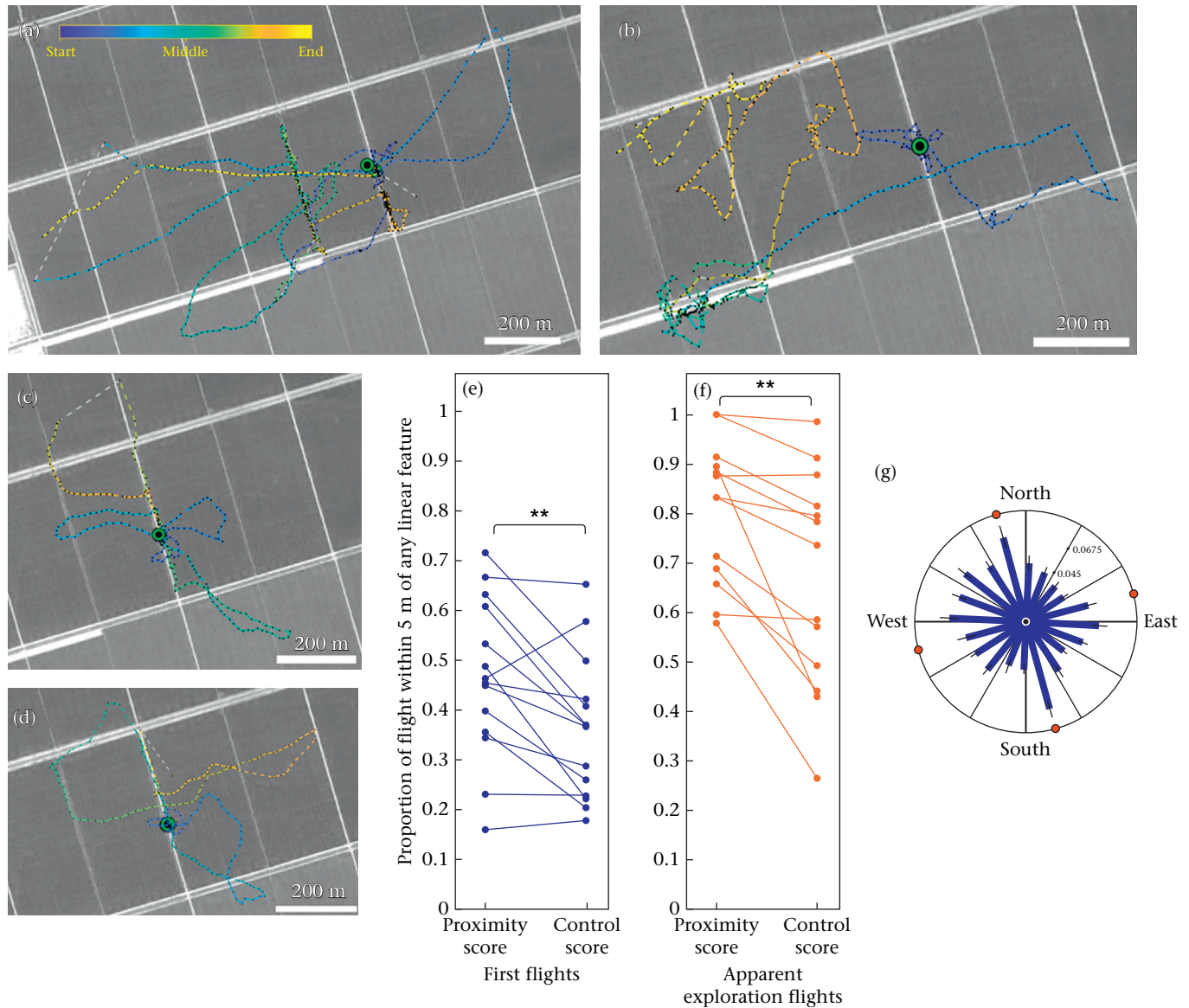


Figure 2. First exploration flights. (a–d) Example first flights of individual bees. Tracks begin dark blue, turn green then yellow by the end of the recorded track. Grey dashed lines indicate a period where the bee's position was unknown. The hive is represented by a green circle. Initial portions of flight were close to the hive and frequently followed the associated footpath. Subsequent loops took the bee further afield, often in directions along or parallel to linear features, showing distinctive right angle turns that matched the surrounding grid (b, d). (a) Example of aliasing error, where the bee appears to mistake an incorrect linear feature for the footpath associated with its hive. The satellite image used is from Google Earth, ©2018 Google. (e, f) Proximity of bee to linear features (proximity score) compared to the track-specific control (control score) for (e) first flights ($N = 14$) and (f) apparent exploration flights ($N = 13$). Each set of two linked points represents a single individual's scores. Asterisks indicate P values from paired-samples t tests: $**P < 0.01$. (g) Circular bar chart (bins of 18°) representing the mean proportion of each first and apparent exploration flight spent flying in each direction ($N = 32$). Black lines represent standard error of the mean for each bar. Red dots on the perimeter show the orientations of linear features in the environment.

Flight bearings of bees during searching flights were significantly nonuniformly distributed (Kuiper's test of uniformity: $V = 4.9107$, $N = 7$, $P < 0.01$), with more flight in directions associated with prevalent ground level features (Fig. 5e). These flights offer a striking contrast to the variety of azimuthal directions adopted by bees searching in landscapes less dominated by regular linear features (Reynolds et al., 2007).

In contrast, when searching for unpredictably located feeders within a field, where the hive and feeders were located away from linear features, bees largely restricted their flight within the field, often approaching field borders without crossing them (Appendix, Experiment A3). The structure of searching flights thus appears to be flexible, with bees using linear features strategically, based on individual experience.

DISCUSSION

Our experimental manipulation of hive and feeder location in a landscape with a featureless, flat horizon and a regular grid of ground level linear features demonstrates that bees made extensive use of these features during their exploration, foraging and searching flights. Their utilization of ground information was strikingly strategic, linked directly to each individual's goals and experience.

Exploration flights performed by bees in our field site were vastly different to those observed in environments with a variety of skyline and ground level cues (Osborne et al., 2013; Woodgate et al., 2016). Flights previously recorded in landscapes less dominated by linear features show distinct looping patterns in all directions (see

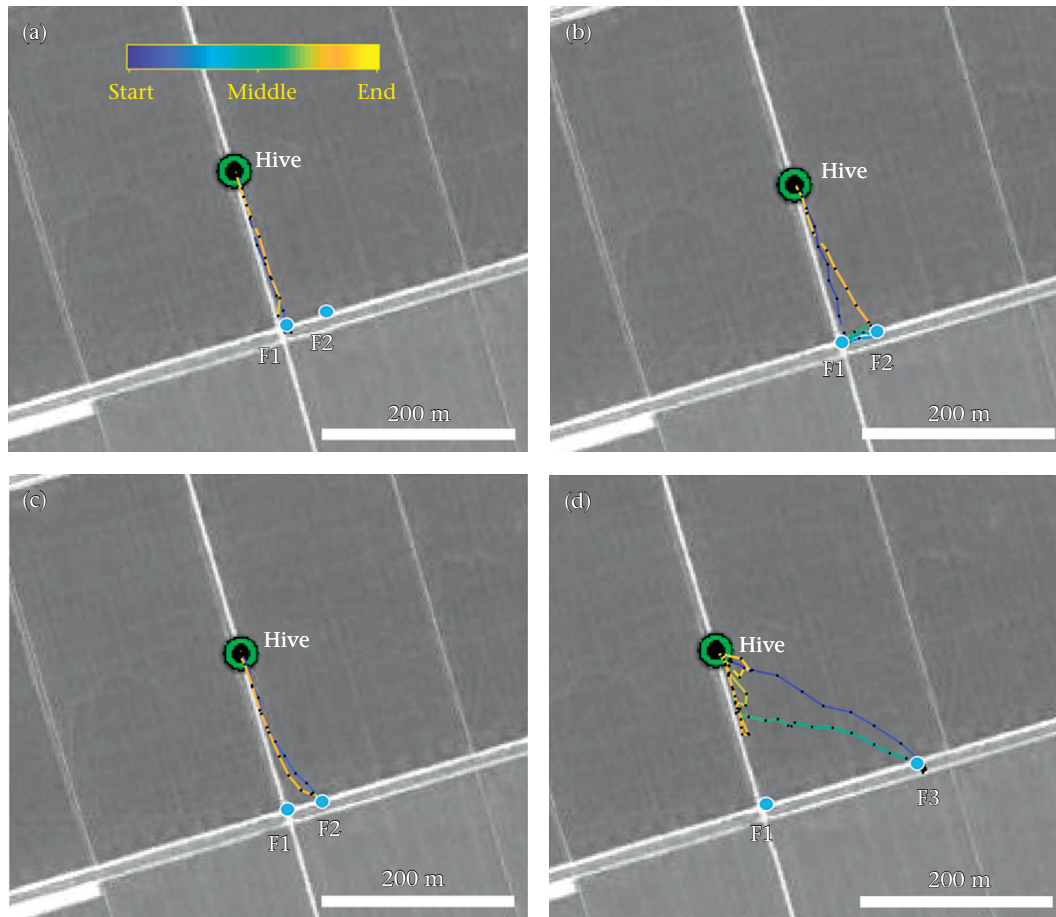


Figure 3. Foraging flights to feeders. (a–d) Example flights of bees to experimental feeders F1, F2 and F3. Tracks begin dark blue, turn green then yellow by the end of the recorded track. Grey dashed lines indicate a period where the bee's position was unknown. The hive is represented by a green circle, and feeders are represented by light blue dots. (a) Example flight to F1, showing a direct, straight route along the path. (b) Example flight to F1 and F2. The bee followed the footpath to F1, followed the dirt road at a right angle to reach F2, zigzagged between the feeders multiple times before cutting across the corner of the field from F2 to return to the hive. (c) Example flight to F2. The bee followed the footpath connected to the hive for about a third of the distance before crossing over the corner of the field to the feeder in a curved route. (d) Example flight to F3. The bee flew directly to the feeder from the hive. On the return flight the bee joined and followed the footpath connected to the hive until it reached its goal. The satellite image used is from Google Earth, ©2018 Google.

Figure 2A in Osborne et al., 2013 and Figure 2 in Woodgate et al., 2016). Our flight tracks show, instead, high proportions of flight along and parallel to linear features (Fig. 2), demonstrating that the shape of exploration flights is determined by the structure of the surrounding landscape. Since bumble bees use exploration flights to discover forage (Osborne et al., 2013; Woodgate et al., 2016), we predict that linear structures will bias floral resource discovery and pollination at a colony level. Support for this comes from our observations of bee visit rates to feeders, where a huge majority of total visits were to the feeder along a footpath rather than to the identical feeder in the centre of a field (Appendix, Experiment A1). This was all the more striking since bumble bees do not communicate forage locations with each other (Dornhaus & Chittka, 1999), so that this was the result of individual level exploration and foraging decisions. Furthermore, when we simulated a foraging source no longer being available (for example, flowers running dry or a tree being cut down) by removing the feeders (experiment 4), bees used their previous experience of linear features to limit their search. The first food sources found may thus shape the discovery and pollination of floral resources in the long term. This effect of landscape structure on exploration flights could also have important implications for bee spatial distribution, by biasing the discovery of new nest sites by queens (Makinson et al., 2019).

Bees flying to experimental feeders often flew distinct, detouring routes along linear features (experiment 2). This was reminiscent of previous observations by Chittka, Kunze, Shipman, and Buchmann (1995), where bees detoured via a salient landmark to reach a feeder. One bee, observed over many flights, gradually refined her route from cutting across the corner of a field but still following linear features, to eventually bypassing linear features altogether (experiment 3). Likewise, the formation of efficient routes between multiple food sources (termed traplines) are refined over time as the bee gains experience (Woodgate et al., 2017). Our data suggest that while inexperienced, the bee relied on linear features for accuracy, switching to using vector memories and/or more detailed visual memory once she was sufficiently experienced (Collett, Chittka, & Collett, 2013; Menzel, Geiger, Joerges, Müller, & Chittka, 1998). If bees rely more on linear features when they have less certainty about their position, this could explain previous observations in which displaced honey bees (Collett & Graham, 2015; Menzel et al., 2019; Wolf et al., 2014) and pigeons (Lipp et al., 2004) showed extensive detouring along linear features.

Why do bees preferentially follow pathways, field edges and roads? Experiments with humans and rats have suggested that beacon cues (that indicate a nearby target location) are

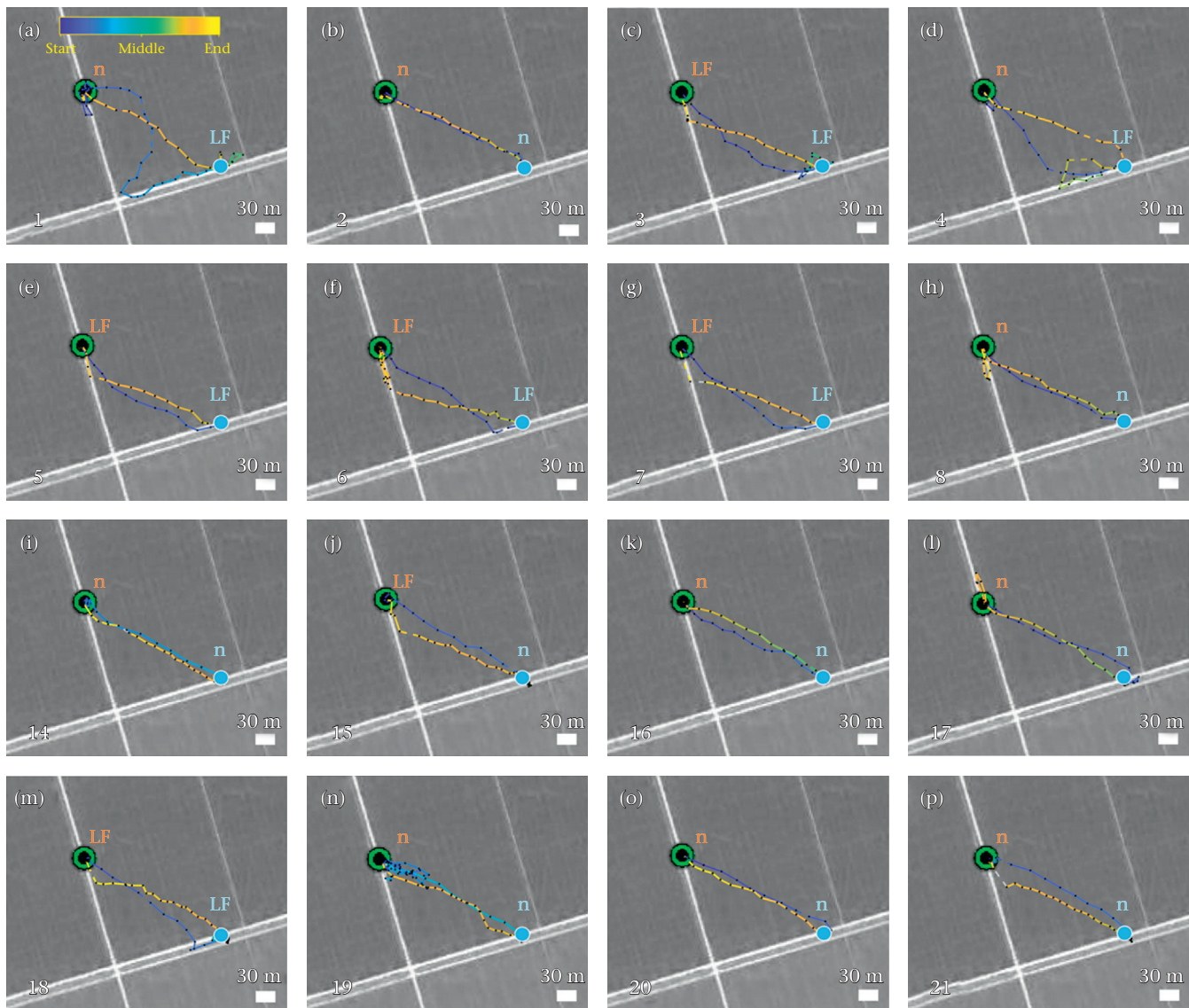


Figure 4. Development of direct route to feeders with experience. (a–p) Radar tracks of the first eight (a–h) and last eight (i–p) flights of a single bee foraging at F3. Flight bouts are indicated by white numbers at the bottom left of each track. Each flight is split into an outbound (blue to green) and inbound (green to yellow) portion of flight. Portions of flight are labelled ‘LF’ if the bee followed a linear feature for the last 30 m of flight approaching the goal (F3 or the hive); portions of flight without line following are marked ‘n’. Outbound portions of flight are labelled in blue, next to the feeder. Inbound portions of flight are labelled in orange, next to the hive. The hive is represented by a green circle, and the feeder is represented by a light blue dot. The satellite image used is from Google Earth, ©2018 Google.

preferentially used over more complex visual cues (for example, cues that inform on the next direction in a route, but do not directly indicate the goal; Chan, Baumann, Bellgrove, & Mattingley, 2012; Waller & Lippa, 2007). Following a linear feature to a desired goal may be similar to using a beacon cue, in providing an easier alternative to navigating directly to an otherwise isolated point, an example of ‘cognitive offloading’ (Risiko & Gilbert, 2016). The last part of a bee’s return to the hive often relies on beacons and other landmarks (Turner, 1908), because path integration lacks small-scale accuracy (Collett & Collett, 2000; Menzel et al., 1998). In our experiments, bees may have implemented a strategy in which they switched to following linear features before they expected to reach their hive. This could allow them to compensate for errors and imprecisions in their path integration and avoid missing their goal.

Path integration is an orientation mechanism that relies on compass cues and distance information to form vector memories (Chittka et al., 1995; Collett & Collett, 2000). When compass cues

are placed in conflict with skyline landmarks, bees prioritize flying in the learnt compass directions rather than using the landmarks to guide them (Chittka & Geiger, 1995; von Frisch, 1967). Intriguingly, the opposite effect has been described in experiments where the landmarks were instead linear features: honey bees prioritized flying along a treeline, a coastline or a road over flying in the correct compass directions (von Frisch & Lindauer, 1954; von Frisch, 1967). Linear features, unlike skyline landmarks, may thus be prioritized over other path integration cues. Our field site presented bees with a regular grid of linear features which provided bees with accurate direction and distance information (Fig. 1b). Future experiments could manipulate ground level linear features on a large scale (e.g. by using tractors to ‘draw’ lines in the earth) to further investigate the role of linear features in setting travel directions.

Geomagnetic cues are another potential source of compass information (Lindauer & Martin, 1972; Wajnberg et al., 2010). We used a magnet to attach the radar transponder to the backs of our

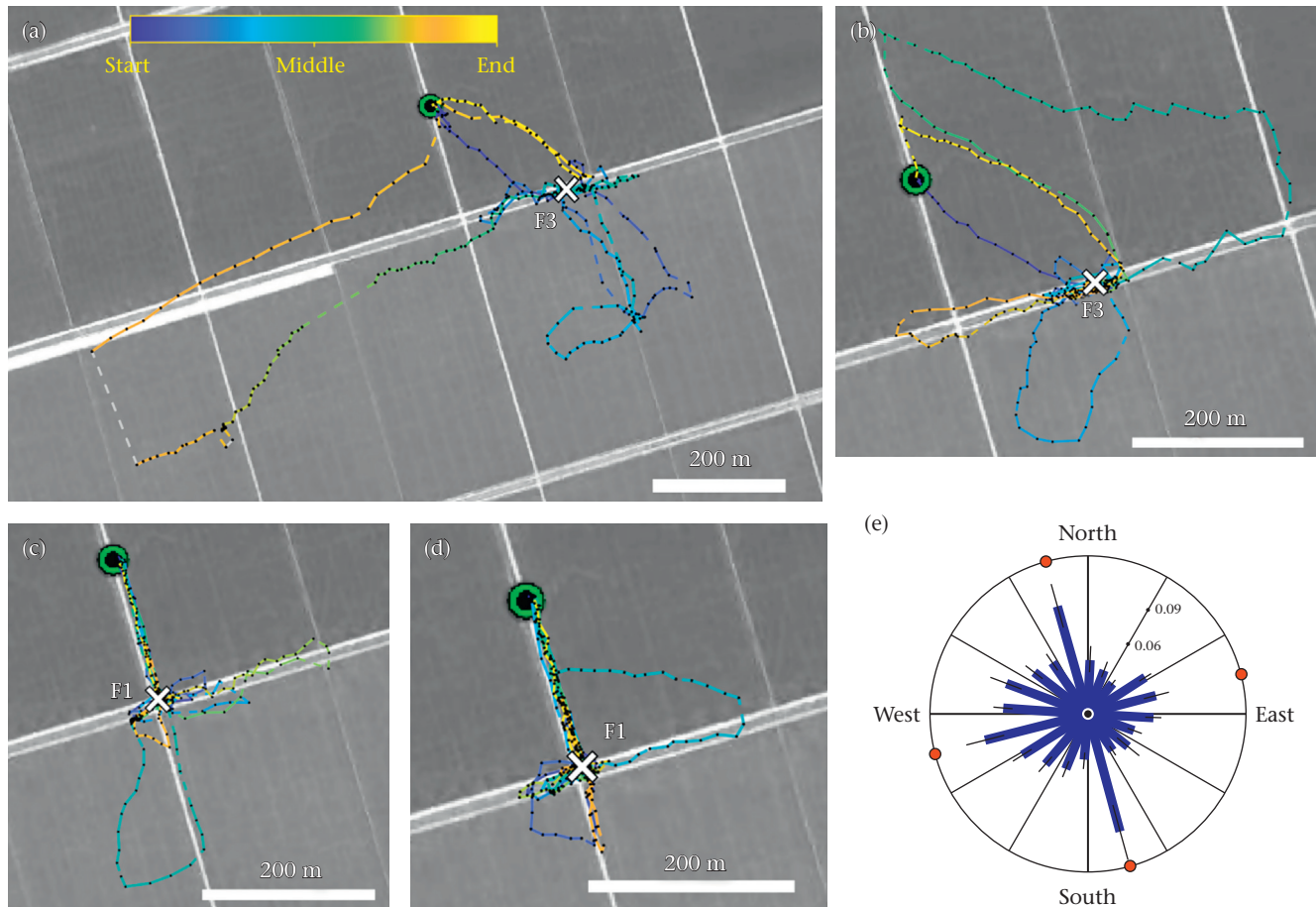


Figure 5. Searching flights. Every bee had prior experience foraging at the feeder (labelled F1 or F3) and was tracked searching after all feeders were removed from the field site. (a–d) Example flight paths of bees searching for the experimentally removed feeder. The location of this feeder before its removal is indicated by a white cross. Tracks begin dark blue, turn green then yellow by the end of the recorded track. Grey dashed lines indicate a period where the bee's position was unknown. The hive is represented by a green circle. The satellite image used is from Google Earth, ©2018 Google. (e) Circular bar chart representing the mean proportion of each searching flight ($N = 7$) spent flying in each direction. Black lines represent standard error of the mean for each bar. Red dots on the perimeter show the orientations of linear features in the environment.

bees, which may theoretically have affected the detection of magnetic information (Walker & Bitterman, 1989). However, during navigation, bees clearly prioritize celestial and visual cues (always available in our study) over any magnetic information (Frier, Edwards, Smith, Neale, & Collett, 1996; Collett & Baron, 1994; Lindauer & Martin, 1972; von Frisch, 1967); as our observations show no evidence of any disorientation, we do not believe the magnet affected the reported results.

Visual-familiarity-based mechanisms are thought to play an important role in insect navigation, where the insect's heading is determined by a measure of similarity between the current view and a relevant subset of memories (Baddeley, Graham, Husbands, & Philippides, 2012; Collett et al., 2013; Zeil, 2012). In this linear-feature-rich landscape, footpaths and roads might all present similar visual cues. When returning or searching for a location on a linear feature, using familiarity to guide the bee's route might always direct the bee towards the closest linear features, causing the line following we observed. If this is the case, we would consequently also predict frequent aliasing errors, where one linear feature is mistaken for another. This was not the case: one single aliasing error was observed, from a bee on her first flight (Fig. 2a). Additionally, foraging bees readily flew away from linear features, even reducing their flight along linear features with experience (experiment 3, Fig. 4). Instead, bees changed how they used linear features depending on their goal and experience: following them to

reach a feeder placed along a footpath (experiment 2); relying on them more when inexperienced (experiment 3); flying along them when searching for a feeder previously placed along a road (experiment 4); using linear features as visual boundaries within which to focus their flight when searching for feeders within a field (Appendix, Experiment A3). This flexible, goal-dependent use of linear features strongly suggests that bees strategically use the information available to them for efficient navigation.

Bees monitor their movements using visual feedback from their own motion, termed 'optic flow' (Srinivasan, 2011). For example, position control can be achieved by matching the speed of optic flow received from left and right (Srinivasan, Lehrer, Kirchner, & Zhang, 1991). If linear features were less visually rich (and thus provided less optic flow) than the surrounding fields, bees might move closer to them to increase the visual input from that direction. Alternatively, bees have been shown to preferentially fly over visually rich ground (Linander, Baird, & Dacke, 2017). If linear features were instead richer in visual feedback, bees might prefer to fly over them. A small difference in visual richness is unlikely, however, to affect bee position, since variations in frequency and visual contrast of optic flow have no effect (Srinivasan et al., 1991). In our field site, linear features and fields were visually similar and can be expected to provide similar levels of visual feedback (see Fig. 1a). We thus believe flight along linear features is not caused by a response to optic flow but instead reflects an adaptive use of available landmarks.

Ground level linear features are of underappreciated importance to animal navigation, especially flying insects. Understanding their utilization by bees will have practical consequences of considerable importance for agriculture and conservation, in the context of the pollination crisis. Insect pollinators are responsible for 35% of global food production (Klein et al., 2007; Potts et al., 2016) and beehives are routinely transported for pollination services in intensive agricultural terrains characterized by regular artificial linear features that could be used to direct pollination outcomes (Alger, Burnham, Lamas, Brody, & Richardson, 2018). We predict, for example, that placing a hive along a linear feature will predispose the bees to travelling along this feature whereas placing a hive in the centre of a field might instead predispose bees to concentrate their pollination efforts within the field's borders. Linear features could also be used for conservation purposes to influence the movements of bees in semiurban and urban environments and facilitate the localization of suitable habitats or foraging areas by wild species.

Author Contributions

J. Brebner: Conceptualization, Methodology, Investigation, Data Curation, Software, Formal Analysis, Visualization, Writing – Original Draft; J. Makinson: Conceptualization, Methodology, Investigation, Writing – Review and editing; O. Bates: Data Curation, Writing – review and editing; N. Rossi: Data curation, Visualization, Writing – review and editing; K. Lim: Resources; T. Dubois: Investigation; T. Gómez-Moracho: Investigation; M. Lihoreau: Methodology (Experiment A3), Investigation, Writing – Review and editing, Funding acquisition; L. Chittka: Conceptualization; Writing – review and editing, Project administration, Funding acquisition; J. Woodgate: Conceptualization, Methodology, Investigation, Software, Formal Analysis, Visualization, Writing – review and editing.

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Appendix

Field Site Panorama

The rice farm Finca Casudis was set in extremely level land, with an almost completely flat horizon and almost no above ground level landmarks. The highest point on the skyline was an installation with grain silos and grain elevator at the farm (dark shape to the right of the panoramic image, Fig. 1a). We assessed whether this could provide a landmark for bees using a panoramic image from Hive Location 1 (since this was closer to the farm than Hive Location 2, see [Methods](#), Fig. 1b). A 360° panoramic photograph was taken using a Samsung Galaxy A3 mobile phone (Fig. 1a). From this panorama, assuming pixels were square (so that the height of the image was scaled identically to the width), we calculated that the top of the grain silos was 1.86° above the adjacent ground level. The maximum resolution of bumble bees has been placed between 2.3° (Dyer et al., 2008) and 4.8° (Chakravarthi, Baird, Dacke, & Kelber, 2016). Thus, it is unlikely that bumble bees could detect the grain silos or any other artificial structure from anywhere close to the

hive. It is harder to determine whether there was enough variation around the skyline to provide useful cues, because there is some distortion in the panoramic image: the skyline was visibly flatter than it appears on the image. The maximum difference in horizon level on the panoramic image was 3.30°, but we believe this is an overestimation. We used heywhatsthat.com (Kosowski, 2021) to calculate the projected skyline as seen from 6 ft above ground level at the hive's GPS position (Hive Location 1), and estimated the maximum variation to be 1.03°, suggesting that there was little or no variation detectable by bumble bees.

Experiment A1

Do linear features affect feeder visits? Between 7 and 8 May 2018, we compared the visit rates between two identical feeders by bees from two bumble bee colonies without prior experience of the field site. Feeder F1 was located 168 m from the hive along a path, while feeder F4 was located 168 m from the hive in the middle of the adjacent field (100 m from the closest path; Fig. 1b). Bees were allowed to come and go freely from the hive from 1735 to 2135 on 7 May and from 1100 to 1740 on 8 May 2018. Visits at each feeder were filmed (Sony Handicam HDR-CX240, Sony Corporation, Tokyo, Japan) between these times, for a total of 10 h and 45 min. The footage was analysed to record the timing of every visit by a bee.

Bees visited F1 far more often than F4 (F1: 341 visits over 10 h 45 min; F4: eight visits over the same period; F1 received 97.7% of all visits; Fig. A1). The visit frequency to F1 was on average 3.22 visits over a 5 min period, with the highest frequency of 10 visits in any consecutive 5 min period, while F4 received on average 0.06 visits per 5 min and never more than one visit in any consecutive 10 min period. The first visit to F1 occurred only 10 min after bees were allowed access to the field site, while the first visit to F4 was 35 min after that (45 min since the hives were opened).

Experiment A2

Do bees use linear features when flying to distant natural forage sources? During experiment 2, not all bees used our experimental feeders. Some were tracked flying to distant locations, out of range of the radar ($N = 39$ tracks, 29 bees; four were from unknown individuals and were excluded from statistical analysis). These locations were presumed to be natural forage, since these individuals frequently returned with pollen and tended to fly towards the river (along which were natural foraging sources).

Bees flying to distant locations spent significantly more of the flight close to linear features than expected by chance (Kolmogorov–Smirnov test of normality: $P = 0.009$ for proximity scores, $P = 0.024$ for control scores; Wilcoxon related-samples signed-rank test: $P < 0.001$). Within these flights, those that included clear inbound and outbound portions of flight showed lower proximity scores in their outbound journeys compared to the corresponding inbound journeys (paired-samples t test: $t_{19} = -2.391$, $P = 0.027$). During the final approach (last 30 m) to a feeder or their hive, bees very often flew along the closest linear feature to reach their goal, suggesting that linear features play an important role in finding the precise location of a goal (Fig. A2). We tracked a total of 39 flights to distant locations, from 29 bees, of which 22 flights were complete enough to examine the inbound portions of flight. Bees clearly followed the footpath adjacent to the hive during their final approach in 21 of these 22 flights ($N = 20$ bees; Fig. A2).

Experiment A3

Do bees use linear features when their hive is in the centre of a field? We investigated whether linear features influenced feeder

Table A1
Details of experimental data

Experiment	Hive location	No. of colonies used	No. of flight tracks	Details*
Experiment 1 Exploration flights	1	2 (C3, C6)	32	First flights: 24 April 2018 ($N = 11$), no feeders 4 May 2018 ($N = 3$), feeders F1, F3, F4 Apparent exploration flights: 30 April 2018 ($N = 2$), feeders F1, F3 4 May 2018 ($N = 1$), feeders F1, F3, F4 7–8 May 2018 ($N = 10$), feeders F1, F4
Experiment 2 Foraging flights	1	6 (C1–C6)	79	20–23 April 2018 ($N = 12$), feeders F1, F2 26–30 April 2018 ($N = 3$), feeders F1, F3 5–8 May 2018 ($N = 6$), feeders F1, F4
Experiment 3 Route formation	1	1 (C3)	21	26, 27 and 30 April 2018 ($N = 1$), feeder F3
Experiment 4 Searching flights	1	4 (C3–C6)	7	27–30 April 2018 ($N = 3$), feeder F3 5–8 May 2018 ($N = 3$), feeder F1
Experiment A1 Visit rates to feeders	1	2 (C5, C6)	N/A	5–8 May 2018, 349 visits recorded from video footage at feeders F1 and F4
Experiment A2 Foraging flights to natural forage	1	6 (C1–C6)	39	20–23 April 2018 ($N = 2$) 26–30 April 2018 ($N = 17$) 2 May 2018 ($N = 10$) 5–8 May 2018 ($N = 4$)
Experiment A3 Searching flights from hive in centre of field	2	2 (C7, C8)	20	9–17 May 2018 ($N = 13$), three feeders present within the field in random positions

'Hive Location 1' was along a linear feature and 'Hive Location 2' was in the centre of a field; see Methods and Fig. 1b. Individual colonies used were labelled C1–C8. The number of flights tracked indicates tracks used solely for that experiment, and for no other, except for experiment 3, as these data were also included in experiment 2. N = number of individual bees tracked during each period for each experiment. Some individuals were tracked during several periods. All data from flights tracked were averaged per individual within relevant categories irrespective of date tracked before statistical analysis. Where relevant, the number and position of feeders are indicated for each period.

* Dates carried out, number of individuals tracked in that time and presence or absence of feeders.

discovery when feeders were not associated with linear features. For this experiment, the hives were in the centre of a field (Hive Location 2, Methods), 100 m from the closest linear features. Bees were trained and tracked individually searching for an array of three feeders from a hive position in the centre of a field (Hive Location 2, Methods).

Pretraining

All bees were given free access to the field site and trained to feed from three feeders near the hive entrance providing ad libitum 40% sucrose solution. Once bees were accustomed to this, the feeders were each placed 5 m from the hive in a triangle formation for another 4 h. Individual bees were then trained one by one on all three feeders, each containing 10 μ l 40% sucrose solution, refilled regularly using an electronic pipette (HandyStep, BrandTech

Scientific Inc., Essex, CT, U.S.A.). The average crop content was determined for each individual bee (from three measures).

Experiment

The three feeders were then arranged in random arrays, where the feeder locations were determined by an algorithm, with the limitation that no two feeders could be closer than 5 m from each other, all feeders were located within the surrounding field and no new array positions used could be within 5 m of any previously used array. Individuals were then tracked searching for the three feeders, each containing one-third of the focal bee's crop capacity of 40% sucrose solution. Every bee ($N = 13$) was tracked during several foraging trips searching for such arrays. Some flights were excluded from analysis due to missing data within the tracks, so that a total of 20 tracks from 13 bees were analysed.

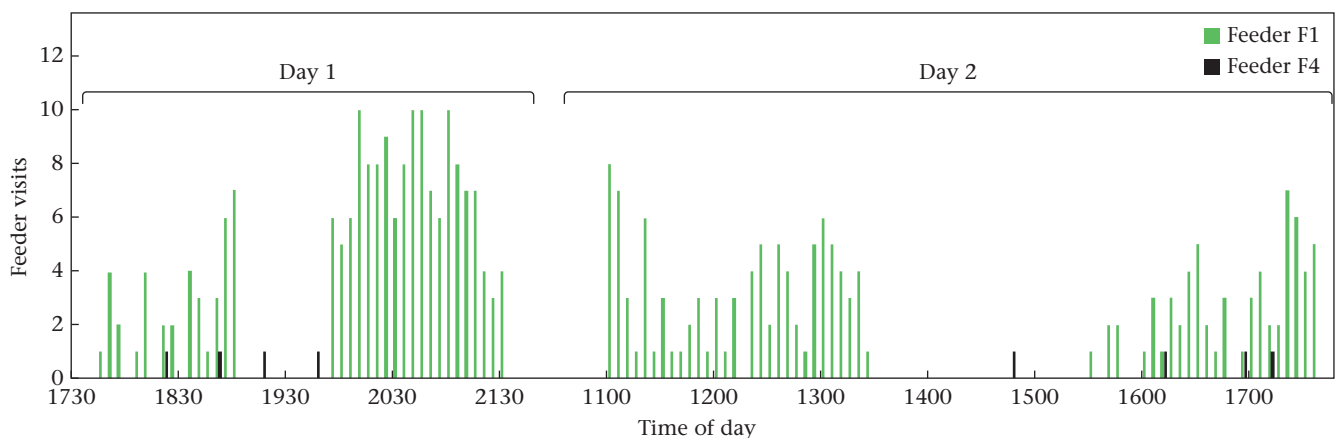


Figure A1. Histogram showing the number of visits to two feeders in 5 min periods over 2 days. Feeder F1 was located along a linear feature adjacent to the hive location. Feeder F4 was placed 100 m away from the closest linear feature. Both feeders were otherwise identical and equidistant from the hive. Feeder visits were counted from video footage.

Results

Even when searching for feeders placed randomly within a field from a hive located in the centre of that same field, bees showed a higher proportion of flight within 5 m of linear features than expected by chance (Kolmogorov–Smirnov test of normality: $P < 0.05$ for initial scores, $P = 0.061$ for control score; Wilcoxon matched-pair signed-rank test: $N = 13$, $P = 0.006$; Fig. A3a). The bees' flight bearings were not uniformly distributed (Kuiper's test of uniformity: $V = 3.5925$, $N = 20$, $P < 0.01$), showing a bias in directions corresponding to ENE–WSW linear features (Fig. A3b).

Of 20 tracks, the bee left the field in half of them. Of these 10, five only made a single flight loop outside the field borders while

three showed extensive exploration beyond the field. Of the 10 tracks in which the bee remained within the field, the bee flew up to but not beyond the field borders in seven, suggesting that bees may have learned to use linear features to bound their search space and turned back when they encountered one (Fig. A3c–d). The last three tracks remained near the hive. Four of the 20 tracks (the three with extensive flight outside the field and one without flight extending beyond the field borders) were long flights showing sustained periods following linear features, suggesting that a learned association between the hive and a linear feature is not the sole cause of line-following behaviour.

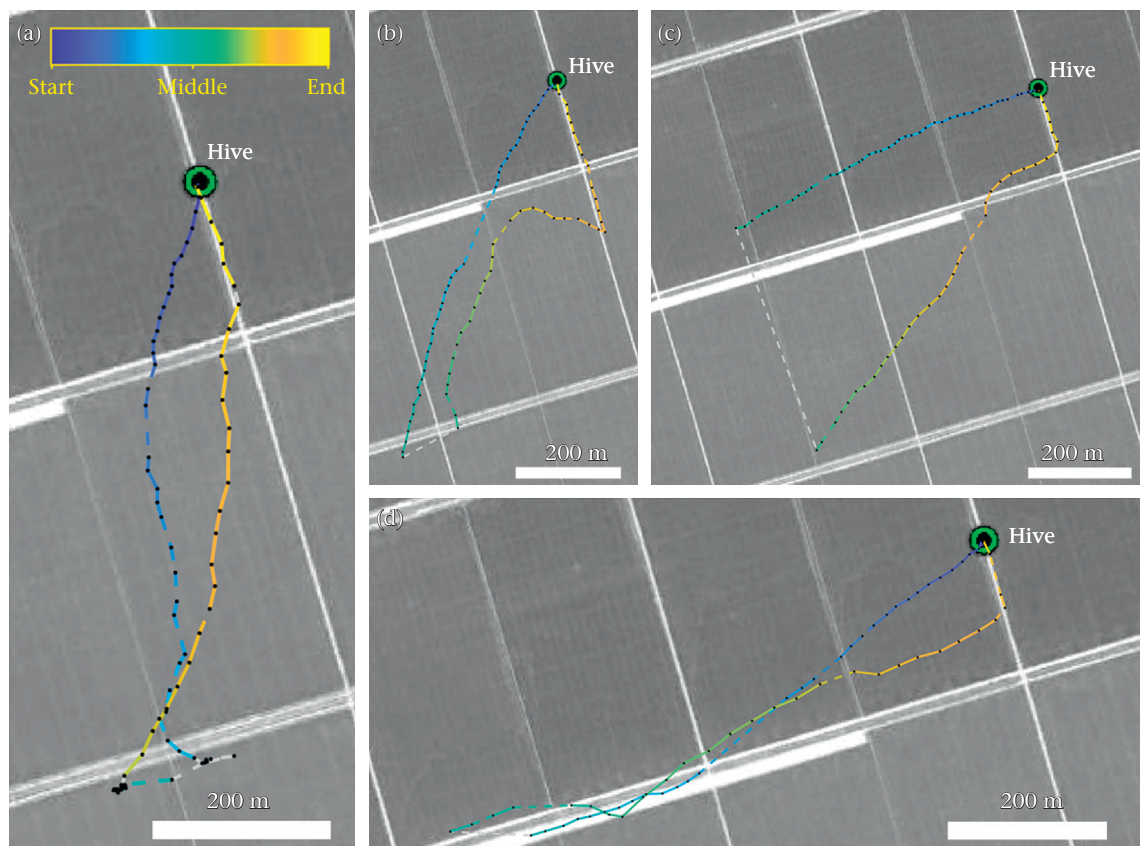


Figure A2. Flights to distant locations. (a–d) Example flight paths of bees flying to and from distant foraging sources. Tracks begin dark blue, turn green then yellow by the end of the recorded track. Tracks cut off when bees fly beyond the range of the radar and restart when bees re-enter the range. Grey dashed lines indicate a period where the bee's position was unknown. The hive is represented by a green circle. Most outbound flights (in blue) cut across linear features and did not follow them for extended periods. The satellite image used is from Google Earth, ©2018 Google.

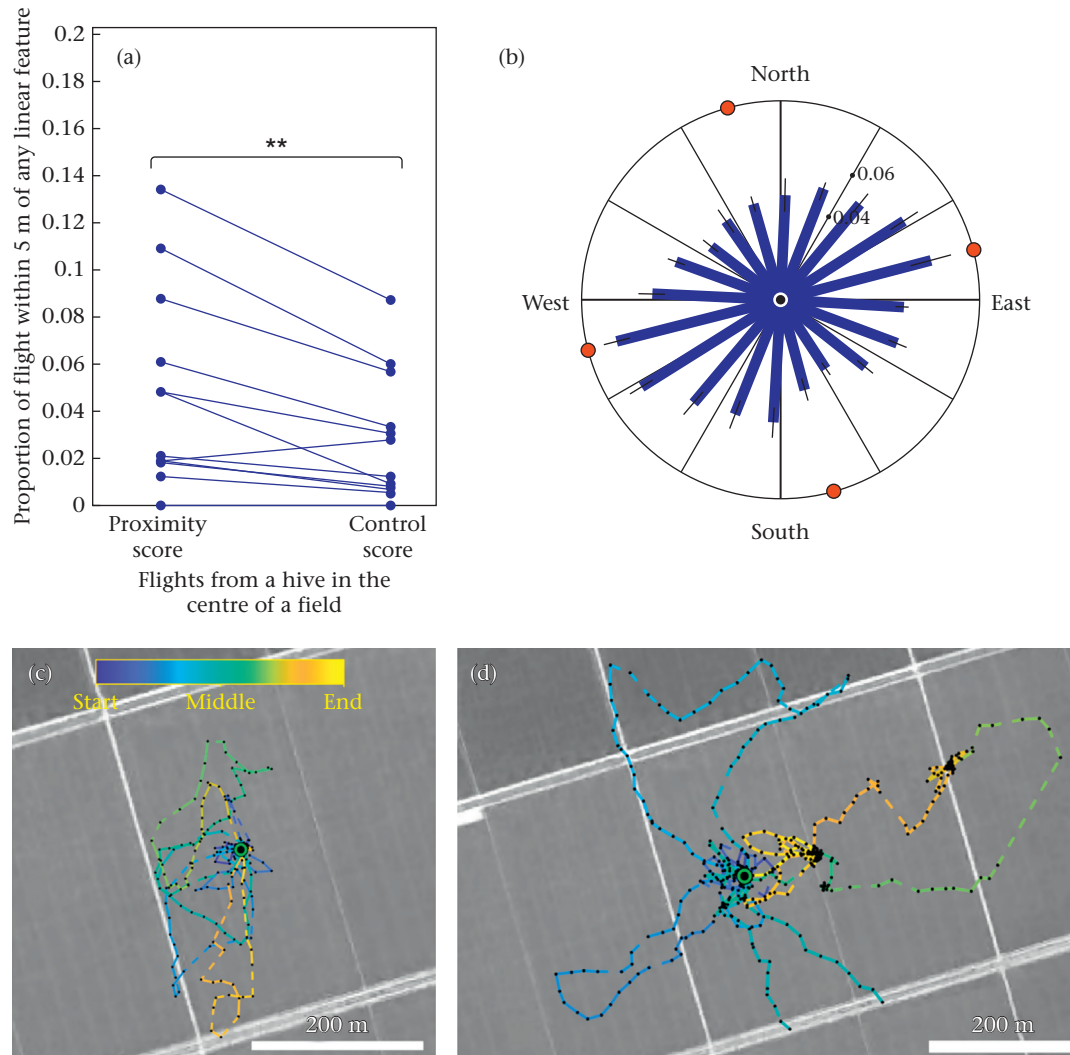


Figure A3. Searching flights for unpredictable feeders. Bees were trained to a protocol in which three feeders were moved at random within the field between foraging bouts. (a) Proximity of bee to linear features (proximity score) compared to the track-specific control (control score) of bees performing searching flights for unpredictable feeders. Each set of two linked points represents a single individual's scores. Asterisks indicate P values from a Wilcoxon matched-pair signed-rank test: $**P < 0.01$. (b) Circular bar chart representing the mean proportion of each searching flight spent flying in each direction. Black lines represent standard error of the mean for each bar. Red dots on the perimeter show the orientations of linear features in the environment. (c-d) Example flight paths of bees searching for feeders in unpredictable locations. Tracks begin dark blue, turn green then yellow by the end of the recorded track. Grey dashed lines indicate a period where the bee's position was unknown. The hive is represented by a green circle. The satellite image used is from Google Earth, ©2018 Google. (c) Example flight in which the bee appeared to bound its search space using linear features, flying between the hive and surrounding paths without crossing them. (d) Example flight in which a bee that did cross linear features to exit the field showed extensive flight along several paths.