Bumblebees increase their learning flight altitude in dense environments

Annkathrin Sonntag[1,*], Mathieu Lihoreau[2], Olivier J.N. Bertrand[1], Martin Egelhaaf[1],

1 Neurobiology, Faculty of Biology, Bielefeld University, 33615 Bielefeld, Germany
2 Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS, University Paul Sabatier - Toulouse III, 31062 Toulouse, France

* Corresponding author: a.sonntag@uni-bielefeld.de

Abstract

Bumblebees rely on visual memories acquired during the first outbound flights to relocate their nest. While these learning flights have been extensively studied in sparse environments with few objects, little is known about how bees adapt their flight in more dense, cluttered, settings that better mimic their natural habitats. Here we investigated how environmental complexity influences the first outbound flights of bumblebees. In a large arena we tracked the bees' 3D positions to examine the flight patterns, body orientations, and nest fixations across environmental conditions characterised by different object constellations around the nest entrance.

In cluttered environments, bees prioritised altitude gain over horizontal distance, suggesting a strategy to overcome obstacles and visual clutter. Body orientation patterns became more diverse in dense environments, indicating a balance between nest-oriented learning and obstacle avoidance. Notably, bees consistently preferred to fixate the location of the nest entrance from elevated positions above the dense environment across all conditions.

Our results reveal significant changes in 3D flight structure, body orientations, and nest fixation behaviours as object density increases. This highlights the importance of considering three-dimensional space and environmental complexity in understanding insect navigation.

Keywords*: Bumblebees; 3D flight pattern; learning flights; clutter; spatial learning*

Summary Statement

Dense environments influence bumblebees' first flights, during which they develop visual memories of their nest entrance. Manipulating nest-surrounding objects showed bees prioritise altitude gain over horizontal distance in cluttered environments.

Introduction

Bees use visual memories to return to their nest after foraging trips. These visual memories are thought to be acquired during the first outbound flights during which the bees do not forage but perform convoluted manoeuvres consisting of many loops and arcs (e.g. Capaldi et al., 2000; Philippides et al., 2013; Woodgate et al., 2016; for reviews see: Collett and Zeil, 2018; Collett and Hempel de Ibarra, 2023). During these first flights, the bees use a “turn back and look” behaviour to regularly face the nest and presumably learn the visual surroundings (Lehrer, 1991, 1993). Bees need to perform these learning flights in order to efficiently return to their nest and avoid risky time-consuming search at the end of every foraging bout (Degen et al., 2016). After a short walking phase, the bees gradually increase their flight altitude and distance to the nest by progressively enlarge the size of their loops, the area covered by these flights expands (Capaldi et al., 2000; Osborne et al., 2013; Woodgate et al., 2016; Lobecke et al., 2018; Bertrand and Sonntag, 2023).

Typically, the learning flights were investigated at a detailed level in simple environments with only few cylindrical objects surrounding the nest (Hempel de Ibarra et al., 2009; Collett et al., 2013; Robert et al., 2018; Collett et al., 2023). For instance, it was found that bumblebees adjust their body orientation toward specific directions like compass direction or towards visual features in the surroundings (de Ibarra et al., 2009; Collett et al., 2013, 2023). Additionally, studies have focused on the coordination between head and body movements during these flights (Odenthal et al., 2020; Doussot et al., 2021).

In nature, however, the nests of bees are often located in cluttered environments such as grasslands, forests or agricultural cropland (O’connor et al., 2017; Liczner and Colla, 2019). These natural habitats might provide obstacles the bees have to overcome or detour. For inexperienced bees, this can be particularly challenging due to their not fully tuned flight control skills. Additionally, visual clutter and occlusions complicate orientation by eliminating reliable visual cues like a visual compass and making it harder to learn the environment.

Consequently, dense and cluttered environments may require more specific learning and navigation strategies for the bees. While the development of the flight altitude played a minor role in aforementioned studies in sparse environments, the described challenges in more ecologically realistic dense environments highlight the importance of understanding how the 3D structure of learning flights are affected.

Here, we investigated how the features of the environment shape the 3D characteristics of the first outbound flights of bumblebees in the immediate vicinity of their nest hole. Objects surrounding the nest entrance may serve as landmarks indicating the nest's position, but they also present challenges such as occlusion, higher collision risks, and dramatic visual changes when transitioning from a dense environment to a more open one. Therefore, we examined how during the initial outbound flights the increase in altitude and distance from the nest is influenced by the features of the surroundings such as the density of these objects' constellations, and the distance between the objects and the nest. In denser environments, the bees could increase their horizontal distance to the nest while keeping their flight altitude low (small altitude distance ratio) as we found in a previous study that homing bees prefer to enter dense environments at low altitudes (Sonntag et al., 2024). Alternatively, the bees could increase their flight altitude while keeping the distance to the nest small (large altitude distance ratio) because the bees may try to 'escape' the clutter to gain an overview of the environment. Such altitude gain in dense environments is expected by homing models based on visual memories (Sonntag et al., 2024).

We also investigated the relationship between the positions where bees fixate on the nest entrance — possibly acquiring the visual memories necessary for their return—and their flight altitude in relation to the height of surrounding objects. In sparse environments bees showed a clear body orientation towards the nest location while the flight direction deviated from this direction (Collett et al., 2023, 2013; Philippides et al., 2013). A dense environment will pose a challenge in keeping the body axis oriented towards the nest due to a higher collision risk and might thus interrupt fixation behaviour. In addition, the body orientation and flight direction could change with altitude when they are affected by the visual clearance or occlusion of the objects.

Material and Methods

Animal handling

We used sequentially three healthy hives of *Bombus terrestris* provided by Koppert B.V., The Netherlands. After arrival, the bees were transferred under red light (non visible to bees (Skorupski and Chittka, 2010)) into an acrylic box (30x30x30cm). This box was covered with black cloth to mimic the natural, underground nesting conditions of *B. terrestris* (Goulson, 2010). The nest box was connected to the flight arena via a system of six small boxes (6cmx6cmx6cm) and plastic tubes (2.5cm in diameter). One of the boxes contained a micro-gravity feeder that gave the hive direct access to sugar solution. The feeder consisted of a bottle with a small plate at the bottom where the bees could feed on sugar solution (30% sugar in volume) *ad libitum* through small slits in the plate. Pollen balls (50ml ground, commercial pollen collected by honeybees (W. Seib, Germany) and 10ml water) were provided *ad libitum* directly into the nest box. After their first outbound flight, the bees were marked with numbered plastic tags glued on their thorax with a melted resin. The temperature in the experimental room was constantly kept at 20° degrees and artificial light from above was provided in a 12h/12h day-night cycle.

Experimental design

The flight arena was similar to Sonntag et al. (2024). It consisted of a cylinder with 1.5m in diameter and 0.8m in height. The walls and the floor were covered with a red and white pattern (perceived as dark and white by the bees (Skorupski and Chittka, 2010)) with a spatial frequency distribution of 1/f characteristic of natural sceneries (Schwegmann et al., 2014). This pattern provided enough contrast for the bees to use optic flow for flight control but did not give any directional information for navigation. To facilitate the video recording and tracking of the bees, the arena was lit from below with 18 neon tubes (36W Osram + Triconic, light spectrum in Fig. S3). This light was filtered by a red acrylic plate (Antiflex ac red 1600 ttv) so that the bees were undisturbed by the lighting outside their perceptual range (Skorupski and Chittka, 2010). The arena was covered with a transparent acrylic ceiling at a height of 0.6m. The ceiling allowed lighting from above by 8 neon tubes (52 W Osram + Triconic) and 8 LEDs

(5W GreenLED, light spectrum in Fig. S3; as in Sonntag et al., 2024), and recording via six high-speed cameras with different viewing angles.

A bee crossed two of the small boxes to enter the arena through a hole from below through a plastic tube (2.5cm in diameter). We used these boxes to separate single bees for the recordings while the other bees could forage undisturbed. The entrance to the flight arena was surrounded by red, cylindrical objects (30cm in height and 2cm in diameter), the exact number and positions depended on the test condition.

We used four environments by varying the number and arrangement of objects in the arena: The 1st environment, “three objects environment” was sparse with three objects arranged close to the nest entrance (distance < 0.1m, Fig. 1A) similar to previously tested environments for learning flights (Doussot et al., 2021; Lobecke et al., 2018; Robert et al., 2018). The three other environments varied in number and density of the objects. These dense environments (clutter) covered a circular area around the nest entrance with a diameter of 0.8m. The 2nd environment of our investigation, “full density”, consisted of 40 randomly placed objects (Fig. 1 B, the three nearest objects to the nest were the same as in the “three object” test) that provided a challenge for the bees but it provided enough space for the bees to fly through (Gonsek, et al 2021; Sonntag et al. 2024). The 3rd environment, “half density”, consisted of 20 objects (Fig. 1C, the three nearest objects to the nest were the same as in the “three objects” test) with half the density of the “full density” environment. The 4th environment, “outer ring”, consisted of 30 objects at the border of the circular area around the nest with the same density as in the “full density” environment (Fig. 1D). While the “outer ring” environment tested an increased distance between the nest and the objects, it also yields similarities to Niko Tinbergen’s famous pine cone ring to test visual learning in wasps (Tinbergen, 1932; Tinbergen and Kruyt, 1938).

Throughout the tests, the objects were initially arranged as in the "full density" setup. For the "three objects," "half density," and "outer ring" environments, a subset of this original object arrangement was used. In the “full density” and “half density” environments we challenged the bees with different densities but a similar area that was covered. In contrast, in the “outer ring” environment we tested if the density alone influenced the behaviour or if it is also influenced by the distance of the objects to the nest entrance. The cylindrical arena could be

accessed by the experimenter by a door in the wall to change the objects of the different environmental conditions.

We tested 22 bees once in each of the four environments, resulting overall in 80 different individuals tested. Only one bee at a time was allowed to enter the flight arena. Between the flights, the arena was cleaned with 70% Ethanol to remove potential chemical markings (Cederberg, 1977; Foster and Gamboa, 1989; Chittka et al., 1999; Eckel et al., 2023). Each bee was recorded for 2min after entering the arena. If a bee took longer than 1min to take-off, the bee was captured, released back to the hive and the recording was discarded.

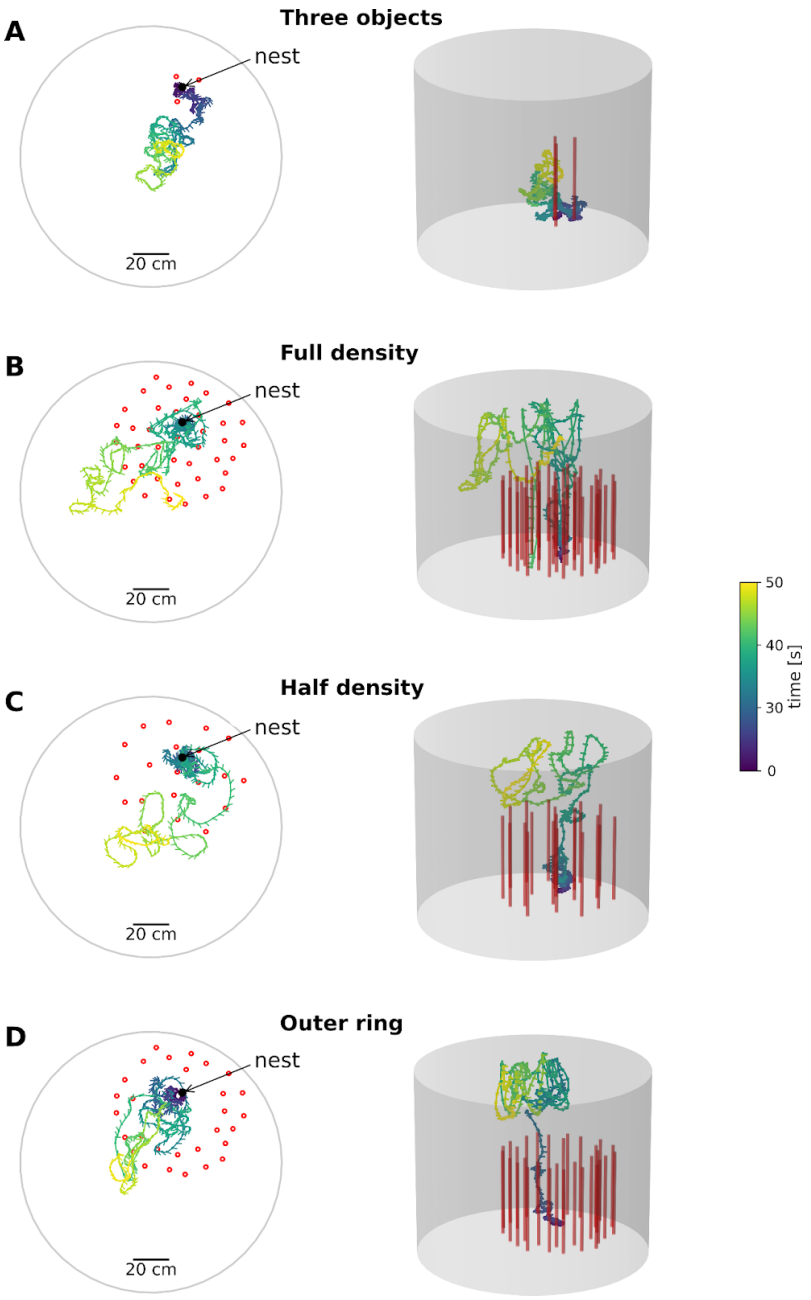

Fig. 1: Exemplary flights in the four environments (the trajectories are colour coded by the time, blue indicating the entry to the area and yellow after 30 seconds of flight after take-off). **A:** *Three objects environment:* three objects surrounding the nest. **B:** *Full density environment*: 40 Objects surrounding the nest. **C:** *Half density environment*: 20 objects surrounding the nest **D:** *Outer ring environment:* A ring of 30 objects surrounding the nest with the same density as with 40 objects. The objects are indicated by red circles in the 2D plots (left column) and red cylinders in the 3D plots (right column). The nest indicated by an arrow is the nest entrance to the flight arena.

3D flight trajectories

The bee trajectories in the arena were recorded at a frequency of 62.5 Hz (16 ms between consecutive frames) using six synchronised Basler cameras (Basler acA 2040um-NIR) positioned at different angles, similar to previous studies (Odenthal et al., 2020; Doussot et al., 2021; Sonntag et al., 2024). One camera was mounted above the centre of the arena to capture the bees' planar movements. Another one was positioned above the centre of the cluttered area around the nest, while the remaining four cameras were arranged around the arena to record the bees' positions from different perspectives, reducing triangulation errors in 3D positioning. Recording commenced prior to the bees' entry into the setup, and the initial 60 frames were utilised to generate a background image of the arena. During a recording, only image sections (40x40 pixels) exhibiting significant differences from the background image (potentially indicating the presence of a bee) were saved to the hard drive along with their coordinates. The recording scripts were written in C++. A custom neural network analysed the image sections to determine whether they contained bees. Any crops showing non-biological speeds (above 4 m/s (Goulson, 2010)) or implausible positions (outside the arena) were manually reviewed. The trajectories were analysed using Python (version 3.8.17), primarily with OpenCV. DeepLabCut (Nath et al., 2019) was used to identify the head and abdomen positions to determine the orientation of the bee's body-length axis. A comprehensive list of the packages used is available in the data publication (https://gitlab.ub.uni-bielefeld.de/a.sonntag/bumblebeelearningflights3d).

Statistical analysis for hypothesis testing

To investigate how the environment influenced the structure of the learning flights of bees, we calculated the ratio between the 2D distance of the bee to the nest (x-y plane) and her altitude. We hypothesised that in denser environments, bees may increase their horizontal distance from the nest while maintaining a low flight altitude to enter dense areas similar to their homing behaviour (Sonntag et al., 2024). Alternatively, in denser environments, bees may increase their flight altitude while keeping close to the nest, potentially to gain a better overview of the environment as homing models based on visual memories suggest (Sonntag et al., 2024). We calculated the mean ratio for 10s after the first take-off (at least 0.01m above the ground) and excluded positions where the bees were walking (z <0.01m). Since we wanted to investigate the initial period of the altitude increase before individual bees might start to descend or hit the ceiling, we chose a time window of 10s during which the majority of bees performed their typical altitude increase (Fig. S1). For a statistical comparison of the altitude distance ratios we used the Kurskal Wallis test and the post-hoc Dunn test because the data was not normally distributed.

We hypothesised that the orientation of the bees' body and their flight direction will be influenced by the nest surrounding object constellations. Furthermore, the body orientation and flight direction could be adapted differently at different altitudes if they were influenced by the visual clearance or occlusion of the objects. Therefore, we looked at the yaw angle of the bees relative to the nest during the first 30 seconds after the first take-off (at least 0.01m above the ground) separated into five altitudes: low altitude within clutter, z < 0.15m; intermediate altitude within clutter, 0.15m =< z < 0.3m; high altitude above clutter, z >= 0.3; low altitude in the arena, z < 0.15m; high altitude in the arena, z >= 0.3m). We used the area of the clutter for all conditions to compare similar regions between all conditions.

Then we calculated the fixations of the nest for the full duration of the recorded learning flight (we only excluded positions 5cm below the ceiling to remove possible positions where the bees collided with the ceiling) similar to Robert et al (2017). The nest fixations are defined as the times the nest position was stationary on the retina (Robert et al., 2018). Frames with a yaw angle of +- 12.5 deg relative to the nest entrance were taken as one sample. The following frames were added to this sample if the absolute angle difference was smaller than 2.4°deg (150 deg /second as in Robert et al, 2017). One sample was only valid if it contained at least 10 consecutive frames (160ms, twice as in Robert et al (2017) to exclude false detections of nest

fixations). These fixations were separated into the same five altitude sections (low altitude within clutter, intermediate altitude within clutter, high altitude above clutter, low altitude in the arena, high altitude in the arena). These altitude sections of the nest fixations were compared with a two-way Anova within each condition and between conditions. In addition, we used the Tukey HSD post-hoc test for pairwise comparisons of each altitude section. In addition we calculated the elevation angle between the bees body position and the nest position in the arena to check for the feasibility of the nest fixations.

For all of these analyses python 3.8 and the packages statsmodels, bioinfokit and scipy were used.

Results

Bees increase flight altitude in dense environments

During learning flights, bees increase their altitude and distance to the nest (Capaldi et al., 2000; Lobecke et al., 2018; Osborne et al., 2013; Woodgate et al., 2016). We investigated how these flight characteristics are influenced by different constellations of objects. The initial part of the first learning flight was compared between the four environmental conditions (Fig. 2). When the nest entrance was surrounded by only three objects, an environment similar to other studies (Cartwright and Collett, 1983; Dittmar et al., 2010; Linander et al., 2018; Lobecke et al., 2018; Robert et al., 2018), we found a median altitude distance ratio of 0.61 (SD = 0.35) indicating that the bees increased more their 2D distance to the nest entrance than their altitude. In contrast, in the full density environment we find a median altitude distance ratio of 1.73, indicating a larger increase in altitude than in 2D distance, and a larger variance (SD = 1.2). To determine whether the density of the objects, and thus the distance between them reflecting the degree of challenge of the environment for flight control, was responsible for this effect, we reduced the object density to half of the number of objects in the same area. In the half density environment, we found a median altitude distance ratio of 1.15 indicating an increase of ratio between altitude and distance between the full density environment and the environment with three objects. The variance with the half density environment (SD = 0.8) was also in between that obtained for the full density environment and the three object environment. Lastly, we tested how the distance of the objects to the nest influences the altitude and distance increase. The outer ring environment resulted in intermediate values of

the median ratio (M = 1.16) and the variance (SD = 0.51) compared to the three objects and the full density environments. Statistically, the altitude distance ratio differed between the "three objects" environment and all others (Kruskal Wallis test: H = 24.277, $p < 0.001$; Dunn's post hoc test: $p_{\text{full density-three objects}} < 0.001$, $p_{\text{half density - three objects}} = 0.006$, $p_{\text{outer ring - -three objects}} = 0.043$).

These results show that the flight structure of the bees' first learning flight depends on the environmental features around the nest as bees increase their altitude with increasing number of objects.

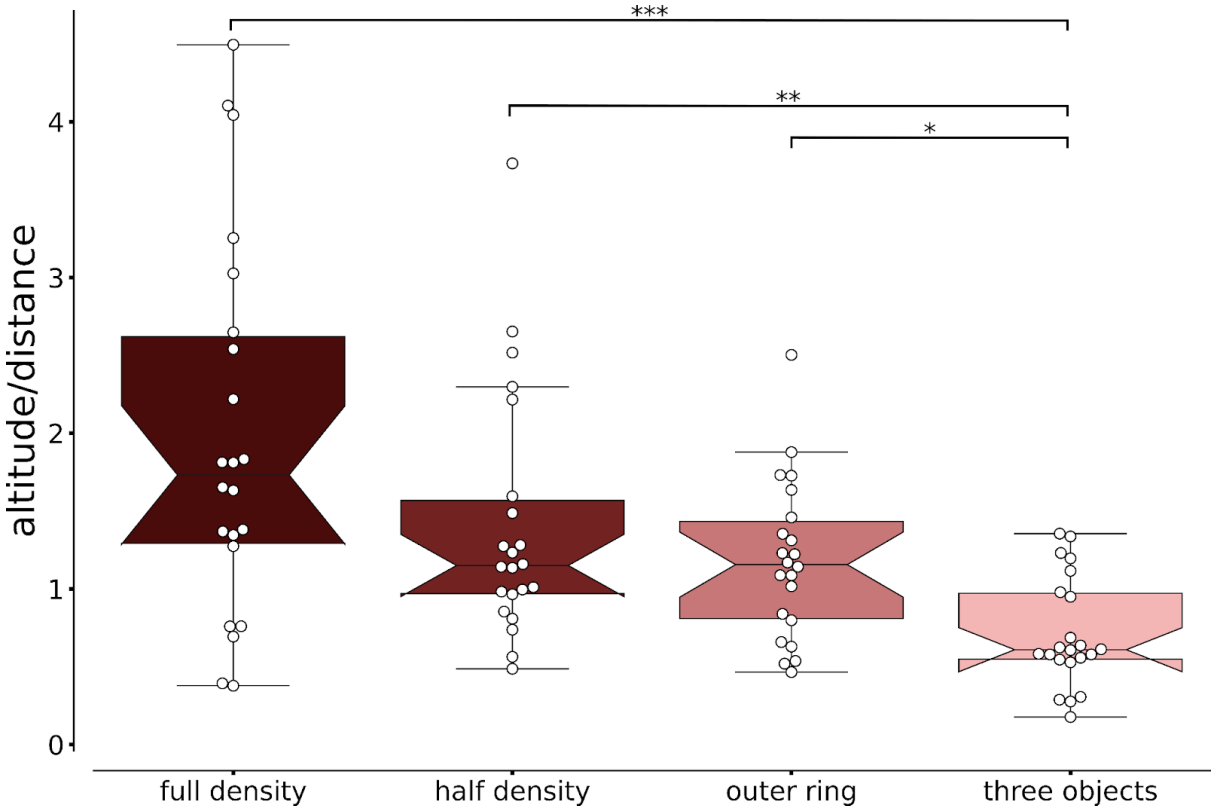

Fig 2: Altitude and distance ratio for the initial part of the learning flights for the four environmental conditions: full density, half density, outer ring, three objects. The altitude distance ratio for each bee is shown as white circles.he hatched boxplots display the median and the whiskers show the lower and upper range of 1.5 times the interquartiles. The star code shows different levels of significance following the Dunn post hoc test. The ratios decreased with decreasing number of objects and differed statistically between the three object environment and the other environments (Kruskal Wallis test: H = 24.277, $p < 0.001$;

Dunn's post hoc test: $p_{\text{full density-three objects}} < 0.001$, $p_{\text{half density - three objects}} = 0.006$, $p_{\text{outer ring - -three objects}} = 0.043$).

Dense environments disrupt nest orientation

During their first outbound flights, bees regularly turn back and look toward the nest entrance (Lehrer, 1991, 1993). Therefore we analysed the yaw angle of the bee's body relative to the nest entrance. The orientations were separated into five categories in regard to their altitude and 2D distance to the nest.

With three objects, the bees oriented most towards the nest in the area of the clutter, irrespective of their flight altitude. Outside this clutter area, the bees oriented mostly away from the nest, probably exploring the environment. In the full density environment the bees did not show an overall clear orientation towards the nest entrance. In the category "low altitude arena" the bees tended to look more away from the nest. In the half density environment, the bees' orientations were similarly distributed as in the full density environment, i.e. the bees oriented similarly in different directions, within the clutter, or face more away from the nest, above the clutter and outside it (Fig. 3). In the outer ring environment the bees faced mostly towards the nest entrance within the clutter at the upper height of the objects. Outside the area of the clutter they faced more away from the nest (SI Fig. S2).

Statistically, we found that all distributions were not uniformly distributed except the area "below outside" in the half density environment (Tab. S1).

Thus, taken together, our results show that the bees oriented themselves towards the nest hole during their learning flights in sparse environments but in dense ones it was less dominant.

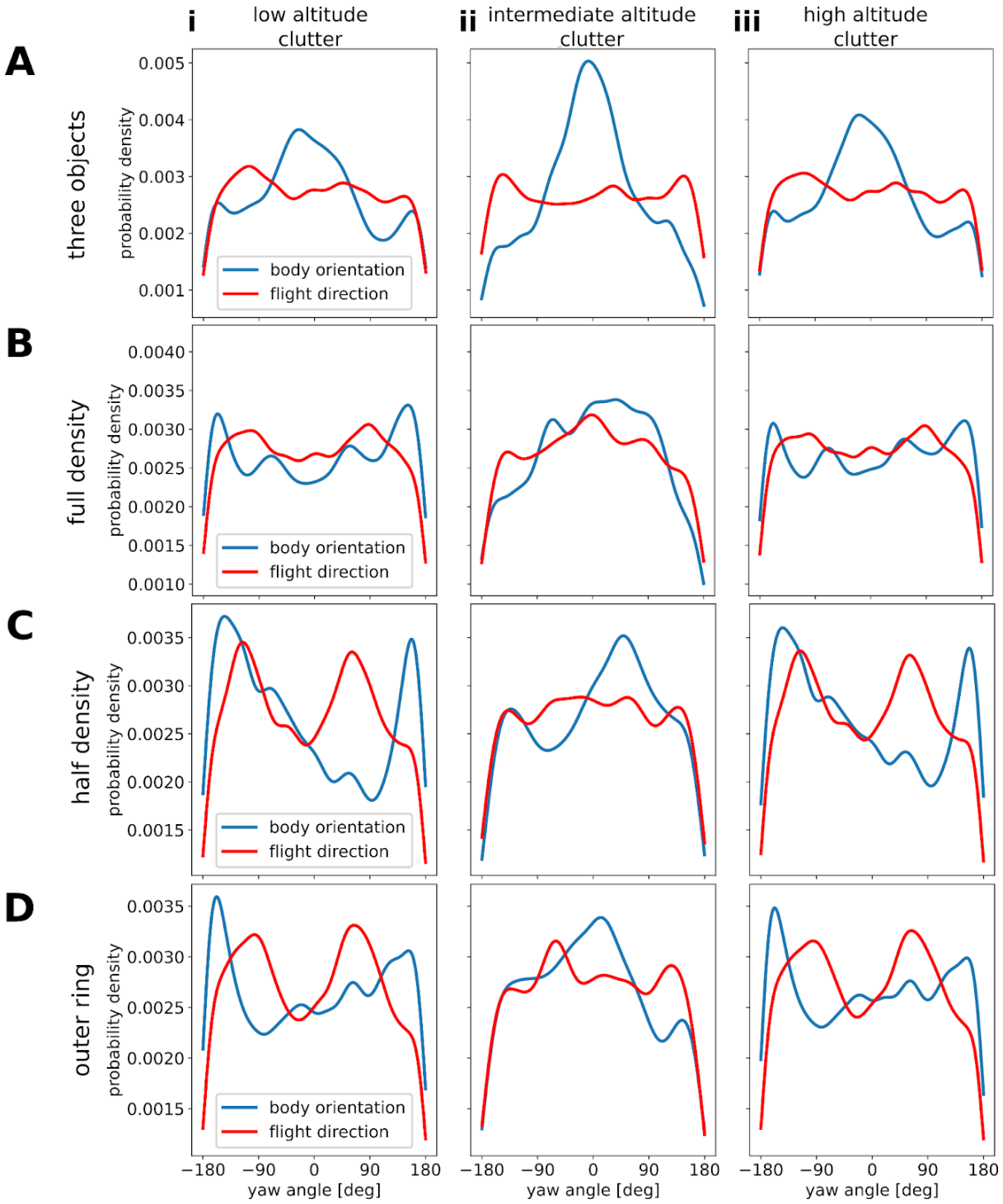

Fig. 3: Kernel density estimation distributions of the body orientation (blue line) of the bees along the yaw angle and their flight direction (red line) relative to the nest (0 deg) for the four environments (A, i: N (number of bees) = 22, n (number of data points) = 31952; ii: N = 22, n = 8236; iii: N = 22, n = 40188), full density (B, i: N = 22, n = 32803, ii: N = 22, n = 4724; iii: N = 22, n = 37527), half density (C, i: N = 22, n = 41532; ii: N = 22, n = 4703; iii: N = 22, n = 46235) and the outer ring (D, i: N = 22, n = 40678; ii: N = 22, n = 4963, iii: N = 22, n = 45641). For each

environment the body orientation and flight direction are shown for different layers in the area of the objects' arrangement (clutter) (**i** low, **ii** intermediate and **iii** above the objects).

Bees fixate of the nest mostly above the objects

To examine how the positions where the bees fixated the nest were influenced by their environment we separated the bees' nest entrance fixations (for a definition, see Methods) in spatial categories, like we did for the body orientations.

In the three objects' environment, similar amounts of nest fixations appeared in the area of the clutter, both within and above (Fig. 4A). In the area outside the clutter, less nest fixations occurred. In the full density environment, the bees mostly fixated the nest when they were flying above the clutter. This is followed by the positions in "low altitude clutter" and "high altitude arena". The least nest fixations occurred in the area outside the clutter and above object height. In the half density and outer ring environments, the nest fixations were distributed similarly as in the full density environment. We saw most nest fixations within the clutter area, most fixations were found above the clutter or at the bottom of the object heights. In the "intermediate altitude clutter" area and outside the clutter, the bees looked similarly often towards the nest.

Overall, we found a significant difference between the spatial categories of nest fixation (two-factor ANOVA, $F(4) = 119.2$, $p < 0.001$) but not between the tested environments (two-factor ANOVA, $F(3) = 0.165$, $p = 0.932$). The interaction of the spatial categories and the environments was significant ($F(12) = 5.098$, $p < 0.001$). We found that the bees fixate the nest more often above the clutter than within the clutter or outside it (Tukey HSD post hoc test, for all comparisons $p < 0.001$). We also found more fixations in the area of the clutter, within or above, than outside it (Tukey HSD post hoc test, "low altitude clutter" - "out below" $p < 0.001$, "clutter above" - "out above" < 0.001, "clutter above" - "out below" $= 0.007$). However, we found only a trend that the bees fixated the nest more in the area "intermediate altitude clutter" and than in the area "high altitude arena" (Tukey HSD post hoc test, $p = 0.088$). Also the nest fixations did not differ statistically between the areas outside the clutter, above or below the height of the objects (Tukey HSD post hoc test, $p = 0.584$). The environmental conditions did not statistically influence the fixation proportion in the different spatial areas but we found an influence between the spatial categories and the environmental conditions.

Since we could not resolve the position and orientation of the bees' head, we used the elevation of the bees' position relative to the nest location in the arena as a proxy for the elevation angle of the nest within the visual field of the bees. An angle of zero degree means that the body axis of the bees was horizontally aligned with the height of the nest, thus directly facing the nest. Decreasing elevation angles mean that the nest appeared lower within the bee's field of view. Most of the elevation angles of the nest within the field of view of the bees ranged from 0 to -60 degrees while some outliers are found < -60 degrees, especially for intermediate and high altitude levels. Thus all in all, the bees fixated the nest mostly at higher altitudes, in close proximity to the nest while the fixation pattern was influenced by the combination of the spatial areas and the features of the environments.

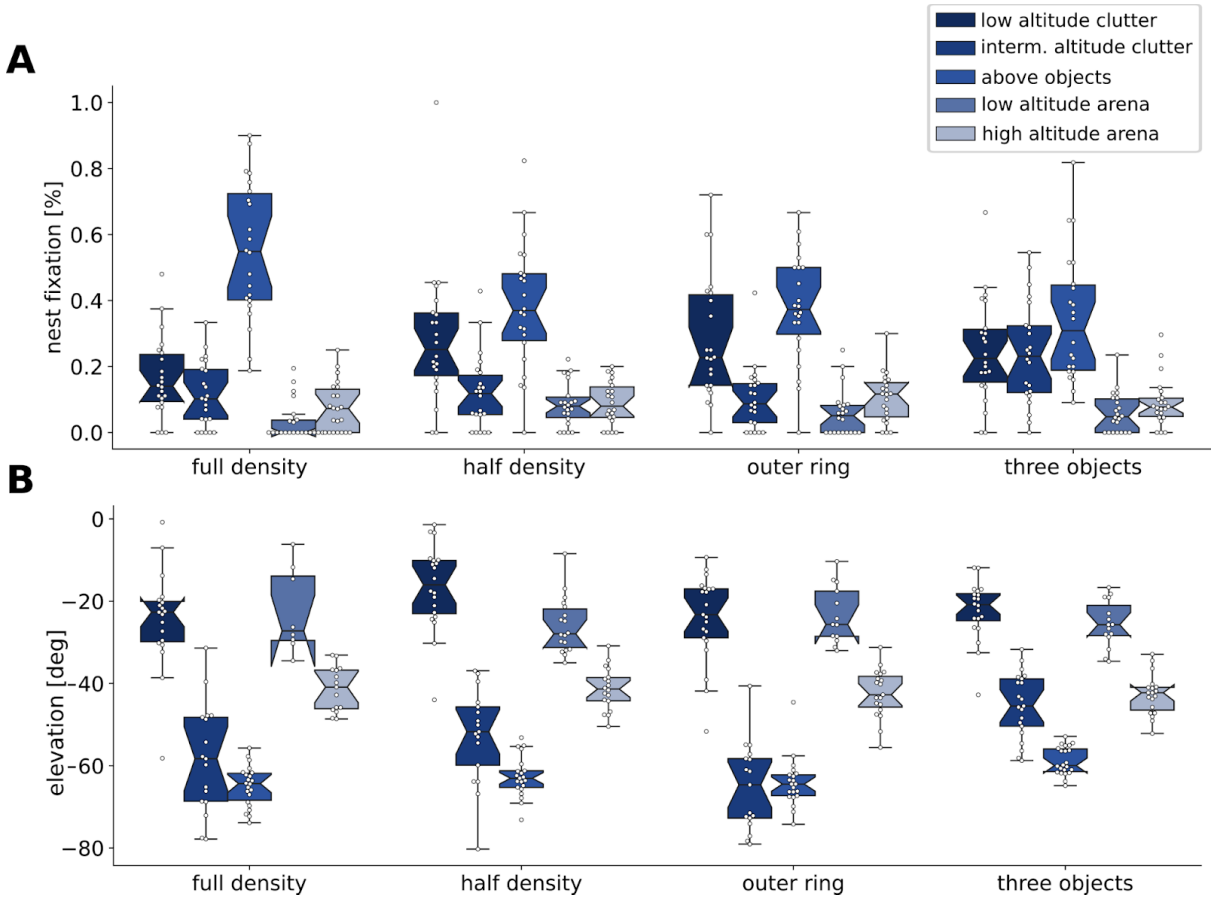

Fig. 4: Nest fixations (**A**) and the elevation angle of these fixations (**B**) for five spatial areas (low altitude clutter, intermediate altitude clutter, high altitude clutter, low altitude arena, high altitude arena) in the four environments. Individual data are shown as white circles, hatched

boxplots display the median and the whiskers show the lower and upper range of 1.5 times the interquartiles. N = 22 bees per environment.

Discussion

Insect learning flights have been analysed so far mainly in sparse environments (e.g. Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Robert et al., 2018; Doussot et al., 2021) which do not represent realistic habitats of bumblebee nests (O'connor et al., 2017; Liczner and Colla, 2019). By manipulating object constellations around a nest entrance hole, we found that bees change their flight pattern in response to environmental challenges, prioritising altitude gain in cluttered environments and modifying their orientation patterns to balance nest-directed learning with obstacle avoidance. We observed a shift towards greater altitude gain in cluttered environments, and more diverse orientation patterns in more dense environments compared to sparse ones. Bees showed a consistent preference for nest entrance fixations from elevated positions above the object arrangements.

Bees increase flight altitude in dense environments

Our study revealed significant differences in how bees adjust their altitude and distance from the nest depending on the environmental complexity. In a sparse environment with only three objects, bees increased their 2D distance to the nest more than their altitude. However, in dense environments with more objects, they prioritised gaining altitude over 2D distance. This demonstrates that bees adapt their flight strategies based on environmental constraints. In cluttered environments, gaining altitude may provide better view points for visual learning from above. Indeed, high-altitude views offer a broad perspective of the landscape, which bees have been shown to use to identify ground-level landmarks and navigate over long distances (Collett and Graham, 2015; Degen et al., 2016; Menzel et al., 2019; Brebner et al., 2021). Flying above the clutter may also be a means to reduce the necessity of obstacle avoidance. The increase in flight altitude in our study might therefore also be influenced by the change in ventral optic flow due to the changes in the environmental features. Previously, it was found that bees adapt their altitude in response to ventral spatial textures (Linander et al., 2018). The higher variance in altitude-distance ratios in complex environments compared to the sparse environment might point towards more diverse individual strategies when navigating challenging landscapes.

Dense environments disrupt bees' nest orientation

We also found that the patterns of body orientation varied significantly across the different environmental conditions. In the three objects' environment, bees showed a clear preference for orienting towards the nest within the object area, regardless of altitude. This aligns with previous findings in simple environments (e.g. Collett et al., 2023, 2013; Hempel de Ibarra et al., 2009; Lobecke et al., 2018; Philippides et al., 2013). However, in cluttered environments, the orientation patterns became more complex. Bees showed less pronounced nest-directed orientations, particularly in the full and half density environments. This suggests that in complex environments, with many objects and visual occlusions, bees may need to balance nest-oriented learning with obstacle avoidance and broader environmental scanning. The environment containing an outer ring of objects, produced an interesting intermediate pattern, with strong nest-oriented behaviour at the upper height of the objects within the clutter area. This might indicate a strategy of using the ring of objects as a reference frame for nest location.

Bees fixate the nest mostly above the objects

Across all our environmental conditions, bees showed a preference for fixating on the location of the nest entrance when flying above the clutter, even though the nest entrance has been hidden and its location may have been occluded by the clutter. This finding is particularly interesting as it suggests that bees use elevated positions to gain clearer views of the nest and its surroundings, potentially creating more reliable visual memories. Fixations at high altitudes might raise the question of where the nest position was in the field of view of the bee. A modelling study on honeybee vision indicated that the resolution of a bee's eye decreases when observing at wide angles, especially as the vertical angles between ommatidia become smaller (Stürzl et al., 2010). In addition, a morphological study on bumblebees showed the limitations of the perception in the ventral direction (Taylor et al., 2019). Therefore, observations of nest fixations at very low elevation angles should be interpreted with caution. For the categories of low altitude within the dense environment and the areas outside it, the elevation angles lay well in the perception range. For the intermediate altitude within the dense environment and above the objects the nest fixations need to be taken with caution as they are rather at the perceivable edge. Some fixations at high altitudes might be at the border of perception of the visual field of bumblebees (Taylor et al., 2019) and

need further evaluation. Since we only investigated the yaw angle of the body orientation of the bees, further investigations that include the tracking of the head position and orientation in 3D are needed (e.g. Doussot et al., 2021; Hateren and Schilstra, 1999). In the dense environments we tested, occlusions of the nest position by objects have been very likely. It is therefore interesting to note that we still found fixations of the nest. The consistent pattern of more fixations within or above the clutter area compared to outside implies that bees focus their learning efforts in the immediate vicinity of the nest, even in such complex environments with a forest of objects.

Implications and future directions

Our findings demonstrate that bumblebees possess remarkable flexibility in their navigation strategies: they alter their flight patterns and orientations according to the environmental features while the fixation of the nest entrance depends on the combination of altitude and object density. The preference for nest fixations from elevated positions, especially in cluttered environments, suggests that bees adapt their flight altitude for acquiring visual memories for their returns depending on the environmental features. This adds a new dimension to our understanding of insect navigation, which has for long studied movements only in two dimensions (Buehlmann et al., 2020; Zeil, 2022). The varied orientation patterns in cluttered environments indicate a dynamic balance between exploring new areas and maintaining familiarity with the nest location. This balance may be crucial for efficient foraging in complex natural habitats with dense clutter. Our study focused only on the initial learning flight, thus future research could examine how these patterns change over multiple outbound trips. In a previous study (Sonntag et al., 2024), investigating the homing abilities of bumblebees in a dense environment, we found that snapshot-models of local homing (Dittmar et al., 2010; Doussot et al., 2020; Zeil, 2022) suggest that snapshot views taken at high altitudes, i.e. above the surrounding objects yield better performances than snapshot views taken at lower altitudes. Our analyses of the learning flights in the current study are consistent with this modelling result with the observed increase in height as opposed to distance in dense environments and also nest fixations at heights above the objects. Interestingly, behavioural experiments on the return flights to the nest entrance have shown that the bees do not need views at high altitude above the objects, in order to successfully return to their nest (Sonntag et al., 2024). One possible explanation is that low altitude flights are more direct and efficient to return to the nest location. The bees might decrease their flight altitude

during consecutive learning flights to learn low altitude views around the nest close to the ground, thus gradually switching memories. While we have investigated here only the first learning flight of naive bees, future studies investigating consecutive learning flights within dense environments are needed to understand the development of learning within complex surroundings. Investigating the energetic costs of different flight strategies in various environments could provide insights into the efficiency of these adaptive behaviours. Our analysis of bumblebee learning flights highlights the importance of studying navigational behaviours of flying insects in 3D and in more diverse settings.

Acknowledgements

We would like to thank Yigit Yargili and Melissa Vera Finke for their help during the data collection.

Competing interests

The authors declare no competing or financial interests.

Funding

We acknowledge the financial support of the 3DNaviBee project by the collaborative funding of the German Research Foundation (DFG) and the French National Research Agency (ANR) (reference code: EG 82/22-1).

Data and resource availability

The data-sets and analysis pipeline for this study can be found in the repository ”BumblebeeLearningFlights3D” (https://gitlab.ub.uni-bielefeld.de/a.sonntag/bumblebeelearningflights3d).

References

Bertrand, O., JN, Sonntag, A., 2023. The potential underlying mechanisms during learning flights. J. Comp. Physiol. A. https://doi.org10.1007/s00359-023-01637-7

Brebner, J.S., Makinson, J.C., Bates, O.K., Rossi, N., Lim, K.S., Dubois, T., Gómez-Moracho, T., Lihoreau, M., Chittka, L., Woodgate, J.L., 2021. Bumble bees strategically use ground

level linear features in navigation. Anim. Behav. 179, 147–160. https://doi.org/10.1016/j.anbehav.2021.07.003

Buehlmann, C., Mangan, M., Graham, P., 2020. Multimodal interactions in insect navigation 23, 1129–1141. https://doi.org/10.1007/s10071-020-01383-2

Capaldi, E.A., Smith, A.D., Osborne, J.L., Farris, S.M., Reynolds, D.R., Edwards, A.S., Martin, A., Robinson, G.E., Poppy, G.M., Riley, J.R., 2000. Ontogeny of orientation flight in the honeybee revealed by harmonic radar 403. https://doi.org/10.1038/35000564

Cartwright, B.A., Collett, T.S., 1983. Landmark learning in bees. J. Comp. Physiol. 151, 521–543. https://doi.org/10.1007/BF00605469

Cederberg, B., 1977. Evidence for trail marking in Bombus terrestris workers (Hymenoptera, Apidae). Zoon 5, 143–146.

Chittka, L., Williams, N.M., Rasmussen, H., Thomson, J.D., 1999. Navigation without vision: bumblebee orientation in complete darkness. Proc. R. Soc. Lond. B Biol. Sci. 266, 45–50. https://doi.org/10.1098/rspb.1999.0602

Collett, T.S., De Ibarra, N.H., Riabinina, O., Philippides, A., 2013. Coordinating compass-based and nest-based flight directions during bumblebee learning and return flights. J. Exp. Biol. 216, 1105–1113. https://doi.org/10.1242/jeb.081463

Collett, T.S., Graham, P., 2015. Insect navigation: Do honeybees learn to follow highways? Curr. Biol. 25, R240–R242. https://doi.org/10.1016/j.cub.2014.11.003

Collett, T.S., Hempel de Ibarra, N., 2023. An 'instinct for learning': the learning flights and walks of bees, wasps and ants from the 1850s to now. J. Exp. Biol. 226, jeb245278. https://doi.org/10.1242/jeb.245278

Collett, T.S., Robert, T., Frasnelli, E., Philippides, A., Hempel De Ibarra, N., 2023. How bumblebees coordinate path integration and body orientation at the start of their first learning flight. J. Exp. Biol. 226, jeb245271. https://doi.org/10.1242/jeb.245271

Collett, T.S., Zeil, J., 2018. Insect learning flights and walks. Curr. Biol. 28, R984–R988. https://doi.org/10.1016/j.cub.2018.04.050

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblofsky, M., Winter, S., Georgieva, P.B., Nguyen, H., Chamkhi, H., Meyer, H., Singh, P.K., Manz, G., Greggers, U., Menzel, R., 2016. Honeybees Learn Landscape Features during Exploratory Orientation Flights. Curr. Biol. 26, 2800–2804. https://doi.org/10.1016/j.cub.2016.08.013

Dittmar, L., Stürzl, W., Baird, E., Boeddeker, N., Egelhaaf, M., 2010. Goal seeking in honeybees: Matching of optic flow snapshots? J. Exp. Biol. 213, 2913–2923. https://doi.org/10.1242/jeb.043737

Doussot, C., Bertrand, O.J.N., Egelhaaf, M., 2021. The Critical Role of Head Movements for Spatial Representation During Bumblebees Learning Flight. Front. Behav. Neurosci. 14, 606590. https://doi.org/10.3389/fnbeh.2020.606590

Doussot, C., Bertrand, O.J.N., Egelhaaf, M., 2020. Visually guided homing of bumblebees in ambiguous situations: A behavioural and modelling study. PLoS Comput. Biol. 16. https://doi.org/10.1371/journal.pcbi.1008272

Eckel, S., Egelhaaf, M., Doussot, C., 2023. Nest-associated scent marks help bumblebees localizing their nest in visually ambiguous situations. Front. Behav. Neurosci. 17.

Foster, R.L., Gamboa, G.J., 1989. Nest Entrance Marking with Colony Specific Odors by the Bumble Bee Bombus occidentalis (Hymenoptera: Apidae). Ethology 81, 273–278. https://doi.org/10.1111/j.1439-0310.1989.tb00773.x

Goulson, D., 2010. Bumblebees : behaviour, ecology, and conservation, Oxford biology. Oxford University Press.

Hateren, J.H.V., Schilstra, C., 1999. Blowfly flight and optic flow: II. Head movements during flight. J. Exp. Biol. 202, 1491–1500. https://doi.org/10.1242/jeb.202.11.1491

Hempel de Ibarra, N., Philippides, A., Riabinina, O., Collett, T.S., 2009. Preferred viewing

directions of bumblebees (Bombus terrestrisL.) when learning and approaching their nest site. J. Exp. Biol. 212, 3193–3204. https://doi.org/10.1242/jeb.029751

Lehrer, M., 1993. Why do bees turn back and look? J. Comp. Physiol. A 172, 549–563. https://doi.org/10.1007/BF00213678

Lehrer, M., 1991. Bees which turn back and look. Naturwissenschaften 78, 274–276. https://doi.org/10.1007/BF01134357

Liczner, A.R., Colla, S.R., 2019. A systematic review of the nesting and overwintering habitat of bumble bees globally. J. Insect Conserv. 23, 787–801. https://doi.org/10.1007/s10841-019-00173-7

Linander, N., Dacke, M., Baird, E., Hempel de Ibarra, N., 2018. The role of spatial texture in visual control of bumblebee learning flights. J. Comp. Physiol. A 204, 737–745. https://doi.org/10.1007/s00359-018-1274-0

Lobecke, A., Kern, R., Egelhaaf, M., 2018. Taking a goal-centred dynamic snapshot as a possibility for local homing in initially naïve bumblebees. J. Exp. Biol. 221, jeb168674. https://doi.org/10.1242/jeb.168674

Menzel, R., Tison, L., Fischer-Nakai, J., Cheeseman, J., Balbuena, M.S., Chen, X., Landgraf, T., Petrasch, J., Polster, J., Greggers, U., 2019. Guidance of navigating honeybees by learned elongated ground structures. Front. Behav. Neurosci. 12. https://doi.org/10.3389/fnbeh.2018.00322

Nath, T., Mathis, A., Chen, A.C., Patel, A., Bethge, M., Mathis, M.W., 2019. Using DeepLabCut for 3D markerless pose estimation across species and behaviors. Nat. Protoc. 14, 2152–2176. https://doi.org/10.1038/s41596-019-0176-0

O'connor, S., Park, K.J., Goulson, D., 2017. Location of bumblebee nests is predicted by counts of nest-searching queens. Ecol. Entomol. 42, 731–736. https://doi.org/10.1111/een.12440

Odenthal, L., Doussot, C., Meyer, S., Bertrand, O.J.N., 2020. Analysing Head-Thorax Choreography During Free-Flights in Bumblebees. Front. Behav. Neurosci. 14, 610029. https://doi.org/10.3389/fnbeh.2020.610029

Osborne, J.L., Smith, A., Clark, S.J., Reynolds, D.R., Barron, M.C., Lim, K.S., Reynolds, A.M., 2013. The ontogeny of bumblebee flight trajectories: From Naïve explorers to experienced foragers. PLoS ONE 8. https://doi.org/10.1371/journal.pone.0078681

Philippides, A., de Ibarra, N.H., Riabinina, O., Collett, T.S., 2013. Bumblebee calligraphy: the design and control of flight motifs in the learning and return flights of Bombus terrestris. J. Exp. Biol. 216, 1093–1104. https://doi.org/10.1242/jeb.081455

Robert, T., Frasnelli, E., Hempel de Ibarra, N., Collett, T.S., 2018. Variations on a theme: bumblebee learning flights from the nest and from flowers. J. Exp. Biol. 221, jeb172601. https://doi.org/10.1242/jeb.172601

Schwegmann, A., Lindemann, J.P., Egelhaaf, M., 2014. Temporal Statistics of Natural Image Sequences Generated by Movements with Insect Flight Characteristics. PLoS ONE 9, e110386. https://doi.org/10.1371/journal.pone.0110386

Skorupski, P., Chittka, L., 2010. Photoreceptor Spectral Sensitivity in the Bumblebee, Bombus impatiens (Hymenoptera: Apidae). PLoS ONE 5, e12049. https://doi.org/10.1371/journal.pone.0012049

Sonntag, A., Sauzet, O., Lihoreau, M., Egelhaaf, M., Bertrand, O., 2024. Switching perspective: Comparing ground-level and bird's-eye views for bees navigating clutter. eLife 13. https://doi.org/10.7554/eLife.99140.1

Taylor, G.J., Tichit, P., Schmidt, M.D., Bodey, A.J., Rau, C., Baird, E., 2019. Bumblebee visual allometry results in locally improved resolution and globally improved sensitivity. eLife 8, e40613. https://doi.org/10.7554/eLife.40613

Tinbergen, N., 1932. Über die Orientierung des Bienenwolfes (Philanthus triangulum Fabr.). Z. Für Vgl. Physiol. 16, 305–334. https://doi.org/10.1007/BF00338750

Tinbergen, N., Kruyt, W., 1938. Über die Orientierung des Bienenwolfes (Philanthus triangulum Fabr.). Z. Für Vgl. Physiol. 25, 292–334. https://doi.org/10.1007/BF00339640

Woodgate, J.L., Makinson, J.C., Lim, K.S., Reynolds, A.M., Chittka, L., 2016. Life-long radar tracking of bumblebees. PLoS ONE 11. https://doi.org/10.1371/journal.pone.0160333

Zeil, J., 2022. Visual navigation: properties, acquisition and use of views. J. Comp. Physiol. A. https://doi.org/10.1007/s00359-022-01599-2

Supplementary Material

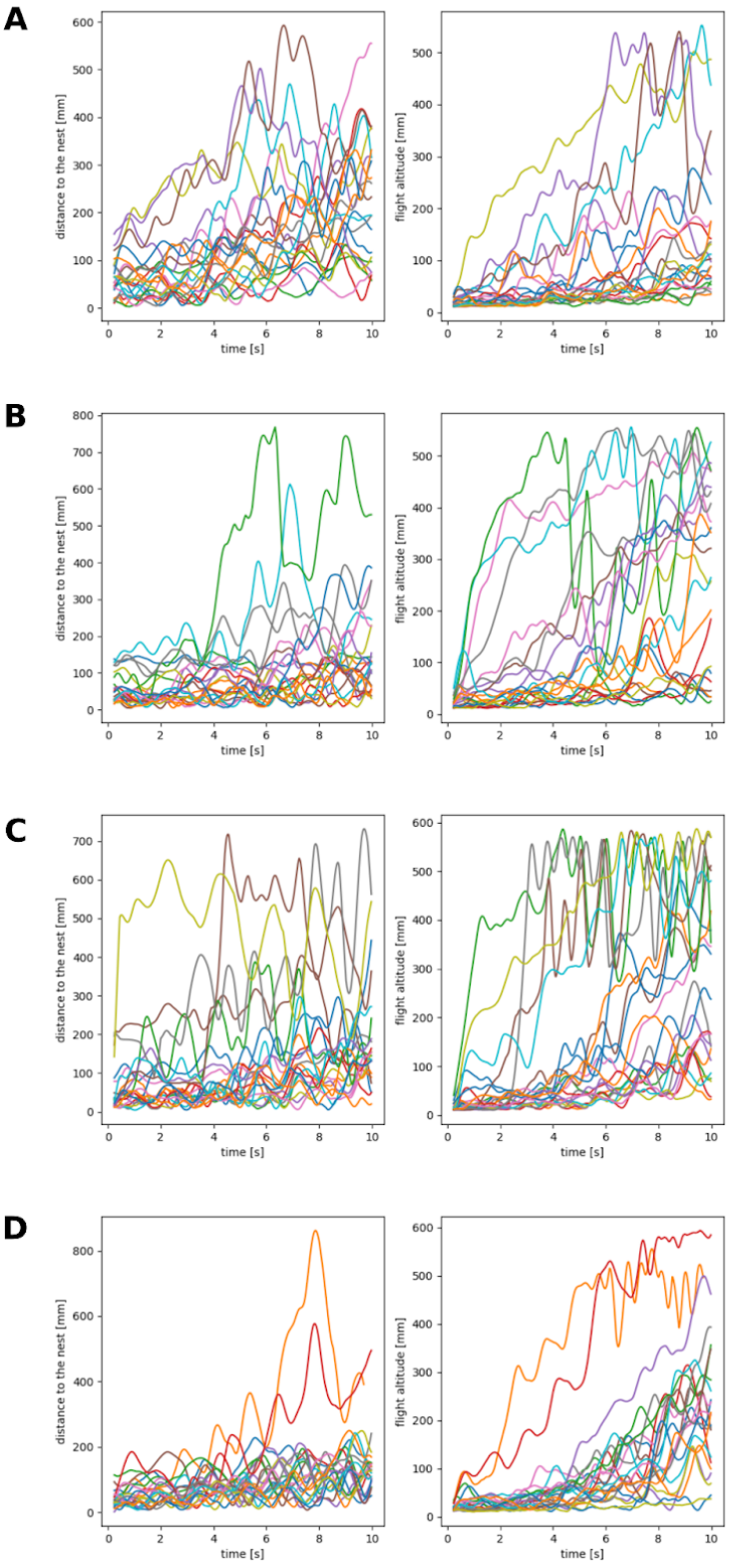

Fig. S1: Distance to the nest and height for each learning flight (z > 0.01m) over time within the time window of 10 seconds after take-off for the four test conditions (A: three objects, B: full density, C: half density, D: outer ring).

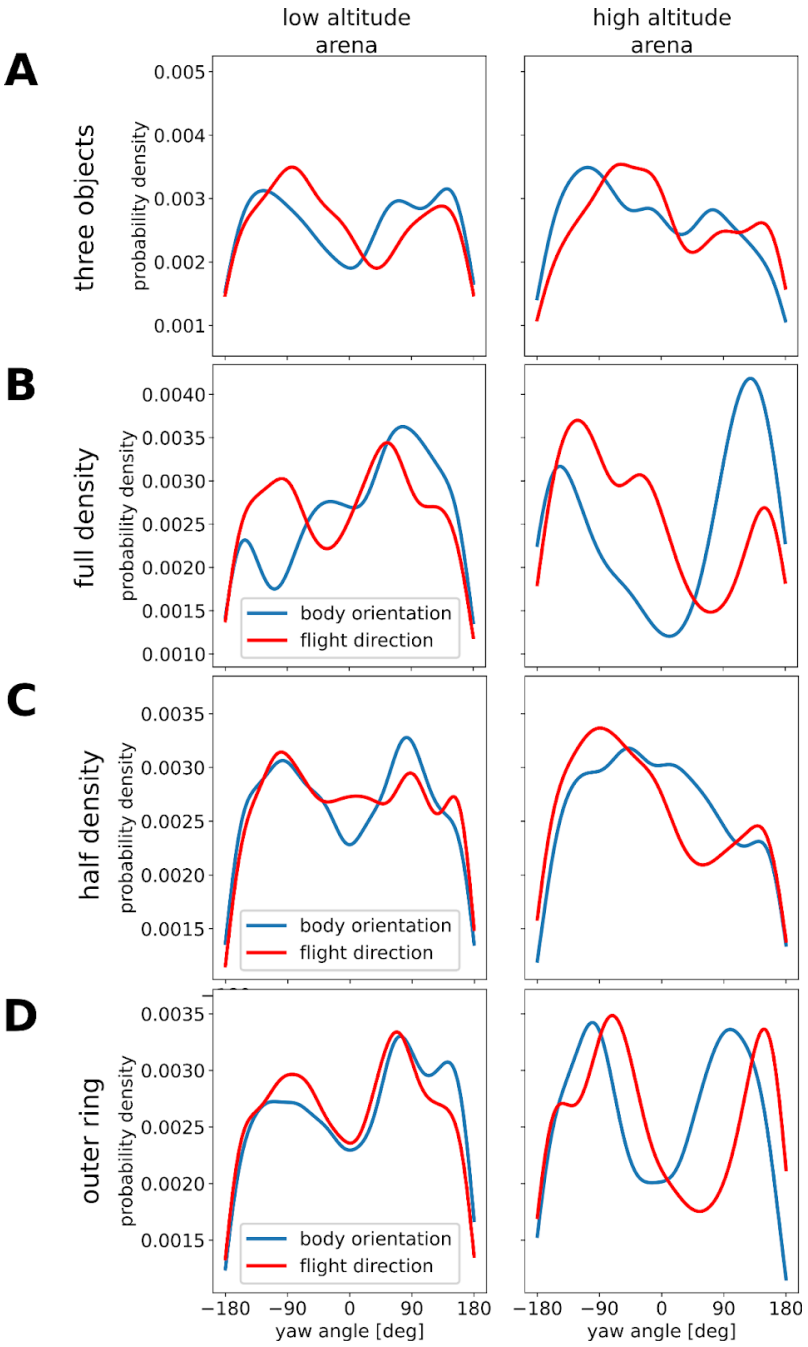

Fig S2: Kernel density estimation distributions of the body orientation (blue line) of the bees along the yaw angle and their flight direction (red line) relative to the nest (0 deg) for the three objects (A, i N = 16, n = 3415; ii N = 16, n = 2335), full density (B, i N = 14, n = 4271; ii N = 9, n =

1742), half density (C, i N = 20, n = 7780; ii N = 16, n =1836) and the outer ring (D, i N = 21, n = 6735; ii N = 12, n = 2553) environments. For each environment the body orientation and flight direction are shown for different layers in the area outside the objects' arrangement (low and high altitude, below and above the height of the objects).

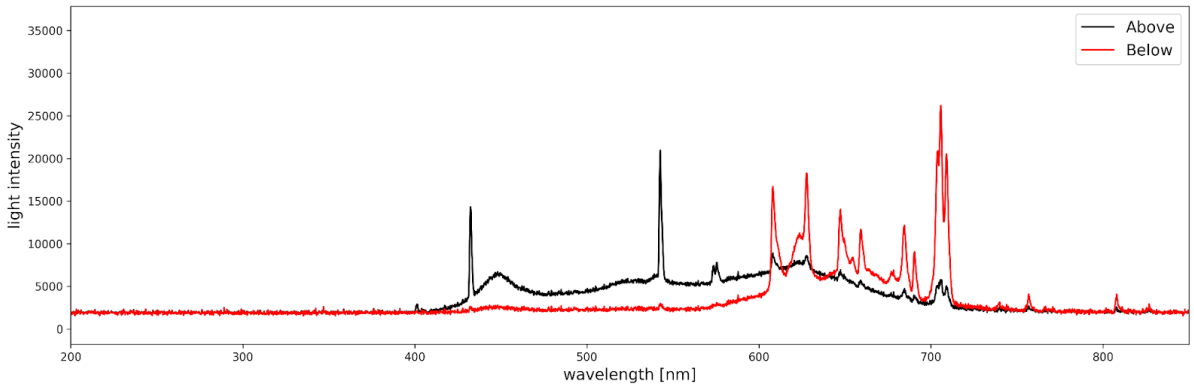

Fig. S3: The light spectrum recorded within the flight arena from above (black) and from below (red). The light from above the arena ranges between 400 and 700 nm which is within the range of visible light to humans. The light from below, used for tracking the bee position, ranges between 600 and 700nm which is outside the perception of bees (Skorupski and Chittka, 2010).

Tab. S1: Results of the Rayleigh test for circular uniformly distributed data. The statistical results of the z-value, p-value and significance level are given for each spatial area and the tested environmental condition.

condition	Area	z	P-value	significance level
Three objects	low_clutter	389.684	1.38E-170	***
	high_clutter	609.359	1.83E-270	***
	above_clutter	825.417	0	***
	below_outside	356.904	2.85E-16	***
	above_outside	137.680	1.03E-06	***
Full density	low_clutter	221.989	2.42E-97	***
	high_clutter	661.197	1.53E-29	***
	above_clutter	123.114	3.01E-54	***
	below_outside	548.840	1.16E-24	***

condition	Area	z	P-value	significance level
	above_outside	182.583	1.77E-82	***
Half density	low_clutter	770.239	0	***
	high_clutter	247.969	1.65E-11	***
	above_clutter	570.329	1.61E-249	***
	below_outside	212.517	0.119	ns
	above_outside	900.812	0.0001	**
Outer ring	low_clutter	287.988	4.40E-126	***
	high_clutter	296.394	1.29E-13	***
	above_clutter	190.498	1.45E-83	***
	below_outside	188.734	6.26E-09	***
	above_outside	148.903	3.34E-07	***