Bumblebees locate goals in 3D with absolute height estimation from ventral optic flow

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ABSTRACT

Introduction: When foraging, flying animals like bees are often required to change their flight altitude from close to the ground to above the height of the vegetation to reach their nest or a food source. While the mechanisms of navigating towards a goal in two dimensions are well understood, the explicit use of height as a source for navigation in three dimensions remains mostly unknown. Our study aims to unravel which strategies bumblebees use for height estimation and whether they rely on global or local cues.

Methods: We expanded a 2D goal localization paradigm, where a goal location is indicated by cylindrical landmarks, to the third dimension by using spherical landmarks to indicate a feeder's position in 3D and examined the search pattern of bumblebees. Additionally, we assessed the ability of bees to estimate the height of a feeder based on local landmarks and global references such as the ground floor.

Results: The search distribution for a feeder's position in 3D was less spatially concentrated compared to in 2D. Assessing the bees' height estimation ability, we found that bees could estimate a feeder's height using the ground floor as a reference. However, the feeder needed to be sufficiently close to the ground floor for the bees to choose correctly.

Discussion: When bumblebees are faced with the challenge of foraging in a 3D environment where the height of a food source and landmark cues are important, they demonstrate the ability to learn and return to a specific flower height. This suggests they rely on ventral optic flow for goal height estimation in bumblebees.

Keywords: navigation, goal localization, vision, optic flow, insect, bumble bee (*Bombus terrestris*)

1 INTRODUCTION

Bees have remarkable navigational abilities in three-dimensional space (Brebner et al., 2021; Bullinger et al., 2023; Menzel, 2023; Osborne et al., 2013; Woodgate et al., 2016). While previous research has extensively explored cue utilization and navigation strategies in two dimensions (Buehlmann et al., 2020; Collett et al., 2013; Kheradmand and Nieh, 2019)(, bees fly at various heights. For example, they feed on flowers ranging from close to the ground to blossoms on trees at the height of multiple meters. They can

also fly at heights of hundreds of meters over more considerable distances (Dillon and Dudley, 2014; Gibo, 1981).

Height estimation can be divided into two key components: estimating the own height and estimating the height of a goal. Previous research has revealed that insects employ various strategies to determine their own flight altitude. Optic flow, for instance, has been suggested as a potential cue for flight height estimation, as the rate of image motion on the retina is influenced by the distance to objects and the insect's velocity (Srinivasan et al., 1991; Esch and Burns, 1996). However, relying solely on ventral optic flow based on the ground floor can be challenging, as changes in the insect's velocity can alter the perceived optic flow, leading to height judgment ambiguities (Srinivasan et al., 1996; Baird et al., 2005). To overcome this problem, insects may use additional cues, such as texture gradients or optic flow information of different sources such as near-by objects (Linander et al., 2016). Mechanisms for height estimation are not only crucial for determining the insect's own flight height but also for estimating the height of other goals, e.g. food sources like blossoms of trees or the home like the nests in trees that can be located above the ground. In principle, estimating the height of goals may be accomplished in different manners. The insect could use absolute height relative to the ground (i.e. a ground-based allocentric estimation of the height of a goal) or the height of the goal relative to their flight altitude (i.e. an egocentric estimation of the goal's height). An allocentric estimation may not only be ground-based, but also relative to objects. In such a case the vertical distance between objects and the goal would be estimated (i.e. an object-based allocentric estimation). Studies have demonstrated that honeybees can accurately estimate height on a small spatial scale of a few centimeters (Lehrer et al., 1988; Srinivasan et al., 1989). During a transfer test, bees trained on the highest flower at 5 cm chose the flower at the trained height of 5 cm instead of the highest flower in the test at 10 cm. Thus they may have used ground-based allocentric estimation or an egocentric estimation of the goal, but do not seem to use an object-based allocentric estimation at this scale. The ability of bees to discriminate height at a small spatial scale raised the question on whether and how they use such information to reach a 3D goal at a larger scale.

Studies in 2D indicate bees can learn the position of a goal using landmarks. Honeybees were trained to learn a feeder position on the floor surrounded by three cylinders (Cartwright and Collett, 1983; Cheng et al., 1987). When the feeder was removed, the bees showed a concentrated search at the previous location of the feeder using landmarks as reference points. Movements and cue utilization in 2D and 3D environments pose distinct challenges to navigating organisms. While navigational strategies may be similar on a 2D plane, navigating in 3D introduces additional complexities due to the need to account for vertical movement and more significant potential for error possibilities. For instance, a study comparing bees' preference for flowers arranged horizontally versus vertically revealed differing performance levels based on cue presentation orientation (Wolf et al., 2015). This suggests that bees exhibit variations in performance when searching for food based on cues presented in different spatial orientations.

In the current study we wanted to transfer this 2D setting (Cartwright and Collett, 1983; Cheng et al., 1987) into the third dimension. We asked whether bumblebees can learn a feeder position in 3D and whether they estimate the goal height based on absolute distance to ground vs relative distance to landmark.We hypothesized that after learning a feeder position indicated by landmarks, bees would show a concentrated search pattern around the feeder position in 3D when the feeder is removed. As the results of the first experiment deviated from this expectation, we hypothesized that the bees may use an absolute height estimate of the feeder relative to the ground and use relative height information of the spherical landmarks relative to the feeder. By analyzing the flight trajectories and search behavior of bumblebees, we provide new insights into goal localization while focusing on height estimation of flying insects in 3D environments.

2 METHODS

2.1 Animal handling

Three *Bombus terrestris* colonies were used, provided by Koppert B.V., France. We tested one colony after the other from September 2022 to January 2023. The bees, arriving in a small box, were transferred under red light (not visible to the bees (Skorupski and Chittka, 2010) into a dark gray acrylic box (0.24 x 0.24 x 0.4 m) with a transparent lid easing the monitoring of the colony health. The hive box was covered with a black cloth to mimic the natural lighting conditions of *B. terrestris* nests underground (Goulson, 2010). We provided pollen balls *ad libitum* in the hive box. For these pollen balls, 50 ml commercial ground pollen collected by honeybees (W. Seip, Germany) were mixed with 10 ml water. Sugar water, a sweet aqueous solution (30% saccharose, 70% water in volume), was provided *ad libitum* to the bees in a micro-gravity feeder in the flight arena. The micro-gravity feeder consisted of a falcon tube screwed on a 3D-printed blue landing platform with small slits where the bees could land on and suck the sugar solution out of the small slits. The landing platform of the feeder had a diameter of 6 cm. The bees were tagged with individually numbered plastic tags (W. Seip, Germany) glued on their thorax with melted resin to discriminate individuals.

2.2 Flight arena

The flight arena was a windowless, indoor room (4 m x 4 m, height: 2 m). The floor was covered with a red and white pattern with a frequency of 1/f (pink noise), mimicking the natural surroundings (a distribution observed in nature; (Schwegmann et al., 2014)), providing enough contrast for the bees to use optic flow. The walls and the ceiling were covered by white tarpaulin. The light was provided by 18 light bulbs placed behind the white tarp. The colony was connected via small boxes (6 x 6 x 6 cm) to a flight arena. The small boxes had closable doors to select bees individually. The bees could access the flight arena via a small tube (2.5 cm diameter) in one corner (Fig. 1A) at a height of 1.25 m. The experimenter could access the arena through a cutout door in the tarpaulin fixated with velcro. Once we saw regular traffic of bumblebee foragers between the colony and the feeder, the experimental tests were started. For the tests, the bees were manually removed from the flight arena and only one bee at a time was allowed to enter it using doors at the colony entrance tube. One bee at a time was allowed into the arena and its search for the feeder was recorded for three minutes. After entering the arena, the bee walked on a platform at a height of 1 m and had to take off that platform to enter the flight arena. The bee had a maximum of two minutes to take off otherwise the trial was discarded. Each bee was tested only once per recording session (one session in the morning, one in the afternoon).

2.3 Video tracking

The bees' flight trajectories were recorded with a custom-written script in C++ inside the flight arena by four synchronized Basler cameras (Basler acA 2040um-NIR) with a frame-rate of 62.5 Hz (as in Sonntag et al. (2024)). These were placed in each corner of the arena, facing upwards towards the center of the arena. Before a bee entered the arena, the recording had already started, and the first 60 seconds were used to calculate a background image of the arena (a bee-less image). After this, only crops, i.e., sections of the full-frame image (42 x 42 pixels) containing large differences between the background image and the current image, were saved to the hard drive with the crop's position in the image. In the next step, these image crops were analyzed with a custom-written neural network to classify whether the crop contained a bee. Finally, the trajectories were reviewed to check for non-biological speed (above 4m/s Goulson (2010)),

or positions outside the arena were detected; in these cases, the neighboring crops were manually reviewed. The analysis of the collected data was performed using Python (3.8.17).

2.3.1 3D goal localization

We transferred the 2D paradigm for 3D goal localization to the nest or a feeder (Cartwright and Collett, 1983; Cheng et al., 1987; Collett et al., 2013; Doussot et al., 2020; Wehner et al., 1996) into 3D to investigate how bumblebees locate a 3D goal position indicated by landmarks (Fig. 1A). The bees (N = 13) were trained to a feeder position surrounded by three landmarks and then tested if they could find the feeder position based on the landmark cues when the feeder was removed (Fig. 1A). We hypothesized that after learning a feeder position indicated by landmarks, the bees would show a concentrated search pattern around the feeder position when the feeder is removed. Three hanging acrylic spheres (diameter of 8 cm) were placed in one corner of the arena surrounding a micro-gravity feeder (consisting of a plate with small slits and a 50 ml Falcon tube containing the sugar water). The landing platform of the feeder was placed at a height of 1.20 m above ground. The distance between the feeder and each of the spheres was similar (0.34 m, 0.37 m, 0.35 m) and they were hanging at three different heights (1.51 m, 1.28 m and 0.99 m). The spheres were coloured in red to be perceived as black by the bees (Skorupski and Chittka, 2010) but provided enough contrast for tracking the bee in front of them with the cameras. When no recordings were taken, this was the training condition where the bees could travel freely between the feeder and the colony. For the test with the shifted constellation of landmarks (Fig. 2A), we calculated the time the bees spent in the area of the training constellation and the shifted constellation (spherical areas with a radius of 0.53 m around the feeder which includes the center of the spheres). We used the Mann-Whitney U test to compare if the bees spent more time at the position of the shifted constellation than at the training position of the constellation.

2.3.2 Height estimation

In the 3D goal localization experiment, the bees did not show the expected concentrated search at the feeder location. Therefore, we adapted the setup to introduce multiple feeders, i.e. just a landing platform without any reward, at different heights during the test (Fig. 3A). We hypothesized that the bees may use an absolute height estimate of the feeder relative to the ground and use relative height information of the spherical landmarks relative to the feeder. The spherical landmark constellation and the feeder were placed in the center of the arena to minimize the influence of global cues present in the arena even if we tried to remove them as far as possible. Surrounding the feeder (1.25 m height), three hanging, acrylic spheres were placed with the same two-dimensional distance to the feeder. One sphere was placed higher than the feeder (1.45 m, high sphere), one at the same height (1.25 m, intermediate sphere) and one sphere was placed below the feeder (0.9 m, low sphere). The experiment was started once we saw regular traffic of foragers between the colony and the feeder. The general test condition consisted of three landing platforms (similar to the feeder but without the tube with sugar water) and one or no sphere. The three landing platforms provided visual goal locations. They were positioned at the planar position of the hanging spheres during the training phase but varied in height. Thereby we could test if the bees can distinguish the landing platforms by their height. Their xy position did not provide any information in regard to the training position. If a sphere was present, it was hanging in the center of the arena like the feeder during the training phase. If the bees used relative height estimation between the sphere and the ground floor to estimate the feeder's height, the sphere would be required as a local height reference. With the shift of the sphere to the center of the arena, we ensured that the feeder height could not be identified by its planar position but only by its height. Four tests were conducted, which differed in the feeder height and the presented sphere. In

the high-sphere test (Fig. 3B), the highest sphere was presented (1.44 m), and the three feeders were placed at 1.84m, 1.56m, and 1.26 m height. In the intermediate-sphere test (Fig. 3D), the intermediate sphere was presented (1.14 m), and the three feeders were placed at 1.51 m, 1.25 m, and 0.95 m height. In the low-sphere test (Fig. 3F), the lowest sphere was presented (0.9 m), and the three feeders were placed at 1.24 m, 1 m, and 0.7 m height. Lastly, no sphere was presented in the ”feeders-only” test, but the feeders were placed as they were in the low-sphere test (Fig. 3H). Before each recording, the landing platforms were cleaned with 70% ethanol to remove chemical markings. To investigate if the bees were searching for the feeder, we used the Mann-Whitney U test to test speed and sinuosity in the regions around the feeders and between the feeders. A lower speed and a higher sinuosity would indicate that the bees show a search behavior. We used a one-way ANOVA and the Tukey post-hoc test to compare the search time at the three feeder positions for each test condition (high sphere, intermediate sphere, low sphere, feeders-only).

3 RESULTS

3.1 3D goal localization

We hypothesized that bees can learn the position of a feeder in 3D. To test this hypothesis, we modified a 2D goal localization paradigm by replacing the cylindrical landmarks with spherical landmarks and hanging a feeder (i.e. the goal) between the constellations of the landmarks. For the control test, the spherical landmarks remained at the same position as during training, but the feeder was removed. We investigated whether bees show a concentrated search at the 3D position of the feeder relative to the landmarks in 3D, even if the feeder itself was removed. In contrast to this expectation, the kernel density probabilities of the bees' search showed that the bees spent much time just before the first sphere (Fig. 1**A**, as seen from the bees' entrance to the arena). The kernel density probability shows a peak on the z-axis just below the highest sphere. The bees did not search in the center of the constellation where the feeder was placed in the training situation, but rather undershot the position by flying not far enough.

For the test with the shifted constellation, the spheres were shifted towards the other side of the arena to minimize the influence of unintentional cues that might exist in the flight arena. We investigated whether bees show a concentrated search at a 3D position of a feeder relative to the landmarks in 3D, even if the feeder itself was removed. We found that the bees searched around the spheres and did not search in the empty corner where the constellation was placed during training (Fig. 2 **E**, Mann-Whitney U-test, $n1 = n2 = 13$, $p = 0.0005$, cohen's d = 1.498). As in the control test, we still see much exploratory behavior in the center of the arena, just below the ceiling, which might have been caused by the artificial lighting above the ceiling.

3.2 Height estimation

The experiment of 3D goal localization has shown that the bees search around the object constellation, but the search did not appear concentrated around the goal. To test whether this effect may be due to the lack of a visual goal or a landing platform, we adapted the experiment to provide the bees different landing platforms during the tests (Fig. 3**A**). These landing platforms could be only differentiated by their height but not by their xy position. We hypothesized that the bees would select the landing platform corresponding to the training situation by using the relative height between the sphere and the floor.

We first tested if the bees were searching at the landing platforms. Since during search bees lower their speed and perform many loops or sinuous maneuvers (Lihoreau et al., 2016), we tested the flight speed and how sinuous their flight paths were at the feeders and between them. We found a lower speed

(Mann-Whitney U-test, n_1 = n_2 = 13, p = $1.663e^{-38}$, cohen's d = -1.302, Fig. 4A) and higher sinuosity (Mann-Whitney U-test, n_1 = n_2 = 13, p = $2.352e^{-08}$, cohen's d = 0.403, Fig. 4B) in the areas around the feeders than in the areas between the feeders . A more tortuous path indicates search behavior. In contrast, a straighter path is assumed to result from a more goal-directed behavior. Thus, we can conclude that the bees were searching at the feeders and quickly transitioning between them. Therefore, comparing the time spent at each feeder will be used as a quantitative indicator if they can discriminate the training feeder height from other heights.

In order to learn the relation between the feeder and the sphere, we conducted three tests with of one the spherical landmarks in each test: high-sphere, intermediate-sphere, and low-sphere. We also provided three landing platforms, each height corresponding to the relative height of each sphere. As such, if the bees learned the feeder location relative to each spherical landmark, they would search for the low feeder with the high-sphere, the intermediate feeder with the intermediate-sphere, and the high feeder with the low-sphere. The search distributions of the bees around the feeder positions and the local landmark cue give a first insight into the motivation of the bees, i.e. whether they searched for the feeder location. Kernel density estimation (KDE) distributions of the bees' search show for the high sphere test, search positions around the lowest feeder (Fig. 3C). However, we also observed some searches around the highest and intermediate feeder positions, indicating that the bees did not fully discriminate the highest and intermediate feeder from the height of the feeder used during training (i.e.the lowest feeder height).

Similar to the high-sphere test, in the other test, the intermediate sphere was placed in the center of the arena, and three feeders were placed around it either higher than the sphere, at an intermediate height, the training height, or lower than the sphere. The KDE distributions show much search around the highest feeder and much less around the intermediate and lowest feeders (Fig. 3E). Since the intermediate feeder was positioned at the training height, the bees searched most at the feeder higher than the training height. As we tested the high and intermediate spheres, we tested the lowest sphere. The lowest sphere was hanging in the center, and the highest feeder was placed at the training height, while the intermediate and lowest feeders were generally lower than the training height. The search distributions show in this test a clear peak of search at the highest feeder at the training height and only minimal search at the other two feeders.

In the low-sphere constellation, the bees searched at the trained feeder height (Fig. 3G). In order to test whether the bees used the sphere as a reference cue, we conducted a fourth test without a sphere, but the three landing platforms corresponded to the test with the lowest sphere to test if the bees could estimate the correct height even with a landmark. In the "feeders-only" test, no sphere was placed in the center, but only the three feeders from the low-sphere test were placed, resulting again in the highest feeders being placed at the training height. The search distributions are similar to those from the low-sphere test, meaning bees also searched at the feeder at the training height (Fig. 3I).

All in all, the search distributions show that the bees searched for a feeder placed at its height during the training period. A comparison of time spent at the three feeder heights in the four tests confirms these observations (Fig. 3J). In the high sphere test, the bees searched the least at the highest feeder and a similar portion of their time at the intermediate and lowest feeder. However, statistically, we found no difference between the search at the three feeders (ANOVA: df = 2.0, F = 0.089, Tukey: p = 0.9). In the intermediate sphere condition, the proportion of search is higher at the high and the intermediate feeder than the lowest feeder, but there is no significant difference between the highest and the intermediate feeder (ANOVA: df = 2.0, F = 5.798, Tukey: $p_{\text{high-low}}$ = 0.021, $p_{\text{intermediate-low}}$ = 0.007). For the low sphere test, we found a significant difference between the proportion of time spent at the highest feeder and at the lowest feeder, indicating that the bees could discriminate between the highest feeder, at the training height, and the lowest

feeder (ANOVA: df = 2.0, F = 14.287, Tukey: $p_{\text{high-intermediate}} = 0.003$, $p_{\text{high-low}} = 0.001$). The search proportion at the intermediate feeders shows a trend of less search than at the highest feeder. In the fourth test, with only the feeders from the low sphere condition but without the sphere in the centre, the bees spent most time searching at the highest feeder and significantly less at the other two feeders (ANOVA: df = 2, F = 15.398, Tukey: $p_{\text{high-intermediate}} = 0.001$, $p_{\text{high-low}} = 0.001$). Since the bees chose most clearly the correct height when the constellation of feeders without the sphere being placed closest to the ground floor, we assume that the bees used the ground floor to estimate their absolute height, most likely based on ventral optic flow. The single spheres that were placed higher (high and intermediate tests) seem to be more like distractors for height estimation. Additionally, the absolute height estimation based on ventral optic flow might not have worked precisely due to the larger distance between the ground floor and the landing platforms. Taken together, we found that bees can find a food location only based on its height, and they would use global cues such as their distance to the ground floor rather than local cues like their spatial relation to the landmarks.

4 DISCUSSION

We investigated how bees locate a goal in 3D space when the goal location is indicated by surrounding local landmark cues, i.e. spheres placed at different locations in 3D space around a feeder. We found that the bees associated the cues with the goal, similar to 2D paradigms. However, we do not observe a concentrated search around the feeder position as found in those studies investigating the goal localization of a food goal in 2D (Cartwright and Collett, 1983; Cheng et al., 1987). In the 2D experiments, when the feeder was removed, the bees searched around this position, but in our 3D, the bees did not search at the location where the feeder was positioned in the training situation. This suggests that bees do not - as is generally assumed for the corresponding 2D situation - use the positions of the landmarks alone to localize a goal position, even if the goal itself is not visible. However, since the animals still coarsely search for the goal location with reference to the landmarks, we hypothesize that they might need additional visual cues to determine the search area.

To find out what additional cues the bees might use to choose a search area, we further assessed the ability of bumblebees to learn just the height of a food source and which cues they are using to find it back. We showed that the bees could find a previously learnt absolute goal height based on global references like the ground floor. This suggests the use of ventral optic flow for absolute height estimation of the goal based on the estimation of distance to the ground. However, the local landmarks and a larger distance to the global references, worked as distractors that degraded bees' search accuracy.

4.1 Differences in 2D and 3D

Transferring an experimental paradigm from 2D to 3D is not trivial, as we found that the bees did not exhibit such a clear search pattern in 3D as shown in 2D (Cheng et al., 1987). By providing landing platforms in the second experiment, we could reduce the complexity of an open space between three landmarks to three distinct positions in 3D. Understanding these differences is crucial for deciphering the mechanisms underlying navigation in bees across various spatial dimensions. In 3D environments, bees face additional challenges beyond those encountered in 2D spaces. Bees engage in various flight maneuvers, including pivoting, turning, sideways movement, hovering, and backward flight (e.g. Linander et al. (2018); Doussot et al. (2021)). This dynamic range of movements suggests that bees adjust their body and head positions to memorize views that guide them back to nest locations or other goals (Doussot et al., 2021). Previous studies have highlighted the importance of this adaptive behavior, indicating that bees employ

spatial awareness strategies to navigate effectively in complex 3D landscapes (Linander et al., 2018). Our findings further contribute to this understanding, revealing that while bees demonstrate the ability to localize goals vertically, success is limited when the landmark-goal arrangement is closer to ground level. This insight emphasizes the nuanced challenges bees face in 3D navigation and the importance of considering spatial context in understanding their behaviors.

4.2 Use of optic flow for flight control

In the height estimation experiment, we observed that the bees searched around the trained feeder height without requiring the use of local landmarks. This indicates that the bees used ventral optic flow for goal height estimation. The utilization of optic flow for flight control is a crucial aspect of bees' navigation in 3D (Lecoeur et al., 2019; Egelhaaf, 2023). Extensive studies have demonstrated that flying insects rely heavily on optic flow to control their flight (Linander et al., 2018; Frasnelli et al., 2021). Bumblebees, in particular, prioritize motion cues from the ground, using ventral optic flow to control their altitude and lateral position (Linander et al., 2017). When the availability of ventral optic flow cues is limited, bumblebees adjust their flight height to maintain the ability to resolve ground texture (Portelli et al., 2010; Linander et al., 2018). As the bees needed a relatively close (i.e. up to 1.25 m) distance between the goal and the ground floor to estimate the goal height correctly, we assume that they used a ground-based allocentric distance estimation. Srinivasan et al. (1989) have shown that honeybees can discriminate distances of at least 2 cm, and our experiment indicates an upper limit of 1.25 m. These results taken together indicate that the bees use a ground-based allocentric distance estimation and not an egocentric height estimation of goal.

The upper limit of the bumblebees' distance estimation of 1.25 m in our study aligns with electrophysiological findings in flies. Flies could only detect flight distances up to approximately one meter at a slow translational speed of around 0.5 m/s. However, the flies required higher speeds for larger distances due to the limited signal-to-noise ratio in visual information processing (Kern et al., 2005). This low speed fits well with the speed at which we observed that the bees were flying when searching in the area of the feeder constellation. Since the space where the bees could fly was limited, the bees were probably not able to fly at speeds high enough to resolve the required height estimations above 1.25 m. Therefore, absolute height estimation of the goal height based on ventral optic flow is the most likely mechanism.

4.3 Local and global cues

Our search results indicate that bumblebees rely on both local and global visual cues for navigation. Cheng et al. (1987) compared the use of global and more local cues. They placed cylindrical landmarks at various distances to the goal and observed the honeybees' search patterns. The bees were found to search more at the landmarks closer to the goal, even when these were moved in relation to the more global landmarks. Transferred to our 3D-setting, one would have expected that the local landmark cues, i.e. spheres placed in 3D, should have played a more significant role than more global reference structures of the arena, such as the ceiling or the plane of the floor. The use of cues for goal localization on the horizontal plane appears to differ from that along the vertical axis. Additionally, a constant reference like a floor plane might be more reliable than more local landmarks, pointing to a similar strategy as shown in humans' higher weighting of stationary objects (Roy et al., 2022).

4.4 Height estimation with both relative and absolute height in a small range

The topic of localizing a goal based on its height was previously investigated in honeybees on a small scale of a few centimeters, where the authors could show that bees can very precisely estimate the distance

in the range of 1-3 cm Srinivasan et al. (1989). The bees used relative distance cues such as a flower height between a higher and a lower flower because the bees learnt the relative distance to the other two flowers. Transferring this to our study meant that the bees should be able to discriminate the learnt height by using the relative references given by the spheres placed in 3D. However, in the intermediate-sphere test (with the landmark at the intermediate height of the training landmark constellation and three landing platforms as feeders), the bees chose the three feeders equally instead of the intermediate feeder. The height estimation of bees in the range of half a meter might be less precise than in the range of a few centimeters but sufficiently good for foraging. We analyzed the pooled behavioral data on the population level, however the bees might have employed different strategies as we saw some search for the correct feeder's height.

4.5 Conclusion

When challenged during foraging in a 3D world where the height of a food source and landmark cues also play a role, bumblebees can learn and return to a specific flower height. They perform better when the food source and the constellation are closer to the floor, suggesting that the bees use optic flow to estimate goal height. However, surrounding cues might act as a distraction rather than useful information, as we see a similar performance without the sphere placed between the potential feeding places. Further studies should investigate how the bees sample optic flow for height estimation, for example, by systematically and periodically changing their flight altitude (Bergantin et al., 2021). Additionally, it would be interesting to know which information the bees would need to locate the correct height when the feeder/landmark constellation is further away from the ground floor. Manipulating the optic flow by varying patterns, indicating distances different from the learnt one, is needed to strengthen our hypothesis.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

AS: conceptualisation, investigation, data curation, formal analysis, methodology, visualisation, writing - original draft, review, and editing. OJNB: conceptualisation, software, supervision, project administration, writing - review, and editing. ME, ML: funding acquisition, project administration, resources, supervision, writing - review, and editing

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DATA AVAILABILITY STATEMENT

The data-sets and analysis pipeline for this study can be found in the repository ”3D goal localization in bumblebees”.

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FIGURES

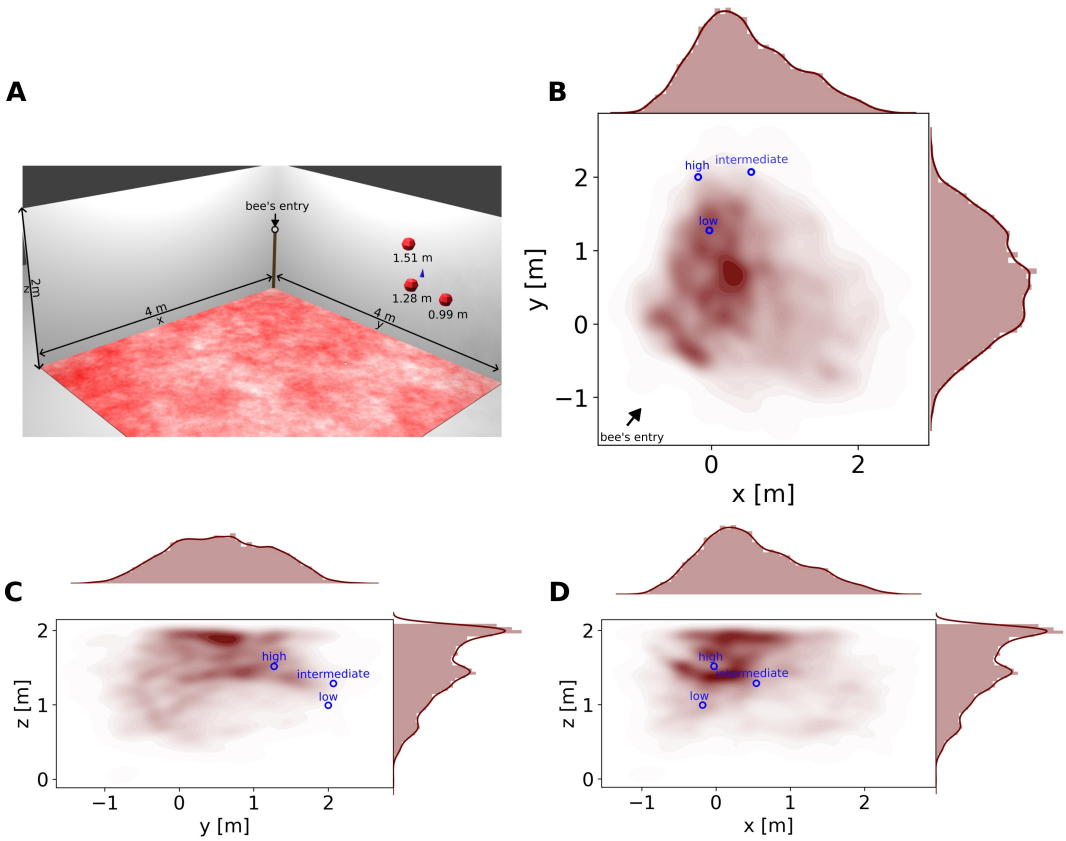

Figure 1. **A** Flight arena with the landmark constellations for 3D goal localization experiment for the training and control test (N = 13). The landmarks are red spheres around the feeder, indicated by the blue cone (only visible for the training condition). In each corner of the arena, one camera was positioned. The bees entered the arena through a tube at the top of the wooden bar in one corner (brown stick). The floor was covered by a red and white pattern (perceived as black and white by the bees (Skorupski and Chittka, 2010)) to provide enough contrast for the bees to use optic flow for flight control. The control test was similar to the training condition, just the feeder was removed. **B - C** Search probability for the control test. The three subfigures show the kernel density probability (given by the color red, the darker the color is the more often the bees visited the position) of the bees' flights in the arena for the control test. The positions of the spheres are indicated by blue circles. **B** shows the bees' flight on the xy plane, **C** on the xz plane and **D** on the yz plane. At the top and right margins, histograms and probability density functions along the axes are shown.

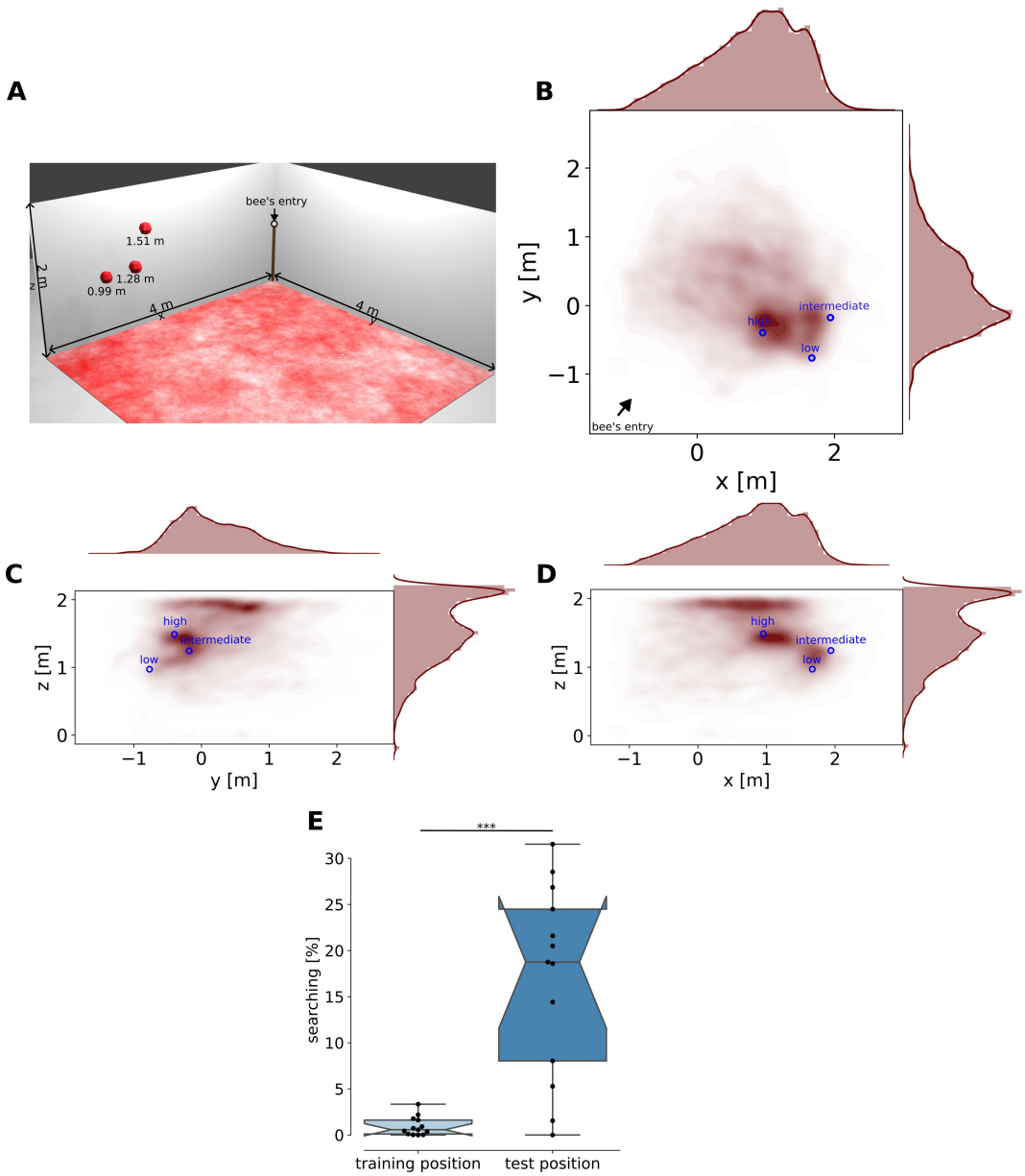

Figure 2. **A** Flight arena with the landmark constellations for 3D goal localization experiment for the shifted constellation test (N = 13, see details in legend of Fig. 1**A**). **B - C** Search probability for the test with the shifted constellation test. The three subfigures show the kernel density probability (given by the color red; the darker the color is, the more often the bees visited the position) of the bees' flights in the arena for the test with the control test (all three spheres shifted to another position). Blue circles indicate the positions of the spheres. **B** shows the bees' flight on the xy plane, **C** on the xz plane and **D** on the yz plane. At the top and right margins, histograms and probability density functions along the axes are shown. **E** The search percentage of the bees at the two locations of either the training or the shifted test position of the landmark constellation. The bees searched more at the position of the shifted test constellation than at the training position (Mann-Whitney U-test, $n_1 = n_2 = 13$, $p < 0.001$).

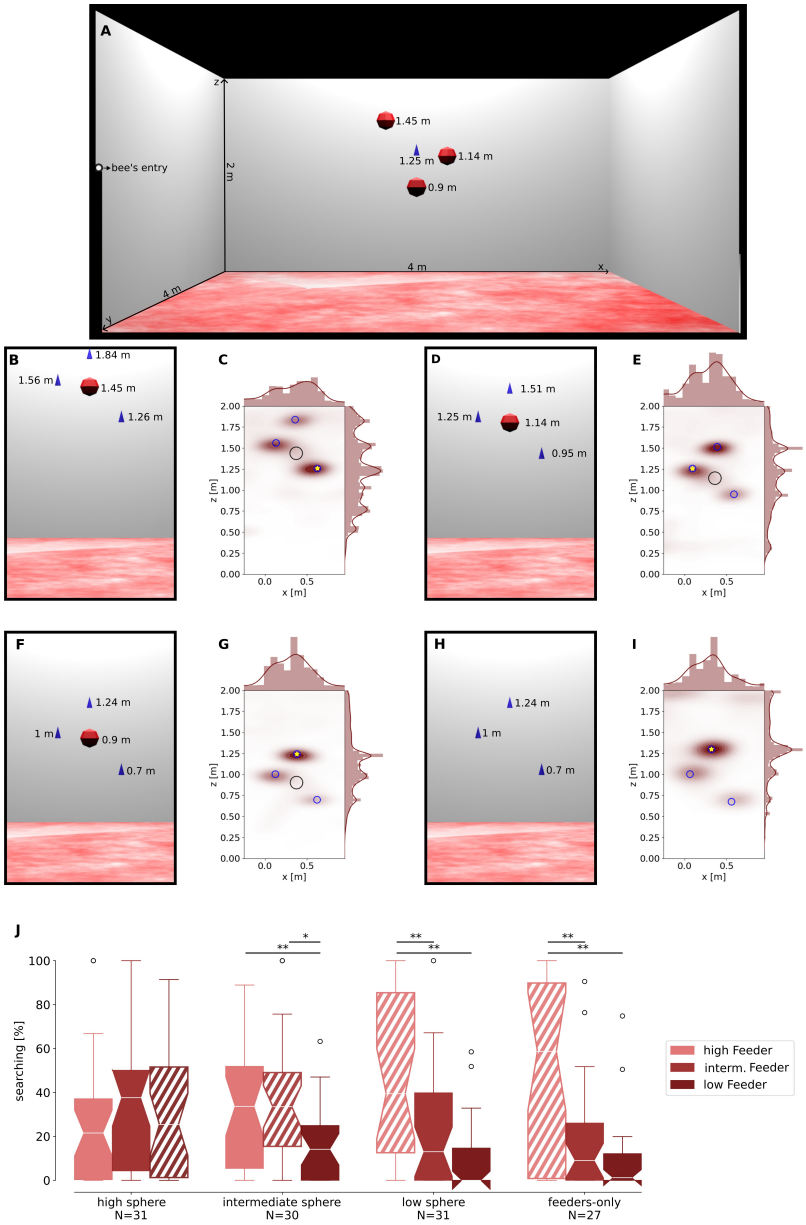

Figure 3. **A - I**: Flight arena for the height estimation experiment with the training constellation (**A**) and all three test constellations with only one of the spherical landmarks (indicated by red circles) and three landing platforms (indicated by blue cones). Test conditions: high sphere (**B**), intermediate sphere (**D**),low sphere (**F**) and feeders-only (**H**). Kernel density estimation of the search distributions in the tests high sphere (**C**), intermediate sphere (**E**) low sphere (**G**) and feeders-only (**I**). The sphere's position is given with a black circle, and the positions of the feeder with blue circles. The correct feeder, in respect to its height for the respective test, is indicated by a yellow star symbol. Heat maps show the highest density of bee position in red, and in white, if no bees were detected, they are seen from the x and z axes given in meters. At the top and right margins, histograms and probability density functions along the x and z axes are shown. **J**: Search proportion at the three feeders (indicated by the color rose for high, red for intermediate feeder, and dark red for the low feeder) in all four tests (high sphere, intermediate sphere, low sphere, feeders-only). The hatched box indicates the feeder at the training height. For the high sphere test, the bees searched a similar amount of time at the three feeders and showed a trend to search more at the lowest feeder than at the highest one. For the intermediate-sphere test, the bees searched more at the high and intermediate feeders than at the low feeders. For the low sphere test, the bees searched the most at the high feeder, which was at the training height. A similar behavior was observed for the test with only feeders without the low sphere.

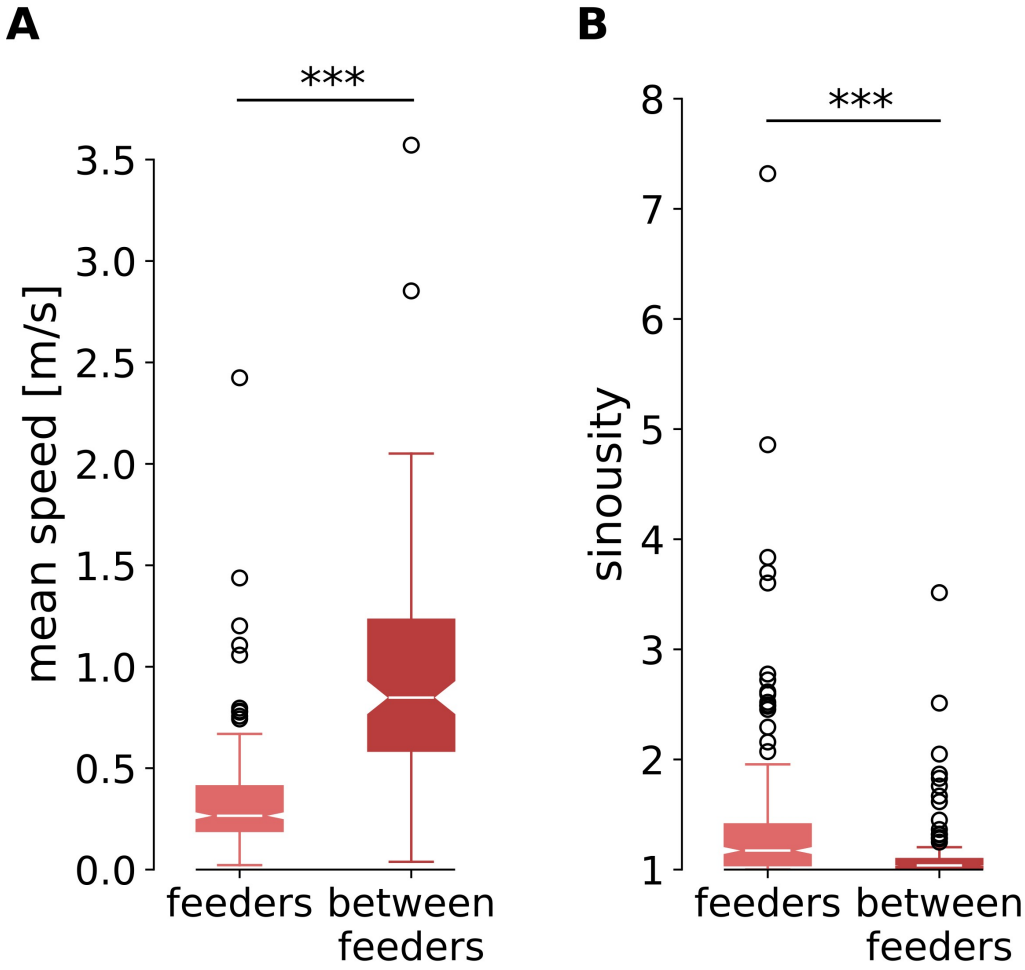

Figure 4. Sinuosity of the bees trajectories in **A** (1 = straight path, 1 ¡ very sinuous path) and the mean speed in **B** of the bees in the areas around the feeders (light red) and between the feeders (dark red). The sinuosity is higher (Mann-Whitney U-test, $n_1 = n_2 = 31$, p < 0.001) and the speed lower in the areas around the feeders than between the feeders (Mann-Whitney U-test, $n_1 = n_2 = 31$, p < 0.001).