

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

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2 ABSTRACT

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

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

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

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

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

1 INTRODUCTION

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

28 also fly at heights of hundreds of meters over more considerable distances (Dillon and Dudley, 2014; Gibo,
29 1981).

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

53 Studies in 2D indicate bees can learn the position of a goal using landmarks. Honeybees were trained to
54 learn a feeder position on the floor surrounded by three cylinders (Cartwright and Collett, 1983; Cheng et al.,
55 1987). When the feeder was removed, the bees showed a concentrated search at the previous location of the
56 feeder using landmarks as reference points. Movements and cue utilization in 2D and 3D environments
57 pose distinct challenges to navigating organisms. While navigational strategies may be similar on a 2D
58 plane, navigating in 3D introduces additional complexities due to the need to account for vertical movement
59 and more significant potential for error possibilities. For instance, a study comparing bees' preference
60 for flowers arranged horizontally versus vertically revealed differing performance levels based on cue
61 presentation orientation (Wolf et al., 2015). This suggests that bees exhibit variations in performance when
62 searching for food based on cues presented in different spatial orientations.

63 In the current study we wanted to transfer this 2D setting (Cartwright and Collett, 1983; Cheng et al.,
64 1987) into the third dimension. We asked whether bumblebees can learn a feeder position in 3D and whether
65 they estimate the goal height based on absolute distance to ground vs relative distance to landmark. We
66 hypothesized that after learning a feeder position indicated by landmarks, bees would show a concentrated
67 search pattern around the feeder position in 3D when the feeder is removed. As the results of the first
68 experiment deviated from this expectation, we hypothesized that the bees may use an absolute height
69 estimate of the feeder relative to the ground and use relative height information of the spherical landmarks
70 relative to the feeder. By analyzing the flight trajectories and search behavior of bumblebees, we provide
71 new insights into goal localization while focusing on height estimation of flying insects in 3D environments.

2 METHODS

72 2.1 Animal handling

73 Three *Bombus terrestris* colonies were used, provided by Koppert B.V., France. We tested one colony
74 after the other from September 2022 to January 2023. The bees, arriving in a small box, were transferred
75 under red light (not visible to the bees (Skorupski and Chittka, 2010) into a dark gray acrylic box (0.24 x
76 0.24 x 0.4 m) with a transparent lid easing the monitoring of the colony health. The hive box was covered
77 with a black cloth to mimic the natural lighting conditions of *B. terrestris* nests underground (Goulson,
78 2010). We provided pollen balls *ad libitum* in the hive box. For these pollen balls, 50 ml commercial
79 ground pollen collected by honeybees (W. Seip, Germany) were mixed with 10 ml water. Sugar water, a
80 sweet aqueous solution (30% saccharose, 70% water in volume), was provided *ad libitum* to the bees in a
81 micro-gravity feeder in the flight arena. The micro-gravity feeder consisted of a falcon tube screwed on a
82 3D-printed blue landing platform with small slits where the bees could land on and suck the sugar solution
83 out of the small slits. The landing platform of the feeder had a diameter of 6 cm. The bees were tagged
84 with individually numbered plastic tags (W. Seip, Germany) glued on their thorax with melted resin to
85 discriminate individuals.

86 2.2 Flight arena

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

102 2.3 Video tracking

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

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

114 2.3.1 3D goal localization

115 We transferred the 2D paradigm for 3D goal localization to the nest or a feeder (Cartwright and Collett,
116 1983; Cheng et al., 1987; Collett et al., 2013; Doussot et al., 2020; Wehner et al., 1996) into 3D to investigate
117 how bumblebees locate a 3D goal position indicated by landmarks (Fig. 1A). The bees (N = 13) were
118 trained to a feeder position surrounded by three landmarks and then tested if they could find the feeder
119 position based on the landmark cues when the feeder was removed (Fig. 1A). We hypothesized that after
120 learning a feeder position indicated by landmarks, the bees would show a concentrated search pattern
121 around the feeder position when the feeder is removed. Three hanging acrylic spheres (diameter of 8 cm)
122 were placed in one corner of the arena surrounding a micro-gravity feeder (consisting of a plate with small
123 slits and a 50 ml Falcon tube containing the sugar water). The landing platform of the feeder was placed
124 at a height of 1.20 m above ground. The distance between the feeder and each of the spheres was similar
125 (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).
126 The spheres were coloured in red to be perceived as black by the bees (Skorupski and Chittka, 2010) but
127 provided enough contrast for tracking the bee in front of them with the cameras. When no recordings were
128 taken, this was the training condition where the bees could travel freely between the feeder and the colony.
129 For the test with the shifted constellation of landmarks (Fig. 2A), we calculated the time the bees spent in
130 the area of the training constellation and the shifted constellation (spherical areas with a radius of 0.53 m
131 around the feeder which includes the center of the spheres). We used the Mann-Whitney U test to compare
132 if the bees spent more time at the position of the shifted constellation than at the training position of the
133 constellation.

134 2.3.2 Height estimation

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

155 the high-sphere test (Fig. 3B), the highest sphere was presented (1.44 m), and the three feeders were placed
156 at 1.84m, 1.56m, and 1.26 m height. In the intermediate-sphere test (Fig. 3D), the intermediate sphere
157 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
158 low-sphere test (Fig. 3F), the lowest sphere was presented (0.9 m), and the three feeders were placed at
159 1.24 m, 1 m, and 0.7 m height. Lastly, no sphere was presented in the "feeders-only" test, but the feeders
160 were placed as they were in the low-sphere test (Fig. 3H). Before each recording, the landing platforms
161 were cleaned with 70% ethanol to remove chemical markings. To investigate if the bees were searching for
162 the feeder, we used the Mann-Whitney U test to test speed and sinuosity in the regions around the feeders
163 and between the feeders. A lower speed and a higher sinuosity would indicate that the bees show a search
164 behavior. We used a one-way ANOVA and the Tukey post-hoc test to compare the search time at the three
165 feeder positions for each test condition (high sphere, intermediate sphere, low sphere, feeders-only).

3 RESULTS

166 3.1 3D goal localization

167 We hypothesized that bees can learn the position of a feeder in 3D. To test this hypothesis, we modified a
168 2D goal localization paradigm by replacing the cylindrical landmarks with spherical landmarks and hanging
169 a feeder (i.e. the goal) between the constellations of the landmarks. For the control test, the spherical
170 landmarks remained at the same position as during training, but the feeder was removed. We investigated
171 whether bees show a concentrated search at the 3D position of the feeder relative to the landmarks in 3D,
172 even if the feeder itself was removed. In contrast to this expectation, the kernel density probabilities of
173 the bees' search showed that the bees spent much time just before the first sphere (Fig. 1A, as seen from
174 the bees' entrance to the arena). The kernel density probability shows a peak on the z-axis just below the
175 highest sphere. The bees did not search in the center of the constellation where the feeder was placed in the
176 training situation, but rather undershot the position by flying not far enough.

177 For the test with the shifted constellation, the spheres were shifted towards the other side of the arena to
178 minimize the influence of unintentional cues that might exist in the flight arena. We investigated whether
179 bees show a concentrated search at a 3D position of a feeder relative to the landmarks in 3D, even if
180 the feeder itself was removed. We found that the bees searched around the spheres and did not search
181 in the empty corner where the constellation was placed during training (Fig. 2 E, Mann-Whitney U -test,
182 $n_1 = n_2 = 13$, $p = 0.0005$, cohen's $d = 1.498$). As in the control test, we still see much exploratory
183 behavior in the center of the arena, just below the ceiling, which might have been caused by the artificial
184 lighting above the ceiling.

185 3.2 Height estimation

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

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

195 (Mann-Whitney U -test, $n_1 = n_2 = 13$, $p = 1.663e^{-38}$, cohen's $d = -1.302$, Fig. 4A) and higher sinuosity
196 (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
197 feeders than in the areas between the feeders. A more tortuous path indicates search behavior. In contrast,
198 a straighter path is assumed to result from a more goal-directed behavior. Thus, we can conclude that the
199 bees were searching at the feeders and quickly transitioning between them. Therefore, comparing the time
200 spent at each feeder will be used as a quantitative indicator if they can discriminate the training feeder
201 height from other heights.

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

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

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

229 All in all, the search distributions show that the bees searched for a feeder placed at its height during
230 the training period. A comparison of time spent at the three feeder heights in the four tests confirms these
231 observations (Fig. 3J). In the high sphere test, the bees searched the least at the highest feeder and a similar
232 portion of their time at the intermediate and lowest feeder. However, statistically, we found no difference
233 between the search at the three feeders (ANOVA: $df = 2.0$, $F = 0.089$, Tukey: $p = 0.9$). In the intermediate
234 sphere condition, the proportion of search is higher at the high and the intermediate feeder than the lowest
235 feeder, but there is no significant difference between the highest and the intermediate feeder (ANOVA: df
236 $= 2.0$, $F = 5.798$, Tukey: $p_{\text{high-low}} = 0.021$, $p_{\text{intermediate-low}} = 0.007$). For the low sphere test, we found a
237 significant difference between the proportion of time spent at the highest feeder and at the lowest feeder,
238 indicating that the bees could discriminate between the highest feeder, at the training height, and the lowest

239 feeder (ANOVA: $df = 2.0$, $F = 14.287$, Tukey: $p_{\text{high-intermediate}} = 0.003$, $p_{\text{high-low}} = 0.001$). The search
240 proportion at the intermediate feeders shows a trend of less search than at the highest feeder. In the fourth
241 test, with only the feeders from the low sphere condition but without the sphere in the centre, the bees
242 spent most time searching at the highest feeder and significantly less at the other two feeders (ANOVA:
243 $df = 2$, $F = 15.398$, Tukey: $p_{\text{high-intermediate}} = 0.001$, $p_{\text{high-low}} = 0.001$). Since the bees chose most clearly
244 the correct height when the constellation of feeders without the sphere being placed closest to the ground
245 floor, we assume that the bees used the ground floor to estimate their absolute height, most likely based
246 on ventral optic flow. The single spheres that were placed higher (high and intermediate tests) seem to be
247 more like distractors for height estimation. Additionally, the absolute height estimation based on ventral
248 optic flow might not have worked precisely due to the larger distance between the ground floor and the
249 landing platforms. Taken together, we found that bees can find a food location only based on its height, and
250 they would use global cues such as their distance to the ground floor rather than local cues like their spatial
251 relation to the landmarks.

4 DISCUSSION

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

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

269 4.1 Differences in 2D and 3D

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

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

285 **4.2 Use of optic flow for flight control**

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

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

307 **4.3 Local and global cues**

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

318 **4.4 Height estimation with both relative and absolute height in a small range**

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

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

330 **4.5 Conclusion**

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

CONFLICT OF INTEREST STATEMENT

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

AUTHOR CONTRIBUTIONS

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

FUNDING

347 The work was supported by the collaborative funding of the 3DNavIBee project of the German Research
348 Foundation (DFG, 431346812) and the French National Research Agency (ANR, ANR-19-CE37-0024),
349 the Prof. Bingel scholarship from the German Academic Exchange Service foundation (DAAD e.V.) and
350 the ERC (European Research Council) through the Cog Bee-Move project (GA101002644). We also
351 acknowledge the support for the publication costs by the Open Access Publication Fund of Bielefeld
352 University.

ACKNOWLEDGMENTS

353 We would like to thank Leo Werner, Paula Bräuer und Madelene Dombrowski for their help during the
354 data collection.

DATA AVAILABILITY STATEMENT

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

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FIGURES

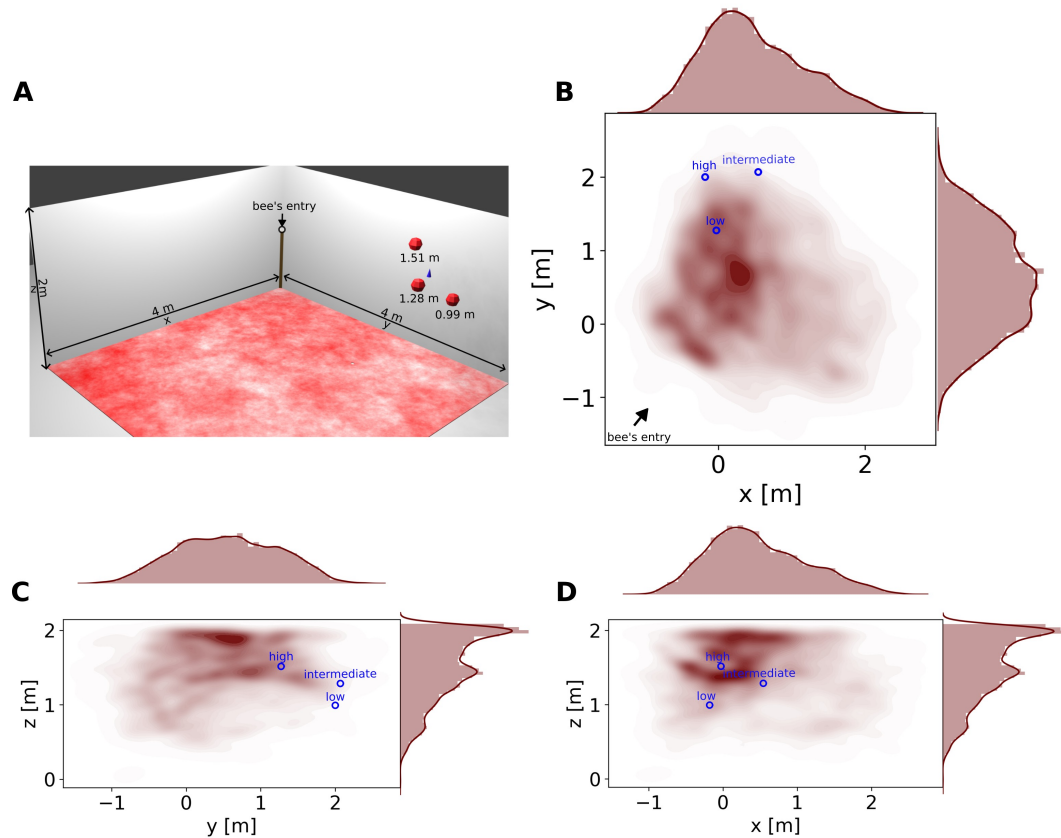


Figure 1. **A** Flight arena with the landmark constellations for 3D goal localization experiment for the training and control test ($N = 13$). The landmarks are red spheres around the feeder, indicated by the blue cone (only visible for the training condition). In each corner of the arena, one camera 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.

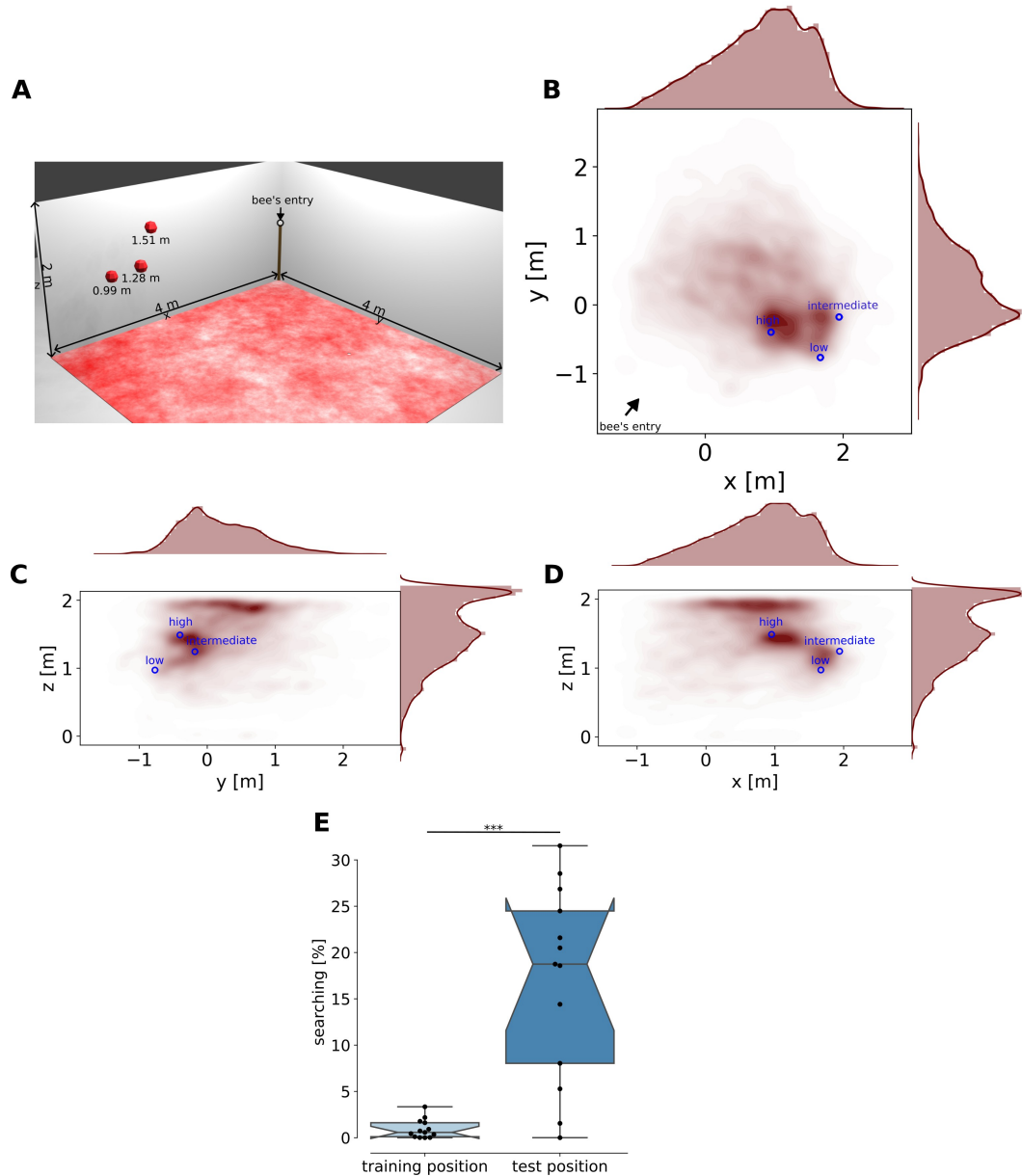


Figure 2. **A** Flight arena with the landmark constellations for 3D goal localization experiment for the shifted constellation test ($N = 13$, see details in legend of Fig. 1A). **B - C** Search probability for the test with the shifted constellation test. The three subfigures show the kernel density 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, $n_1 = n_2 = 13$, $p < 0.001$).

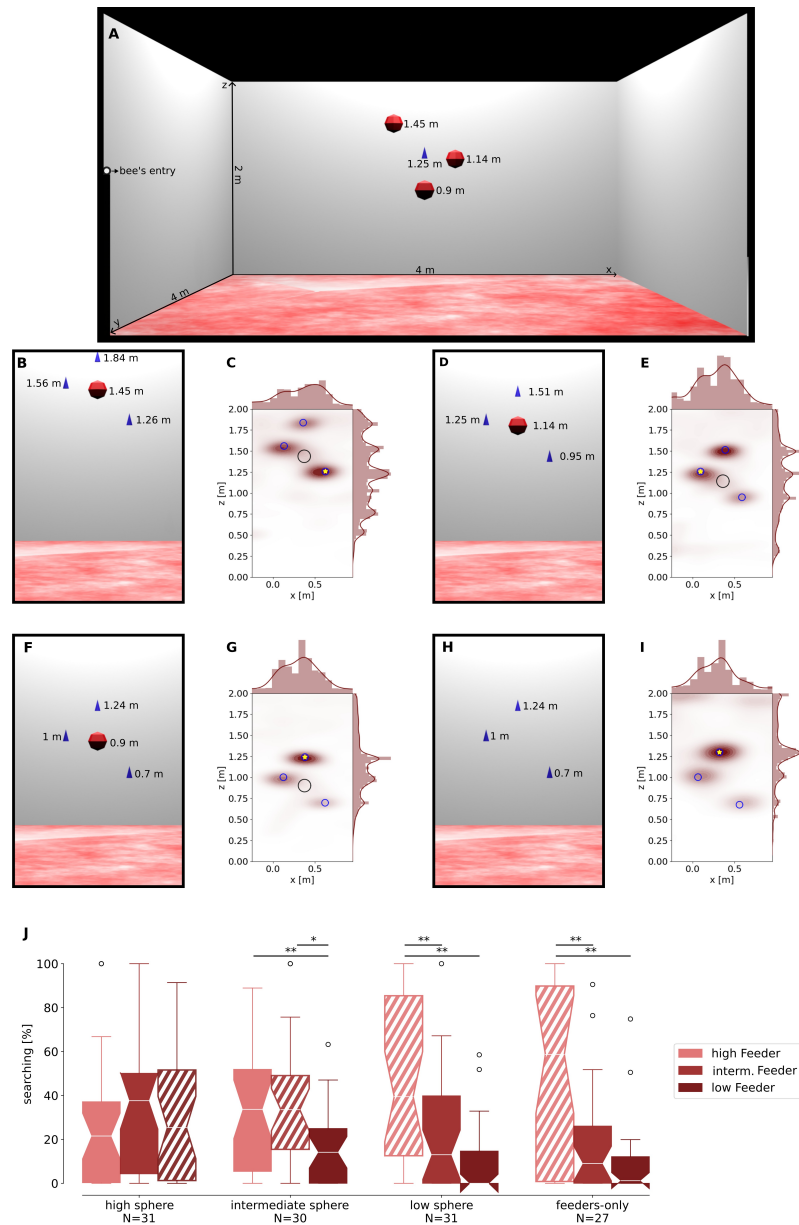


Figure 3. A - I: Flight arena for the height estimation experiment with the training constellation (A) and all three test constellations with only one of the spherical landmarks (indicated by red circles) and three landing platforms (indicated by blue cones). Test conditions: high sphere (B), intermediate 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.

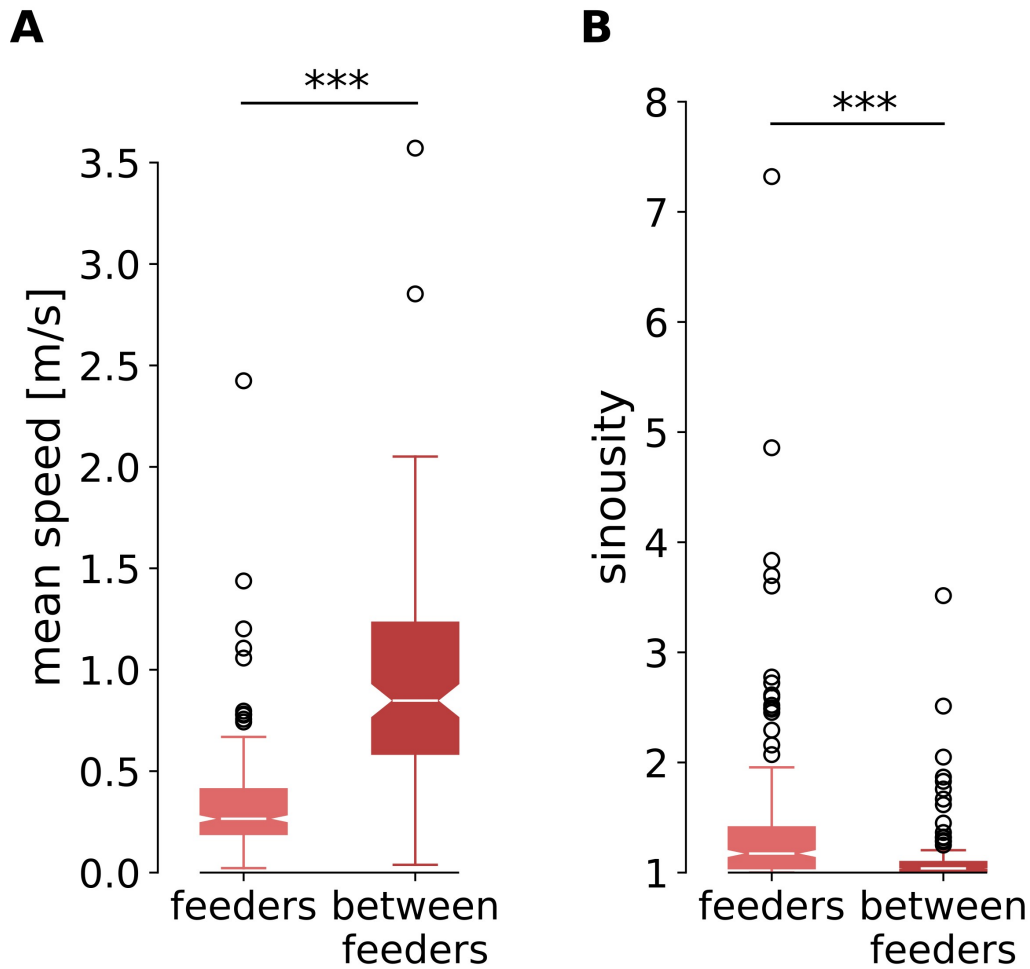


Figure 4. Sinuosity of the bees trajectories in **A** (1 = straight path, 1 ; very sinuous path) and the mean speed in **B** of the bees in the areas around the feeders (light red) and between the feeders (dark red). The sinuosity is higher (Mann-Whitney U -test, $n_1 = n_2 = 31$, $p < 0.001$) and the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