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1 Bumblebees increase their learning 2 flight altitude in dense environments

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9 Abstract

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

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

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

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

27 Summary Statement

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

31 Introduction

32 Bees use visual memories to return to their nest after foraging trips. These visual memories
33 are thought to be acquired during the first outbound flights during which the bees do not
34 forage but perform convoluted manoeuvres consisting of many loops and arcs (e.g. Capaldi et
35 al., 2000; Philippides et al., 2013; Woodgate et al., 2016; for reviews see: Collett and Zeil,
36 2018; Collett and Hempel de Ibarra, 2023). During these first flights, the bees use a “turn back
37 and look” behaviour to regularly face the nest and presumably learn the visual surroundings
38 (Lehrer, 1991, 1993). Bees need to perform these learning flights in order to efficiently return to
39 their nest and avoid risky time-consuming search at the end of every foraging bout (Degen et
40 al., 2016). After a short walking phase, the bees gradually increase their flight altitude and
41 distance to the nest by progressively enlarge the size of their loops, the area covered by
42 these flights expands (Capaldi et al., 2000; Osborne et al., 2013; Woodgate et al., 2016;
43 Lobecke et al., 2018; Bertrand and Sonntag, 2023).

44 Typically, the learning flights were investigated at a detailed level in simple environments with
45 only few cylindrical objects surrounding the nest (Hempel de Ibarra et al., 2009; Collett et al.,
46 2013; Robert et al., 2018; Collett et al., 2023). For instance, it was found that bumblebees
47 adjust their body orientation toward specific directions like compass direction or towards
48 visual features in the surroundings (de Ibarra et al., 2009; Collett et al., 2013, 2023).
49 Additionally, studies have focused on the coordination between head and body movements
50 during these flights (Odenthal et al., 2020; Doussot et al., 2021).

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

57 Consequently, dense and cluttered environments may require more specific learning and
58 navigation strategies for the bees. While the development of the flight altitude played a minor
59 role in aforementioned studies in sparse environments, the described challenges in more
60 ecologically realistic dense environments highlight the importance of understanding how the
61 3D structure of learning flights are affected.

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

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

86 Material and Methods

87 Animal handling

88 We used sequentially three healthy hives of *Bombus terrestris* provided by Koppert B.V., The
89 Netherlands. After arrival, the bees were transferred under red light (non visible to bees
90 (Skorupski and Chittka, 2010)) into an acrylic box (30x30x30cm). This box was covered with
91 black cloth to mimic the natural, underground nesting conditions of *B. terrestris* (Goulson,
92 2010). The nest box was connected to the flight arena via a system of six small boxes
93 (6cmx6cmx6cm) and plastic tubes (2.5cm in diameter). One of the boxes contained a
94 micro-gravity feeder that gave the hive direct access to sugar solution. The feeder consisted
95 of a bottle with a small plate at the bottom where the bees could feed on sugar solution (30%
96 sugar in volume) *ad libitum* through small slits in the plate. Pollen balls (50ml ground,
97 commercial pollen collected by honeybees (W. Seib, Germany) and 10ml water) were provided
98 *ad libitum* directly into the nest box. After their first outbound flight, the bees were marked
99 with numbered plastic tags glued on their thorax with a melted resin. The temperature in the
100 experimental room was constantly kept at 20° degrees and artificial light from above was
101 provided in a 12h/12h day-night cycle.

102 Experimental design

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

114 (5W GreenLED, light spectrum in Fig. S3; as in Sonntag et al., 2024), and recording via six
115 high-speed cameras with different viewing angles.

116

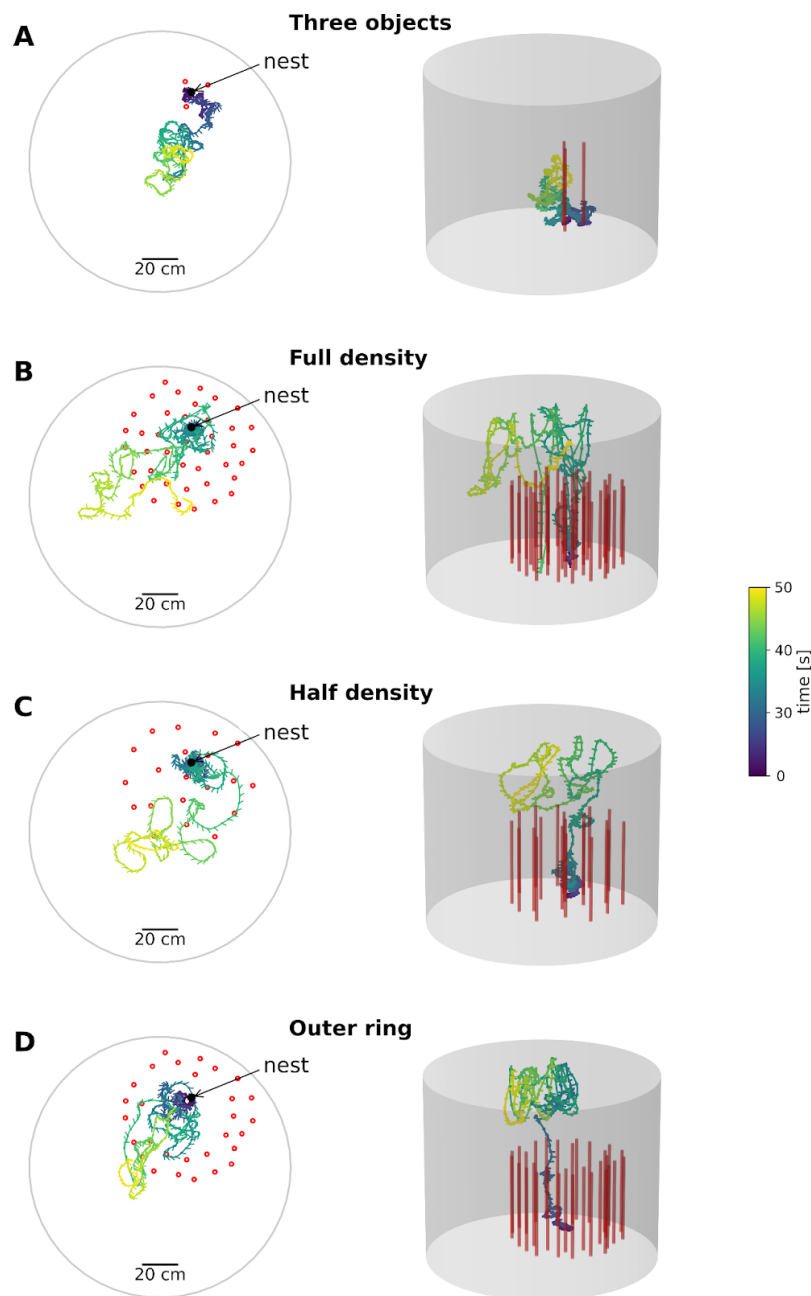
117 A bee crossed two of the small boxes to enter the arena through a hole from below through a
118 plastic tube (2.5cm in diameter). We used these boxes to separate single bees for the
119 recordings while the other bees could forage undisturbed. The entrance to the flight arena
120 was surrounded by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

122 We used four environments by varying the number and arrangement of objects in the arena:
123 The 1st environment, “three objects environment” was sparse with three objects arranged
124 close to the nest entrance (distance < 0.1m, Fig. 1A) similar to previously tested environments
125 for learning flights (Doussot et al., 2021; Lobecke et al., 2018; Robert et al., 2018). The three
126 other environments varied in number and density of the objects. These dense environments
127 (clutter) covered a circular area around the nest entrance with a diameter of 0.8m. The 2nd
128 environment of our investigation, “full density”, consisted of 40 randomly placed objects (Fig. 1
129 B, the three nearest objects to the nest were the same as in the “three object” test) that
130 provided a challenge for the bees but it provided enough space for the bees to fly through
131 (Gonsek, et al 2021; Sonntag et al. 2024). The 3rd environment, “half density”, consisted of
132 20 objects (Fig. 1C, the three nearest objects to the nest were the same as in the “three
133 objects” test) with half the density of the “full density” environment. The 4th environment,
134 “outer ring”, consisted of 30 objects at the border of the circular area around the nest with the
135 same density as in the “full density” environment (Fig. 1D). While the “outer ring” environment
136 tested an increased distance between the nest and the objects, it also yields similarities to
137 Niko Tinbergen’s famous pine cone ring to test visual learning in wasps (Tinbergen, 1932;
138 Tinbergen and Kruyt, 1938).

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

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

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



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

162 3D flight trajectories

163 The bee trajectories in the arena were recorded at a frequency of 62.5 Hz (16 ms between
164 consecutive frames) using six synchronised Basler cameras (Basler acA 2040um-NIR)
165 positioned at different angles, similar to previous studies (Odenthal et al., 2020; Doussot et
166 al., 2021; Sonntag et al., 2024). One camera was mounted above the centre of the arena to
167 capture the bees' planar movements. Another one was positioned above the centre of the
168 cluttered area around the nest, while the remaining four cameras were arranged around the
169 arena to record the bees' positions from different perspectives, reducing triangulation errors
170 in 3D positioning. Recording commenced prior to the bees' entry into the setup, and the initial
171 60 frames were utilised to generate a background image of the arena. During a recording,
172 only image sections (40x40 pixels) exhibiting significant differences from the background
173 image (potentially indicating the presence of a bee) were saved to the hard drive along with
174 their coordinates. The recording scripts were written in C++. A custom neural network
175 analysed the image sections to determine whether they contained bees. Any crops showing
176 non-biological speeds (above 4 m/s (Goulson, 2010)) or implausible positions (outside the
177 arena) were manually reviewed. The trajectories were analysed using Python (version 3.8.17),
178 primarily with OpenCV. DeepLabCut (Nath et al., 2019) was used to identify the head and
179 abdomen positions to determine the orientation of the bee's body-length axis. A
180 comprehensive list of the packages used is available in the data publication
181 (<https://gitlab.ub.uni-bielefeld.de/a.sonntag/bumblebeelarningflights3d>).

182 Statistical analysis for hypothesis testing

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

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

206 Then we calculated the fixations of the nest for the full duration of the recorded learning flight
207 (we only excluded positions 5cm below the ceiling to remove possible positions where the
208 bees collided with the ceiling) similar to Robert et al (2017). The nest fixations are defined as
209 the times the nest position was stationary on the retina (Robert et al., 2018). Frames with a yaw
210 angle of ± 12.5 deg relative to the nest entrance were taken as one sample. The following
211 frames were added to this sample if the absolute angle difference was smaller than 2.4°
212 (150 deg /second as in Robert et al, 2017). One sample was only valid if it contained at least 10
213 consecutive frames (160ms, twice as in Robert et al (2017) to exclude false detections of nest

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

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

223 Results

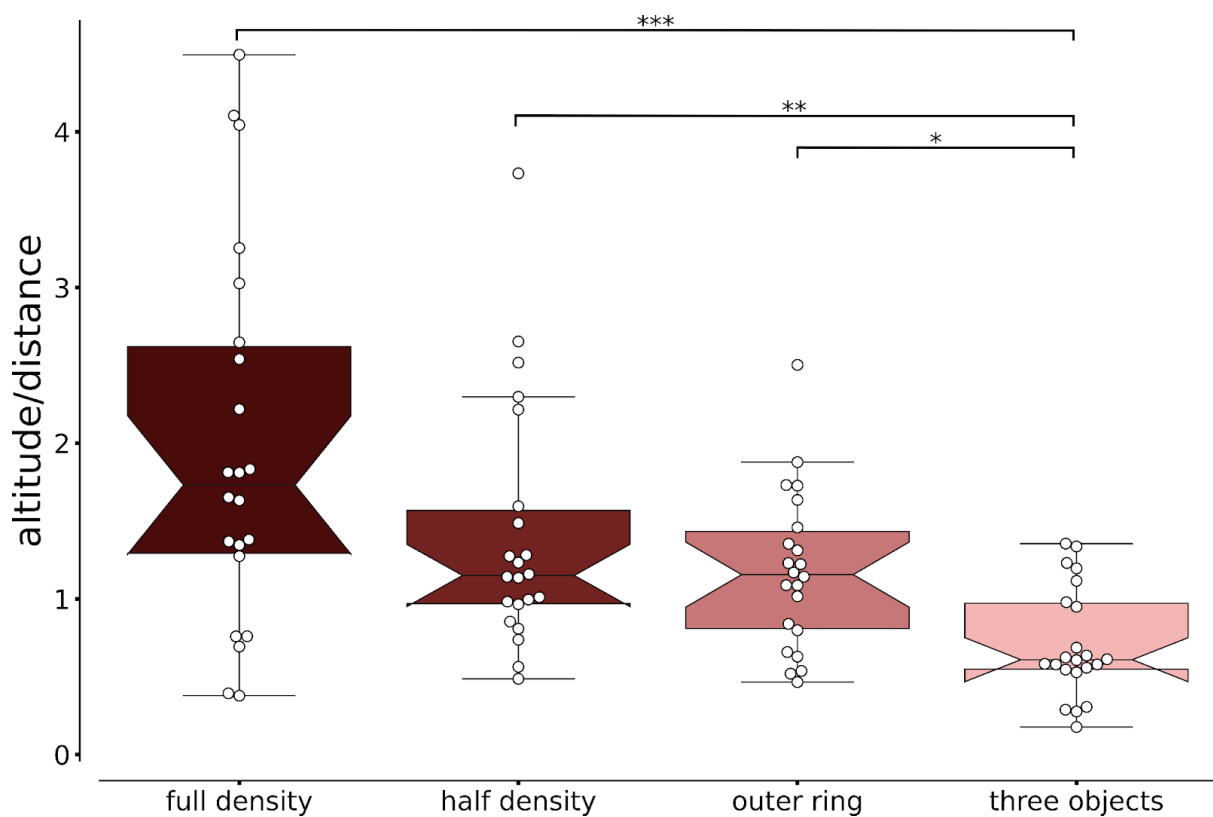
224 Bees increase flight altitude in dense environments

225 During learning flights, bees increase their altitude and distance to the nest (Capaldi et al.,
226 2000; Lobecke et al., 2018; Osborne et al., 2013; Woodgate et al., 2016). We investigated how
227 these flight characteristics are influenced by different constellations of objects. The initial part
228 of the first learning flight was compared between the four environmental conditions (Fig. 2).
229 When the nest entrance was surrounded by only three objects, an environment similar to
230 other studies (Cartwright and Collett, 1983; Dittmar et al., 2010; Linander et al., 2018; Lobecke
231 et al., 2018; Robert et al., 2018), we found a median altitude distance ratio of 0.61 (SD = 0.35)
232 indicating that the bees increased more their 2D distance to the nest entrance than their
233 altitude. In contrast, in the full density environment we find a median altitude distance ratio of
234 1.73, indicating a larger increase in altitude than in 2D distance, and a larger variance (SD =
235 1.2). To determine whether the density of the objects, and thus the distance between them
236 reflecting the degree of challenge of the environment for flight control, was responsible for
237 this effect, we reduced the object density to half of the number of objects in the same area. In
238 the half density environment, we found a median altitude distance ratio of 1.15 indicating an
239 increase of ratio between altitude and distance between the full density environment and the
240 environment with three objects. The variance with the half density environment (SD = 0.8) was
241 also in between that obtained for the full density environment and the three object
242 environment. Lastly, we tested how the distance of the objects to the nest influences the
243 altitude and distance increase. The outer ring environment resulted in intermediate values of

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

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

251



252

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

260 Dunn's post hoc test: $p_{\text{full density-three objects}} < 0.001$, $p_{\text{half density - three objects}} = 0.006$, $p_{\text{outer ring - three objects}} =$
261 0.043).

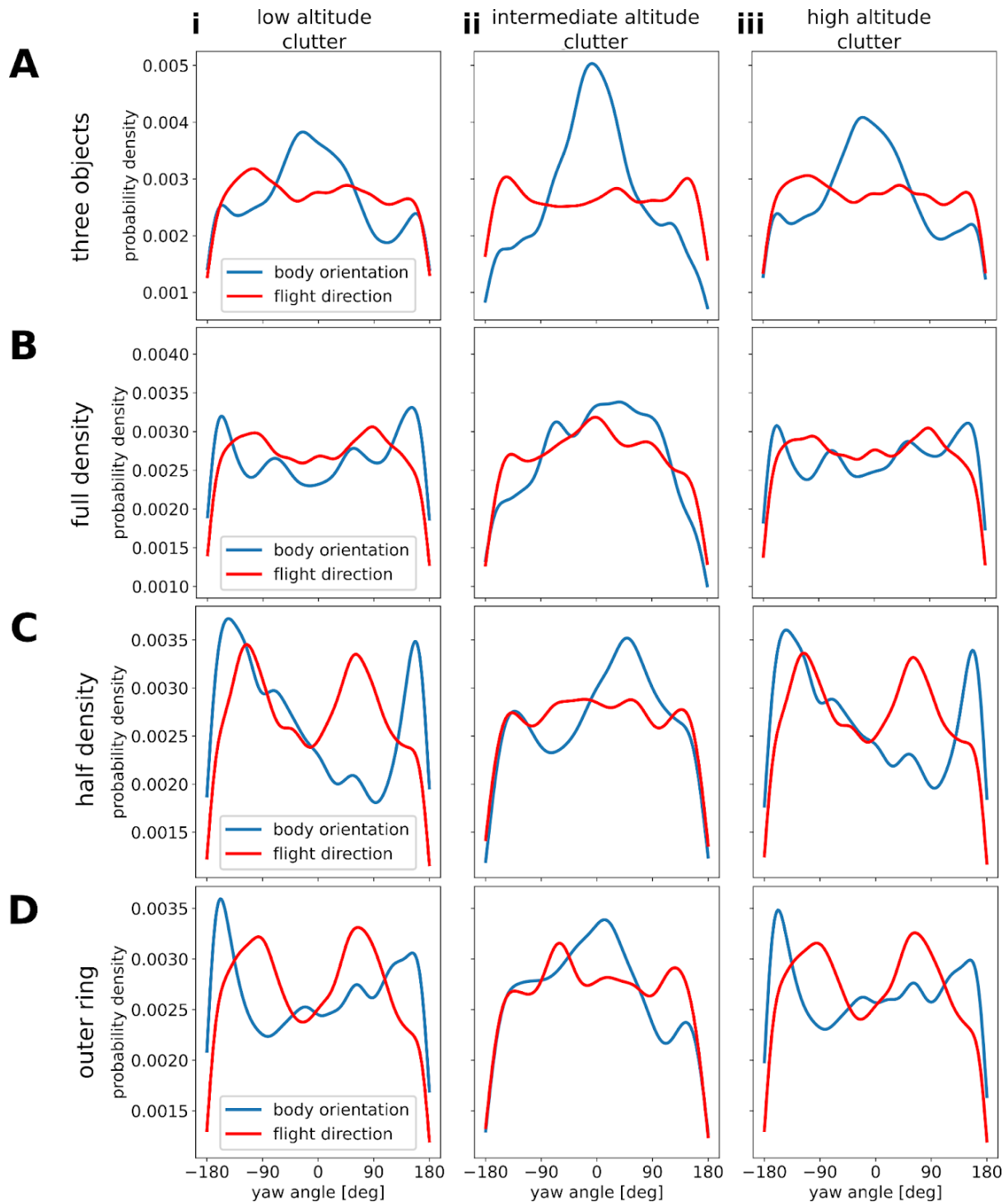
262 Dense environments disrupt nest orientation

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

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

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

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



283

284 Fig. 3: Kernel density estimation distributions of the body orientation (blue line) of the bees
 285 along the yaw angle and their flight direction (red line) relative to the nest (0 deg) for the four
 286 environments (A, i: N (number of bees) = 22, n (number of data points) = 31952; ii: N = 22, n =
 287 8236; iii: N = 22, n = 40188), full density (B, i: N = 22, n = 32803, ii: N = 22, n = 4724; iii: N = 22,
 288 n = 37527), half density (C, i: N = 22, n = 41532; ii: N = 22, n = 4703; iii: N = 22, n = 46235) and
 289 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

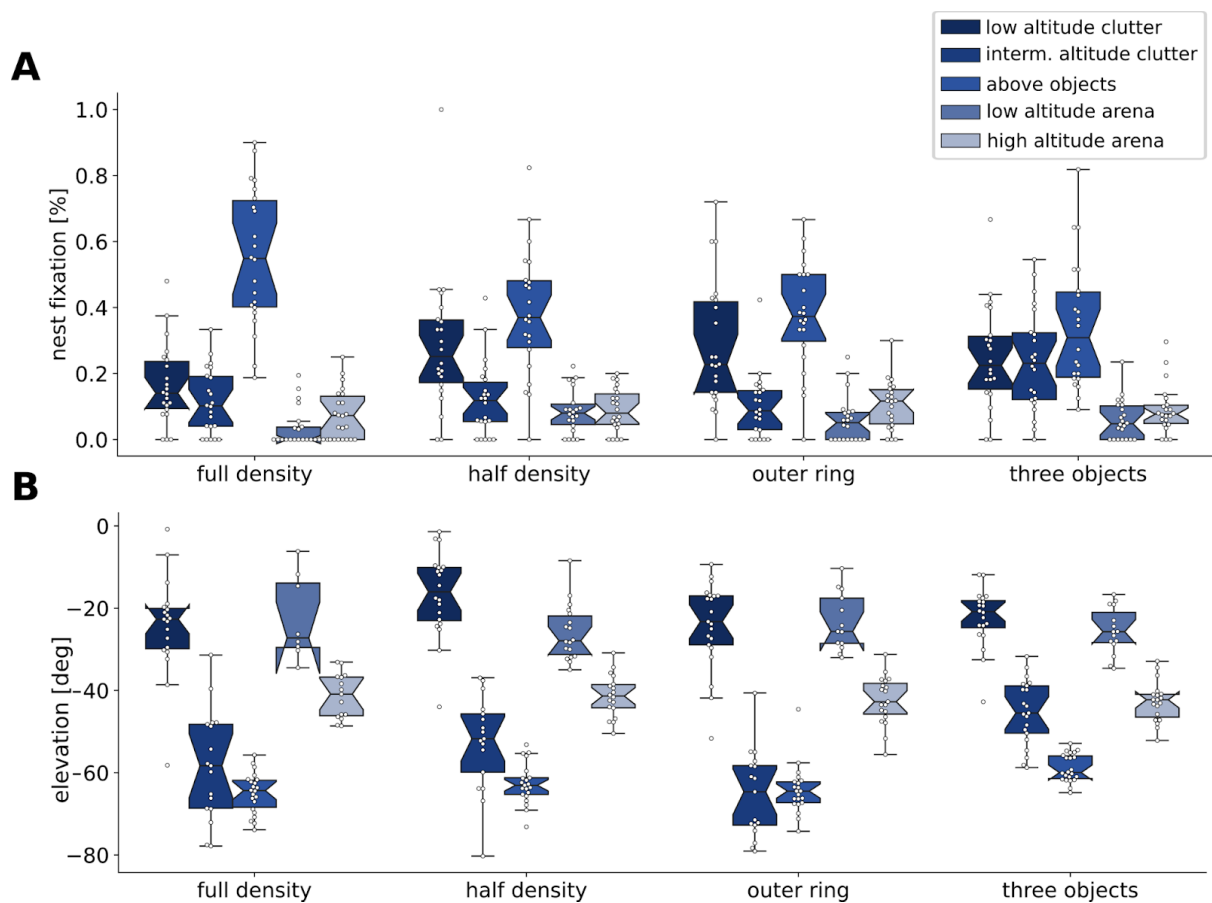
To examine how the positions where the bees fixated the nest were influenced by their environment we separated the bees' nest entrance fixations (for a definition, see Methods) in spatial categories, like we did for the body orientations.

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.

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

330



331

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

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

337 Discussion

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

348 Bees increase flight altitude in dense environments

349 Our study revealed significant differences in how bees adjust their altitude and distance from
350 the nest depending on the environmental complexity. In a sparse environment with only three
351 objects, bees increased their 2D distance to the nest more than their altitude. However, in
352 dense environments with more objects, they prioritised gaining altitude over 2D distance. This
353 demonstrates that bees adapt their flight strategies based on environmental constraints. In
354 cluttered environments, gaining altitude may provide better view points for visual learning
355 from above. Indeed, high-altitude views offer a broad perspective of the landscape, which
356 bees have been shown to use to identify ground-level landmarks and navigate over long
357 distances (Collett and Graham, 2015; Degen et al., 2016; Menzel et al., 2019; Brebner et al.,
358 2021). Flying above the clutter may also be a means to reduce the necessity of obstacle
359 avoidance. The increase in flight altitude in our study might therefore also be influenced by
360 the change in ventral optic flow due to the changes in the environmental features. Previously,
361 it was found that bees adapt their altitude in response to ventral spatial textures (Linander et
362 al., 2018). The higher variance in altitude-distance ratios in complex environments compared
363 to the sparse environment might point towards more diverse individual strategies when
364 navigating challenging landscapes.

365 Dense environments disrupt bees' nest orientation

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

379 Bees fixate the nest mostly above the objects

380 Across all our environmental conditions, bees showed a preference for fixating on the location
381 of the nest entrance when flying above the clutter, even though the nest entrance has been
382 hidden and its location may have been occluded by the clutter. This finding is particularly
383 interesting as it suggests that bees use elevated positions to gain clearer views of the nest
384 and its surroundings, potentially creating more reliable visual memories. Fixations at high
385 altitudes might raise the question of where the nest position was in the field of view of the
386 bee. A modelling study on honeybee vision indicated that the resolution of a bee's eye
387 decreases when observing at wide angles, especially as the vertical angles between
388 ommatidia become smaller (Stürzl et al., 2010). In addition, a morphological study on
389 bumblebees showed the limitations of the perception in the ventral direction (Taylor et al.,
390 2019). Therefore, observations of nest fixations at very low elevation angles should be
391 interpreted with caution. For the categories of low altitude within the dense environment and
392 the areas outside it, the elevation angles lay well in the perception range. For the intermediate
393 altitude within the dense environment and above the objects the nest fixations need to be
394 taken with caution as they are rather at the perceivable edge. Some fixations at high altitudes
395 might be at the border of perception of the visual field of bumblebees (Taylor et al., 2019) and

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

404 Implications and future directions

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

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

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439 Competing interests

440 The authors declare no competing or financial interests.

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445 Data and resource availability

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

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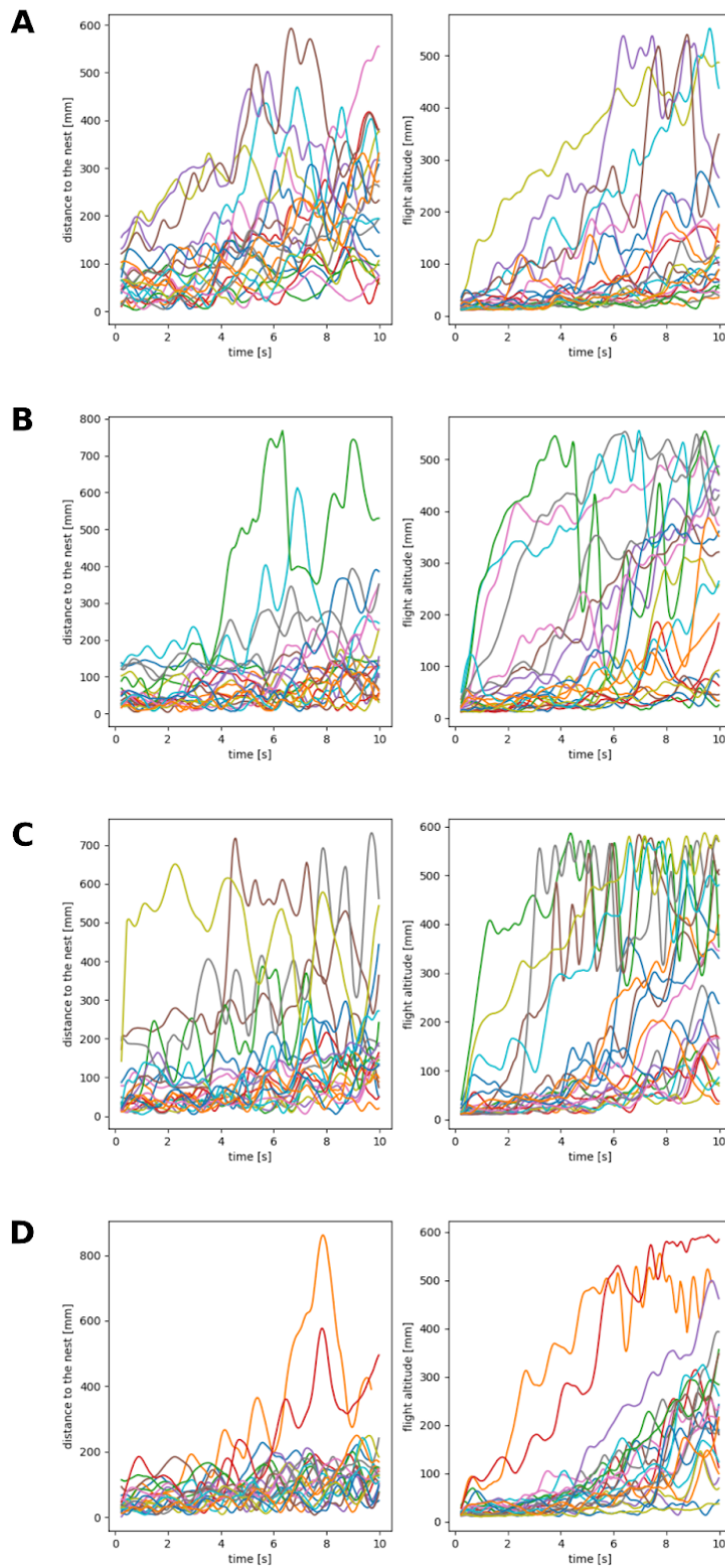
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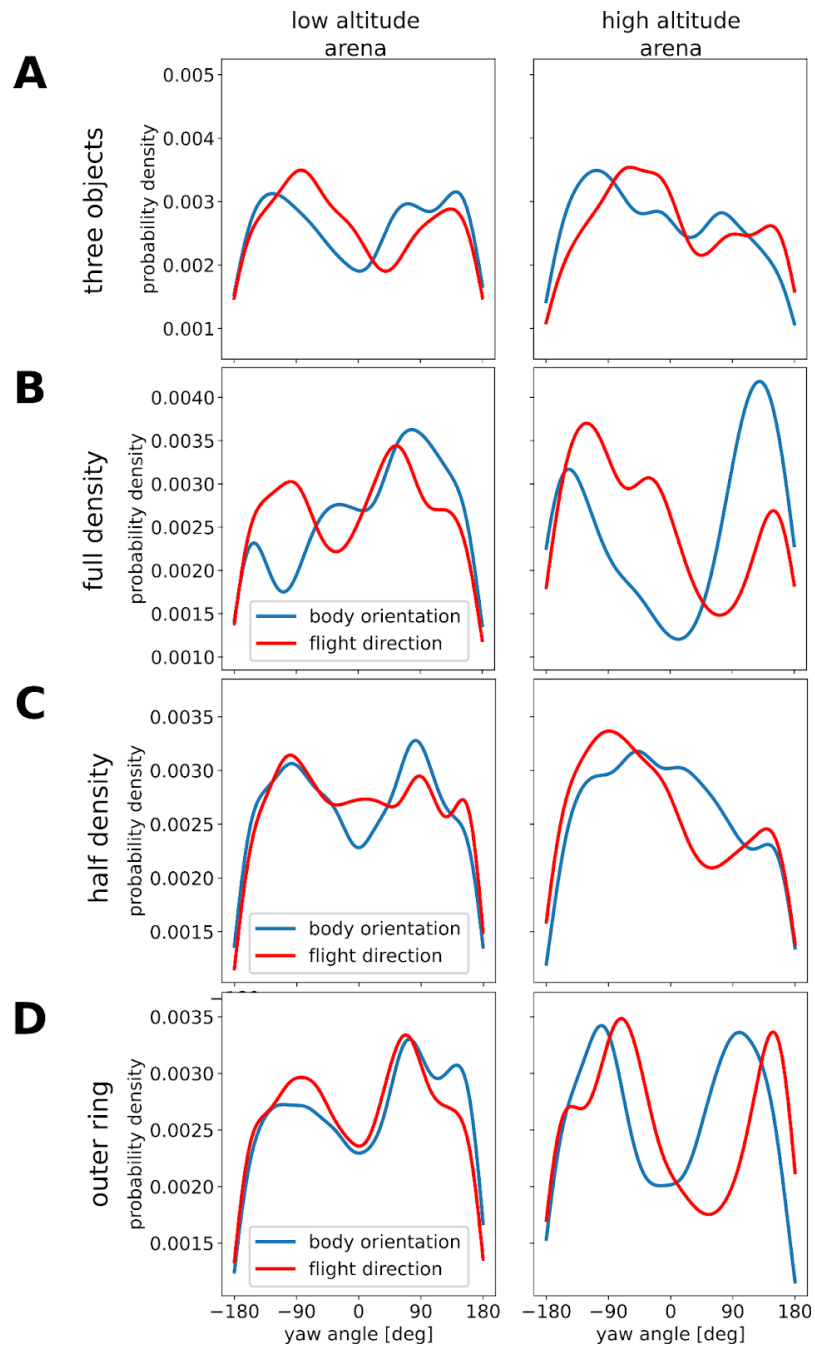
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560 Supplementary Material



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

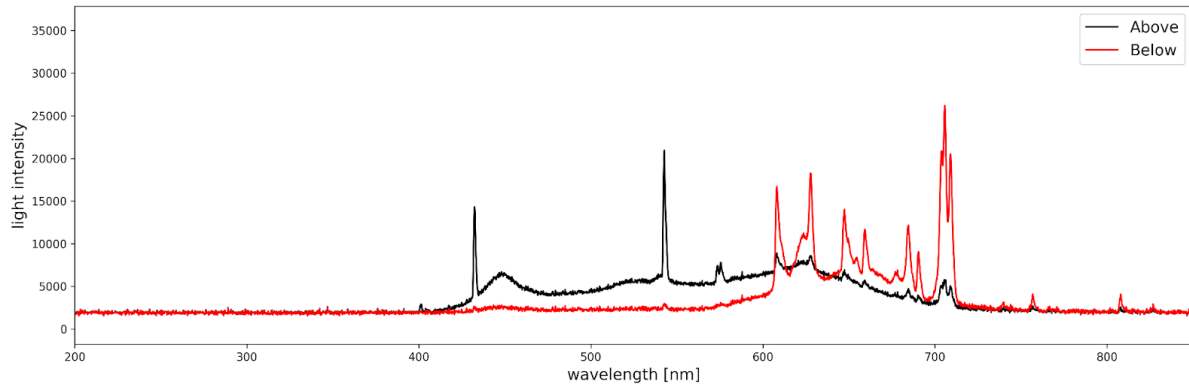
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566

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

570 1742), half density (C, i N = 20, n = 7780; ii N = 16, n =1836) and the outer ring (D, i N = 21, n =
 571 6735; ii N = 12, n = 2553) environments. For each environment the body orientation and flight
 572 direction are shown for different layers in the area outside the objects' arrangement (low and
 573 high altitude, below and above the height of the objects).



574

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

580

581 Tab. S1: Results of the Rayleigh test for circular uniformly distributed data. The statistical
 582 results of the z-value, p-value and significance level are given for each spatial area and the
 583 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	***

584

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586