

1 Landmark knowledge overrides optic flow in honeybee waggle dance distance
2 estimation

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4 Running title: Landmark memory and waggle dance distance code

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13 **Summary statement**

14 The distance code in the honeybee waggle dance is embedded in the landscape memory that bees
15 establish during their exploratory and their foraging flights.

16

17 **Abstract**

18 Honeybees encode in their waggle dances the vector (distance and direction) of an outbound
19 flight to a food source or a new nest side. Optic flow has been identified as the major source
20 of information in the distance estimation. Additional components of distance estimation were
21 also identified, e.g. the sequence of experienced landmarks. Here we address the question of
22 whether bees also use the landscape memory developed during exploratory orientation flights
23 to estimate distance. We took advantage of the fact that flights in a narrow tunnel lead to
24 further distance measures due to higher optic flow. We find that this effect is lost when bees
25 had explored the area in which the tunnel is located and when they have somewhat restricted
26 visual access to the surrounding environment through the mesh on top of the tunnel. These
27 data are interpreted in the context of other findings about the structure of navigational
28 memory in bees that develops during exploratory orientation flights. In particular, the data
29 suggest that bees embed distance measures into a representation of navigational space that
30 stores previously experienced landscape features.

31

32 **Key words:**

33 honeybees, waggle dance, optic flow, distance code, landscape memory, exploratory learning

34

35 **Introduction**

36

37 The waggle dance of honeybees encodes the distance and direction of the flight from the hive
38 to a food source or to a new nest site (von Frisch, 1967). A most important discovery about
39 the symbolic encoding of distance is the finding that the odometer of bees relies on optic flow
40 during the outbound flight (Esch and Burns, 1995; Esch and Burns, 1996; Srinivasan et al.,
41 2000). This was discovered by training bees in narrow tunnels that create higher optic flow
42 than what is experienced during flight in the open environment. Increased optic flow leads to
43 higher values of the distance code. In most experiments the distance measured by the bee was
44 determined by the duration of the waggle run during the bees' dances within the hive.

45 Additional and supportive information comes from experiments in which a feeder was located
46 inside the tunnel and bees were videotaped during their search flight when the feeder was
47 removed (Srinivasan et al., 1997). Combining these experimental approaches, it was possible
48 to exclude alternative measures of flown distance, e.g. energy consumption (Heran, 1956;
49 Heran, 1963), duration of flight, measuring and integrating airspeed, or some yet unknown
50 measure of wing movement. Accumulating all this rich and supporting evidence (review:
51 (Srinivasan, 2011)) it appears to be a well-established conclusion that the bee's odometer
52 receives its information only or predominantly from optic flow.

53 However, several observations indicate that additional or even alternative processes may also
54 contribute to distance estimation. (1) Bees trained along serially placed landmarks fly to both
55 the real distance of the feeder and the serially correct location if the distances between the
56 landmarks were either increased or decreased (Chittka and Geiger, 1995; Menzel et al.,
57 2010). Similar "counting" effects of serially arranged marks were found when these marks
58 were shown inside a tunnel (Dacke and Srinivasan, 2008a), indicating that both outside and
59 inside the tunnel landmarks provide additional reference points for distance estimation. (2)
60 Arranging a 6 m long tunnel in the open field at an angle of 90° to the direction of the
61 approach flight did not lead to an accompanying shift of the danced waggle direction,
62 rejecting the possibility that the flight in the tunnel contributes to a global vector based on a
63 path integration process only (De Marco and Menzel, 2005). (3) Interestingly, such a global
64 vector resulting from path integration was demonstrated by performing tunnel experiments in
65 which the bees flew in the first half-length under a transversely oriented polarization filter
66 (simulating a solar position that was directly ahead or behind the direction of flight), and the
67 second half-length under an axially oriented polarization filter (simulating a solar position that
68 was 90 deg to the left or the right of the flight direction) (Evangelista et al., 2014). These bees

69 signaled a food source direction of 45 deg in their waggle dances, indicating an L-shaped
70 flight with equal arm length, and thus integration of two paths under 90° direction. The
71 waggle run duration of around 230 ms was found to be within the range of the results of
72 (Srinivasan et al., 2000). Other than in the experiments of De Marco et al. (De Marco et al.,
73 2008), the bees performed their outbound and inbound flights inside the tunnel and had most
74 likely no access to external landmarks. (4) Srinivasan et al. (Srinivasan et al., 1997) found in
75 experiments with the tunnel that a landmark inside the tunnel enhances the accuracy with
76 which the bees searched for food, thus leading to a reduction of the error accumulation
77 process in optic flow measures. (5) It is known that feeders closer to the hive are more
78 attractive than more distant feeders of similar quality (e.g. sucrose concentration). Shafir et al.
79 (Shafir and Barron, 2010) arranged two tunnels such that one was shorter than the other
80 tunnel but induced higher optic flow (and thus should appear longer). Bees qualified the
81 shorter tunnel better in their dances although it was associated with higher optic flow. (6)
82 Dacke and Srinivasan (Dacke and Srinivasan, 2008b) concluded from their data that bees
83 appear to have two odometers, one that drives waggle dance communication and one they use
84 to estimate the total distance in their flights to a feeder they had visited before.
85 In all of these studies bees were trained to fly to a feeder in such a way that additional
86 parameters besides optic flow competed with the distance estimation. Here, we have taken a
87 different approach: we ask how the information from optic flow is integrated into what bees
88 have learned during their previous exploratory flights at the beginning of their lives as
89 foragers. Exploration of the environment is essential for bees before they start foraging
90 (Capaldi et al., 2000; Degen et al., 2015). Sequential learning flights increasing to distances >
91 100 m and varying in direction lead to a knowledge of the environment surrounding the hive
92 that allows them to find home from anywhere within the explored area via direct flights
93 (Degen et al., 2016). The memory established during exploration is best understood as
94 integrating egocentric, allocentric and compass information including local as well as global
95 guiding cues (Menzel, 2023). Such a memory would potentially allow extracting a flown
96 distance from this highly integrated form of spatial memory.
97 One may ask, therefore, how these different reference systems for distance estimation interact
98 and under which conditions one dominates the other or whether compromises are made when
99 information is inconsistent and bees have to communicate distance in the waggle dance. We
100 address this question by setting up tunnel experiments under conditions in which the bees
101 were differently familiar with the terrain in which the experiments were performed. For most
102 of the experiments the colonies were positioned in the environment more than 4 weeks before

103 the experiments started, ensuring that the foragers tested had explored the environment
104 outside of the tunnel. The surroundings were characterized by rich landmarks (trees, bushes,
105 houses). In one experiment they had explored a different environment and were relocated just
106 before the experiment. We found that the familiarity with the environment resulting from
107 exploratory flights (and possibly additionally from foraging flights to natural food sources),
108 rather than optic flow information, dominated the distance communicated in the waggle
109 dance.

110

111 **Materials and methods**

112 The experiments were performed with observation hives (containing approximately 3,000
113 bees each) in the summers 2022 and 2023. An IR camera (Raspberry Pi) monitored the dance
114 area close to the entrance/exit of the hive. The experimental site was a highly structured
115 domestic area in the village Amöneburg (Germany, 50°47'35.7"N 8°55'36.9"E) with trees,
116 bushes, houses, roads.

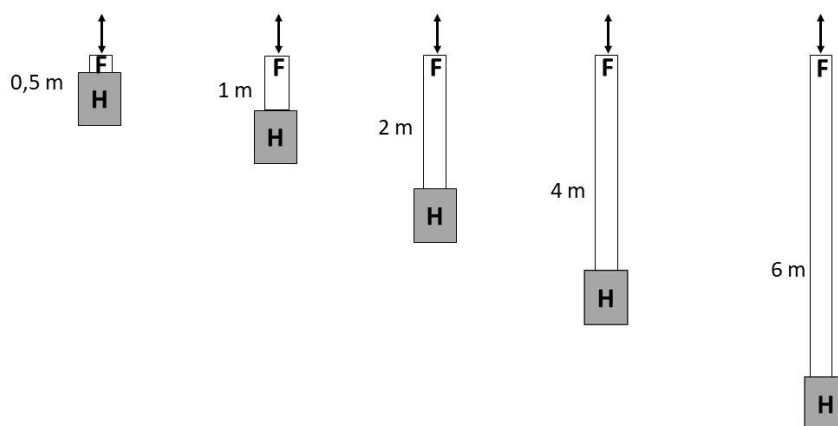
117 The flight tunnels were rather similar to those used by Srinivasan et al. (Srinivasan et al.,
118 2000), with length varying between 0.5 m and 6 m (in the preliminary experiment) and 6 m
119 (in the main experiments 1 to 7) with an inner width of 11 cm, and a height of 30 cm. The top
120 of the tunnel was covered with a metal-colored insect-screen (Fig. 1). Bees saw the sky above
121 them and rising landmarks in the surrounding within an angle approx. up to 60° during their
122 flights in the tunnel. In the main experiment bees saw the surrounding environment only
123 during the flight in the tunnel and not at all when they were feeding at the feeder F, because a
124 light tight box was mounted at the end of the tunnel containing the feeder allowing to observe
125 and monitor the marked bees (Fig. 1). The floor and the sides of the tunnel were covered with
126 a black and white random texture with pixel size of 1 cm by 1 cm. Bees were trained to a
127 feeder located outside, at the entrance of the tunnel or the end of the tunnel depending on
128 experimental design (Fig. 2, 3).



129

130 *Fig. 1 Left: view from within the tunnel as used during the preliminary experiments and the*
131 *experiments 1 – 6 of the main experiment. Right: view of the tunnel from outside. Note the*
132 *concealed ending: the feeder was in the dark, and the experimenter could enter the cover.*
133

134 Two sets of experiments were run. In the first set (preliminary experiments, Fig. 2) the tunnels
135 were of different length (0.5, 1, 2, 4 or 6 m) and attached to the entrance/exit of the hive such
136 that the end of the tunnel was always at the same location relative to the external landmarks.
137 The length of the tunnel was changed several times by moving the colony accordingly. A
138 feeder was always located at the end of the tunnel. The far end of the tunnel was open to
139 allow foraging bees not taking part in the experiment to fly in and out freely. Bees visiting the
140 feeder were marked with a white dot at the abdomen. Within the hive, dances of the marked
141 bees were visually observed via a video camera. A monitor displaying the images of these
142 recordings was set up behind the hive. The colony in the observation hive had long time
143 experience (at least 4 weeks) with their environment.

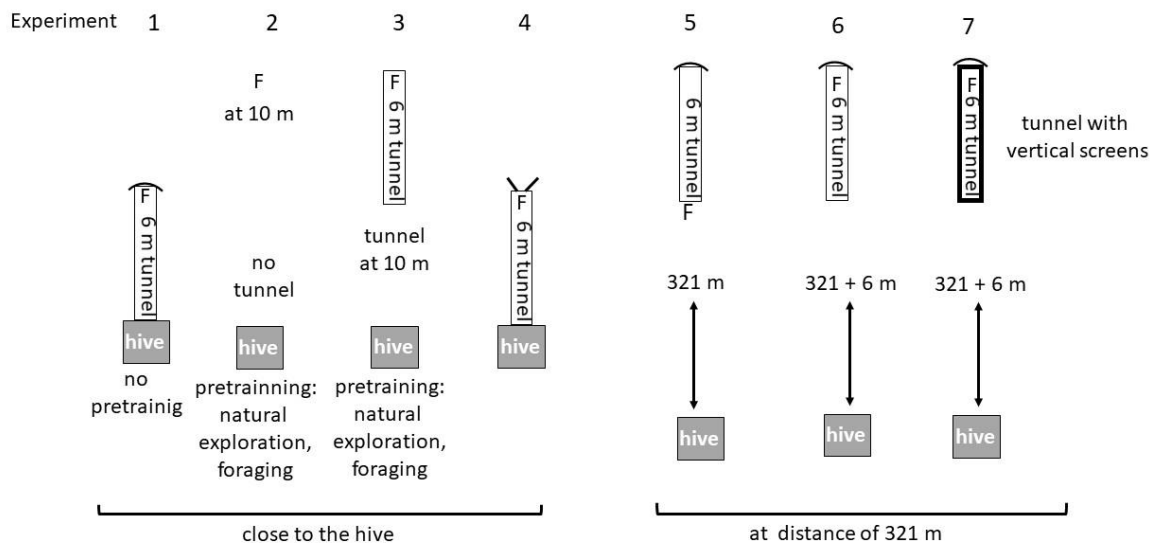


144
145 *Fig. 2 Preliminary experiments. The colony in the observation hive had long time experience*
146 *with the environment. Tunnels of different length (0.5, 1, 2, 4 or 6 m) were attached to the*
147 *front of the hive with the entrance hole such that the entrance/exit to the tunnel was always at*
148 *the same location. The feeder was close to the end inside the tunnel. The length of the tunnel*
149 *was changed several times by moving the colony accordingly. Bees visiting the feeder were*
150 *marked with a white dot at the abdomen. A monitor displaying the images of the IR video*
151 *camera recording the bees' dances within the hive was set up behind the hive, and dances of*
152 *the marked bees were visually observed and evaluated. Bees not trained to the feeder (thus*
153 *not marked) were free to move in and out at the end of the tunnel and were not included in the*
154 *on-line evaluated dances.*
155

156 In the main experiments the location of the tunnel, the location of the feeder and the far end of
157 the tunnel (open or closed) varied according to the individual design of the different
158 experiments (experiments 1 – 7, Fig. 3). Bees visiting the feeder were individually marked
159 with dual digit black and white number tags (or only pre-marked for experiment 1). The
160 number range for differently marked bees was enhanced to 470 by positioning the tags in 4
161 different directions on the thorax. The dances on the dance floor were video-taped using an IR
162 Raspberry Pi camera. The videos were analyzed off-line with the help of a custom written
163 video analysis script in Python that detected the location and the time of a waggle run,
164 stopped the video and opened a window that allowed to mark the start and the end of the
165 waggle run as detected by the first and the last frame in which the bee was unsharp due to her
166 fast-wagging movement. The video was recorded with 50 frames per second, and bees
167 appeared somewhat unsharp during the waggle run but not during normal walking or return
168 runs, allowing to set the frames for start and end of the waggle run accurately. The video
169 frame was calibrated for space and time and the following data were noted in the pop-up
170 window of the program and saved to file: duration of the waggle run, its length and the
171 number of waggles performed, and the direction of the waggle run relative to gravity. The
172 latter was used by the script to derive the angle to north in reference to the location of the
173 hive, the date and the time of the day. This procedure led to efficient and precise
174 measurements of large numbers of waggle runs. Furthermore, these data allowed to compare
175 the variance and the correlation of two possible codes of distance: the duration and the
176 number of waggles per waggle run. We found that number of waggles per waggle run varied
177 less than waggle duration, and therefore used this metric (see results section, Fig. 4). The
178 correlation between number of waggles and duration of the waggle run allowed us to relate
179 our data to published data that used duration as the distance code.

180 The tunnels of the main experiment were close to the hive in the experiments 1 – 4 and at a
181 further distance (321 m, coordinates: 50°47'46.1"N 8°55'35.5"E) in the experiments 5 – 7. As
182 mentioned above the bees flying in the tunnel could see the external environment within an
183 angle of approximately 60° during their flights in the tunnel because they always flew close
184 underneath the mesh. They did not see the environment during feeding. In experiment 1, the
185 colony was located first in an area about 4.5 kilometers from the experimental area behind a
186 hill (50°48'52.3"N 8°52'20.7"E). Many foraging bees were marked with a white dot at the
187 abdomen before the colony was moved. Then they were moved over night into the
188 experimental area, the 6 m long tunnel was attached to the hive and the far end closed. Thus,
189 the colony was naïve to the environment because it was moved into the test environment

190 shortly before the experiment started and the bees had not explored the test area yet. The
 191 feeder could be inserted and refilled without allowing bees to fly out or to approach it from
 192 the outside.
 193 Thus, foragers feeding at the end of the tunnel in experiment 1 had only experience with the
 194 tunnel and not with the environment around the tunnel. This was different in experiments 2 to
 195 7. The foragers in these experiments had explored the environment. They could reach the
 196 feeder only by flying through the tunnel. Experiments 2 and 5 were control experiments with
 197 a feeder at 10 m distance from the hive (experiment 2) or at 321 m distance (experiment 5)
 198 and no tunnel flight. In experiment 3 the entrance of the 6 m tunnel was at a distance of 10 m
 199 and the feeder was located at the end of the tunnel. In experiment 4 the tunnel was attached to
 200 the hive. The difference to experiment 1 was that the bees had experience with the
 201 surroundings before flying through the tunnel to the feeder at the end of the tunnel. In
 202 experiment 6, bees visited the feeder at the end of the tunnel. In experiment 7, two screens
 203 (2.5 m high) tightly attached to the right and left of the tunnel excluded the view of landmarks
 204 outside the tunnel but left the view to the sky.
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 207

208 *Fig. 3 Design of the experiments in the main experiment. Experiment 1: The tunnel was*
 209 *attached to the entrance of the hive prior to any exploratory flights, foraging flights or feeder*
 210 *(F) training. Experiment 2: The foraging bees were trained to a feeder at a distance of 10 m*
 211 *from the hive. Experiment 3: Experienced foragers were trained to the end of the 6 m tunnel.*
 212 *The entrance to the tunnel was at 10 m from the hive. Experiment 4: The tunnel was attached*
 213 *to the entrance of the hive after the bees had explored the environment and foraged at natural*
 214 *food sources. Experiment 5: experienced foragers were trained to a feeder (F) at a distance of*

215 *321 m and subsequently fed at this location. Experiment 6: bees from experiment 5 were*
216 *trained to a feeder (F) at the end of the 6 m tunnel. Experiment 7: The animals from*
217 *experiment 5 and 6 were further trained to the feeder of the tunnel but two screens (2.5 m*
218 *high) were attached to the side walls of the feeder. In this situation the animals could see the*
219 *sky but no landmarks surrounding the feeder. \blacktriangledown in experiment 4 indicates that the*
220 *entrance/exit to the hive via the tunnel is open for other foragers not feeding at F, \curvearrowright marks*
221 *the closed end of the tunnel that is covered with a box allowing access to the feeder and*
222 *blocking the view of the surrounding during feeding at F.*

223

224 Statistics and plotting were done using *Python* 3.9.15, *Pandas* 2.1.4, *Seaborn* 0.13.2,
225 *Statsmodel* 0.14.0, *SciPy* 1.11.3. Boxplots show quartiles, whiskers the full distribution,
226 except for outliers that are determined using a method in *Seaborn* that is a function of the
227 inter-quartile range.

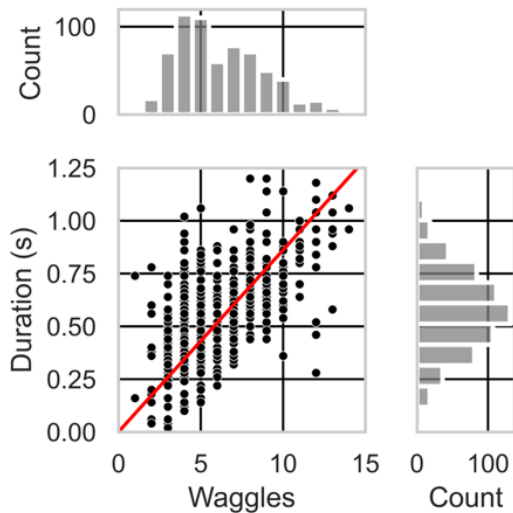
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229 **Results**

230 Number of waggles per waggle run as a code for distance

231 Our initial objective was to compare various parameters of the waggle run to identify which
232 of them had the least variation, and thus which would deliver the most accurate distance code
233 (recent review (Kohl and Rutschmann, 2021)). The number of dance rounds in 15 seconds,
234 commonly utilized by Karl von Frisch (von Frisch, 1967) in many experiments, exhibited
235 high variability; the length of the waggle run varied also considerably (data not shown).
236 Consequently, both measures were excluded from our analysis. Instead, we concentrated on
237 two parameters for the same waggle runs: the number of waggles per run and the duration of a
238 run. Both parameters were measured for the same waggle runs through video analysis in a
239 subset of our data, as described above. The duration of the waggle run displayed slightly more
240 variability than the number of waggles per run ($p < 0.01$, Levene Test). It is noteworthy that the
241 frequency distribution of the durations was close to a single Gaussian distribution and did not
242 show any indication of a double peaked distributions (Fig. 4, right histogram), while the
243 frequency distribution of waggle count/run showed two distinct peaks, one for experiments 1
244 and 6, and another one for experiment 7 (Fig. 4, top histogram), revealing that the lower
245 variability in this metric kept the two distinct distributions visible. Therefore, for the bulk of
246 our measurements, we evaluated the waggle count metric. A linear regression gave a slope of
247 0.086 s per waggle.

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249 .

250 *Fig. 4 Plot of waggles/run against duration/run, for experiments 1, 6 and 7 of the main*
251 *experiment group, with histograms for both at the side. Note that the histogram for waggles*
252 *has two peaks: at 5 waggles/run corresponding to experiments 1 ($n=84$, $\text{mean}\pm\text{std}$: 4.1 ± 1.1)*
253 *and 6 ($n=233$, $\text{mean}\pm\text{std}$: 4.3 ± 1.3), and at 7 waggles/run corresponding to experiment 7*
254 *($n=337$, $\text{mean}\pm\text{std}$: 8.1 ± 2.1). The higher variability in duration smears the distribution to a*
255 *single peak ($\text{mean}\pm\text{std}$ for duration, Exp. 1, 6, 7: 0.43 ± 0.18 , 0.48 ± 0.19 , 0.67 ± 0.18).*

256

257 Dances during the preliminary experiments

258 The design of the preliminary experiment allowed to distinguish between round dances and
259 waggle dances. The observation hive was positioned on a trolley, allowing for quick mobility
260 of the hive while the entrance/exit of the tunnel and the feeder at the end of the tunnel
261 remained stationary. This is a necessary requirement because bees learn the surroundings of
262 the hive very accurately, and in these experiments all bees accessed the hive via the tunnel
263 irrespective of its length and whether they were trained to a feeder at the end of the tunnel or
264 flew to natural food sources. Outbound and inbound bees not feeding at the feeder
265 accommodated very quickly to the changing length of the tunnel. Thus, the entrance/exit to
266 the observation hive via the tunnel remained stable, while the length of the tunnel varied (see
267 Fig. 2). These test conditions facilitated the alteration of tunnel length, ensuring that bees
268 encountered the access to the hive via the tunnel while maintaining rather constant spatial
269 relations to the environment. Based on the literature we expected waggle runs under 4 of the 5
270 test conditions (i.e. in all conditions with the tunnels longer than 0.5 m).

271 Prior to the start of the preliminary experiments, foragers from the colony experienced a
272 condition with a short tunnel (0.5 m) for several weeks. A feeder was positioned 10 cm from
273 the entrance/exit within the tunnel, and bees visiting the feeder were identified by marking

274 them with a white dot on the abdomen. The tunnel's length was modified once at least 50
275 dances were observed. Multiple rounds of semi-random insertions of tunnels with varying
276 lengths were conducted, and the feeder was always 10 cm from the entrance/exit of the tunnel
277 irrespective of its length. Only round dances, and no waggle dances, were observed in marked
278 bees for all 5 test conditions. This is an important and rather surprising finding because one
279 would have expected that under the conditions of this experiment either only waggle dances
280 were performed in four of the five test conditions, or an increasing number of waggle runs
281 with increasing tunnel lengths. Thus, our results falsified the hypothesis we had in mind when
282 we started this experiment (see above).

283 A substantial difference to the experiments of (Srinivasan et al., 2000) was that our tunnel
284 flying bees could see the surrounding environment which they had learned before during their
285 exploratory orientation flights. Furthermore, although the bees feeding at the feeder inside the
286 tunnel and close to its far end predominantly shuttled between the feeder and the access to the
287 hive at the other end of the tunnel, some of them may have flown out of the tunnel from time
288 to time since the far end of the tunnel was not closed. These considerations lead to designing
289 the main experiment in which the potential effects of the exploratory experience with the
290 natural environment prior to the tunnel flights were systematically tested.

291 Dances during experiments 1 – 7 in the main experiment

292 Seven experiments were run in the main experiment (Fig. 3). In experiment 1, the colony in
293 the observation hive was first located for 5 weeks in an area approximately 4.5 km away from
294 the experimental area (50°48'52.3"N 8°52'20.7"E). The landscape here (agricultural fields,
295 grass land) was very different from that of the experimental area (domestic area in the
296 village). In the last week before moving the hive, many foragers were marked with a white
297 dot on the abdomen. The foragers were not trained to a feeder, and the natural food supply was
298 scattered over larger distances (> 200 m) and rather scarce. Before the experiment, the hive
299 was moved during the night, a 6 m tunnel was attached to the entrance/exit, and the tunnel
300 was closed at the far end. A feeder was placed at the far end within the tunnel, and a small
301 shelter allowed to examine the feeder and refill it. Videos of the dance floor were recorded
302 with an IR camera and the dances analyzed off-line using the procedure described in the
303 Method section. In experiment 2 – 7 the colony in the observation hive was located in the
304 experimental area for 3 weeks before the experiment started.

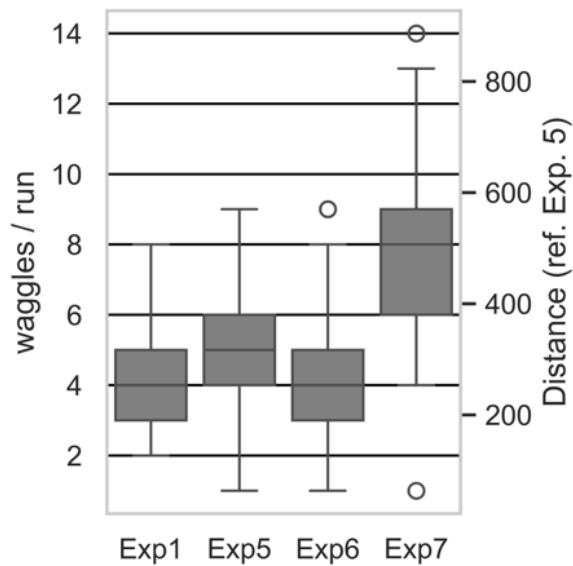
305 In experiment 1 three sessions of 30 minutes recording each were analyzed. The marked
306 bees predominantly performed waggles dances (n=298, waggles/run mean±std: 4.1±1.1, Fig.
307 5); 2 round dances were observed. Thus, flights in the 6 m tunnel with view to an unexplored

308 environment led to waggle runs, indicating that the optic flow in the tunnel elicited a long-
309 distance dance (see below for calibration).

310 No tunnel was used in experiment 2, and the feeder was located 10 m from the hive in the
311 direction of 351° to N. Four sessions of 30 minutes each were video recorded. We observed
312 only round dances but no waggle runs, in accordance to the short distance flown. However,
313 the same result was found also for experiments 3 and 4: only round dances. In experiment 3
314 the entrance/exit to the 6 m tunnel was at distance of 10 m from the hive, direction 354° to
315 N, feeder at the end of the tunnel, and in experiment 4, the 6 m tunnel was attached to the
316 hive and the feeder at the end of the tunnel. This latter experiment required a different
317 colony because a new colony had to be brought into the experimental area, and the foragers
318 learned to access the hive through the tunnel. Therefore, experiment 4 was carried out at
319 the time when colony 1 was exposed to a different environment (experiment 1). The results
320 show that in experiments 3 and 4, bees danced for a location in the immediate vicinity,
321 despite of having flown through the tunnel (which simulated long-distance in experiment 1).
322 Therefore, in the next experiments 5-7, we asked under which conditions does a tunnel
323 simulate a long-distance, and when does it not.

324 In experiments 5 – 7, foragers familiar with the landscape were trained to a distant location
325 (321 m, direction 354° to N). In experiment 5 the feeder was at the entrance of the 6 m
326 tunnel (bees did not fly through the tunnel) and in experiment 6 at the end of the 6 m
327 tunnel. In experiment 7 the feeder was also at the end of the tunnel but two screens (2.5 m
328 high) excluded the view of landmarks outside the tunnel and left the view to the sky. As
329 expected, foragers performed waggle dances in all these conditions (Fig. 5).

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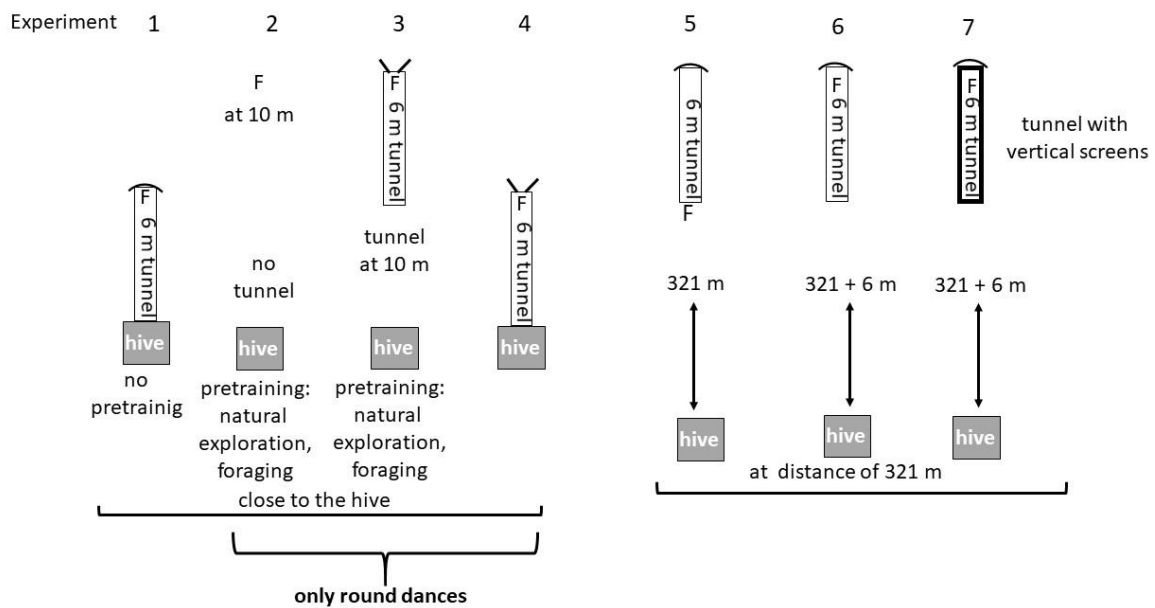
333 *Fig 5. Distribution of waggles/run in experiments 1, 5, 6 and 7. Boxes show quartiles,*
334 *whiskers rest of distributions, except for outliers (see methods). Right ordinate axis shows*
335 *distances in meters indicated by the waggle dance, calibrated with experiment 5 (no tunnel).*
336 *(see S2 for data and statistics).*

337

338 In experiment 5, bees danced about 5 waggles/run to indicate the 321 m distance (n=227,
339 mean±std: 5.1±1.5); in experiment 6, with the added 6 m tunnel, the waggles did not
340 increase (n=547, mean±std: 4.2±1.2); in experiment 7, when shielding the 6 m tunnel from
341 the surroundings, the waggles increased (n=456, mean±std: 7.6±2.2; Fig. 5). A generalized
342 linear model analysis (Poisson model family, log link function, IRLS method with post-hoc
343 testing) showed no significant difference between the results in experiment 1 and 6.
344 Experiment 5 and experiment 7 differed significantly ($p < 0.001$). Even though the difference
345 between experiments 5 against 1 and 6 is significantly different, the ranges are strongly
346 overlapping (see Fig. 5), suggesting that this difference may not have a biological relevance.
347 However, the distribution ranges of experiment 7 are clearly distinct (Fig. 5): here flying
348 through the tunnel led to a highly relevant and significant increased distance as signaled in
349 the waggle dance.

350 We used the data from experiment 5 to calibrate the distance code for number of waggles:
351 each waggle/run indicated a 63.3 m distance (approx. 5 waggles/run for the known 321 m
352 distance, see Fig.5). Applying this calibration, bees indicated a fictive distance for the 6 m
353 tunnel of 262 m in experiment 1. A similar distance was danced in experiment 6 (265 m,

354 short of the really flown distance of 321+6 m). In experiment 7, bees danced 481 m. If we
 355 subtract the open distance of 321 m, this indicates a danced tunnel length of 220 m, i.e.,
 356 slightly shorter than in exp. 1 (see right ordinate scale in Fig. 5). This experiment also shows
 357 that bees add up distance in free flight with the distance within the tunnel, when flying both
 358 sequentially. Taken together, these data suggest that the environment surrounding the
 359 tunnel provides information for distance coding if the dancer is familiar with the
 360 environment (in experiment 6 the bees did not experience the tunnel as a long distance,
 361 while in experiment 7, with no view of the environment, the tunnel was experienced as a
 362 long distance).



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Exp. 1		Exp. 5	Exp. 6	Exp. 7
4.1 ± 1.1	waggles	5.1 ± 1.5	4.2 ± 1.2	7.6 ± 2.2
298	n	227	547	456
0.356 s	duration (calc)	0.437 s	0.360 s	0.655 s
	m/waggle	63.3 m	78.1 m	
	danced distance (ref exp 5)	321 m	265 m	481 m
262 m	danced tunnel length (5)	59 m	3 m	220 m
	danced distance (ref exp 6)	396 m	327 m	594 m
323 m	danced tunnel length (6)	73 m	4 m	271 m

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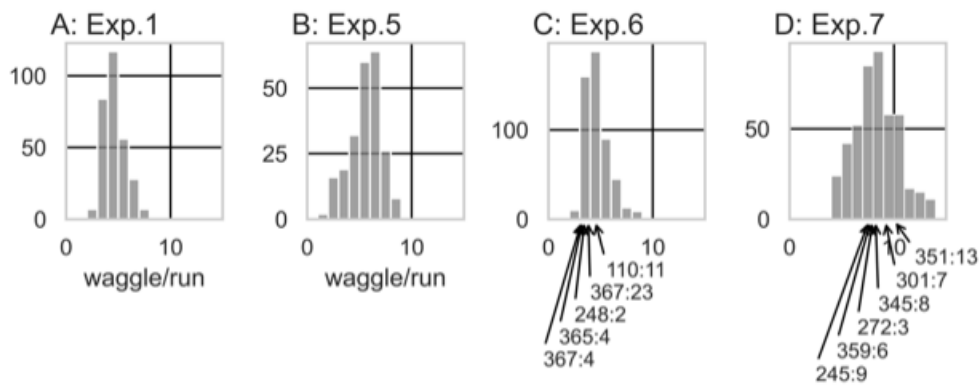
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369 *Fig. 6 Summary of the results on dances in the main experiment. The upper part of the figure*
370 *repeats the design of the 7 experiments, the lower part gives the data for the experiments 1,*
371 *5, 6 and 7 together with the calculations of distances from the number of waggles and the*
372 *corresponding durations as calculated from Fig. 4.*

373

374 The observations in the preliminary experiments (Fig. 3) and the video analyses of
375 experiments 2, 3, 4 and 6 showed clearly that the input from the environment dominated
376 the distance coding when the dancers had explored the environment. However, it could be
377 that the average values of waggles per run in these experiments may have resulted from
378 some sort of switching between the competing inputs. We plotted the distribution of
379 waggles/run for each experiment in order to investigate whether there was any evidence for
380 independent dual information (Fig. 7). We found that the frequency distribution was close to
381 Gaussian with no indication of double peaked distributions in any of the experiments.

382



383

384 *Fig. 7 Histograms of waggles/run in experiments 1, 5, 6 and 7. In each experiment, a single*
385 *prominent peak is visible. Note the high values in experiment 7 as compared to experiments*
386 *1, 5 and 6. In experiments 6 and 7, the values for identified bees are indicated with arrows*
387 *toward the abscissa, the label indicates the bee identity, and the number of averaged*
388 *(observed) waggle dances (e.g.: bee number 110 had 11 evaluated dances in experiment 6).*

389

390 Marking individual bees with number tags allowed us to further address the question
391 whether individual dancers exposed to competing conditions may differ in coping with this
392 situation. We had marked 368 foragers with number tags and hoped that they would forage

393 in both experiments 6 and 7 allowing us to see at the individual level whether they would
394 deal with the test conditions differently (Fig. 7). Unfortunately, no tagged dancers were seen
395 in our videos that were exposed to both test conditions. However, calculating the average of
396 waggles per waggle run for each of the individuals separately for the two test conditions
397 allowed us to reject the possibility that some individuals may have weighted the two inputs
398 differently.

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402 **Discussion**

403 The flight through the 6 m tunnel simulated a flight distance of 262 m (experiment 1, tunnel
404 close to the hive) or a distance of 220 m (experiment 7, tunnel further away from the hive) if
405 environmental information at the test site was excluded, thus confirming the finding that optic
406 flow is a major factor for bees to estimate flight distance. The data reported in (Srinivasan et
407 al., 2000) (their Fig. 2, experiments 2 and 4) indicated optic-flow induced distances of a
408 similar 6 m tunnel of 184 m (close to the hive) and 230 m (further away from the hive). These
409 results are rather close to the result reported here given the condition that different colonies
410 were used and the tunnels had different heights. Our tunnel was 30 cm high and their tunnel
411 20 cm high. The corresponding durations of waggle runs in (Srinivasan et al., 2000) were 441
412 ms for the tunnel close to the hive and 529 ms for the tunnel further away from the hive,
413 which is in the same order as the results found here: applying the conversion of number of
414 waggles to duration of 0.086 s per waggle (Fig. 4) we obtained 356 ms waggle run duration
415 for the close tunnel (Exp. 1) and 408 ms for experiment 7 (Fig. 6).

416 The data presented here add an important component to distance estimation in honeybees that
417 goes beyond the measurement and the encoding/decoding of distance in the waggle dance.

418 We show that knowledge of the environment surrounding the tunnel can override the optic
419 flow effect. This partly corroborates findings cited in the introduction with respect of effects
420 of serial landmark learning (Chittka et al., 1995; Menzel et al., 2010), “counting” phenomena
421 (Dacke and Srinivasan, 2008a), reduced error accumulation through serial landmarks
422 (Srinivasan et al., 1997), and importantly the discovery of two odometers in bees (Dacke and
423 Srinivasan, 2008b). In addition, the study by (De Marco and Menzel, 2005) showed that large
424 range path integration collapses when bees fly out of the tunnel that was arranged 90° to the
425 access flight to the feeder. Interestingly, unlike the results presented here, the optic flow effect
426 was not overridden in that study when the bees continued flying the same direction inside of

427 the tunnel as in the access flight, even though the view of the environment was not blocked.
428 This can be explained by the special conditions of the environment around the tunnel in their
429 study. The experiments were carried out in a large, flat and horizontal grassland without rising
430 objects and a flat horizon. The bees saw the environment only in the moment when they left
431 the tunnel, and in that moment their knowledge of the environment took over. The differences
432 between (Evangelista et al., 2014) and (De Marco and Menzel, 2005) on path integration can
433 also be resolved on the basis of our data reported here. Flights only inside the tunnel with
434 little or no view of the environment as in the case of the Evangelista 2014 (Evangelista et al.,
435 2014) study restrict the distance measure to optic flow, whereas the moment the bees leave
436 the tunnel and return back to the hive in flight through the open they will refer their distance
437 measure to the landscape memory.

438 What we have termed “knowledge of the environment” and “landscape memory” here should
439 be understood as a technical term catching the consequences of exploratory learning.
440 Conceptually, this memory could have two forms within the bees’ neural networks: either,
441 each point of the known environment is elementally associated to a homing vector, or,
442 different points of the known environment are connected in a navigational map. The
443 elemental hypothesis would mean that in our experiments bees referred to a list of
444 independent memories storing associations between single and specified landscape features
445 associated with the homing vector. Such an elementary form of landscape memory cannot
446 strictly be excluded by these experiments. However, this might be quite costly for the bee
447 brain in terms of necessary memory capacity, and offers little flexibility in natural
448 environments with all their daily and seasonal changes. We argue, therefore, that the data
449 reported here can be explained in a more parsimonious way, i.e. using a navigational map.
450 Exploratory learning of a navigational map differs from elemental target associative learning
451 in several important aspects (Birke and Archer, 1983; Gallistel, 1990; Renner, 1988; Tolman,
452 1948). The process of exploration is an attention inducing and rewarding process in itself
453 accompanied with active movement. Sequentially experienced and spatially separated objects
454 are bound together leading to a representation of organized space, and multiple experiences of
455 similar cues (both of the egocentric and allocentric domain) will make the spatial memory
456 richer and more precise (Chen and Mou, 2024; Hilton and Wiener, 2023). It has been argued
457 that multiple exploratory flights lead to memory storage and retrieval processes that appear to
458 bind together separate memories through generalization process, memory updating,
459 completion and correction (Menzel, 2023). Taken together these multiple and independent
460 data sets allow to interpret the memory structure resulting from exploration as being

461 substantially different from memory resulting from associative learning. In our view, this
462 form of spatial/temporal representation is best captured by the term navigational map,
463 sometimes referred to as cognitive map (Jeffery et al., 2024; Tolman, 1948). Extraction of
464 elementary components from such global representations, e.g. the distance between objects in
465 explored space, requires the retrieval of specified memory of the appropriate part of space and
466 its spatial connection to other locations, e.g. the center of life (nest) or recently visited
467 locations (feeding places). The accumulating supporting evidence in favor of such a form of
468 spatial representation in waggle dance followers allows to conclude that the endpoint of the
469 symbolically encoded flight vector (distance and direction) is represented as a location in this
470 spatial memory (Wang et al., 2023). The kind of cognitive operations are performed most
471 likely both in waggle dancers and in waggle dance followers because dancers frequently
472 switch between foraging, dance following and dancing. Taken together, we conclude that the
473 measure of distance as expressed in the dance is embedded in the global representation of the
474 explored space. Phenomena like dancing for a food source after a detour flight (e.g. around a
475 mountain (von Frisch, 1967) , p. 174 – 178) or uphill could mean that dancers and followers
476 estimate the true distance (further distance) by referring their flight to the learned
477 characteristics of the landscape. In an ecological context, trips need to be planned taking into
478 account changing properties of the environment and weather conditions.

479

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483 cameras.

484

485

486 **Competing interests**

487 No competing interests are declared.

488

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491

492 **Data availability**

493 All data will be placed in a public repository upon publication.

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497 **References**

498 **Birke, L. I. and Archer, J.** (1983). Some issues and problems in the study of animal
499 exploration. *Exploration in animals and humans*, 1-21.

500 **Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R.,**
501 **Edwards, A. S., Martin, A., Robinson, G. E., Poppy, G. M. et al.** (2000). Ontogeny of orientation flight
502 in the honeybee revealed by harmonic radar. *Nature* **403**, 537-540.

503 **Chen, Y. and Mou, W.** (2024). Path integration, rather than being suppressed, is used to
504 update spatial views in familiar environments with constantly available landmarks. *Cognition* **242**,
505 105662.

506 **Chittka, L. and Geiger, K.** (1995). Can honeybees count landmarks? *Anim. Behav* **49**, 159-164.

507 **Chittka, L., Geiger, K. and Kunze, J.** (1995). The influences of landmarks on distance
508 estimation of honey bees. *Anim. Behav* **50**, 23-31.

509 **Dacke, M. and Srinivasan, M. V.** (2008a). Evidence for counting in insects *Anim Cogn* **11**, 683-
510 689.

511 **Dacke, M. and Srinivasan, M. V.** (2008b). Two odometers in honeybees? *J Exp. Biol* **211**,
512 3281-3286.

513 **De Marco, R. J., Gurevitz, J. M. and Menzel, R.** (2008). Variability in the encoding of spatial
514 information by dancing bees *J Exp. Biol* **211**, 1635-1644.

515 **De Marco, R. J. and Menzel, R.** (2005). Encoding spatial information in the waggle dance. *J.*
516 *Exp. Biology* **208**, 3885-3894.

517 **Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblöfsky, M.,**
518 **Winter, S., Georgieva, P. B., Nguyen, H. et al.** (2016). Honeybees Learn Landscape Features during
519 Exploratory Orientation Flights. *Current Biology* **26**, 2800 - 2804.

520 **Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblöfsky, M.,**
521 **Winter, S., Georgieva, P. B., Nguyen, H. et al.** (2015). Exploratory behaviour of honeybees during
522 orientation flights. *Animal Behaviour* **102**, 45-57.

523 **Esch, H. E. and Burns, J. E.** (1995). Honeybees use optic flow to measure the distance of a
524 food source. *Naturwiss* **82**, 38-40.

525 **Esch, H. E. and Burns, J. E.** (1996). Distance estimation by foraging honeybees. *Journal of*
526 *Experimental Biology* **199**, 155-162.

527 **Evangelista, C., Kraft, P., Dacke, M., Labhart, T. and Srinivasan, M.** (2014). Honeybee
528 navigation: critically examining the role of the polarization compass. *Philosophical Transactions of the*
529 *Royal Society B: Biological Sciences* **369**, 20130037.

- 530 **Gallistel, C. R.** (1990). *The Organization of Learning*. Cambridge, Mass., London: MIT Press.
- 531 **Heran, H.** (1956). Ein Beitrag zur Frage nach der Wahrnehmungsgrundlage der
532 Entfernungsweisung der Bienen. *Z. vergl. Physiol* **38**, 168-218.
- 533 **Heran, H.** (1963). Wie beeinflusst eine zusätzliche Last die Fluggeschwindigkeit der
534 Honigbiene? *Verh. Dt. Zool. Ges. Wien* **26**, 346-354.
- 535 **Hilton, C. and Wiener, J.** (2023). Route sequence knowledge supports the formation of
536 cognitive maps. *Hippocampus* **33**, 1161-1170.
- 537 **Jeffery, K. J., Cheng, K., Newcombe, N. S., Bingman, V. P. and Menzel, R.** (2024). Unpacking
538 the navigation toolbox: insights from comparative cognition. *Proceedings of the Royal Society B* **291**,
539 20231304.
- 540 **Kohl, P. L. and Rutschmann, B.** (2021). Honey bees communicate distance via non-linear
541 waggle duration functions. *PeerJ* **9**, e11187.
- 542 **Menzel, R.** (2023). Navigation and dance communication in honeybees: a cognitive
543 perspective. *Journal of Comparative Physiology A*, 1-13.
- 544 **Menzel, R., Fuchs, J., Nadler, L., Weiss, B., Kumbischinski, N., Adebisi, D., Hartfil, S. and**
545 **Greggers, U.** (2010). Dominance of the odometer over serial landmark learning in honeybee
546 navigation *Naturwissenschaften* **97**, 763-767.
- 547 **Renner, M. J.** (1988). Learning During Exploration: The Role of Behavioral Topography During
548 Exploration in Determining Subsequent Adaptive Behavior in the Sprague-Dawley Rat (*Rattus*
549 *norvegicus*). *International Journal of Comparative Psychology* **2**.
- 550 **Shafir, S. and Barron, A. B.** (2010). Optic flow informs distance but not profitability for
551 honeybees. *Proceedings of the Royal Society B: Biological Sciences* **277**, 1241-1245.
- 552 **Srinivasan, M. V.** (2011). Honeybees as a model for the study of visually guided flight,
553 navigation, and biologically inspired robotics. *Physiological reviews* **91**, 413-460.
- 554 **Srinivasan, M. V., Zhang, S., Altwein, M. and Tautz, J.** (2000). Honeybee Navigation: Nature
555 and Calibration of the "Odometer". *Science* **287**, 851-853.
- 556 **Srinivasan, M. V., Zhang, S. W. and Bidwell, N. J.** (1997). Visually mediated odometry in
557 honeybees. *Journal of Experimental Biology* **200**, 2513-2522.
- 558 **Tolman, E. C.** (1948). Cognitive maps in rats and men. *Psychol. Rev* **55**, 189-208.
- 559 **von Frisch, K.** (1967). *The dance language and orientation of bees*. Cambridge: Harvard
560 Univ.Press.
- 561 **Wang, Z., Chen, X., Becker, F., Greggers, U., Walter, S., Werner, M., Gallistel, C. R. and**
562 **Menzel, R.** (2023). Honey bees infer source location from the dances of returning foragers.
563 *Proceedings of the National Academy of Sciences* **120**, e2213068120.

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565

566 **Figure legends**

567

568 *Fig. 1 Left: view from within the tunnel as used during the preliminary experiments and the*
569 *experiments 1 – 6 of the main experiment. Right: view of the tunnel from outside. Note the*
570 *concealed ending: the feeder was in the dark, and the experimenter could enter the cover.*

571

572 *Fig. 2 Preliminary experiments. The colony in the observation hive had long time experience*
573 *with the environment. Tunnels of different length (0.5, 1, 2, 4 or 6 m) were attached to the*
574 *front of the hive with the entrance hole such that the entrance/exit to the tunnel was always at*
575 *the same location. The feeder was close to the end inside the tunnel. The length of the tunnel*
576 *was changed several times by moving the colony accordingly. Bees visiting the feeder were*
577 *marked with a white dot at the abdomen. A monitor displaying the images of the IR video*
578 *camera recording the bees' dances within the hive was set up behind the hive, and dances of*
579 *the marked bees were visually observed and evaluated. Bees not trained to the feeder (thus*
580 *not marked) were free to move in and out at the end of the tunnel and were not included in the*
581 *on-line evaluated dances.*

582

583 *Fig. 3 Design of the experiments in the main experiment. Experiment 1: The tunnel was*
584 *attached to the entrance of the hive prior to any exploratory flights, foraging flights or feeder*
585 *(F) training. Experiment 2: The foraging bees were trained to a feeder at a distance of 10 m*
586 *from the hive. Experiment 3: Experienced foragers were trained to the end of the 6 m tunnel.*
587 *The entrance to the tunnel was at 10 m from the hive. Experiment 4: The tunnel was attached*
588 *to the entrance of the hive after the bees had explored the environment and foraged at natural*
589 *food sources. Experiment 5: experienced foragers were trained to a feeder (F) at a distance of*
590 *321 m and subsequently fed at this location. Experiment 6: bees from experiment 5 were*
591 *trained to a feeder (F) at the end of the 6 m tunnel. Experiment 7: The animals from*
592 *experiment 5 and 6 were further trained to the feeder of the tunnel but two screens (2.5 m*
593 *high) were attached to the side walls of the feeder. In this situation the animals could see the*
594 *sky but no landmarks surrounding the feeder. ▼ in experiment 4 indicates that the*
595 *entrance/exit to the hive via the tunnel is open for other foragers not feeding at F, ◐ marks*
596 *the closed end of the tunnel that is covered with a box allowing access to the feeder and*
597 *blocking the view of the surrounding during feeding at F.*

598

599 *Fig. 4 Plot of waggles/run against duration/run, for experiments 1, 6 and 7 of the main*
600 *experiment group, with histograms for both at the side. Note that the histogram for waggles*
601 *has two peaks: at 5 waggles/run corresponding to experiments 1 (n=84, mean±std: 4.1±1.1)*
602 *and 6 (n=233, mean±std: 4.3±1.3), and at 7 waggles/run corresponding to experiment 7*
603 *(n=337, mean±std: 8.1±2.1). The higher variability in duration smears the distribution to a*
604 *single peak (mean±std for duration, Exp. 1, 6, 7: 0.43±0.18, 0.48±0.19, 0.67±0.18).*

605
606 *Fig 5. Distribution of waggles/run in experiments 1, 5, 6 and 7. Boxes show quartiles,*
607 *whiskers rest of distributions, except for outliers (see methods). Right ordinate axis shows*
608 *distances in meters indicated by the waggle dance, calibrated with experiment 5 (no tunnel).*
609 *(see S2 for data and statistics).*

610
611 *Fig. 6 Summary of the results on dances in the main experiment. The upper part of the figure*
612 *repeats the design of the 7 experiments, the lower part gives the data for the experiments 1,*
613 *5, 6 and 7 together with the calculations of distances from the number of waggles and the*
614 *corresponding durations as calculated from Fig. 4.*

615
616 *Fig. 7 Histograms of waggles/run in experiments 1, 5, 6 and 7. In each experiment, a single*
617 *prominent peak is visible. Note the high values in experiment 7 as compared to experiments*
618 *1, 5 and 6. In experiments 6 and 7, the values for identified bees are indicated with arrows*
619 *toward the abscissa, the label indicates the bee identity, and the number of averaged*
620 *(observed) waggle dances (e.g.: bee number 110 had 11 evaluated dances in experiment 6).*

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