

1 **Making honey bees lie: experimental dissociation of flight experience and** 2 **dance communication**

3 4 **Authors:**

5 Arumoy Chatterjee^{1,2}, Prabhudev M.V.^{1,3}, Ebi A. George¹, Pallab Basu⁴, and Axel
6 Brockmann^{1*}

7 8 **Affiliations:**

9 1 National Centre for Biological Sciences, Tata Institute of Fundamental Research,
10 Bangalore, 560056, India.

11 2 School of Chemical & Biotechnology, SASTRA University, Thanjavur, 613401, India.

12 3 University of Mysore, Mysore, 570006, India.

13 4 International Center for Theoretical Sciences, Tata Institute of Fundamental Research,
14 Bangalore, 560012; India.

15
16 ***Correspondence to:** axel.ncbs@gmail.com

17 18 **ORCID:**

19 A.C.: 0000-0003-4078-5984

20 E.A.G.: 0000-0002-5533-5428

21 A.B.: 0000-0003-0201-9656

22 23 **Abstract**

24 Honey bees use their dance to communicate flight distance and direction of a food source to
25 their nest mates in the hive. How bees transpose flight information to generate a
26 corresponding walking (dance) behavior is still unknown. We now present a detailed study of
27 the changes in dance duration of individual bees after shifting feeder distance. Our
28 experiments indicated that most bees needed two or more foraging trips to the new position
29 before showing an updated dance duration. In addition, only a few bees significantly changed
30 dance duration immediately, whereas most bees first produced intermediary durations.
31 Double shift experiments showed that under certain conditions bees do not update dance
32 duration but continued to perform dance duration for the previously visited feeder position.
33 We propose that generation of dance information involves two memory contents one for
34 newly acquired and one for previously stored distance information.

35 36 **One Sentence Summary**

37 Generation of dance information is temporally separated from immediate flight experience
38 and involves two different memory contents.

39
40 **Keywords:** *Apis mellifera*, foragers, spatial memory, social communication.

41 **Introduction**

42 Honey bee foragers returning from a foraging trip communicate flight distance and direction
43 to the food source to their nest mates using a small-scale walking pattern, the so-called
44 waggle dance (1). Duration and orientation of the waggle run correlates with the flight
45 distance and direction to the food source from the hive, respectively (1-4). Exploring how
46 honey bees use flight information to produce waggle dances and vice versa is central for
47 identifying neural mechanisms underlying dance communication. The time dynamics of these
48 processes will provide insights about how tightly navigational and dance information
49 processing are coupled.

50 In this study, we asked two questions: (i) How many foraging trips do honey bee foragers
51 need to update their waggle dance duration? (ii) Do foragers use only information from the
52 most recent foraging trip or do they also include information from earlier flight experiences
53 to generate dance duration?

54

55 **Single shift experiment**

56 First, we measured waggle dance durations for individually marked foragers visiting an
57 unscented sugar-water feeder at a distance of 300m from the hive for 1-2 hrs. Then, the
58 feeder was shifted for 100m, either forward (400m) or backward (200m) and we monitored
59 the changes in dance behavior (Fig 1A; table S1; supplementary materials).

60

61 Altogether, we trained 190 foragers (forward: $n = 112$, backward: $n = 53$), but only 35
62 individuals continued dancing after finding the feeder at the new distance. Fifteen out of
63 these 35 foragers (43%) continued dancing after the first visit, whereas 20 foragers (57%)
64 stopped dancing for one or more foraging trips before they resumed dancing (Fig. 2A). We
65 determined the first occurrence of a significant change in dance duration (= change point) for
66 each individual (Fig. 2A-B; results of the change point analyses are provided in table S3). A
67 total of 34 individuals showed a significant change-point in their post shift dance duration
68 (table S3). Among the bees that continued dancing after the shift, 3 out of 15 (20%) foragers
69 immediately showed a change point after the first feeder visit (= immediate update) while 12
70 foragers needed two or more foraging trips to update dance duration (= delayed update, Fig.
71 2B-C; table S3). Among the foragers that stopped dancing, 12 out of 19 (63%) foragers
72 showed a change point in their first dance whereas 7 foragers still showed a delayed update
73 (Fig. 2B; table S3). The proportion of bees showing change point in the very first post-shift
74 dance was significantly higher in the group which stopped and resumed dancing (χ^2 -test:
75 63% vs 20%, $\chi^2 = 3.84$, $df = 1$, $p = 0.03$). The magnitude of change in dance duration was
76 similar in the forward and backward shift (Fig. S2A).

77

78 Further, we asked whether the change in dance duration occurred gradually or abruptly. A
79 gradual update would include dances with intermediary durations (i.e. durations between the
80 mean dance duration for pre-shift and post-shift dances). The occurrence of intermediary
81 dances would indicate that bees use information not only from the most recent foraging trip
82 but also from earlier foraging trips. We compared differences between consecutive dances in
83 the pre-shift phase and post-change-point phase with those of the dances during the
84 intermediary phase (= dance after the shift till the change point; supplementary materials).

85 Among the individuals which showed intermediary dances (n=19), 15 (78.9%) of them
86 showed a gradual change in dance duration, while 4 (21.1%) showed an abrupt change (Fig.
87 S3A-C; analysis results in table S4). Within the group of bees that continued dancing, 10 out
88 of 12 bees (83.3%) showed a gradual change and 2 out of 12 showed an abrupt change in
89 dance duration (Fig. S3C; table S4). Second, we fitted two sigmoidal curves with different
90 slopes to identify gradual and abrupt changes during the intermediary dances (supplementary
91 materials, methods). Nine out of the total 19 (47.3%) bees showed a better fit with the
92 gradual and 3 (15.7 %) a better fit with the abrupt model (Fig. S4). Six bees could not be
93 grouped to either category. Among the group of continuously dancing bees, 6 out of 12 bees
94 (50%) showed a better fit with the gradual model and 2 out of 12 showed a better fit with the
95 abrupt model (Fig. S4; analysis results in table S4).

96

97 **Double Shift experiment**

98 In a second set of experiments, we tested how honey bee foragers would adjust their dance
99 behavior when confronted with shifting the feeder position twice, in opposite directions:
100 either “backward-forward” (300m-200m-300m) or “forward-backward” (300m-400m-300m)
101 (Fig. 1B; supplementary methods). Our question was whether or not the bees would show
102 similar dance durations for the first and the second 300m feeder distance. Differences in the
103 dance duration would indicate an effect of prior experiences.

104

105 In the backward-forward shift experiment, all foragers (n = 11) showed significant changes in
106 the waggle dance duration after the first and second shift (Fig. 3A-B). However, 7 out of the
107 11 foragers showed significantly shorter waggle run durations for the 300m(2) compared to
108 the 300m(1) (number in brackets indicates first or second test at 300m distance; Fig. 3B,
109 results from linear mixed effect modeling followed by general linear hypothesis testing are
110 provided in table S6).

111

112 In the forward-backward shift experiment, 8 foragers (n = 9) showed a significant change in
113 the dance duration after the first shift, but 6 foragers did not show any change in dance
114 duration after the second shift (Fig. 3A-B). All foragers showed significantly longer dance
115 duration for the first 300m(2) compared to the 300m(1). In fact, the dance durations for the
116 300m(2) were very similar to those of the previously visited 400m distance ($-1.04 \pm 2.82\%$
117 change in dance duration, Fig. S5; table S7). We also did not find any change point after the
118 second shift (Fig. 3A, table S5). An additional double shift experiment using different
119 distances (forward-backward: 200m-300m-200m, n = 2) suggested that our finding is
120 independent of the flight distances (Fig S6).

121

122 **Discussion**

123 To summarize, our single shift experiments demonstrated that the majority of honey bee
124 foragers needed multiple foraging trips to generate significantly changed, i.e. updated, dance
125 duration. These results are supported by an observation by Tautz et al. (5), who mentioned,
126 but did not analyze, a higher variability in waggle run duration during the first dances after a
127 feeder shift. As there is ample evidence that ants and honey bees instantaneously know the
128 walking or flight distance of a newly found food location (6-8), we propose that the

129 additional trips (or additional time) are needed to exclusively generate the corresponding
130 dance duration information. Thus, flight distance estimation and generation of waggle run
131 duration are temporally separated.

132

133 Our double shift experiments provide two results. First, in the backward-forward experiment
134 foragers showed shorter dance durations for the same feeder distance after feeder shifts.
135 Second, in the forward-backward experiment bees did not change dance durations after the
136 second shift. The forager basically ignored the new flight distance information and continued
137 to perform the dance durations that they showed for the 400m. So far, the discussion on
138 memory processes involved in generation of dance information have been focused on the
139 sun-compass system and dance direction information (9,10). F. Dyer (11) proposed a model
140 in which the generation of dance direction information involves two separate memories, a
141 memory of a newly acquired navigational information from the most recent foraging trip and
142 a memory of previously stored information from earlier flight experiences (Fig. 3C). The
143 “newly acquired” memory will get incorporated in the “previously stored” memory, but both
144 memories can be independently used to generate waggle dance duration. Some of the foragers
145 in Dyer’s experiments showed an abrupt change in dance orientation ignoring previous
146 experiences. Our forward-backward experiments showed foragers that did not change dance
147 duration ignoring newly acquired navigational information. Similar to Dyer’s (11) ideas we
148 propose that the generation of dance distance information is based on “newly acquired” and
149 “previously stored” memory contents.

150

151 Recent radar tracking experiments showed that dance recruits compare newly received
152 information from dancers with their own previous foraging experiences and use both
153 information to decide where to search for food (12). We think that the results of our
154 experiments point to a similar process in the dancers. First, the time delay between
155 calculating flight distance and updating waggle run duration indicates that both information
156 processing can be experimentally decoupled. Second, the double shift experiments showed
157 that dancers are capable of generating dance information that is not related to the flight
158 distance of the last foraging trip. Our results are supported by earlier studies that showed that
159 foragers are able to perform appropriate dances during the night without a prior foraging trip
160 (13). Together, all these experiments indicate that generation of dance information genuinely
161 involves memory processes.

162

163 Dacke and Srinivasan (14) recently suggested that honey bee forager might have two
164 different odometers, one for their personal use (i.e. flight navigation) and one for social
165 (dance) communication. In our forward-backward experiments the foragers knew the correct
166 flight distance otherwise they would not have continuously arrived at the 300m feeder
167 position. However, for the communication of the distance they used their previously stored
168 memory of the 400m feeder distance. We propose that both experiments report a similar
169 phenomenon, if bees get confused or become uncertain about the distance of a feeder, they
170 use the most recent navigational memory for their own orientation but communicate the
171 previously stored (“confirmed”) navigational information to their nest mates.

172

173 Still the question remains why the foragers in the backward-forward experiment updated the
174 waggle dance duration whereas the foragers in the forward-backward experiments did not.
175 All earlier feeder shift experiments including the generation of distance calibration curves
176 indicated that honey bees do not have any major difficulties with multiple forward or
177 backward shifts of a feeder (1,2,8,15). If honey bees do not have any problem with double
178 shift experiments, the difference in the behavior might have something to do with the spatial
179 arrangement of the feeders or the order of feeder shifts. In the backward-forward experiments
180 the new feeder position (200m) is actually on the way to the starting feeder position, whereas
181 in the forward-backward experiments the new feeder position (400m) was not known before.
182 We favor the idea that the novelty of the flight experience affected the response after the
183 backward shift, but more experiments need to be done.

184

185 Finally, so far all attempts to identify molecular processes involved in dance communication
186 failed because it was not possible to experimentally dissociate foraging flight and dance
187 behavior (16). Our finding that most foragers need 2-3 foraging trips to update dance
188 information opens a window to study molecular brain processes specifically involved in
189 generating dance information (17,18).

190

191 **References**

- 192 1. K.v. Frisch, The dance language and orientation of bees. Harvard University Press,
193 Cambridge (1967).
- 194 2. M.V. Srinivasan, S. Zhang, M. Altwein, J. Tautz, Honeybee navigation: nature and
195 calibration of the 'odometer' Science 287 851-853 (2000).
- 196 3. J.R. Riley, U. Greggers, A.D. Smith, D.R. Reynolds, R. Menzel, The flight paths of
197 honeybees recruited by the waggle dance. Nature 435 205–207 (2005).
- 198 4. F.C. Dyer, The Biology of the dance language. Annu. Rev. Entomol 47, 917-949 (2002).
- 199 5. J. Tautz, S. Zhang, J. Spaethe, A. Brockmann, A. Si, M. Srinivasan, Honeybee odometry:
200 performance in varying natural terrain. PLoS Biol. 2 E211 (2004).
- 201 6. R. Wehner, M.V. Srinivasan, Path integration in insects. In K.J. Jeffery (Ed.), The
202 Neurobiology of Spatial Behaviour, Oxford University Press, Oxford pp. 9-22 (2003).
- 203 7. M.V. Srinivasan, Where paths meet and cross: navigation by path integration in the desert
204 ant and the honeybee. J. Comp. Physiol. A 201 533- (2015).
- 205 8. Tomer J. Czaczkes and Jürgen Heinze, Ants adjust their pheromone deposition to a
206 changing environment and their probability of making errors. Proc. R. Soc. B 282:20150679
207 (2015).
- 208 9. M. Lindauer, Kompassorientierung. Ergeb. Biol. 26 158-181 (1963).
- 209 10. J.L. Gould, Processing of sun-azimuth information by honey bees. Anim. Behav. 32 149-
210 152.
- 211 11. F. Dyer, Memory and sun compensation by honey bees. J. Comp. Physiol. 160 621-633
212 (1987)
- 213 12. R. Menzel, Navigation and communication in honeybees. In R. Menzel, J. Fischer Eds.
214 Animal Thinking – Contemporary Issues in Comparative Cognition. MIT Press, Cambridge
215 Mass. p 9-21 (2010)

- 216 13. Lindauer M. (1954) Dauertaenze im Bienenstock und ihre Beziehung zum Sonnenbahn.
217 Naturwissen. 41. 506-507.
- 218 14. Dacke M., Srinivasan M.V. Two odometers in honeybee? J Exp Biol. 211 3281-3286
219 (2008).
- 220 15. R. Schürch, F.L.W. Ratnieks, E.E.W. Samuelson, M. J. Couvillon, Dancing to her own
221 beat: honey bee foragers communicate via individually calibrated waggle dances. J. Exp.
222 Biol. 219 1287-1289 (2016).
- 223 16. T. Kiya, T. Kubo, Dance type and flight parameters are associated with different
224 mushroom body neural activities in worker honeybee brains. *PLoS One* 6 e19301 (2011).
- 225 17. A.S. Singh, A. Shah, A. Brockmann. Honey bee foraging induces upregulation of early
226 growth response protein 1, hormone receptor 38 and candidate downstream genes of the
227 ecdysteroid signalling pathway. *Insect Mol. Biol.* 27 90-98 (2018).
- 228 18. M Sen Sarma, Rodriguez-Zas SL, Gernat T, Nguyen T, Newman T, Robinson GE.
229 Distance-responsive genes found in dancing honey bees. *Genes Brain Behav.* 2010 Oct;
230 9(7):825-30. doi: 10.1111/j.1601-183X.2010.00622.x. Epub 2010 Sep 1.

231

232 **Authors' Contributions.**

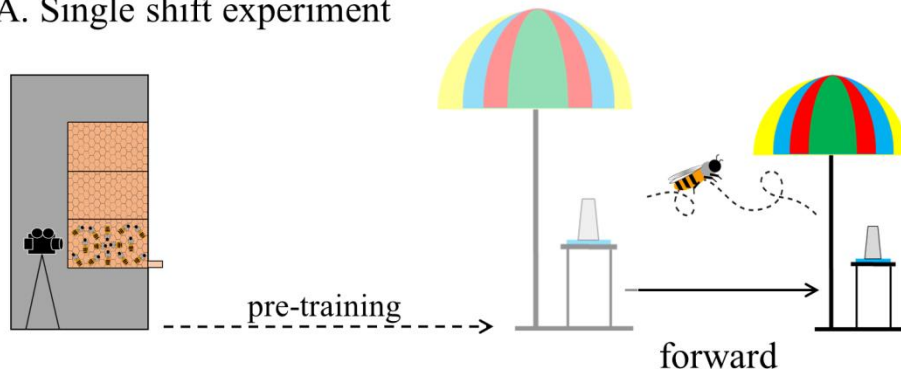
233 Experiments were designed by AC and AB and experiments were performed by AC and
234 PMV. Dance analysis was done by AC. The statistical analyses were done by AC, EAG and
235 PB. The paper was written by AB, AC and PB.

236

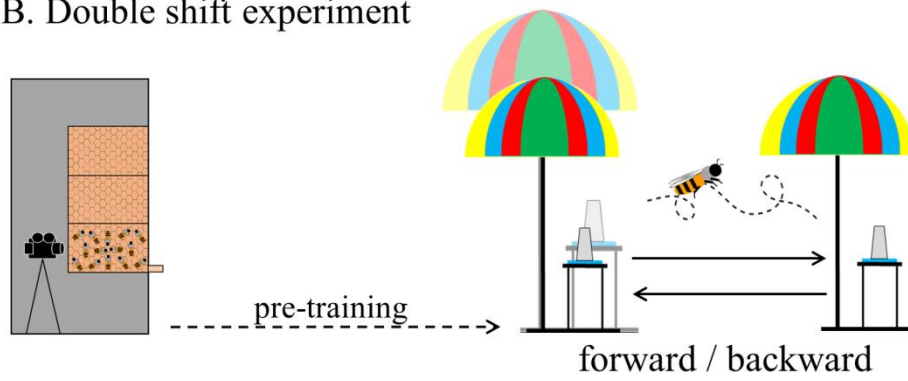
237 **Acknowledgments.**

238 We would like to thank B. Krishnan, S.K. Sethy, A. Sengupta, A. Suryanarayanan, S.
239 Unnikrishnan, N. Thulasi, A. Johnny, R. Fatima, A. Dey for their help with the behavioral
240 experiments. We would like to thank UAS-GKVK, Bangalore for agreeing to let us use their
241 campus. AC was supported by a fellowship from University Grants Commission; AB is
242 supported by NCBS-TIFR institutional funds No. 12P4167.

A. Single shift experiment



B. Double shift experiment

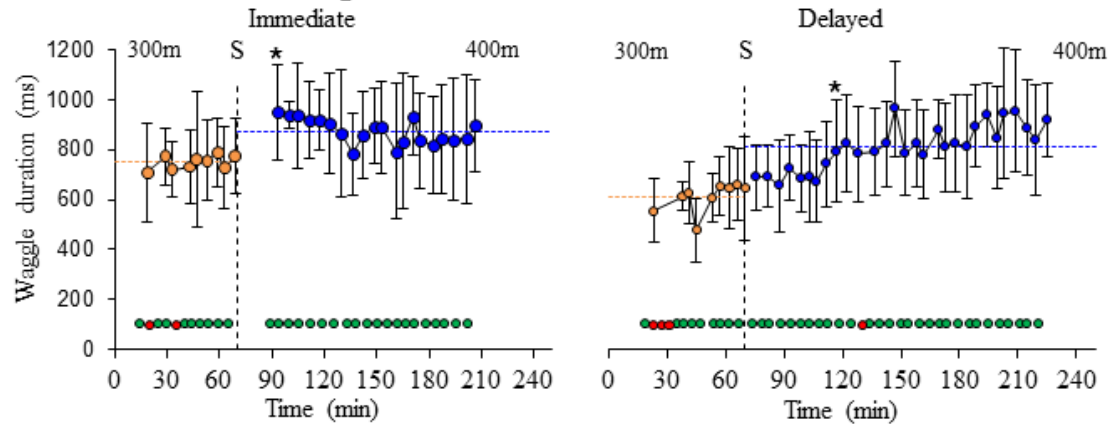


C. Experiment set up

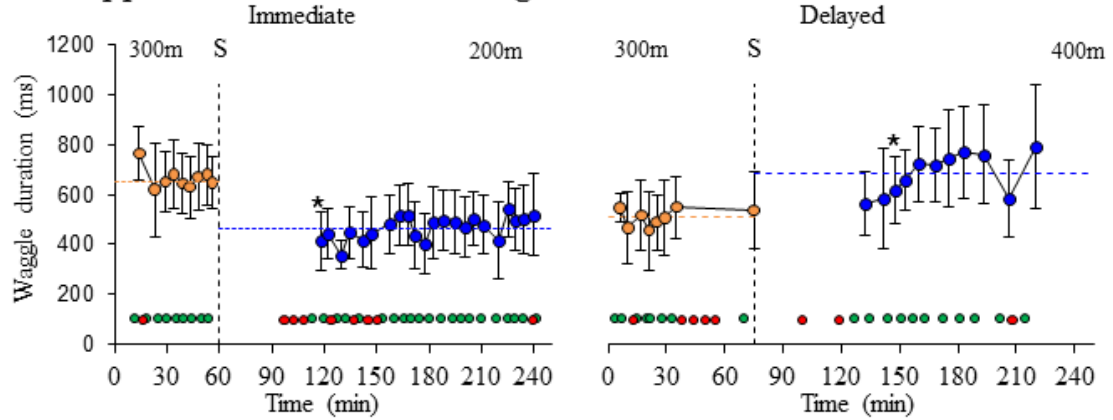


243 **Fig. 1. Honey bee foragers communicate the change in feeder location in waggle dance.**
244 (A) Single shift experiment: Foragers were trained to visit a feeder with non-scented sugar
245 water 300m away from the hive (see also supplementary materials). During the experiment,
246 individually marked foragers were allowed to visit the feeder for 1-2 hours; then the feeder
247 was linearly displaced for 100m forward (400m) or backward (200m). (B) Double shift
248 experiment: Similar experimental procedure as in the single shift experiment. In addition, the
249 feeder was shifted back to the initial location after 1.5-2 hrs. (C) Google Image of the training
250 path of the bees (left). H is the hive location and the red dots showed 100m, 200m, 300m and
251 400m feeder distances respectively. The training path offered necessary optic flow (middle).
252 Feeder advertised for the bees with a multicolored umbrella (right).

A Continued dancing

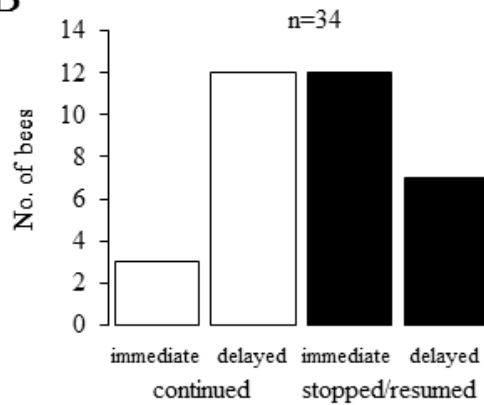


Stopped and resumed dancing

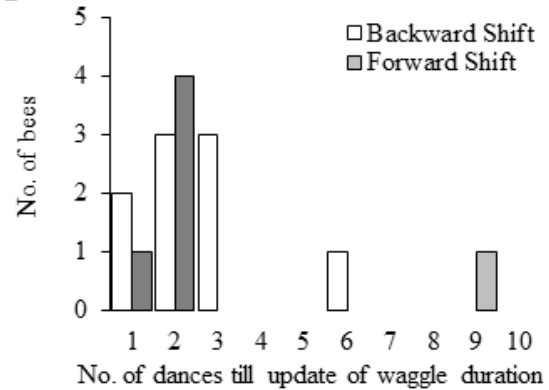


● Foraging trips with dance. ● Pre-shift dance duration. * Change point in dance duration.
 ● Foraging trips with no dance. ● Post-shift dance duration. --- Mean dance duration S Feeder shift time

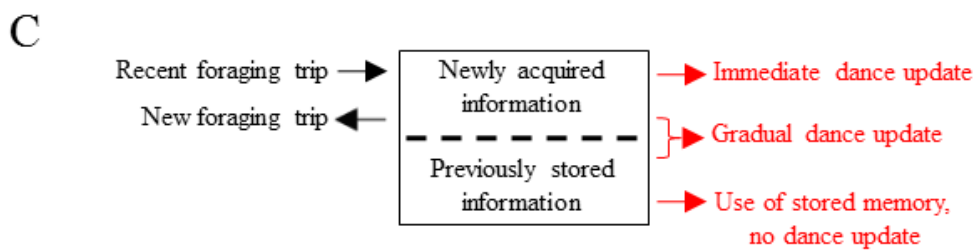
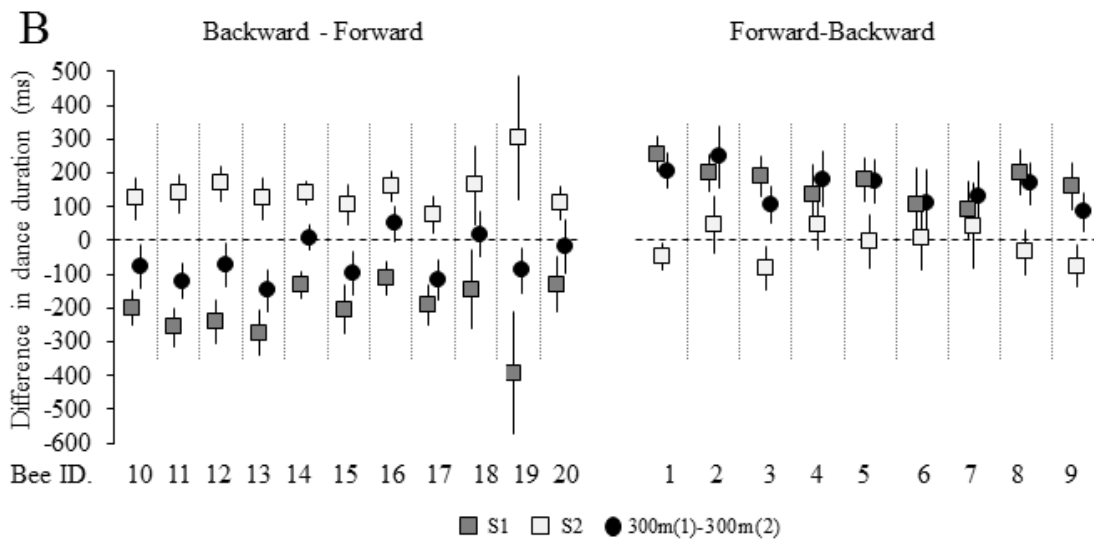
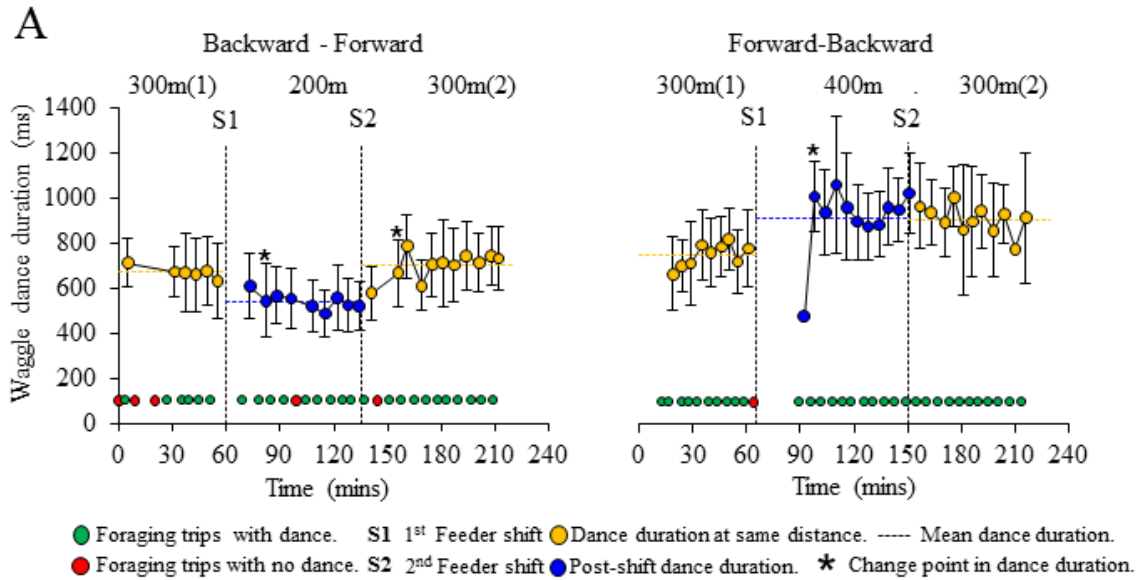
B



C



253 **Fig. 2. Single feeder shift experiments.** A) After finding the new feeder location, foragers
254 either continued dancing or first stopped and later resumed dancing. The change point
255 determined the first post-shift dance with significantly different dance duration
256 (supplementary materials). Individuals updated dance duration either on the very first post
257 shift dance (immediate) or after one or more dances (delayed). Yellow and blue colored
258 circles show pre- and post-shift dance duration. Error bars show standard deviation of waggle
259 run duration. Green and red circles show feeder visit timings with or without following
260 dances. Vertical dashed lines represent the timing of the feeder shift. Horizontal dashed line
261 represents average dance duration before and after feeder shift (S). The asterisk indicates the
262 change-point. B) 34 out of 35 foragers showed the change point on post-shift dance duration
263 (table S3). We did not find any bias for post shift dance behavior (continued/stopped and
264 resumed dancing, immediate/delayed update in dance duration) between forward or backward
265 shift (χ^2 -test: $\chi^2=0.682$, $df=3$, $p=0.8775$). White bars for bees continuing dancing and black
266 bars for those who stopped and resumed dancing, after feeder shift. C) 15 foragers continued
267 dancing after finding the feeder at the new distance. Majority of foragers (12) needed
268 multiple (>1) foraging trips to update dance duration, only 3 foragers updated dance-duration
269 on the very first post shift dance. White and grey bars represents backward and forward shift
270 respectively.



271 **Fig. 3. Double feeder shift experiments showed the role of past experience in shaping**
272 **waggle dance duration.** A) Individual forager updated dance duration (change point)
273 following both feeder shifts (left) for backward-forward experiment but did not show any
274 change in dance duration after the second shift (right) for forward-backward experiment
275 (supplementary materials, table S5). Yellow colored circles show dance duration at same
276 distance, blue circles show dance duration after first shift. Error bars show standard deviation
277 of waggle run duration. Green circles show foraging trips followed by dances and red circles
278 show same without dances. Horizontal dashed line represents average dance duration before
279 and after first (S1) and second (S2) feeder shifts. The asterisk shows the change-points of
280 dance duration. B) All foragers (n=11) showed significant change in dance duration after
281 each shift (left) in the backward-forward experiments, yet 7 foragers (Bee ID. 10-13,
282 15,17,19) showed shorter dance duration while dancing for same distance (300m) after
283 double feeder shift. While, in the forward-backward experiments, 8 foragers (n=9) updated
284 dance duration after the first shift but none showed any change in dance duration after the
285 second shift (right). Symbol shape represents difference in dance duration: solid rectangle =
286 before and after first shift, hollow rectangle = before and after second shift and circles = same
287 distance. Error bars show confidence intervals of the change in dance duration. C) Heuristic
288 model of updating dance duration. We propose that flight navigational memory contains at
289 least two different memory contents: (1.) “*newly acquired information*”, i.e. the most recent
290 flight information, and (2.) “*previously stored information*”. In most cases both memories
291 interact to guide dance behavior shortly after having found a new feeder position. During
292 following trips, a new memory of “*previously stored information*” is generated. In the cases
293 in which bees immediately updated they used the memory of “*newly acquired information*”.
294 After the second feeder shift in the forward-backward experiment the bees communicated the
295 “*previously stored information*” without updating dance information.