1   **Effects of landmark distance and stability on accuracy of reward relocation**

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27   **Abstract**

28   Although small-scale navigation is well studied in a wide range of species, much of what is

29   known about landmark use by vertebrates is based on laboratory experiments. To investigate

30   how vertebrates in the wild use landmarks, we trained wild male rufous hummingbirds to

31   feed from a flower that was placed in a constant spatial relationship with two artificial

32   landmarks. In the first experiment, the landmarks and flower were 0.25m, 0.5m or 1m apart

33   and we always moved them 3-4m after each visit by the bird. In the second experiment, the

34   landmarks and flower were always 0.25m apart and we moved them either 1m or 0.25m

35   between trials. In tests, in which we removed the flower, the hummingbirds stopped closer to

36   the predicted flower location when the landmarks had been closer to the flower during

37   training. However, while the distance that the birds stopped from the landmarks and

38   predicted flower location was unaffected by the distance that the landmarks moved between

39   trials, the birds directed their search nearer to the predicted direction of the flower, relative to

40   the landmarks, when the landmarks and flower were more stable in the environment. In the

41   field, then, landmarks alone were sufficient for the birds to determine the distance of a reward

42   but not its direction.

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Introduction

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55 Mobile animals have to travel to find food, shelter and other resources, and often return to

56 these at a later time. Some animals may have to navigate between locations separated by tens

57 of metres or even thousands of kilometres (Dyer 1998) while others, such as food-storing

58 birds or rodents, must also accurately pinpoint a location to within a few centimetres (Cheng

59 et al. 2006; Gould et al. 2010). For diurnal animals navigating over relatively short distances,

60 visual features of the environment near to the goal are often important cues (Shettleworth

61 2009). Furthermore, when the appearance of the goal is variable or hidden from competitors

62 or predators, landmarks can allow animals to relocate the goal reliably without requiring cues

63 from the goal itself.

64 Our understanding of how vertebrates learn and use landmarks is largely based on

65 laboratory studies (Shettleworth 2009; Gould et al. 2010) and it is rare to examine cue use in

66 the wild. It is not clear, however, whether the laboratory results accurately represent the

67 behaviour of wild animals. There are at least two reasons to expect that they may not.

68 Firstly, landmark experiments are conducted in rooms or mazes that are walled and rarely

69 exceed 3 or 4 metres in diameter (Cheng 1988; Biegler and Morris 1993; Gould-Beierle and

70 Kamil 1996). Secondly, animals experience the experimental arenas only during training and

71 testing, spending the intervening time in holding cages. Wild vertebrates, in contrast, can

72 inhabit a territory of multiple hectares, without walls or other all-encompassing boundaries

73 that significantly restrict their movements, and must balance the demands of navigation with

74 other requirements, such as finding mates, avoiding predation and defending their territory

75 (Healy & Hurly 2003).

76   Two variables that are often manipulated in laboratory landmark experiments are the

77   distance of the landmarks from the goal and the reliability of the landmarks relative to other

78   cues in the arena. When multiple landmarks are provided around a goal, animals often use

79   some landmarks in favour of others. These preferences are often related to distance: some

80   species appear to prefer to use closer, or "proximal", landmarks while others prefer to use

81   further, or "distal", landmarks (Gould et al. 2010). It is common to differentiate between

82   these two types of landmarks on the basis of distance but as it is not clear how notions of

83   "proximal" or "distal" landmarks in the laboratory translate to the cues animals use in the

84   field, where animals inhabit larger, less constrained environments than experienced in the

85   laboratory. It is possible that this differentiation is, in fact, a laboratory artefact. It is not

86   clear, for example, how cues that are considered to be distal or global in the laboratory

87   compare with landmarks that are more than a couple of metres from a rewarded location in

88   the field or to large, distant landmarks such as mountains.

89   To determine which of the provided landmarks laboratory animals use to relocate a

90   goal, experimenters often move the landmarks and the goal between visits to ensure that other

91   possible cues in the maze or room do not reliably indicate the goal location (e.g. Jones et al.

92   2002). If an animal can effectively locate a goal using only a pair of landmarks, then the

93   distance that the landmarks and goal are moved between trials should not reduce the accuracy

94   with which an animal searches for a goal. Rather, the farther the landmarks and goal move

95   between trials, the better the landmarks should predict the goal location compared to other,

96   global cues, and so moving the landmarks may be expected to increase the weight that

97   animals would give to the landmarks (Wagner et al 1968).

98   To determine how these two key components of landmark use in the lab (distance and

99   reliability) might differ from landmark use in the wild, we examined how wild rufous

100   hummingbirds (*Selasphorus rufus*) used a pair of landmarks we provided to remember flower

101   locations. In the first experiment, we examined whether the accuracy with which

102   hummingbirds stopped in the vicinity of a pair of landmarks for a rewarded location varied

103   depending on the distance from the landmarks to the flower. In the second experiment, we

104   manipulated the distance that the landmarks and a reward moved between visits to see

105   whether the reliability of cues other than the experimental landmarks influenced the accuracy

106   with which the hummingbirds searched for the rewarded location. In both experiments, the

107   birds experienced all of the experimental treatments. As we do not know what larger scales

108   cues hummingbirds might use to orient themselves (e.g. mountains, magnetic variations), we

109   used a repeated-measures design to compare the effect of the different landmark distances

110   and stabilities while controlling for differences in the environment and between birds.

111

112   **Methods**

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114   ***Subjects and Experimental Site***

115   The experiment was carried out along the Westcastle Valley, which is located within the

116   Eastern Range of the Canadian Rockies in south-western Alberta (49°29'N, 114°25'W),

117   where feeders containing 20% sucrose solution were placed along the valley in early May

118   2012. By late May male rufous hummingbirds had arrived from their over-wintering grounds

119   in Mexico and established territories around individual feeders. As the weather got warmer,

120   the sucrose concentration was lowered to 14% and this remained constant for the remainder

121   of the season while testing was carried out. Testing continued until early July, when the

122   males abandoned their territories.

123   The subjects of this experiment were three territorial male rufous hummingbirds,

124   which we distinguished both by their territorial behaviour and by applying a mark to their

125 chest using coloured, non-toxic ink. To mark the birds, we trapped them using a wire mesh

126 trap surrounding the feeder. The mark lasted for the six weeks of the field season.

127   The University of St Andrews Ethical Committee and the University of Lethbridge

128 Animal Welfare Committee approved all of the work described here, which was also

129 conducted according to the requirements of the Canadian Council on Animal Care and under

130 permits from Alberta Sustainable Resource Development and Environment Canada.

131

132 *Experiment 1: Inter-landmark distance*

133 To test the effects of landmark distance on the ability of the birds to learn a rewarded

134 location, we trained the birds to feed from a flower that was positioned in an equilateral

135 triangle with a pair of landmarks. The distance between the flower and each of the landmarks,

136 was 0.25m, 0.5m, or 1m. In every training trial the landmark-flower array was arranged with

137 the flower placed due south from the mid-point of the two landmarks and we filled the flower

138 with 25% sucrose. The landmarks we used in this experiment were two vertical Plastic pipes

139 (910mm tall x 90mm diameter), one covered in grey tape, the other in blue tape, secured into

140 the ground using sharpened dowling rods. The "flower" was a 700µl Eppendorf tube

141 attached to the top of a 610mm rod of 10mm diameter doweling. During training trials we

142 arranged the triangular array of landmarks and flower in a $36m^2$ experimental open arena or

143 "pitch", aligned north to south. We organised the pitch into four equal quadrants and marked

144 each quadrant with a small rock at each corner (see Figure 1). We set up camcorders on 1.3m

145 tripods 6m south and either east or west of a pre-chosen "test quadrant". Each camera faced

146 the centre of the test quadrant, with the bottom of its visual field aligned to a point halfway

147 (3m) between the tripod and the quadrant centre. The experimenter sat a further 3 metres

148 behind one of the cameras.

149

150   *Training trials*

151   We considered every visit to the flower by the birds a trial, and we trained birds for 180 trials

152   each. We organised the 180 trials into three blocks of 60 trials in each of which the landmarks

153   were 0.25 m, 0.5m or 1m from the flower. We pseudo-randomised the order of presenting the

154   three different blocks across birds such that the order differed for each bird. Between trials,

155   we moved the landmarks and flower to a different quadrant on the pitch. We pseudo-

156   randomised the order of quadrants so that birds visited all quadrants at least twice before they

157   experienced a test trial. Within each quadrant we always placed the array in a different

158   position so that at no point during the experiment did we return the flower or the landmarks

159   to a previously rewarded location.

160

161   *Test trials*

162   On every 10th visit within a 60-trial block the birds experienced a test trial. For a test trial we

163   presented the landmarks in the test quadrant but removed the flower. We set the cameras to

164   record and the calibrated the video with a 0.4m x 0.4m chequerboard of 40mm x 40mm

165   squares placed 1m above the ground at 3m and 6m from each camera. We made a sharp

166   noise so that we could synchronise the videos for later analysis. When the bird returned, we

167   recorded the flight of the bird and the stops he made around the landmarks.

168   Following the test trial, we moved the landmark-flower array to the next quadrant to

169   start the next set of 10 trials. After the test that marked the 60th trial, we returned the bird's

170   feeder and stopped training for at least three hours before we began the next block.

171

172   **Experiment 2: Landmark Stability**

173   The second experiment immediately followed the first for each subject. Our aim was to

174   determine whether the distance that we moved the array between visits affected the accuracy

175   with which the birds searched. For this experiment we trained and tested all birds on a

176   smaller pitch, 4m^2, which we established at least 4m from that we had used for Experiment 1.

177

178   *Training trials and test trials*

179   We trained and tested birds in two 60-trial blocks, varying the order of blocks across birds.

180   In this experiment, we again arranged the landmarks and reward flower in a triangular array,

181   but this time the distance between each of the three array components was always 0.25m. We

182   manipulated only the distance we moved the array after each trial. In one condition, we

183   moved the array of landmarks and flower 1 metre, to a different quadrant, in a pseudo-

184   randomised order. Within each quadrant the landmarks and flower were never placed in the

185   same location. For the other condition, we moved the landmarks and flower 0.25m in one of

186   eight directions (N, NE, E, SE, S, SW, W, NW), the order of which was pseudorandomised

187   with the condition that the flower was never in the same location twice but that the landmarks

188   and flower always remained on the pitch.

189   As before, every tenth trial was a test in which we moved the landmarks to the next

190   location and removed the flower. Each bird was tested six times in each of the two

191   conditions, resulting in twelve tests per bird, and eighteen tests in each condition. In the

192   0.25m shift condition, the landmarks were moved 0.5m for the test trial rather than 0.25m.

193   This was to ensure that during the test the previous location of the flower was more than

194   0.25m from the location that would be predicted by the landmarks. In the 1m shift condition,

195   we moved the landmarks 1m before each test, as during training, because we considered that

196   1m was a distance sufficient to allow us to distinguish between the predicted flower location

197   and the location of the flower in the previous training trial.

198

199   **Data analysis**

200   From the video data, we extracted the frames that contained the chequerboard at both 3m and

201   6m from the camera, as well as the first and second stops by the birds (hummingbirds will

202   hover in 3-D space and we termed these 'stops'). We identified the location of the birds by

203   placing a red dot over the head of the hummingbird. We used a custom code written in

204   Python to cycle through the images, storing the x,y pixel coordinates of the landmarks, the

205   corners of the chequerboard, the location 6m from each camera, and the locations of the

206   birds. We used a custom R script (R Development Core Team 2012) that used the pixel

207   dimensions of the chequerboard at the two distances to estimate the field of view of the

208   camera as well as the position of the camera, as viewed from above, by locating the

209   intersection of the edges of the field of view. Before training and testing began we arranged

210   the cameras at 90 degrees to each other and using the camera locations and the field of view

211   we reconstructed the position of the landmarks and hummingbird stops. We did this by fitting

212   a line perpendicular to the direction of view from each camera, running from one edge of the

213   camera's field of view to the other. We called each line an "image plane" as they

214   corresponded to the x-axis of the images from each camera. Next we determined the position

215   of features, such as the hummingbird and either side of the landmarks, on the image plane by

216   dividing the x-coordinate of each feature by the total pixel width. Finally, for each camera,

217   we determined the equation for a line, referred to as the "feature-line", which passes through

218   both the camera position and the position of a particular feature on the image plane. We

219   considered the point at which the feature lines from both cameras intersected to mark the

220   position of that feature. The x,y location of the landmarks was based on the centre of the

221   landmarks with the radius of the landmarks based on the mean distance of the sides from both

222   cameras.

223   We did not consider the raw x,y data to be exact reconstructions of the locations of

224   the landmarks or hummingbird as we could not correct the distortion of the image produced

249   moved between visits) as a fixed factor and bird as a random factor. We tested the distances

250   that the birds stopped from the flower location, and from the landmarks, for normality using

251   Shapiro-Wilks tests, and when these variables were not normally distributed and were

252   positively skewed, we log-transformed the variables prior to analysis. We analysed the

253   directions of the stops using a Rayleigh test (or "V test" (Batschelet 1981)), which enabled us

254   to determine whether the distribution of a set of directions was significantly oriented in a

255   specific direction, in this case the direction of the flower, to that expected from a uniform

256   distribution. In later analyses we used GLMs to compare how the direction that the birds

257   stopped from the landmarks differed between treatments (distance of the flower from the

258   landmarks, or distance moved between visits), using the observed directional component (V)

259   as an independent variable. As V scores are bounded between -1 and 1, we added 1 to each

260   score, and then divided each score by 2 to convert the data to a proportion. We then arcsine-

261   transformed the data to make them suitable for analysis. Values are presented as mean $\pm$ SE.

262

263   **Results**

264

265   ***Experiment One: Landmark Distance***

266   During the test trials, the birds stopped and hovered near the landmarks a mean of 3.87$\pm$0.26

267   times before leaving the pitch across all test trials, of these stops we only examined the first

268   two. The number of stops did not differ within each treatment (One way ANOVA of number

269   of stops with test number (1-6): $F_{5,48} = 1.22$, $P = 0.314$) or between treatments (ANOVA of

270   number of stops with landmark distance : $F_{2,51} = 1.17$, $P = 0.318$).

271   We excluded from further analysis two of the 54 first stops (one from Bird 2, one

272   from Bird 3) and two of the 54 second stops (both from Bird 3) because they were more than

273   2 standard distances away from the mean stop location for each bird.

274

275   *Use of the landmarks*

276   Our first question was whether the hummingbirds paid any attention to the landmarks.

277   Potentially, the birds could just have learned to beacon to the flower rather than learning an

278   association between the landmarks and the flower. If this was the case, the birds should have

279   stopped first in the quadrant in which they had previously found the flower. Alternatively, if

280   the birds used the landmarks to return to the flower, they should have followed the landmarks

281   to the test quadrant. Two of the three birds stopped within 1.5m of the flower location,

282   within the test quadrant, while Bird 3 searched further away but outside of the experimental

283   pitch (Figure 2 & 3a). The birds did not, therefore search in the previous location of the

284   flower before the test but instead stopped near the current location of the landmarks, often in

285   the test quadrant, during the test trials. The birds had apparently learned to associate the

286   landmarks with the flower.

287   Our next question was whether the distance of the landmarks from the flower affected

288   the accuracy with which the birds searched for the flower. In order to measure the accuracy

289   with which the birds searched, we calculated the distance that the hummingbirds stopped

290   from the predicted location of the flower based on the location of the landmarks, i.e. at the

291   third point of a triangle with the two landmarks. We then ran a GLM of distance from the

292   flower location, with the distance of the landmarks from the flower (1m, 0.5m or 0.25m), the

293   order of the stop (first, or second), and the test number within each distance treatment (1-6) as

294   factors and bird identity as a random factor. The accuracy of the birds' stops depended on the

295   distance that the flower had been positioned from the landmarks during training: all of the

296   birds stopped between 0.3m to 2m of the predicted flower location, but stopped closer to the

297   predicted flower location when the landmarks had been closer to the flower during training

298   (mixed GLM of log distance from the flower location with landmark distance, stop order

299   (first or second), test number, and bird as a random factor: landmark distance, $F_{2,3.61} = 10.23$,

300    $P = 0.033$; Figures 2 & 3a). When the landmarks had been 0.25m from the flower all of the

301   birds stopped 0.5-0.6m closer to the actual flower location than they did when the landmarks

302   had been 1m from the flower (mean $\pm$ SE of distance of stops from the flower location:

303    $0.57m \pm 0.026$). The birds did not stop consistently closer or further from the flower location

304   on their second stops (stop order, $F_{1,1.96} = 5.80$, $P = 0.14$) or become more or less accurate

305   with repeated tests (test number, $F_{5,9.46} = 1.73$, $P = 0.22$). Although in most of the tests, the

306   birds increased in accuracy when the distance between the landmarks and the flower became

307   smaller, in some tests individual birds stopped further away from the flower location when

308   the landmarks were 0.5m from the flower, than when they were 1m away (Birds 1 & 3, first

309   test; Bird 1 second test), or further when the landmarks were 0.25m away than when they

310   were 0.5m away (Bird 1, second and sixth tests; Bird 2, first test; Bird 3, fourth test), or when

311   the closest to the flower location when the landmarks were 1m from the flower, than when

312   the landmarks were 0.5m or 0.25m (Birds 1 & 2, fifth test) (landmark distance x test number

313   x bird, $F_{19,14} = 2.52$, $P = 0.042$; all other interactions $P > 0.05$).

314   These results by themselves are not straightforward to interpret. Although, intuitively,

315   the distance that the birds stopped from the flower location is an appropriate measure of

316   accuracy, there may be other reasons why the birds stopped closer to the location of the

317   flower when the landmarks had been closer to the flower during training. For example, if the

318   birds had learned neither the distance nor the direction of the flower, but just searched around

319   the landmarks, this would also have lead the birds to stop closer to the predicted flower

320   location than when the landmarks had been closer to the flower because the landmarks

321   themselves were closer to that location. The distance from the predicted flower location that

322   the birds stopped cannot, then, allow us to discriminate between these alternative

323   explanations. To better describe how the birds used the landmarks, we analysed the distance

324   and direction of their first and second stops from the landmarks. If the birds searched only

325   around the landmarks, they should have searched at the same distance and direction from the

326   landmarks irrespective of the distance between the landmarks and flower. Alternatively, if the

327   birds had learned about the distance and direction of the flower from the landmarks, and

328   searched more accurately when the landmarks were closer to the flower, we might expect the

329   birds to stop closer to the correct distance from the landmarks when the landmarks had been

330   0.25m from the flower than when they had been 1m from the flower. We might also expect

331   that the birds were searching more in the correct direction from each landmark, as measured

332   in degrees. Although, as the error in degrees around a bearing would cover a smaller area

333   0.25m from the landmarks than it would 1m from the landmarks, the birds could also appear

334   more accurate while not actually reducing their directional error.

335

336   *Direction from landmarks*

337   To determine whether the distance of the landmarks from the flower during training affected

338   the direction in which the birds stopped during the test trials, we first calculated the direction

339   of each first and second stop from both the left and the right landmarks. If the birds searched

340   more accurately when the landmarks were closer to the flower, the directions that the birds

341   stopped from each landmark should have clustered more in the trained direction of the flower

342   when the landmarks were 0.25m from the flower, than when the landmarks were 1m from the

343   flower. The directional component (V) is a statistic that measures the degree to which a

344   collection of direction cluster in a given reference direction. We calculated V statistics for the

345   first and second stops from each landmark for each treatment (1m, 0.5m, 0.25m), using the

346   trained direction of the flower as the reference direction. Using the V statistics as a measure

347   of direction accuracy, we then ran a GLM on the directional accuracy (V) with the distance of

348   the landmarks from the flower, and the order of the stops (first or second) as fixed factors.

349   Although the distance between the landmarks and the flower did not affect the accuracy of

350   the birds' stop direction (GLM: landmark distance and stop order: landmark distance, $F_{2,6} =$

351   2.50, $P = 0.162$), the birds were more accurate on their second stops (stop order, $F_{1,6} = 18.17$,

352    $P = 0.005$). We did not find, therefore any evidence that the birds stopped more accurately in

353   the direction of the flower when the landmarks were closer to the flower during training

354   (Figure 4).

355

356   *Distance to the landmarks*

357   Although the distance of the flower from the landmarks did not affect how accurately the

358   birds searched in the direction of the flower from the landmarks, we might expect that the

359   distance of the flower from the landmarks to have affected the distance that the birds stopped

360   from the landmarks. This is because although the flower was always in the same direction

361   from the landmarks, the distance of the flower from the landmarks changed across treatments.

362   In order to determine whether the distance that the birds stopped from the landmarks was

363   affected by the distance of the flower from the landmarks during training, we first calculated

364   the distance of the stops from each landmark, and then ran a GLM of the distance from the

365   landmarks, with landmark distance during training (1m, 0.5m, 0.25m), stop order (first or

366   second), landmark identity (right or left), and test number with the trial (1-6) as factors. When

367   the flower had been closer to the landmarks, the birds also stopped closer to the landmarks

368   (mixed GLM of log distance of stops from the landmarks, with landmark distance, stop order,

369   landmark (right or left), and test number, and bird as a random factor. Landmark distance:

370    $F_{2,3.63} = 15.44$, $P = 0.017$; Figure 3b). There were no significant differences in the distances

371   from the landmarks between the first and second stops made by the birds (stop order: $F_{1,1.92} =$

372   6.14 $P = 0.14$) and no difference in the distances from right or left landmarks of the

373   hummingbirds' stops (landmark: $F_{1,1.96} = 3.88$, $P = 0.19$). The birds appear to have learned

374   the distance from the landmarks within 10 visits as they were no more accurate after 60 trials

375   than after 10 trials (test number: $F_{5,9.61} = 1.03$, $P = 0.45$. All interactions $P > 0.05$).

376

377   *Coefficient of Variation*

378   As the hummingbirds appeared to have learned something about the distance of the

379   landmarks from the flower and searched closer to the flower location when the flower was

380   closer to the landmarks, we next asked how the hummingbirds may have estimated the

381   distance from the landmarks. Pigeons search more precisely when trained with a reward a

382   short distance from landmarks, than when trained to a location a greater distance away

383   (Cheng 1990). It is possible that the hummingbirds may also estimate the distance to the

384   closer landmarks better because small differences between small distances are more

385   discriminable than are the same differences between further distances (Weber's Law:

386   Shettleworth 2009). If this is the case then we would expect the standard deviation of the

387   distance that birds stopped from the landmarks to increase the further the landmarks from the

388   flower but the coefficient of variation, the ratio of the standard deviation to the mean, should

389   remain constant. To examine this, we combined the standard deviation and coefficient of

390   variation into a single variable, which we called "variation", in which half of the values were

391   standard deviations for each landmark distance, and half were coefficients of variation. We

392   then ran a GLM of "variation" with the source of the variation (whether the value was a

393   standard deviation, or a coefficient of variation), and landmark distance as factors. If the

394   standard deviation and coefficient of variation differed in their response to the landmarks, we

395   predicted that we would see an interaction between the distance of the landmarks and whether

396   the "variation" was a standard deviation or the coefficient of variation. The standard

397   deviation and coefficient of variation did not respond differently to the distance of the flower

398   from the landmarks (mixed GLM of variation in distance from the landmarks with the source

399   of variation (Standard Deviation or log Coefficient of Variation) and distance, and bird as a

400   random factor: source of variation, $F_{1,2} = 14.761$, $P = 0.062$; distance, $F_{2,4} = 3.58$, $P = 0.13$;

401   source of variation x distance, $F_{2,4} = 3.59$, $P = 0.13$; Figure 3c). The birds did not search

402   more precisely when they had learned the location of a flower 0.25m from landmarks than

403   when they had learned the location of a flower 0.5m or 1m away from the landmarks.

404

405   **Experiment Two: Landmark Stability**

406   In Experiment 2, we, again, analysed only the location of the first two stops made by the

407   birds in each test. There was no difference in the number of stops after 10 visits or 60 visits

408   (one-way ANOVA: $F_{5,30} = 1.74$, $P = 0.155$) but they stopped significantly more often when

409   we moved the landmarks 1m between visits rather than 0.25m (mean stops 1m: 4.17 ± 0.73,

410   mean stops 0.25m: 2.78 ± 0.58; one-way ANOVA: $F_{1,34} = 5.20$, $P = 0.029$). No stops were

411   more than 2 standard distances from the mean stop location for each bird and we did not

412   exclude any of the data points from the analyses.

413

414   *Use of the landmarks*

415   As in the first experiment, we first determined whether the birds followed the landmarks or

416   continued to search at the previous location of the flower. The previous flower location was

417   1m or 0.5m away in the 1m and 0.25m conditions respectively. In both conditions, the birds

418   stopped closer to the predicted flower location than to the previous location (moved 1m: 0.47

419   ± 0.06m; moved 0.25m: 0.29 ± 0.03m). Again, the birds did not search at the previous flower

420   location.

421   Our next question was whether the movement of the landmarks and flower between

422   visits affected how close the birds stopped to the predicted flower location. As in Experiment

423   1, we ran a GLM of the distance that the birds stopped from the flower's predicted location,

424   with the distance that the landmark-flower array moved between visits (1m or 0.25m), the

425   stop order (first or second), and the test number in the treatment (1-6) as factors, and bird

426   identity as a random factor. Birds did not stop significantly closer to the flower's location

427   when we had moved the array 0.25m between visits than when we had moved the array 1m

428   (mixed GLM of log distance of stops from the flower location, with distance moved, stop

429   order and test number, and bird as a random factor, distance moved: $F_{1,1.96}= 5.52$, $P = 0.15$;

430   Figures 5 & 6a). The birds stopped closer to the flower location on their second stops than

431   they had on the first stop (stop order: $F_{1,1.38} = 280.67$, $P = 0.016$). Although there was no

432   consistent difference across tests (test number: $F_{5,9.97} = 0.40$, $P = 0.839$), individual birds did

433   differ in their accuracy across tests, depending on the distance that we had moved the array

434   between visits (distance moved x test number x bird: $F_{10,7} = 6.55$, $P = 0.01$; all other

435   interactions $P > 0.05$).

436

437   *How did the birds use the landmarks?*

438   Although we did not find that the stability of the landmarks and flower affected the proximity

439   at which birds stopped relative to the flower location, this does not mean that the stability of

440   the landmarks had no effect on the birds' search locations. To look at the effect of landmark

441   on where the birds searched in more detail, we examined whether the distance that the

442   landmarks and flower moved between visits affected the distance and direction that the birds

443   stopped from the landmarks.

444   Examining first the effect of the distance that the landmark-flower array had been

445   moved between visits on the distance that the birds stopped from the landmarks, we ran a

446   GLM of the distance of the stops from each landmark, with the distance that the landmarks

447   moved between visits (1m, 0.25m), the stop order (first or second), the landmark identity (left

448   or right), and the test number in the treatment (1-6) as factors. The distance that we moved

449   the landmarks between trials had no effect on the distance that the birds stopped from the

450   landmarks (mixed GLM of log distance of stops from landmarks with distance moved (0.25m

451   or 1m), stop order, landmark, and test number, and bird as a random factor; distance moved:

452    $F_{1,0.99} = 0.95$, $P = 0.51$). The birds consistently stopped closer to the landmarks on their

453   second stops than they had on their first stop (stop order : $F_{1,1.75} = 72.47$, $P = 0.02$). The birds

454   did not, however, stop at a different distance from the landmarks after 60 trials than they had

455   after 10 trials (test number (without Experiment 1 data): $F_{5,9.98} = 0.046$, $P = 0.10$), and they

456   did not stop consistently closer to one landmark than the other (landmark: $F_{1,1.94} = 0.93$, $P =$

457   0.44; All other interactions $P > 0.05$). Landmark stability did not, therefore, appear to have an

458   effect on the distance at which the birds stopped from the landmarks.

459   Finally, we looked to see whether landmark stability affected the direction that the

460   birds searched from the landmarks. In order to examine the effect on direction, we again

461   calculated V statistics, to represent the degree of cluster of the stops in the direction of the

462   flower, for the first and second stops from both the right and left landmarks in each treatment.

463   Following this, as in Experiment 1, we used a GLM of the V statistics with the distance that

464   the landmarks moved between visits (1m or 0.25m) and stop order (first or second) as factors

465   to determine whether the accuracy in stop direction was influenced by the distance that the

466   landmark-flower array was moved. The birds were more accurate when we moved the

467   landmarks 0.25m vs. 1m (two-way GLM of arcsine of V with distance moved (0.25m or 1m)

468   and stop order (first and second): distance moved, $F_{1,4} = 22.23$, $P = 0.009$) and stopped

469   significantly closer to the direction of the flower in their second stops (stop order, $F_{1,4} =$

470   10.16, $P = 0.033$, Figure 6b) .

471

472   **Discussion**

473

474   In Experiment 1, the birds stopped closer to the landmarks when they had been trained to

475   visit a flower 0.25m from the landmarks than when they had been trained with landmarks 1m

476   from a flower. The direction in which the birds stopped relative to the landmarks was not,

477   however, affected by the distance of the landmarks from the flower: birds did not stop in the

478   direction of the flower on their first stops and, in fact, stopped on the other side of the

479   landmarks. Because the birds stopped more in the direction of the flower on their second

480   stops but at the same distance from the landmarks as for their first stop, the birds stopped

481   closer to the predicted flower location on their second stops, compared to their first.

482   In Experiment 2, the birds stopped more accurately in the predicted direction of the

483   flower when we moved the landmarks and flower 0.25m, rather than 1m, between visits by

484   the birds. The landmarks were always 0.25m from the flower in the second experiment, and

485   the distance that we moved the landmarks and flower between visits had no discernible effect

486   on the distance that the birds stopped from the landmarks. The birds also stopped more

487   accurately in the direction of the flower, as well as closer to the landmarks, on their second

488   stops than on their first.

489   One of the apparent differences between these experiments and those conducted on

490   other species in the laboratory is the size and complexity of the environment in which the

491   animals were trained and tested. Unlike the confined, bare environment of a laboratory

492   testing room, we trained the hummingbirds to relocate a location in their natural

493   surroundings, which was an open mountain valley. Despite the availability of large visual

494   features, such as individual trees, forest boundaries and mountains, however, in the absence

495   of the flower, the hummingbirds stopped closest to its relative location when trained with

496   landmarks less than 0.5m away. In the laboratory, other vertebrates also search more

497   accurately when provided with landmarks closer to the reward, often only tens of centimetres

498   away, rather than when provided with landmarks that are further away (Gould-Beierle and

499   Kamil 1999; Chamizo et al. 2006). Furthermore, nearby landmarks in the lab can overshadow

500   those further away, resulting in animals relying on very local landmarks to return to

501   remembered locations (Cheng 1989; Cheng 1990; Spetch and Wilkie 1994; Spetch 1995). As

502   hummingbirds will use visual features within 0.8m to decide between which of multiple

503   flowers to visit (Healy and Hurly 1998; Hurly & Healy 2002; Henderson et al. 2006), the

504   results of the first experiment might suggest that such nearby cues are more useful to the

505   hummingbirds and that the apparent superiority of landmarks within half a metre of the

506   flower results in hummingbirds relying less on further away cues when returning to a flower

507   location.

508   To address these possibilities, we must look at where the hummingbirds stopped in

509   relation to the landmarks and not just at how close to the predicted flower location they

510   stopped. In the lab, nearby cues are thought to result in greater search accuracy as animals

511   estimate short distances from landmarks more accurately than they estimate greater distances

512   (e.g. Cheng, 1990), a phenomenon known as Weber's Law. If Weber's Law is responsible for

513   the hummingbirds' greater accuracy when trained with landmarks within 0.5m of the flower,

514   the relative error in their distance estimates should have increased as the distance of the

515   landmarks from the flower increased. In fact, while the birds did stop at appropriate distances

516   from the landmarks, the distances that they stopped from the landmarks were not more

517   precise when they had been trained with landmarks 0.25m from the flower than when they

518   had been trained with landmarks 1m from the flower. It seems unlikely, therefore, that

519   Weber's Law can explain why the hummingbirds stopped closer to the flower location when

520   trained with landmarks closer to the flower. Instead, it would appear that, by stopping closer

521   to the landmarks when trained with landmarks closer to the flower, the birds searched within

522   a smaller area around the landmarks and were closer to the flower location as a result.

523   Despite the birds stopping closer to the location of the flower when the landmarks were

524   0.25m from the flower during training we cannot say, from the data we present here, that

525   hummingbirds can use nearer landmarks more accurately than they can use landmarks that

526   are further away.

527   We can also describe where the hummingbirds searched relative to the landmarks by

528   examining the direction of their stops in relation to each landmark. In the first experiment, the

529   hummingbirds did not confine their searching to the side of the landmarks where the flower

530   would have been found. Rather they stopped all around the landmarks. Given the results of

531   Experiment 2, it seems likely that moving the landmarks and flower 3-4m between visits may

532   have prevented the birds from orienting themselves relative to the landmarks. Such

533   disorientation would mean that the results from the first experiment may not reflect the

534   accuracy with which the hummingbirds can use landmarks at different distances from a

535   reward. In Experiment 2 the birds stopped more in the direction of the flower when the

536   landmarks were moved 0.25m between visits. To determine whether this greater accuracy,

537   where the landmarks were 0.25m from the flower, would be maintained when the landmarks

538   are 0.5m, or 1m from the flower one would need to test the birds when moving the landmarks

539   and flower only short distances between visits.

540   That the distance that the landmarks and flower were moved between visits affected

541   the direction but not the distance searched suggests two things: first, hummingbirds, similar

542   to bees (Cheng 1998) and other birds (Cheng 1994), may encode distance and direction

543   separately, rather than as a whole vector (Gibson and McGowan 2014), although further

544   experiments are required to confirm this; and second, while landmarks alone appear sufficient

545   for the birds to estimate the distance of a goal from the landmarks, the landmarks alone are

546   not sufficient to provide directional information.

547   Theoretically, the birds could have used the pair of individually distinctive landmarks

548 as a configural cue to orient: as the landmarks are two corners of landmark flower array, the

549 birds could have used those landmarks to find the missing third corner where the flower

550 would have been located. If this had been the case, it seems unlikely that the distance that we

551 moved the landmarks between the birds' visits would have affected the birds' ability to find

552 the flower's location. On the contrary, by establishing the landmarks as the most reliable

553 indicator of a flower's location, moving the landmarks and flower further between visits

554 should have resulted in the birds paying more attention to the landmarks, and searching more

555 accurately when the flower was removed (e.g. Roberts & Pearce 1998).

556   As the hummingbirds did not search more accurately in the direction of the flower

557 when the landmarks were moved 1m rather than 0.25m between visits, and indeed searched

558 less accurately, it appears unlikely that they used the configuration of the two experimental

559 landmarks to remember the direction of the flower. Instead, the birds seem to have relied on

560 other cues, such as the configuration of one of the landmarks and other, non-experimental,

561 global information in the environment. This result conflicts somewhat with findings from

562 animals tested in the laboratory, which, unlike the hummingbirds, readily use the

563 configuration of a set of experimental landmarks when other possible orientation cues are

564 unavailable or made unreliable (Collett et al. 1986; Biegler and Morris 1996; Gould-Beierle

565 and Kamil 1996; Kamil and Jones 2000; McGregor et al. 2004; Gibson et al. 2007; Kelly

566 2010). There are at least two possible reasons why our hummingbirds may have differed from

567 animals tested in the laboratory. First, differences in the scale of experimental arena and cues

568 in the laboratory and the field could mean that other cues are more readily controlled in the

569 laboratory. If in general animals learn to use the configuration of experimental landmarks

570 only when all other cues are unreliable, it may be easier to make other cues unreliable in the

571 smaller, enclosed space of the laboratory room or maze, where moving the landmarks array

572   even small distances will considerably change the relationship between the landmarks, the

573   reward, and global cues. In the field, the relationship between the reward and distant or

574   extended cues may have remained stable enough over the course of training, to possibly

575   result in the nearby, experimental landmarks never becoming solely predictive of a reward's

576   location. The presence of these other cues may, then, have prevented the hummingbirds from

577   learning to use the configuration of the experimental landmarks alone to direct their

578   searching. Confirmation of this would need further experimental manipulations such as

579   rotations of the array coupled with translocations between visits, which would render all other

580   cues unreliable.

581   Secondly, in many of the laboratory studies animals are trained to search for hidden

582   food or a submerged platform rather than for a visible feeder, such as the flower in our

583   experiments. If moving the landmarks 1m between visits interfered with the birds' ability to

584   determine the direction of the reward from the landmarks, the flower itself could have been

585   acting as a beacon and outcompeting the configuration of the landmarks during training.

586   Hummingbirds do not usually rely on beacons to relocate flowers (Hurly & Healy 2002;

587   Hurly et al. 2010) and the birds in our experiment also did not appear to rely solely on a

588   beaconing strategy as when the landmarks were relatively stable (moved 0.25m between

589   visits), the birds appear to have oriented relatively well. Wild hummingbirds will, however,

590   learn to beacon to flowers when all other cues are unreliable (Flores-Abreu et al. 2012), just

591   as rats in the lab will learn to rely on a beacon during navigation, if the beacon and goal are

592   moved every trial (Roberts and Pearce 1999; Gibson and Shettleworth 2003). Regardless of

593   whether moving the landmarks resulted in the birds relying more heavily on a beaconing

594   strategy to relocate the flower, the use of a visible target in our experiment may have affected

595   the search behaviour by the birds. In laboratory experiments where animals search for a

596   hidden goal, the search distributions are often more focussed than are those seen in these field

597   experiments (e.g. Cheng 1988; Cheng 1989; Gould-Beierle and Kamil 1996; Kamil and Jones

598   2000). One reason for this is that may be that if the hummingbirds notice that the flower has

599   gone, they may not search exactly where they think the flower had been but might be more

600   likely to search around the general area in which they remembered they had seen the flower.

601   In conclusion we have demonstrated here firstly that studies of small-scale spatial

602   cognition in vertebrates need not be confined to the laboratory. Firstly, wild free-living

603   hummingbirds can be trained to use experimental landmarks to return to rewarded locations.

604   Secondly, birds can remember the distance of a reward from the landmarks and can use those

605   landmarks alongside other information to direct their search. We cannot say, from the data

606   presented here, however, what other information the hummingbirds require to orient relative

607   to the experimental landmarks or how hummingbirds or other animals in the wild may use

608   landmarks to remember the locations of resources. Although these questions have been well

609   studied in the laboratory (reviewed in e.g. Cheng et al. 2006; Gould et al. 2010) they have

610   not, to our knowledge, attracted much attention in wild vertebrates.

611

612   **Acknowledgements**

618

619   **References**

620   Batschelet E (1981) Circular statistics in biology. Academic Press

621 Biegler R, Morris R (1996) Landmark stability: studies exploring whether the perceived
622   stability of the environment influences spatial representation. J Exp Biol 199:187–193.

623 Biegler R, Morris RG (1993) Landmark stability is a prerequisite for spatial but not
624   discrimination learning. Nature 361:631–633.

625 Chamizo VD, Manteiga RD, Rodrigo T, Mackintosh NJ (2006) Competition between
626   landmarks in spatial learning: the role of proximity to the goal. Behav Processes 71:59–
627   65. doi: 10.1016/j.beproc.2005.11.003

628 Cheng K (1988) Some psychophysics of the pigeon's use of landmarks. J Comp Physiol A
629   162:815–826.

630 Cheng K (1989) The vector sum model of pigeon landmark use. J Exp Psychol Anim Behav
631   Process 15(4):366–375.

632 Cheng K (1990) More psychophysics of the pigeon's use of landmarks. J Comp Physiol A
633   166:857–863. doi: 10.1007/BF00187333

634 Cheng K (1998) Distances and directions are computed separately by honeybees in landmark-
635   based search. Anim Learn Behav 26:455–468. doi: 10.3758/BF03199239

636 Cheng K (1994) The determination of direction in landmark-based spatial search in pigeons:
637   A further test of the vector sum model. Anim Learn Behav 22:291–301. doi:
638   10.3758/BF03209837

639 Cheng K, Spetch ML, Kelly DM, Bingman VP (2006) Small-scale spatial cognition in
640   pigeons. Behav Processes 72:115–127. doi: 10.1016/j.beproc.2005.11.018

641 Collett TS, Cartwright BA, Smith BA (1986) Landmark learning and visuo-spatial memories
642   in gerbils. J Comp Physiol A 158:835–851. doi: 10.1007/BF01324825

643 Dyer F (1998) Cognitive ecology of navigation. In: Dukas R (ed) Cogn. Ecol. University of
644   Chicago Press, Chicago, Illinois, pp 201–260

645 Flores-Abreu IN, Hurly TA, Healy SD (2012) One-trial spatial learning: wild hummingbirds
646   relocate a reward after a single visit. Anim Cogn 15:631–637. doi: 10.1007/s10071-012-
647   0491-0

648 Gibson B, McGowan F (2014) Rats average entire vectors when navigating toward a hidden
649   goal: A test of the vector sum model in rodents. Behav Processes 102:18–24. doi:
650   10.1016/j.beproc.2013.12.009

651 Gibson BM, Shettleworth SJ (2003) Competition among spatial cues in a naturalistic food-
652   carrying task. Anim Learn Behav 31:143–159. doi: 10.3758/BF03195977

653 Gibson BM, Wilks TJ, Kelly DM (2007) Rats (*Rattus norvegicus*) encode the shape of an
654   array of discrete objects. J Comp Psychol 121:130–144. doi: 10.1037/0735-
655   7036.121.2.130

656 Gould KL, Kelly DM, Kamil AC (2010) What scatter-hoarding animals have taught us about
657   small-scale navigation. Philos Trans R Soc Lond B Biol Sci 365:901–914. doi:
658   10.1098/rstb.2009.0214

659 Gould-Beierle KL, Kamil AC (1999) The effect of proximity on landmark use in Clark's
660   nutcrackers. Anim Behav 58:477–488.

661 Gould-Beierle KL, Kamil AC (1996) The use of local and global cues by Clark's nutcrackers,
662   *Nucifraga columbiana*. Anim Behav 52:519–528. doi: 10.1006/anbe.1996.0194

663 Healy SD, Hurly TA (1998) Rufous Hummingbirds' (*Selasphorus rufus*) Memory for
664   Flowers: Patterns or Actual Spatial Locations? J Exp Psychol Anim Behav Process 396–
665   404.

666 Healy SD, Hurly TA (2003) Cognitive ecology: foraging in hummingbirds as a model
667   system. Adv Study Behav 32:325–359.

668 Henderson J, Hurly TA, Healy SD (2006) Spatial relational learning in rufous hummingbirds
669   (*Selasphorus rufus*). Anim Cogn 9:201–5. doi: 10.1007/s10071-006-0021-z

670 Hurly TA, Healy SD (2002) Cue learning by rufous hummingbirds (*Selasphorus rufus*). J
671   Exp Psychol Anim Behav Process 28:209–223. doi: 10.1037//0097-7403.28.2.209

672 Hurly TA, Franz S, Healy SD (2010) Do rufous hummingbirds (*Selasphorus rufus*) use visual
673   beacons? Anim Cogn 13:377–383. doi: 10.1007/s10071-009-0280-6

674 Jones JE, Antoniadis E, Shettleworth SJ, Kamil AC (2002) A comparative study of geometric
675   rule learning by nutcrackers (*Nucifraga columbiana*), pigeons (*Columba livia*) and
676   jackdaws (*Corvus monedula*). J Comp Psychol 116:350–356. doi: 10.1037//0735-
677   7036.116.4.350

678 Kamil AC, Jones JE (2000) Geometric rule learning by Clark's nutcrackers (*Nucifraga
679   columbiana*). J Exp Psychol Anim Behav Process 26:439–453. doi: 10.1037/0097-
680   7403.26.4.439

681 Kelly DM (2010) Features enhance the encoding of geometry. Anim Cogn 13:453–462. doi:
682   10.1007/s10071-009-0296-y

683 McGregor A, Good MA, Pearce JM (2004) Absence of an interaction between navigational
684   strategies based on local and distal landmarks. J Exp Psychol Anim Behav Process
685   30:34–44. doi: 10.1037/0097-7403.30.1.34

686 R Development Core Team (2012) R: A language and environment for statistical computing.

687 Roberts ADL, Pearce JM (1998) Control of spatial behaviour by an unstable landmark. J Exp
688   Psychol Anim Behav Process 24:172–184.

689 Roberts ADL, Pearce JM (1999) Blocking in the Morris swimming pool. J Exp Psychol
690   Anim Behav Process 25:225–235. doi: 10.1037/0097-7403.25.2.225

691   Shettleworth S (2009) Cognition, evolution, and behavior. Oxford University Press

692   Spetch ML, Wilkie DM (1994) Pigeons′ Use of Landmarks Presented in Digitized Images.
693   Learn Motiv 25:245–275. doi: 10.1006/lmot.1994.1014

694   Spetch ML (1995) Overshadowing in landmark learning: Touch-screen studies with pigeons
695   and humans. J Exp Psychol Anim Behav Process 21:166–181. doi: 10.1037/0097-
696   7403.21.2.166

697   Wagner, A R., Logan, F. A, Haberlandt, K., & Price, T. (1968). Stimulus selection in animal
698   discrimination learning. J Exp Psych, *76*(2), 171–180. doi:10.1037/h0030023

699

700

701   **Figure Legends**

702

703   **Figure 1**: Bird's eye diagram of the experimental pitches. In Experiment 1 the pitch was 6m
704   across, with the cameras centred on a test quadrant. In Experiment 2 the pitch was 2m across,
705   with the cameras centred on the centre of the pitch.
706
707   **Figure 2**:
708   a. The location of the first stops around the landmarks by the birds in Experiment 1. The light
709   and dark grey circles indicate the position of the landmarks, while the dashed circle shows the
710   predicted position of the flower relative to the landmarks. Each smaller, black shape shows
711   the location of the first stop by a bird in one of the 6 tests, with each bird represented by a
712   different shape (Bird 1: triangles; Bird 2: circles; Bird 3: squares).

713   b. The location of the second stops around the landmarks by the birds in Experiment 1. All
714   symbols are represented as in Figure 2a.

715
716   **Figure 3**:
717   a. The mean distance (± SE) of the first (closed symbols) and second stops (open symbols)
718   from the location of the flower in Experiment 1. Bird 1 (triangles), Bird 2 (circles) and Bird 3
719   (squares) all stopped closer the flower location when the landmarks were closer to the goal.
720   b. The mean distance (± SE) of the first (closed symbols) and second stops (open symbols)
721   from the landmarks in Experiment 1. The dashed red line shows the distance of the flower
722   from the landmark during training. Bird 1 (triangles), Bird 2 (circles) and Bird 3 (squares) all
723   stopped closer to the left landmark (unbroken error line) and right landmark (dashed error
724   line) when the landmarks were closer to the flower.
725   c. The variation in the distances that the birds stopped from the landmarks. If the birds were
726   more precise at estimating shorter distances, we predicted that the Coefficient of Variation for

727 the distances that the birds stopped should remain constant across difference landmarks
728 distances, while the standard deviation should increase with larger landmark distances. This
729 was not the case. The Coefficient of Variation (left), and the standard deviation (right)
730 responded similarly across different landmark distances, suggesting the birds were not
731 estimating smaller distances more precisely.

733 **Figure 4:** The direction in degrees of the first (black) and second (red) stops from each
734 landmark in Experiment 1. The dashed line indicates the direction of the flower from the
735 landmarks during training. The directions of the central arrows indicate the mean direction of
736 first (black) and second (red) stops while the lengths of the arrows indicate the degree of
737 dispersion around the mean, the mean resultant length, the longer the line, the less dispersed
738 are the data.

740 **Figure 5:** The location of the first and second stops around the landmarks in Experiment 2.
741 See Figure 2 for details (Bird 1: triangles; Bird 2: circles; Bird 3: squares).

743 **Figure 6:**
744 a. The mean distance (± SE) of first and second stops from the flower location in Experiment
745 2. The distance that the landmarks moved between trials did not affect how close Bird 1
746 (triangles), Bird 2 (circles) and Bird 3 (squares) stopped to the flower location.
747 b. The direction in degrees of the first (black) and seconds stops (red) from each landmark in
748 Experiment 2. See Figure 4 for details.

751 **Supplementary material**

752 **Analyses testing the effects of experience**

753 **Experiment 1**

754 Over the three treatments in Experiment 1, each bird experienced 180 trials, consisting of 162

755 training trials and 18 test trials. The order in which each bird experienced the different

756 landmark distances was balanced across birds, such that experience with the experiment was

757 not correlated with landmark distance. Still, if the birds' behaviour changed as a result of

758 their experience across the first experiment, we might expect them to stop closer to the flower

759   locations, closer to the trained distance from the landmarks, or more in the direction of the

760   flower from each landmark in the third treatment rather than their first treatment.

761   To test whether the birds' behaviour changed across the three treatments we ran three

762   different analyses to look at how distance from flower location of their stops, difference

763   between trained distance and stopping distance, and accuracy of direction, changed across the

764   three treatments.

765   **Distance to the flower location**

766   In order to determine whether birds stopped closer to the predicted location of the flower as

767   they had more experience of the experiment, we ran a GLM to examine the effect of

768   landmark distance including treatment order (whether the treatment was the first, second, or

769   third that the bird had experienced) as a factor. If experience had an effect, we would expect

770   to see a significant effect of treatment order, a significant effect of test number within the

771   treatment, or a significant interaction between treatment order and test number. But we did

772   not find significant effects of any of these possibilities (mixed GLM of log distance from the

773   flower location with treatment order (first, second, or third), stop order (first or second) and

774   test number within treatment (1-6), and bird as a random factor: treatment order, $F_{2,3.92} =$

775   0.123, $P = 0.887$; test number, $F_{5,9.40} = 0.722$, $P = 0.194$; treatment order*test number,

776      $F_{10,18.21} = 0.946$, $P = 0.517$). We could not discern an effect on the distance from the goal

777   location that the birds stopped due to their experience gained during the first experiment.

778   **Effect of order on the stop distance from the landmarks**

779   In order to determine whether birds stopped closer to the flower's location from the

780   landmarks with increasing experience of the experiment, we subtracted the distance from the

781   landmarks that the birds stopped from the distance of the flower from the landmarks, for each

782   treatment. For example, when the landmarks were 1m from the flower, we subtracted 1m

783   from the distance that the birds searched from the landmarks. As the differences between the

784   search distance and the training distance were not normally distributed, we log transformed

785   the data prior to analysis. If experience had an effect, we would expect to see a significant

786   effect of treatment order, a significant effect of test number within the treatment, or a

787   significant interaction between treatment order and test number. Again, we could see no

788   significant effects of any of these variables (mixed GLM of log difference between training

789   landmark distance and distance searched from the landmarks, with treatment order (first,

790   second, or third), stop order (first or second) and test number within treatment (1-6), and bird

791   as a random factor: treatment order, $F_{2,3.90} = 0.588$, $P = 0.598$; test number, $F_{5,9.60} = 1.88$, $P =$

792   0.188; treatment order*test number, $F_{10,17.82} = 0.839$, $P = 0.599$). Birds did not stop closer to

793   the training distance as their experience of the experimental set up increased.

794   **Direction of stops from the flower**

795   The final measure of performance we examine was how the accuracy of the birds stop

796   direction changed as they had more experience of the first experiment. As a measure of

797   accuracy we used the V statistic, which represents the degree to which a collection of

798   directions are clustered in a particular reference direction, in this case the direction of the

799   flower during training. For this analysis we calculated V statistics from the directions that the

800   birds searched during their first, second, or third treatment. In order to determine whether the

801   birds searched more accurately in the direction of the flower with more experience of the

802   experiment, we ran a GLM of the calculated V statistics with treatment order (the first,

803   second, or third treatment that the bird experienced) and the stop order by the birds. If the

804   birds were more accurate at estimating the direction of the flower with more experience of

805   the experiment, we would predict a significant effect of treatment order on V, and possibly a

806   significant interaction between treatment order and stop order. We did not find significant

807   effects for any of these (GLM of arcsine of directional component (V), with treatment order

808   (first, second, or third) and stop order: treatment order, $F_{2,6} = 1.19$, $P = 0.365$; treatment

809   order*stop, $F_{2,6} = 1.007$, $P = 0.420$). Birds did not stop more accurately in the direction of the

810   flower with increasing experience of the first experiment.

811

812   **Experiment 2**

813   Assessing the effect of experience in the second experiment was difficult for two reasons.

814   First, by the second experiment, the birds had already experienced 180 trials over which they

815   showed no sign of improvement in any of our measures of performance. Second, in

816   Experiment 2 two of the three birds experienced trials in which we moved the landmarks 1m

817   before they experienced the landmarks being moved 0.25m, which would confound stability

818   and experience for those two birds. It is not perhaps surprising, then, that we found a

819   significant effect of treatment order, with birds stopping more in the direction of the flower in

820   their second treatment compared to their first (GLM of arcsine of directional component (V),

821   with treatment order (first or second) and stop order: treatment order, $F_{1,4} = 32.778$, $P =$

822   0.005; treatment order*stop, $F_{1,4} = 0.026$, $P = 0.881$).

823   To look at the effect sizes for order, we ran two sets of Pearson correlations, one with

824   regard to the accuracy of search direction and the order of treatments, and another with regard

825   to the accuracy of search direction and the distance that the landmarks moved between visits.

826   There was no significant correlation between the accuracy of direction and the order of

827   treatments (Pearson correlation of arcsine of directional component and treatment order (first

828   or second): $r = 0.68$, $p = 0.063$), but there was a significant correlation between the accuracy

829   of direction and the stability of the landmarks, with the birds stopping more accurately in the

830   direction of the flower when the landmarks were moved 0.25m between visits than when they

831   were moved 1m (Pearson correlation of arcsine of directional component and distance moved

832   between visits (0.25m or 1m): $r = -0.77$, $p = 0.025$).

833   While we cannot exclude the possibility that the performance by the birds in the

834   second experiment was the result of experience, the lack of evidence for any effect of

835   experience across the 180 trials by the birds in the first experiment, plus the smaller effect

836   size for treatment order relative to landmark stability, leads us to conclude that the observed

837   variation in the birds' performance is better explained by the experimental treatments than by

838   increased experience.

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