

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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53 **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**

113

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 700 μ l 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 36m² 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, 4m², 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

225 by the lens. Our reconstruction did, however, conserve the relative distance and direction
226 that the birds stopped from the landmarks, which enabled us to compare the effect of
227 landmarks distance and stability on reward relocation by these birds. Following
228 reconstruction, we rotated and scaled the x,y coordinates, standardising the orientation and
229 size of the landmark array within treatments, so that we could compare treatments across the
230 different trials and across multiple birds. The scaling resulted in the distance between the
231 landmarks equalling that in the experiment (1m, 0.5m, 0.25m). This resizing resulted in only
232 modest changes in the data (average difference before and after resizing: $0.028\text{m} \pm 0.03\text{m}$).

233 Following the reconstruction, rotation, and scaling of the stop locations, we calculated
234 three variables from the coordinates for statistical analysis: the distance of stops from the
235 hypothetical location of the flower relative to the landmarks in millimetres, the distance of
236 stops from each of the landmarks and the direction of stops from each landmark.

237 Prior to analysis we determined whether any of the stops could be considered outliers.
238 To do this we calculated the Standard Distance of the stops for each bird in each treatment,
239 and excluded those stops that were more than two standard distances away from the centroid
240 for that bird in that treatment. Standard distance is a measure of variation that is similar to
241 standard deviation but that uses x, y coordinates rather than single values, and therefore we
242 considered it the most appropriate measure of variation around the centroid. The equation for
243 standard distance is given below, where x_i, y_i is the location of the i^{th} point and \bar{X}, \bar{Y} is the
244 mean location of all the points:

$$245 \quad \text{Standard Distance} = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{X})^2}{n} + \frac{\sum_{i=1}^n (y_i - \bar{Y})^2}{n}}$$

246

247 We analysed the distances of stops from the flower location and from the landmarks
248 using GLMs with the treatment (distance of the flower from the landmarks or the distance

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 ± 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 $0.57\text{m} \pm 0.026$). 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 ± 0.06 m; moved 0.25m: 0.29 ± 0.03 m). 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**

613 We thank Ken Cheng and two anonymous reviews for their comments, which greatly
614 improved this manuscript. We also thank Maria Tello Ramos, Rachael Marshall, Caitlin
615 Hamilton and Jamie Dunlop for their assistance over the field season. This work was
616 supported by the University of St Andrews, the University of Lethbridge and the Natural
617 Sciences and Engineering Council of Canada.

618

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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 (\pm 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 (\pm 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.

732

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.

739

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).

742

743 **Figure 6:**

744 a. The mean distance (\pm 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.

749

750

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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