

# Effects of landmark distance and stability on accuracy of reward relocation

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**Abstract**

Although small-scale navigation is well studied in a wide range of species, much of what is known about landmark use by vertebrates is based on laboratory experiments. To investigate how vertebrates in the wild use landmarks, we trained wild male rufous hummingbirds to feed from a flower that was placed in a constant spatial relationship with two artificial landmarks. In the first experiment, the landmarks and flower were 0.25m, 0.5m or 1m apart and we always moved them 3-4m after each visit by the bird. In the second experiment, the landmarks and flower were always 0.25m apart and we moved them either 1m or 0.25m between trials. In tests, in which we removed the flower, the hummingbirds stopped closer to the predicted flower location when the landmarks had been closer to the flower during training. However, while the distance that the birds stopped from the landmarks and predicted flower location was unaffected by the distance that the landmarks moved between trials, the birds directed their search nearer to the predicted direction of the flower, relative to the landmarks, when the landmarks and flower were more stable in the environment. In the field, then, landmarks alone were sufficient for the birds to determine the distance of a reward but not its direction.

## Introduction

Mobile animals have to travel to find food, shelter and other resources, and often return to these at a later time. Some animals may have to navigate between locations separated by tens of metres or even thousands of kilometres (Dyer 1998) while others, such as food-storing birds or rodents, must also accurately pinpoint a location to within a few centimetres (Cheng et al. 2006; Gould et al. 2010). For diurnal animals navigating over relatively short distances, visual features of the environment near to the goal are often important cues (Shettleworth 2009). Furthermore, when the appearance of the goal is variable or hidden from competitors or predators, landmarks can allow animals to relocate the goal reliably without requiring cues from the goal itself.

Our understanding of how vertebrates learn and use landmarks is largely based on laboratory studies (Shettleworth 2009; Gould et al. 2010) and it is rare to examine cue use in the wild. It is not clear, however, whether the laboratory results accurately represent the behaviour of wild animals. There are at least two reasons to expect that they may not. Firstly, landmark experiments are conducted in rooms or mazes that are walled and rarely exceed 3 or 4 metres in diameter (Cheng 1988; Biegler and Morris 1993; Gould-Beierle and Kamil 1996). Secondly, animals experience the experimental arenas only during training and testing, spending the intervening time in holding cages. Wild vertebrates, in contrast, can inhabit a territory of multiple hectares, without walls or other all-encompassing boundaries that significantly restrict their movements, and must balance the demands of navigation with other requirements, such as finding mates, avoiding predation and defending their territory (Healy & Hurly 2003).

Two variables that are often manipulated in laboratory landmark experiments are the distance of the landmarks from the goal and the reliability of the landmarks relative to other cues in the arena. When multiple landmarks are provided around a goal, animals often use some landmarks in favour of others. These preferences are often related to distance: some species appear to prefer to use closer, or “proximal”, landmarks while others prefer to use further, or “distal”, landmarks (Gould et al. 2010). It is common to differentiate between these two types of landmarks on the basis of distance but as it is not clear how notions of “proximal” or “distal” landmarks in the laboratory translate to the cues animals use in the field, where animals inhabit larger, less constrained environments than experienced in the laboratory. It is possible that this differentiation is, in fact, a laboratory artefact. It is not clear, for example, how cues that are considered to be distal or global in the laboratory compare with landmarks that are more than a couple of metres from a rewarded location in the field or to large, distant landmarks such as mountains.

To determine which of the provided landmarks laboratory animals use to relocate a goal, experimenters often move the landmarks and the goal between visits to ensure that other possible cues in the maze or room do not reliably indicate the goal location (e.g. Jones et al. 2002). If an animal can effectively locate a goal using only a pair of landmarks, then the distance that the landmarks and goal are moved between trials should not reduce the accuracy with which an animal searches for a goal. Rather, the farther the landmarks and goal move between trials, the better the landmarks should predict the goal location compared to other, global cues, and so moving the landmarks may be expected to increase the weight that animals would give to the landmarks (Wagner et al 1968).

To determine how these two key components of landmark use in the lab (distance and reliability) might differ from landmark use in the wild, we examined how wild rufous hummingbirds (*Selasphorus rufus*) used a pair of landmarks we provided to remember flower

locations. In the first experiment, we examined whether the accuracy with which hummingbirds stopped in the vicinity of a pair of landmarks for a rewarded location varied depending on the distance from the landmarks to the flower. In the second experiment, we manipulated the distance that the landmarks and a reward moved between visits to see whether the reliability of cues other than the experimental landmarks influenced the accuracy with which the hummingbirds searched for the rewarded location. In both experiments, the birds experienced all of the experimental treatments. As we do not know what larger scales cues hummingbirds might use to orient themselves (e.g. mountains, magnetic variations), we used a repeated-measures design to compare the effect of the different landmark distances and stabilities while controlling for differences in the environment and between birds.

## Methods

### *Subjects and Experimental Site*

The experiment was carried out along the Westcastle Valley, which is located within the Eastern Range of the Canadian Rockies in south-western Alberta (49°29'N, 114°25'W), where feeders containing 20% sucrose solution were placed along the valley in early May 2012. By late May male rufous hummingbirds had arrived from their over-wintering grounds in Mexico and established territories around individual feeders. As the weather got warmer, the sucrose concentration was lowered to 14% and this remained constant for the remainder of the season while testing was carried out. Testing continued until early July, when the males abandoned their territories.

The subjects of this experiment were three territorial male rufous hummingbirds, which we distinguished both by their territorial behaviour and by applying a mark to their

chest using coloured, non-toxic ink. To mark the birds, we trapped them using a wire mesh trap surrounding the feeder. The mark lasted for the six weeks of the field season.

The University of St Andrews Ethical Committee and the University of Lethbridge Animal Welfare Committee approved all of the work described here, which was also conducted according to the requirements of the Canadian Council on Animal Care and under permits from Alberta Sustainable Resource Development and Environment Canada.

### *Experiment 1: Inter-landmark distance*

To test the effects of landmark distance on the ability of the birds to learn a rewarded location, we trained the birds to feed from a flower that was positioned in an equilateral triangle with a pair of landmarks. The distance between the flower and each of the landmarks, was 0.25m, 0.5m, or 1m. In every training trial the landmark-flower array was arranged with the flower placed due south from the mid-point of the two landmarks and we filled the flower with 25% sucrose. The landmarks we used in this experiment were two vertical Plastic pipes (910mm tall x 90mm diameter), one covered in grey tape, the other in blue tape, secured into the ground using sharpened dowling rods. The “flower” was a 700µl Eppendorf tube attached to the top of a 610mm rod of 10mm diameter doweling. During training trials we arranged the triangular array of landmarks and flower in a 36m<sup>2</sup> experimental open arena or “pitch”, aligned north to south. We organised the pitch into four equal quadrants and marked each quadrant with a small rock at each corner (see Figure 1). We set up camcorders on 1.3m tripods 6m south and either east or west of a pre-chosen “test quadrant”. Each camera faced the centre of the test quadrant, with the bottom of its visual field aligned to a point halfway (3m) between the tripod and the quadrant centre. The experimenter sat a further 3 metres behind one of the cameras.

### *Training trials*

We considered every visit to the flower by the birds a trial, and we trained birds for 180 trials each. We organised the 180 trials into three blocks of 60 trials in each of which the landmarks were 0.25 m, 0.5m or 1m from the flower. We pseudo-randomised the order of presenting the three different blocks across birds such that the order differed for each bird. Between trials, we moved the landmarks and flower to a different quadrant on the pitch. We pseudo-randomised the order of quadrants so that birds visited all quadrants at least twice before they experienced a test trial. Within each quadrant we always placed the array in a different position so that at no point during the experiment did we return the flower or the landmarks to a previously rewarded location.

### *Test trials*

On every 10<sup>th</sup> visit within a 60-trial block the birds experienced a test trial. For a test trial we presented the landmarks in the test quadrant but removed the flower. We set the cameras to record and calibrated the video with a 0.4m x 0.4m chequerboard of 40mm x 40mm squares placed 1m above the ground at 3m and 6m from each camera. We made a sharp noise so that we could synchronise the videos for later analysis. When the bird returned, we recorded the flight of the bird and the stops he made around the landmarks.

Following the test trial, we moved the landmark-flower array to the next quadrant to start the next set of 10 trials. After the test that marked the 60<sup>th</sup> trial, we returned the bird's feeder and stopped training for at least three hours before we began the next block.

### ***Experiment 2: Landmark Stability***

The second experiment immediately followed the first for each subject. Our aim was to determine whether the distance that we moved the array between visits affected the accuracy

with which the birds searched. For this experiment we trained and tested all birds on a smaller pitch, 4m<sup>2</sup>, which we established at least 4m from that we had used for Experiment 1.

### *Training trials and test trials*

We trained and tested birds in two 60-trial blocks, varying the order of blocks across birds. In this experiment, we again arranged the landmarks and reward flower in a triangular array, but this time the distance between each of the three array components was always 0.25m. We manipulated only the distance we moved the array after each trial. In one condition, we moved the array of landmarks and flower 1 metre, to a different quadrant, in a pseudo-randomised order. Within each quadrant the landmarks and flower were never placed in the same location. For the other condition, we moved the landmarks and flower 0.25m in one of eight directions (N, NE, E, SE, S, SW, W, NW), the order of which was pseudorandomised with the condition that the flower was never in the same location twice but that the landmarks and flower always remained on the pitch.

As before, every tenth trial was a test in which we moved the landmarks to the next location and removed the flower. Each bird was tested six times in each of the two conditions, resulting in twelve tests per bird, and eighteen tests in each condition. In the 0.25m shift condition, the landmarks were moved 0.5m for the test trial rather than 0.25m. This was to ensure that during the test the previous location of the flower was more than 0.25m from the location that would be predicted by the landmarks. In the 1m shift condition, we moved the landmarks 1m before each test, as during training, because we considered that 1m was a distance sufficient to allow us to distinguish between the predicted flower location and the location of the flower in the previous training trial.

### *Data analysis*



From the video data, we extracted the frames that contained the chequerboard at both 3m and 6m from the camera, as well as the first and second stops by the birds (hummingbirds will hover in 3-D space and we termed these ‘stops’). We identified the location of the birds by placing a red dot over the head of the hummingbird. We used a custom code written in Python to cycle through the images, storing the x,y pixel coordinates of the landmarks, the corners of the chequerboard, the location 6m from each camera, and the locations of the birds. We used a custom R script (R Development Core Team 2012) that used the pixel dimensions of the chequerboard at the two distances to estimate the field of view of the camera as well as the position of the camera, as viewed from above, by locating the intersection of the edges of the field of view. Before training and testing began we arranged the cameras at 90 degrees to each other and using the camera locations and the field of view we reconstructed the position of the landmarks and hummingbird stops. We did this by fitting a line perpendicular to the direction of view from each camera, running from one edge of the camera’s field of view to the other. We called each line an “image plane” as they corresponded to the x-axis of the images from each camera. Next we determined the position of features, such as the hummingbird and either side of the landmarks, on the image plane by dividing the x-coordinate of each feature by the total pixel width. Finally, for each camera, we determined the equation for a line, referred to as the “feature-line”, which passes through both the camera position and the position of a particular feature on the image plane. We considered the point at which the feature lines from both cameras intersected to mark the position of that feature. The x,y location of the landmarks was based on the centre of the landmarks with the radius of the landmarks based on the mean distance of the sides from both cameras.

We did not consider the raw x,y data to be exact reconstructions of the locations of the landmarks or hummingbird as we could not correct the distortion of the image produced

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

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

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

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

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

moved between visits) as a fixed factor and bird as a random factor. We tested the distances that the birds stopped from the flower location, and from the landmarks, for normality using Shapiro-Wilks tests, and when these variables were not normally distributed and were positively skewed, we log-transformed the variables prior to analysis. We analysed the directions of the stops using a Rayleigh test (or “V test” (Batschelet 1981)), which enabled us to determine whether the distribution of a set of directions was significantly oriented in a specific direction, in this case the direction of the flower, to that expected from a uniform distribution. In later analyses we used GLMs to compare how the direction that the birds stopped from the landmarks differed between treatments (distance of the flower from the landmarks, or distance moved between visits), using the observed directional component (V) as an independent variable. As V scores are bounded between -1 and 1, we added 1 to each score, and then divided each score by 2 to convert the data to a proportion. We then arcsine-transformed the data to make them suitable for analysis. Values are presented as mean  $\pm$  SE.

## Results

### *Experiment One: Landmark Distance*

During the test trials, the birds stopped and hovered near the landmarks a mean of  $3.87 \pm 0.26$  times before leaving the pitch across all test trials, of these stops we only examined the first two. The number of stops did not differ within each treatment (One way ANOVA of number of stops with test number (1-6):  $F_{5,48} = 1.22$ ,  $P = 0.314$ ) or between treatments (ANOVA of number of stops with landmark distance :  $F_{2,51} = 1.17$ ,  $P = 0.318$ ).

We excluded from further analysis two of the 54 first stops (one from Bird 2, one from Bird 3) and two of the 54 second stops (both from Bird 3) because they were more than 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 &amp; 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

(first or second), test number, and bird as a random factor: landmark distance,  $F_{2,3.61} = 10.23$ ,  $P = 0.033$ ; Figures 2 & 3a). When the landmarks had been 0.25m from the flower all of the birds stopped 0.5-0.6m closer to the actual flower location than they did when the landmarks had been 1m from the flower (mean  $\pm$  SE of distance of stops from the flower location:  $0.57\text{m} \pm 0.026$ ). The birds did not stop consistently closer or further from the flower location on their second stops (stop order,  $F_{1,1.96} = 5.80$ ,  $P = 0.14$ ) or become more or less accurate with repeated tests (test number,  $F_{5,9.46} = 1.73$ ,  $P = 0.22$ ). Although in most of the tests, the birds increased in accuracy when the distance between the landmarks and the flower became smaller, in some tests individual birds stopped further away from the flower location when the landmarks were 0.5m from the flower, than when they were 1m away (Birds 1 & 3, first test; Bird 1 second test), or further when the landmarks were 0.25m away than when they were 0.5m away (Bird 1, second and sixth tests; Bird 2, first test; Bird 3, fourth test), or when the closest to the flower location when the landmarks were 1m from the flower, than when the landmarks were 0.5m or 0.25m (Birds 1 & 2, fifth test) (landmark distance x test number x bird,  $F_{19,14} = 2.52$ ,  $P = 0.042$ ; all other interactions  $P > 0.05$ ).

These results by themselves are not straightforward to interpret. Although, intuitively, the distance that the birds stopped from the flower location is an appropriate measure of accuracy, there may be other reasons why the birds stopped closer to the location of the flower when the landmarks had been closer to the flower during training. For example, if the birds had learned neither the distance nor the direction of the flower, but just searched around the landmarks, this would also have lead the birds to stop closer to the predicted flower location than when the landmarks had been closer to the flower because the landmarks themselves were closer to that location. The distance from the predicted flower location that the birds stopped cannot, then, allow us to discriminate between these alternative explanations. To better describe how the birds used the landmarks, we analysed the distance

and direction of their first and second stops from the landmarks. If the birds searched only around the landmarks, they should have searched at the same distance and direction from the landmarks irrespective of the distance between the landmarks and flower. Alternatively, if the birds had learned about the distance and direction of the flower from the landmarks, and searched more accurately when the landmarks were closer to the flower, we might expect the birds to stop closer to the correct distance from the landmarks when the landmarks had been 0.25m from the flower than when they had been 1m from the flower. We might also expect that the birds were searching more in the correct direction from each landmark, as measured in degrees. Although, as the error in degrees around a bearing would cover a smaller area 0.25m from the landmarks than it would 1m from the landmarks, the birds could also appear more accurate while not actually reducing their directional error.

#### *Direction from landmarks*

To determine whether the distance of the landmarks from the flower during training affected the direction in which the birds stopped during the test trials, we first calculated the direction of each first and second stop from both the left and the right landmarks. If the birds searched more accurately when the landmarks were closer to the flower, the directions that the birds stopped from each landmark should have clustered more in the trained direction of the flower when the landmarks were 0.25m from the flower, than when the landmarks were 1m from the flower. The directional component (V) is a statistic that measures the degree to which a collection of direction cluster in a given reference direction. We calculated V statistics for the first and second stops from each landmark for each treatment (1m, 0.5m, 0.25m), using the trained direction of the flower as the reference direction. Using the V statistics as a measure of direction accuracy, we then ran a GLM on the directional accuracy (V) with the distance of the landmarks from the flower, and the order of the stops (first or second) as fixed factors.

Although the distance between the landmarks and the flower did not affect the accuracy of the birds' stop direction (GLM: landmark distance and stop order: landmark distance,  $F_{2,6} = 2.50$ ,  $P = 0.162$ ), the birds were more accurate on their second stops (stop order,  $F_{1,6} = 18.17$ ,  $P = 0.005$ ). We did not find, therefore any evidence that the birds stopped more accurately in the direction of the flower when the landmarks were closer to the flower during training (Figure 4).

#### *Distance to the landmarks*

Although the distance of the flower from the landmarks did not affect how accurately the birds searched in the direction of the flower from the landmarks, we might expect that the distance of the flower from the landmarks to have affected the distance that the birds stopped from the landmarks. This is because although the flower was always in the same direction from the landmarks, the distance of the flower from the landmarks changed across treatments. In order to determine whether the distance that the birds stopped from the landmarks was affected by the distance of the flower from the landmarks during training, we first calculated the distance of the stops from each landmark, and then ran a GLM of the distance from the landmarks, with landmark distance during training (1m, 0.5m, 0.25m), stop order (first or second), landmark identity (right or left), and test number with the trial (1-6) as factors. When the flower had been closer to the landmarks, the birds also stopped closer to the landmarks (mixed GLM of log distance of stops from the landmarks, with landmark distance, stop order, landmark (right or left), and test number, and bird as a random factor. Landmark distance:  $F_{2,3.63} = 15.44$ ,  $P = 0.017$ ; Figure 3b). There were no significant differences in the distances from the landmarks between the first and second stops made by the birds (stop order:  $F_{1,1.92} = 6.14$   $P = 0.14$ ) and no difference in the distances from right or left landmarks of the hummingbirds' stops (landmark:  $F_{1,1.96} = 3.88$ ,  $P = 0.19$ ). The birds appear to have learned

the distance from the landmarks within 10 visits as they were no more accurate after 60 trials than after 10 trials (test number:  $F_{5,9,61} = 1.03$ ,  $P = 0.45$ . All interactions  $P > 0.05$ ).

### *Coefficient of Variation*

As the hummingbirds appeared to have learned something about the distance of the landmarks from the flower and searched closer to the flower location when the flower was closer to the landmarks, we next asked how the hummingbirds may have estimated the distance from the landmarks. Pigeons search more precisely when trained with a reward a short distance from landmarks, than when trained to a location a greater distance away (Cheng 1990). It is possible that the hummingbirds may also estimate the distance to the closer landmarks better because small differences between small distances are more discriminable than are the same differences between further distances (Weber's Law: Shettleworth 2009). If this is the case then we would expect the standard deviation of the distance that birds stopped from the landmarks to increase the further the landmarks from the flower but the coefficient of variation, the ratio of the standard deviation to the mean, should remain constant. To examine this, we combined the standard deviation and coefficient of variation into a single variable, which we called "variation", in which half of the values were standard deviations for each landmark distance, and half were coefficients of variation. We then ran a GLM of "variation" with the source of the variation (whether the value was a standard deviation, or a coefficient of variation), and landmark distance as factors. If the standard deviation and coefficient of variation differed in their response to the landmarks, we predicted that we would see an interaction between the distance of the landmarks and whether the "variation" was a standard deviation or the coefficient of variation. The standard deviation and coefficient of variation did not respond differently to the distance of the flower from the landmarks (mixed GLM of variation in distance from the landmarks with the source



of variation (Standard Deviation or log Coefficient of Variation) and distance, and bird as a random factor: source of variation,  $F_{1,2} = 14.761$ ,  $P = 0.062$ ; distance,  $F_{2,4} = 3.58$ ,  $P = 0.13$ ; source of variation x distance,  $F_{2,4} = 3.59$ ,  $P = 0.13$ ; Figure 3c). The birds did not search more precisely when they had learned the location of a flower 0.25m from landmarks than when they had learned the location of a flower 0.5m or 1m away from the landmarks.

## **Experiment Two: Landmark Stability**

In Experiment 2, we, again, analysed only the location of the first two stops made by the birds in each test. There was no difference in the number of stops after 10 visits or 60 visits (one-way ANOVA:  $F_{5,30} = 1.74$ ,  $P = 0.155$ ) but they stopped significantly more often when we moved the landmarks 1m between visits rather than 0.25m (mean stops 1m:  $4.17 \pm 0.73$ , mean stops 0.25m:  $2.78 \pm 0.58$ ; one-way ANOVA:  $F_{1,34} = 5.20$ ,  $P = 0.029$ ). No stops were more than 2 standard distances from the mean stop location for each bird and we did not exclude any of the data points from the analyses.

### *Use of the landmarks*

As in the first experiment, we first determined whether the birds followed the landmarks or continued to search at the previous location of the flower. The previous flower location was 1m or 0.5m away in the 1m and 0.25m conditions respectively. In both conditions, the birds stopped closer to the predicted flower location than to the previous location (moved 1m:  $0.47 \pm 0.06$ m; moved 0.25m:  $0.29 \pm 0.03$ m). Again, the birds did not search at the previous flower location.

Our next question was whether the movement of the landmarks and flower between visits affected how close the birds stopped to the predicted flower location. As in Experiment 1, we ran a GLM of the distance that the birds stopped from the flower's predicted location,

with the distance that the landmark-flower array moved between visits (1m or 0.25m), the stop order (first or second), and the test number in the treatment (1-6) as factors, and bird identity as a random factor. Birds did not stop significantly closer to the flower's location when we had moved the array 0.25m between visits than when we had moved the array 1m (mixed GLM of log distance of stops from the flower location, with distance moved, stop order and test number, and bird as a random factor, distance moved:  $F_{1,1.96} = 5.52$ ,  $P = 0.15$ ; Figures 5 & 6a). The birds stopped closer to the flower location on their second stops than they had on the first stop (stop order:  $F_{1,1.38} = 280.67$ ,  $P = 0.016$ ). Although there was no consistent difference across tests (test number:  $F_{5,9.97} = 0.40$ ,  $P = 0.839$ ), individual birds did differ in their accuracy across tests, depending on the distance that we had moved the array between visits (distance moved x test number x bird:  $F_{10,7} = 6.55$ ,  $P = 0.01$ ; all other interactions  $P > 0.05$ ).

### ***How did the birds use the landmarks?***

Although we did not find that the stability of the landmarks and flower affected the proximity at which birds stopped relative to the flower location, this does not mean that the stability of the landmarks had no effect on the birds' search locations. To look at the effect of landmark on where the birds searched in more detail, we examined whether the distance that the landmarks and flower moved between visits affected the distance and direction that the birds stopped from the landmarks.

Examining first the effect of the distance that the landmark-flower array had been moved between visits on the distance that the birds stopped from the landmarks, we ran a GLM of the distance of the stops from each landmark, with the distance that the landmarks moved between visits (1m, 0.25m), the stop order (first or second), the landmark identity (left or right), and the test number in the treatment (1-6) as factors. The distance that we moved

the landmarks between trials had no effect on the distance that the birds stopped from the landmarks (mixed GLM of log distance of stops from landmarks with distance moved (0.25m or 1m), stop order, landmark, and test number, and bird as a random factor; distance moved:  $F_{1,0.99} = 0.95$ ,  $P = 0.51$ ). The birds consistently stopped closer to the landmarks on their second stops than they had on their first stop (stop order :  $F_{1,1.75} = 72.47$ ,  $P = 0.02$ ). The birds did not, however, stop at a different distance from the landmarks after 60 trials than they had after 10 trials (test number (without Experiment 1 data):  $F_{5,9.98} = 0.046$ ,  $P = 0.10$ ), and they did not stop consistently closer to one landmark than the other (landmark:  $F_{1,1.94} = 0.93$ ,  $P = 0.44$ ; All other interactions  $P > 0.05$ ). Landmark stability did not, therefore, appear to have an effect on the distance at which the birds stopped from the landmarks.

Finally, we looked to see whether landmark stability affected the direction that the birds searched from the landmarks. In order to examine the effect on direction, we again calculated V statistics, to represent the degree of cluster of the stops in the direction of the flower, for the first and second stops from both the right and left landmarks in each treatment. Following this, as in Experiment 1, we used a GLM of the V statistics with the distance that the landmarks moved between visits (1m or 0.25m) and stop order (first or second) as factors to determine whether the accuracy in stop direction was influenced by the distance that the landmark-flower array was moved. The birds were more accurate when we moved the landmarks 0.25m vs. 1m (two-way GLM of arcsine of V with distance moved (0.25m or 1m) and stop order (first and second): distance moved,  $F_{1,4} = 22.23$ ,  $P = 0.009$ ) and stopped significantly closer to the direction of the flower in their second stops (stop order,  $F_{1,4} = 10.16$ ,  $P = 0.033$ , Figure 6b) .

## 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

away, rather than when provided with landmarks that are further away (Gould-Beierle and Kamil 1999; Chamizo et al. 2006). Furthermore, nearby landmarks in the lab can overshadow those further away, resulting in animals relying on very local landmarks to return to remembered locations (Cheng 1989; Cheng 1990; Spetch and Wilkie 1994; Spetch 1995). As hummingbirds will use visual features within 0.8m to decide between which of multiple flowers to visit (Healy and Hurly 1998; Hurly & Healy 2002; Henderson et al. 2006), the results of the first experiment might suggest that such nearby cues are more useful to the hummingbirds and that the apparent superiority of landmarks within half a metre of the flower results in hummingbirds relying less on further away cues when returning to a flower location.

To address these possibilities, we must look at where the hummingbirds stopped in relation to the landmarks and not just at how close to the predicted flower location they stopped. In the lab, nearby cues are thought to result in greater search accuracy as animals estimate short distances from landmarks more accurately than they estimate greater distances (e.g. Cheng, 1990), a phenomenon known as Weber's Law. If Weber's Law is responsible for the hummingbirds' greater accuracy when trained with landmarks within 0.5m of the flower, the relative error in their distance estimates should have increased as the distance of the landmarks from the flower increased. In fact, while the birds did stop at appropriate distances from the landmarks, the distances that they stopped from the landmarks were not more precise when they had been trained with landmarks 0.25m from the flower than when they had been trained with landmarks 1m from the flower. It seems unlikely, therefore, that Weber's Law can explain why the hummingbirds stopped closer to the flower location when trained with landmarks closer to the flower. Instead, it would appear that, by stopping closer to the landmarks when trained with landmarks closer to the flower, the birds searched within a smaller area around the landmarks and were closer to the flower location as a result.

Despite the birds stopping closer to the location of the flower when the landmarks were 0.25m from the flower during training we cannot say, from the data we present here, that hummingbirds can use nearer landmarks more accurately than they can use landmarks that are further away.

We can also describe where the hummingbirds searched relative to the landmarks by examining the direction of their stops in relation to each landmark. In the first experiment, the hummingbirds did not confine their searching to the side of the landmarks where the flower would have been found. Rather they stopped all around the landmarks. Given the results of Experiment 2, it seems likely that moving the landmarks and flower 3-4m between visits may have prevented the birds from orienting themselves relative to the landmarks. Such disorientation would mean that the results from the first experiment may not reflect the accuracy with which the hummingbirds can use landmarks at different distances from a reward. In Experiment 2 the birds stopped more in the direction of the flower when the landmarks were moved 0.25m between visits. To determine whether this greater accuracy, where the landmarks were 0.25m from the flower, would be maintained when the landmarks are 0.5m, or 1m from the flower one would need to test the birds when moving the landmarks and flower only short distances between visits.

That the distance that the landmarks and flower were moved between visits affected the direction but not the distance searched suggests two things: first, hummingbirds, similar to bees (Cheng 1998) and other birds (Cheng 1994), may encode distance and direction separately, rather than as a whole vector (Gibson and McGowan 2014), although further experiments are required to confirm this; and second, while landmarks alone appear sufficient for the birds to estimate the distance of a goal from the landmarks, the landmarks alone are not sufficient to provide directional information.

Theoretically, the birds could have used the pair of individually distinctive landmarks as a configural cue to orient: as the landmarks are two corners of landmark flower array, the birds could have used those landmarks to find the missing third corner where the flower would have been located. If this had been the case, it seems unlikely that the distance that we moved the landmarks between the birds' visits would have affected the birds' ability to find the flower's location. On the contrary, by establishing the landmarks as the most reliable indicator of a flower's location, moving the landmarks and flower further between visits should have resulted in the birds paying more attention to the landmarks, and searching more accurately when the flower was removed (e.g. Roberts & Pearce 1998).

As the hummingbirds did not search more accurately in the direction of the flower when the landmarks were moved 1m rather than 0.25m between visits, and indeed searched less accurately, it appears unlikely that they used the configuration of the two experimental landmarks to remember the direction of the flower. Instead, the birds seem to have relied on other cues, such as the configuration of one of the landmarks and other, non-experimental, global information in the environment. This result conflicts somewhat with findings from animals tested in the laboratory, which, unlike the hummingbirds, readily use the configuration of a set of experimental landmarks when other possible orientation cues are unavailable or made unreliable (Collett et al. 1986; Biegler and Morris 1996; Gould-Beierle and Kamil 1996; Kamil and Jones 2000; McGregor et al. 2004; Gibson et al. 2007; Kelly 2010). There are at least two possible reasons why our hummingbirds may have differed from animals tested in the laboratory. First, differences in the scale of experimental arena and cues in the laboratory and the field could mean that other cues are more readily controlled in the laboratory. If in general animals learn to use the configuration of experimental landmarks only when all other cues are unreliable, it may be easier to make other cues unreliable in the smaller, enclosed space of the laboratory room or maze, where moving the landmarks array

even small distances will considerably change the relationship between the landmarks, the reward, and global cues. In the field, the relationship between the reward and distant or extended cues may have remained stable enough over the course of training, to possibly result in the nearby, experimental landmarks never becoming solely predictive of a reward's location. The presence of these other cues may, then, have prevented the hummingbirds from learning to use the configuration of the experimental landmarks alone to direct their searching. Confirmation of this would need further experimental manipulations such as rotations of the array coupled with translocations between visits, which would render all other cues unreliable.

Secondly, in many of the laboratory studies animals are trained to search for hidden food or a submerged platform rather than for a visible feeder, such as the flower in our experiments. If moving the landmarks 1m between visits interfered with the birds' ability to determine the direction of the reward from the landmarks, the flower itself could have been acting as a beacon and outcompeting the configuration of the landmarks during training. Hummingbirds do not usually rely on beacons to relocate flowers (Hurly & Healy 2002; Hurly et al. 2010) and the birds in our experiment also did not appear to rely solely on a beaconing strategy as when the landmarks were relatively stable (moved 0.25m between visits), the birds appear to have oriented relatively well. Wild hummingbirds will, however, learn to beacon to flowers when all other cues are unreliable (Flores-Abreu et al. 2012), just as rats in the lab will learn to rely on a beacon during navigation, if the beacon and goal are moved every trial (Roberts and Pearce 1999; Gibson and Shettleworth 2003). Regardless of whether moving the landmarks resulted in the birds relying more heavily on a beaconing strategy to relocate the flower, the use of a visible target in our experiment may have affected the search behaviour by the birds. In laboratory experiments where animals search for a hidden goal, the search distributions are often more focussed than are those seen in these field



experiments (e.g. Cheng 1988; Cheng 1989; Gould-Beierle and Kamil 1996; Kamil and Jones 2000). One reason for this is that may be that if the hummingbirds notice that the flower has gone, they may not search exactly where they think the flower had been but might be more likely to search around the general area in which they remembered they had seen the flower.

In conclusion we have demonstrated here firstly that studies of small-scale spatial cognition in vertebrates need not be confined to the laboratory. Firstly, wild free-living hummingbirds can be trained to use experimental landmarks to return to rewarded locations. Secondly, birds can remember the distance of a reward from the landmarks and can use those landmarks alongside other information to direct their search. We cannot say, from the data presented here, however, what other information the hummingbirds require to orient relative to the experimental landmarks or how hummingbirds or other animals in the wild may use landmarks to remember the locations of resources. Although these questions have been well studied in the laboratory (reviewed in e.g. Cheng et al. 2006; Gould et al. 2010) they have not, to our knowledge, attracted much attention in wild vertebrates.

## **Acknowledgements**

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

## **References**

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, Hurlly 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

- Shettleworth S (2009) Cognition, evolution, and behavior. Oxford University Press
- Spetch ML, Wilkie DM (1994) Pigeons' Use of Landmarks Presented in Digitized Images. *Learn Motiv* 25:245–275. doi: 10.1006/lmot.1994.1014
- Spetch ML (1995) Overshadowing in landmark learning: Touch-screen studies with pigeons and humans. *J Exp Psychol Anim Behav Process* 21:166–181. doi: 10.1037/0097-7403.21.2.166
- Wagner, A R., Logan, F. A, Haberlandt, K., & Price, T. (1968). Stimulus selection in animal discrimination learning. *J Exp Psych*, 76(2), 171–180. doi:10.1037/h0030023

## Figure Legends

**Figure 1:** Bird's eye diagram of the experimental pitches. In Experiment 1 the pitch was 6m across, with the cameras centred on a test quadrant. In Experiment 2 the pitch was 2m across, with the cameras centred on the centre of the pitch.

### Figure 2:

a. The location of the first stops around the landmarks by the birds in Experiment 1. The light and dark grey circles indicate the position of the landmarks, while the dashed circle shows the predicted position of the flower relative to the landmarks. Each smaller, black shape shows the location of the first stop by a bird in one of the 6 tests, with each bird represented by a different shape (Bird 1: triangles; Bird 2: circles; Bird 3: squares).

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

### Figure 3:

a. The mean distance ( $\pm$  SE) of the first (closed symbols) and second stops (open symbols) from the location of the flower in Experiment 1. Bird 1 (triangles), Bird 2 (circles) and Bird 3 (squares) all stopped closer the flower location when the landmarks were closer to the goal.

b. The mean distance ( $\pm$  SE) of the first (closed symbols) and second stops (open symbols) from the landmarks in Experiment 1. The dashed red line shows the distance of the flower from the landmark during training. Bird 1 (triangles), Bird 2 (circles) and Bird 3 (squares) all stopped closer to the left landmark (unbroken error line) and right landmark (dashed error line) when the landmarks were closer to the flower.

c. The variation in the distances that the birds stopped from the landmarks. If the birds were more precise at estimating shorter distances, we predicted that the Coefficient of Variation for

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

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

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

**Figure 6:**

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

## Supplementary material

### Analyses testing the effects of experience

#### Experiment 1

Over the three treatments in Experiment 1, each bird experienced 180 trials, consisting of 162 training trials and 18 test trials. The order in which each bird experienced the different landmark distances was balanced across birds, such that experience with the experiment was not correlated with landmark distance. Still, if the birds' behaviour changed as a result of their experience across the first experiment, we might expect them to stop closer to the flower

locations, closer to the trained distance from the landmarks, or more in the direction of the flower from each landmark in the third treatment rather than their first treatment.

To test whether the birds' behaviour changed across the three treatments we ran three different analyses to look at how distance from flower location of their stops, difference between trained distance and stopping distance, and accuracy of direction, changed across the three treatments.

### **Distance to the flower location**

In order to determine whether birds stopped closer to the predicted location of the flower as they had more experience of the experiment, we ran a GLM to examine the effect of landmark distance including treatment order (whether the treatment was the first, second, or third that the bird had experienced) as a factor. If experience had an effect, we would expect to see a significant effect of treatment order, a significant effect of test number within the treatment, or a significant interaction between treatment order and test number. But we did not find significant effects of any of these possibilities (mixed GLM of log distance from the flower location with treatment order (first, second, or third), stop order (first or second) and test number within treatment (1-6), and bird as a random factor: treatment order,  $F_{2,3.92} = 0.123$ ,  $P = 0.887$ ; test number,  $F_{5,9.40} = 0.722$ ,  $P = 0.194$ ; treatment order\*test number,  $F_{10,18.21} = 0.946$ ,  $P = 0.517$ ). We could not discern an effect on the distance from the goal location that the birds stopped due to their experience gained during the first experiment.

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

In order to determine whether birds stopped closer to the flower's location from the landmarks with increasing experience of the experiment, we subtracted the distance from the landmarks that the birds stopped from the distance of the flower from the landmarks, for each

treatment. For example, when the landmarks were 1m from the flower, we subtracted 1m from the distance that the birds searched from the landmarks. As the differences between the search distance and the training distance were not normally distributed, we log transformed the data prior to analysis. If experience had an effect, we would expect to see a significant effect of treatment order, a significant effect of test number within the treatment, or a significant interaction between treatment order and test number. Again, we could see no significant effects of any of these variables (mixed GLM of log difference between training landmark distance and distance searched from the landmarks, with treatment order (first, second, or third), stop order (first or second) and test number within treatment (1-6), and bird as a random factor: treatment order,  $F_{2,3.90} = 0.588$ ,  $P = 0.598$ ; test number,  $F_{5,9.60} = 1.88$ ,  $P = 0.188$ ; treatment order\*test number,  $F_{10,17.82} = 0.839$ ,  $P = 0.599$ ). Birds did not stop closer to the training distance as their experience of the experimental set up increased.

#### **Direction of stops from the flower**

The final measure of performance we examine was how the accuracy of the birds stop direction changed as they had more experience of the first experiment. As a measure of accuracy we used the V statistic, which represents the degree to which a collection of directions are clustered in a particular reference direction, in this case the direction of the flower during training. For this analysis we calculated V statistics from the directions that the birds searched during their first, second, or third treatment. In order to determine whether the birds searched more accurately in the direction of the flower with more experience of the experiment, we ran a GLM of the calculated V statistics with treatment order (the first, second, or third treatment that the bird experienced) and the stop order by the birds. If the birds were more accurate at estimating the direction of the flower with more experience of the experiment, we would predict a significant effect of treatment order on V, and possibly a

significant interaction between treatment order and stop order. We did not find significant effects for any of these (GLM of arcsine of directional component ( $V$ ), with treatment order (first, second, or third) and stop order: treatment order,  $F_{2,6} = 1.19$ ,  $P = 0.365$ ; treatment order\*stop,  $F_{2,6} = 1.007$ ,  $P = 0.420$ ). Birds did not stop more accurately in the direction of the flower with increasing experience of the first experiment.

## Experiment 2

Assessing the effect of experience in the second experiment was difficult for two reasons. First, by the second experiment, the birds had already experienced 180 trials over which they showed no sign of improvement in any of our measures of performance. Second, in Experiment 2 two of the three birds experienced trials in which we moved the landmarks 1m before they experienced the landmarks being moved 0.25m, which would confound stability and experience for those two birds. It is not perhaps surprising, then, that we found a significant effect of treatment order, with birds stopping more in the direction of the flower in their second treatment compared to their first (GLM of arcsine of directional component ( $V$ ), with treatment order (first or second) and stop order: treatment order,  $F_{1,4} = 32.778$ ,  $P = 0.005$ ; treatment order\*stop,  $F_{1,4} = 0.026$ ,  $P = 0.881$ ).

To look at the effect sizes for order, we ran two sets of Pearson correlations, one with regard to the accuracy of search direction and the order of treatments, and another with regard to the accuracy of search direction and the distance that the landmarks moved between visits. There was no significant correlation between the accuracy of direction and the order of treatments (Pearson correlation of arcsine of directional component and treatment order (first 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.

839