



Female hummingbirds do not relocate rewards using colour cues



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Males generally outperform females in spatial tasks. This difference in spatial performance may reflect differences in cue preference because males often use both spatial cues (distance and direction) and feature cues, whereas females prefer to use feature cues. However, studies in birds are few and results are conflicting. As wild male rufous hummingbirds, *Selasphorus rufus*, prefer to use spatial cues to relocate a rewarded flower, in the present study we tested free-flying wild female hummingbirds of three different species (rufous, white-eared, *Hylocharis leucotis*, and magnificent hummingbird, *Eugenes fulgens*) and males of one species (white-eared) for their cue preference in the same task in which rufous males have been tested previously. Birds were allowed to feed once from a four-flower array in which only one flower was rewarded. When the birds returned, the colour and the spatial cue designating the rewarded flower had been dissociated. Although we had expected females to visit the flower of the correct colour (feature cue) first, during the test phase most of the birds (males and females) went to the correct spatial location (spatial cue). It appears, then, that preference for spatial cues is not specific to males and it seems more likely to depend on the relevance or value of a cue to the solution of the task.

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Males typically outperform females on a range of spatial learning tasks. One consistent feature of the males' performance is that they use distance, direction and feature cues whereas females prefer to use mainly feature cues to solve the task (Collins & Kimura, 1997; Gaulin & Fitzgerald, 1986; Jozet-Alves, Modéran, & Dickel, 2008; Kavaliers et al., 1996; Lacreuse, Herndon, Killiany, Rosene, & Moss, 1999; Schmidtke & Esser, 2011; Seymoure, Dou, & Juraska, 1996). This effect appears to hold across both species and tasks. For example, when returning to a platform in a Morris water maze male rats outperform conspecific females when extramaze landmark cues are absent but not when a landmark is made available (Roof & Stein, 1999). Similarly, on a computer screen version of a delayed non-matching-to-sample task, women were more likely to use feature than location cues, whereas men used both types of information equally (Jones & Healy, 2006). Even when describing the location of a place, men tend to provide Euclidean information (distance and direction) whereas women provide information on landmarks (Dabbs, Chang, Strong, & Milun, 1998). Furthermore, for male rats Euclidean information overshadows landmark information but for female rats the opposite is true (Rodríguez, Chamizo, & Mackintosh, 2011).

This male advantage in spatial cognition is so typical that there are multiple evolutionary hypotheses purporting to explain these differences, typically by relating sex differences in spatial abilities to the use of space (e.g. range size: Gray & Buffery, 1971; male foraging: Silverman et al., 2000; see Jones, Braithwaite, & Healy, 2003). In particular, since polygynous males cover a larger home range than their conspecific females, it has been suggested that selection has favoured those males with better spatial abilities (Gaulin & Fitzgerald, 1986, 1989; Perdue, Snyder, Zhihe, Marr, & Maple, 2011).

The sex difference in cue use appears not to be confined to mammals as male domestic chicks, *Gallus gallus domesticus*, used position cues to relocate a food reinforcement whereas female chicks used colour cues more readily (Vallortigara, 1996) and female shiny cowbirds, *Molothrus bonariensis*, retrieved food rewards faster than males only when the food was associated with a colour cue (Astié, Kacelnik, & Rebores, 1998). But the avian data are more mixed than are the data from the mammalian literature. For example, both male and female great tits, *Parus major*, used a position cue to relocate a reward hidden in one of three wells rather than the colour of a cloth on top of each well (Hodgson & Healy, 2005). Furthermore, if cue preference is associated with selection pressure for better spatial ability, then in the cowbirds one would expect the females to be the sex that prefers/uses spatial rather than feature information as it is the females that appear to have the great spatial memory demand (as they alone search for nests to

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parasitize; [Reboreda, Clayton, & Kacelnik, 1996](#)) and the better spatial memory: female brown-headed cowbirds, *Molothrus ater*, outperformed conspecific males in a spatial memory task ([Guigueno, Snow, MacDougall-Shackleton, & Sherry, 2014](#)). The lack of a compelling association between spatial demand/performance and preferential use of spatial information in birds led [Hodgson and Healy \(2005\)](#) to suggest that cue use/preference might be due to the relative value of those cues in the specific context in which the animals were tested, i.e. that cue use might be context dependent rather than favoured by selection.

Hummingbirds are a useful group in which to address sex differences in cue use because in most species the sexes differ in foraging behaviour and their use of space. Males are typically territorial, defending and feeding from hundreds of flowers within their territories every day ([Kodric-Brown & Brown, 1978](#)) and in most species are considered to be polygynous. Females, on the other hand, are not usually territorial, are thought to forage by trap lining ([Temeles, Shaw, Kudla, & Sander, 2006](#)) and provide all of the parental care. Consistent with this expectation, wild male rufous hummingbirds, *Selasphorus rufus*, preferentially used spatial information to return to a previously rewarded artificial flower ([Hurly & Healy, 1996](#)).

To determine whether female hummingbirds preferred to use feature rather than spatial cues we tested female hummingbirds of three different species using a similar task to that used by [Hurly and Healy \(1996\)](#). In this test birds visited a four-flower array in which all four artificial flowers were a different colour and only one of them contained reward. Prior to the birds' return, we emptied the flower and switched it with one of the other flowers. If the birds preferred to use feature cues to choose the flower it expected to contain reward (the original flower contained more sucrose than the bird could consume in a single visit), it should visit the flower of the 'correct' colour. If, however, it preferred to use spatial cues, it should visit the flower in the original location (the 'correct' spatial cue). We tested female rufous hummingbirds at a site in Canada and females of the white-eared hummingbird, *Hylocharis leucotis*, and of the magnificent hummingbird, *Eugenes fulgens*, at a site in Mexico. As we had the opportunity, we also tested male white-eared hummingbirds.

Our expectation was that if space use selects for a preference for using spatial cues, then like female mammals, the female hummingbirds should preferentially use feature information rather than spatial information when returning to a previously rewarded artificial flower. The male white-eared hummingbirds should, however, prefer to use spatial cues.

METHODS

Subjects and Field Site

Eleven female rufous hummingbirds were tested in free-flying field experiments along the Westcastle Valley in Alberta, Canada (49° 21' N, 114° 25' W). Individuals were identified by their unique throat feather patterns. Trials were run between 0700 and 1800 hours Mountain Standard Time from 13 to 24 July 2013. In Mexico, at the National Park 'La Malinche', Tlaxcala, in Central Mexico (19° 14' N, 98° 58' W with a 3000 m elevation) we tested eight males and three female white-eared hummingbirds and seven female magnificent hummingbirds. Individual birds in Mexico were identified by a detailed record of their visit rates to an artificial nectar feeder and identification of perch sites. To avoid retesting individuals only one bird of each species or sex was tested at a particular site. Trials in Mexico were run from 21 September to 11 November 2013.

The study was conducted with ethical permission from the University of St Andrews Ethical Committee, the University of Lethbridge Animal Welfare Committee, Alberta Sustainable

Resource Development and Environmental Canada and with permission from the Scientific Station 'La Malinche' from the Universidad Autónoma de Tlaxcala.

Initial Training

In Canada, we put out feeders containing 14% sucrose solution along the Westcastle Valley during the third week of May. Each feeder had a red plastic base and the birds accessed the sucrose via a hole in a single yellow plastic flower that was secured to the base of the feeder. By the first week of July, females started to feed from the feeders regularly and to defend them.

During that time we trained those females that were defending feeders and had distinctive throat patterns to feed from artificial flowers. To do this we first lowered the feeder 20 cm at a time until it had reached a height of 60 cm above the ground and we then replaced the single yellow plastic flower on the feeder with another artificial 'flower'. This flower comprised a plastic vial full of 25% sucrose solution, which was surrounded by a yellow cardboard circle (2 cm in diameter). After the bird had fed from the cardboard 'flower' once, we replaced it with a larger one, also yellow (6 cm in diameter). Finally, after the bird fed from that flower, the feeder was removed and the experiment began. This training procedure ensured that the bird was exposed to both the spatial and colour cues the same number of times.

The hummingbirds tested in Mexico were trained following the same training protocol as described above. We first placed artificial feeders at locations within 1 km of La Malinche research station and waited for the hummingbirds to visit regularly. Then, birds were trained to feed from an artificial flower. At La Malinche both the white-eared hummingbird and the magnificent hummingbird are year-round residents with both males and females migrating altitudinally to match the blooming schedule of different flower species ([Lara, 2006](#)).

Experimental Trials

For the experiment we presented the bird with an array of four artificial flowers mounted on 60 cm high wooden sticks in an 80 cm square where only one flower was filled with 600 μ l of 25% sucrose solution. The other three flowers were empty. The amount of sucrose solution in the rewarded flower was enough so that a single foraging bout was not sufficient for the birds to empty the flower. The rewarded flower was always the same colour as the feeder's plastic flower and was always placed in the same location as that where the bird had fed from the cardboard flower when it was still attached to the feeder. The other three flowers were each of one of three colours (possible colours were: yellow, red, pink and purple) and the location of these flowers within the array was assigned pseudorandomly.

Once the hummingbird had fed from the rewarded flower (Phase 1) and left the array, we switched the rewarded flower with one of the other flowers in the array so that its colour and its absolute location were in conflict ([Fig. 1](#); Phase 2). We emptied all of the flowers and ensured that the birds did not return to the array for at least 5 min. When the bird returned to the array we recorded the first visit made. Visits were defined as a probe into a flower. Each bird was tested only once.

Statistical Analyses

We compared the distribution of observed first visits made to all flowers during Phase 2 to a distribution expected by chance with a chi-square goodness-of-fit test. Since the expected frequencies of the distribution of the visits were smaller than 5, a randomization

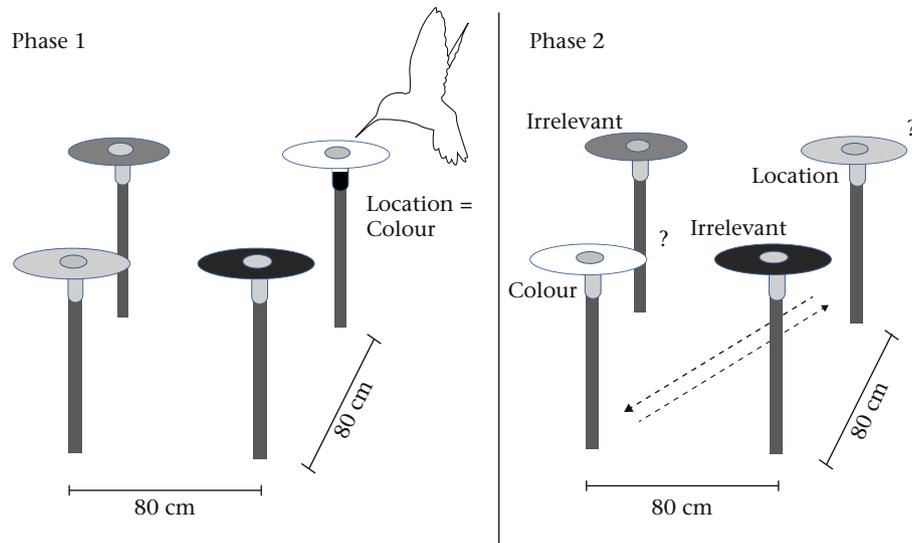


Figure 1. Diagram of the experimental set-up. During Phase 1, a hummingbird had to search for one rewarded flower among four flowers presented in an 80 cm square array. Before the bird returned, we switched the rewarded flower with the flower in the diagonal corner. During Phase 2 all flowers were empty. The dashed arrows indicate the flowers that had been switched in Phase 2.

test with 10 000 permutations was used to determine the probability of obtaining the observed and more extreme results (Sokal & Rohlf, 1995). Since the P values of both the chi-square goodness-of-fit test and the randomization test were similar we present only the P values for the randomization test. If the birds visited flowers in the array randomly the chance of visiting any flower was assumed to be equal. Nevertheless, for the purpose of the analysis the probabilities of visiting the two irrelevant flowers were pooled together so that the probability of visiting an irrelevant flower was 0.5 while the probability of visiting one of the relevant flowers was 0.25 each. Using a binomial test, we also analysed the bird's preference for the three types of flowers by comparing the number of birds that visited each type of flower (location, colour or irrelevant) against the proportion expected if birds were to visit the array at chance level. Again, the expected proportion for both the location and colour flower was 25% whereas the expected proportion for the irrelevant flowers was 50%.

RESULTS

Female Rufous Hummingbirds

The mean \pm SE intertrial interval was 20.6 ± 6.2 min. Since all flowers were empty during Phase 2, the rufous females could have searched all four flowers but only two birds did. Most birds searched only two flowers and for two birds both visits were to the location flower. During Phase 2, six of the 11 birds first visited the correct location, one bird visited the flower of the correct colour first and four birds visited an irrelevant flower first (Fig. 2). The overall distribution of choices was skewed towards location but not significantly different from the frequencies expected by chance (as tested by a chi-square goodness-of-fit test: $\chi^2_2 = 5.364$, with a randomization $P = 0.057$).

Importantly for this experiment, where the birds were expected to visit the flower with the correct colour, in fact, more female rufous hummingbirds returned to the flower at the previous correct location than expected by chance (binomial test with an expected proportion of 0.25 for the location and colour flowers: location: 6/11, $Z = 2.263$, $P = 0.034$; colour: 1/11, $Z = 1.218$, $P = 0.311$; irrelevant flowers with an expected proportion of 0.50: irrelevant: 4/11, $Z = 0.904$, $P = 0.548$).

Female White-eared and Magnificent Hummingbirds

Owing to the small sample size of female white-eared hummingbirds we pooled the visits of both Mexican species of females. The mean \pm SE intertrial interval of the females was 26.8 ± 2.0 min. The majority of females (nine of 10) returned first to the flower at the correct previous location. Only one female first visited the flower with the correct colour and no female went first to an irrelevant flower (Fig. 2). Together, the distribution of first visits to the three different types of flowers was significantly different to the distribution expected if the birds were revisiting the array at random (tested by a chi-square goodness-of-fit test: $\chi^2_2 = 22.8$, with a randomization $P < 0.001$).

This preference for the flower in the original location was significantly higher than expected by chance, while the visit to the flower of the correct colour did not differ from chance (binomial test with an expected proportion of 0.25, 0.25 and 0.50 for the location, colour and irrelevant flowers, respectively; location: 9/10,

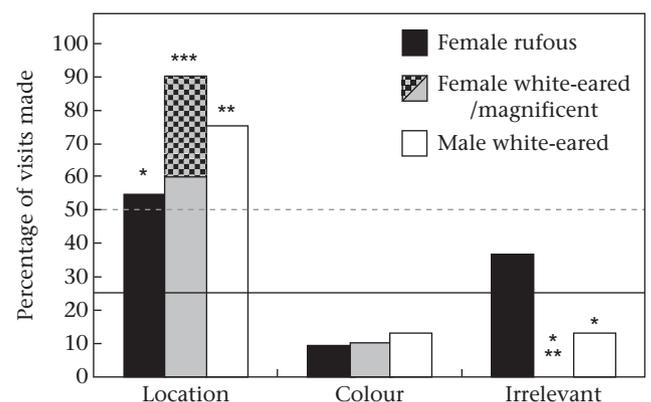


Figure 2. The percentage of first visits made to each flower type during Phase 2 (11 female rufous hummingbirds, three female white-eared hummingbirds, seven female magnificent hummingbirds, eight male white-eared hummingbirds). The black horizontal line represents chance for both the correct location and correct colour whereas the grey dashed line represents chance for the two irrelevant flowers. Asterisks indicate the statistical significance of comparisons between observed and expected values: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$.

$Z = 4.746$, $P < 0.001$; colour: $1/10$, $Z = 1.095$, $P = 0.468$; irrelevant: $0/10$, $Z = 3.162$, $P < 0.001$).

Male White-eared Hummingbirds

The mean \pm SE intertrial interval for the male white-eared hummingbirds was 13 ± 1.0 min. Like the females from all three species, the majority of white-eared males (six of eight birds) returned first to the flower at the correct previous location and not the flower of the correct colour. Only one male visited the flower with the correct colour first while another went first to an irrelevant flower (Fig. 2). The general distribution of first visits to the three different types of flowers was significantly different to the distribution expected if the birds were revisiting the array at random (as tested by a chi-square goodness-of-fit test: $\chi^2_2 = 10.75$, with a randomization $P = 0.007$).

The visits made to the flower at the original location were significantly greater than expected by chance while the visits made to the colour flower were no different from chance. The visits made to the two irrelevant flowers were significantly fewer than expected by chance (binomial test with an expected proportion of 0.25, 0.25 and 0.50; location: $6/8$, $Z = 3.265$, $P = 0.004$; colour: $1/8$, $Z = 0.816$, $P = 0.688$; irrelevant: $1/8$, $Z = 2.121$, $P = 0.035$).

DISCUSSION

Contrary to expectation, female hummingbirds of the three species we tested here did not prefer to use colour cues when returning to a flower array: only one of the 11 rufous females and one of the seven magnificent females returned first to the flower bearing the same colour as the flower rewarded in Phase 1. None of the three white-eared females tested returned first to the flower with the correct colour cue. Rather, like the rufous males tested by Hurly and Healy (1996) the majority of the females first visited the flower at the correct location (55% of the rufous females and 90% of the white-eared and magnificent females). Male white-eared hummingbirds also first visited the flower at the correct previous location (75%). Although the birds tested differed in their species, sex, territoriality, breeding stage (breeding or nonbreeding season) or whether they are latitudinal migrants, most of them preferred to use spatial cues to relocate a reward.

Although our results are not consistent with the previously reported female preference for feature cues in other species (e.g. Astié et al., 1998; Dabbs et al., 1998; Jones et al., 2003) they do concur with a preference for spatial cues in both sexes of great tits (Hodgson & Healy, 2005). As the birds in that study experienced the same location and colour 10 times in a row before they were tested, the authors speculated that it was the repeated nature of the training that led to both sexes preferring spatial cues. That would not, however, explain the outcome we observed because our hummingbirds experienced the rewarded flower only once before they had to choose between colour and spatial cues.

Female hummingbirds, just like male hummingbirds, forage among hundreds of flowers every day. Within a female's foraging range or a male's territory, the colours might differ between flower species (e.g. red paintbrush, *Castilleja* spp. versus yellow columbines, *Aquilegia flavescens*) but colour alone would not be an informative cue for whether a particular flower has been previously visited. Conversely, the location of a flower alone is sufficient information to relocate an exact specific flower. Therefore, perhaps it is not surprising that females also prefer to use spatial cues to relocate a rewarded flower.

There are, however, instances where feature cues might be more informative than spatial cues (for both sexes). While migrating, as hummingbirds arrive at a new stopover site, colour cues are likely

to be helpful initially in locating flowers and at that time we might expect birds to prefer to use feature cues to locate profitable sites. Once the birds learn the locations of rewarding plants in their new territories (which they establish temporarily along their migration route), however, they should switch to using spatial cues. This possibility is supported by evidence that males can be flexible as to their preferred cue use when in their breeding territory: after only three trials in which a rewarded artificial flower was moved after each time the male fed, male rufous hummingbirds switched from using spatial cues to relocate the rewarding site to using colour cues (Flores-Abreu, Hurly, & Healy, 2012). We would need to test hummingbirds' cue preferences along the migration route to confirm this suggestion.

Furthermore, hummingbirds (and probably other animals) use different types of cues at different scales. When male rufous hummingbirds had to return to a single rewarded flower in an experimental array, for example, they used the relative position of the flower within the array when flowers were spaced at 40 cm or less but when flowers were further apart (i.e. 80 cm or more) the birds used the absolute location of the flower (Healy & Hurly, 1998).

In sum, we suggest that these data add weight to the suggestion made by Hodgson and Healy (2005) that sex differences in cue use are context dependent rather than ecological and that it is likely that selection has favoured flexibility in cue use, rather than fixed strategies that differ between the sexes.

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