

Memory for location and visual cues in white-eared hummingbirds *Hylocharis leucotis*

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Abstract In nature hummingbirds face floral resources whose availability, quality and quantity can vary spatially and temporally. Thus, they must constantly make foraging decisions about which patches, plants and flowers to visit, partly as a function of the nectar reward. The uncertainty of these decisions would possibly be reduced if an individual could remember locations or use visual cues to avoid revisiting recently depleted flowers. In the present study, we carried out field experiments with white-eared hummingbirds *Hylocharis leucotis*, to evaluate their use of locations or visual cues when foraging on natural flowers *Penstemon roseus*. We evaluated the use of spatial memory by observing birds while they were foraging between two plants and within a single plant. Our results showed that hummingbirds prefer to use location when foraging in two plants, but they also use visual cues to efficiently locate unvisited rewarded flowers when they feed on a single plant. However, in absence of visual cues, in both experiments birds mainly used the location of previously visited flowers to make subsequent visits. Our data suggest that hummingbirds are capable of learning and employing this flexibility depending on the faced environmental conditions and the information acquired in previous visits [*Current Zoology* 57 (4): 468–476, 2011].

Keywords Visual cues, Location, White-eared hummingbird, Foraging

Memory enhances efficiency with which animals return to different sites to exploit resources and avoid those that may be dangerous or costly (Collett, 2009). The use of spatial memory to guide movement towards a remembered location has been widely documented in animal pollinators. For example, there is good evidence that bumblebees have a strong long-term spatial memory to remember and return to highly rewarding plants (Pyke, 1978a; Burns and Thomson, 2006; Ohashi and Thomson, 2009), and there is ample evidence that bees can recall rewarding locations, especially when in these sites there are abundant nectar supply (Collett et al., 1993; Huber et al., 1994; Menzel et al., 2000).

As insects, hummingbirds are inherently interesting models for studying spatial memory because they are faced with similar complex routing problems when foraging (joining multiple locations in a floral patch to minimizing route length). For these birds the main food source is the nectar obtained of the flowers, and a hummingbird foraging in nature faces a patchily distributed

resource of variable quantity and quality, refilling rate, and color (Healy and Hurly, 2003; Lara, 2006). Likewise, during their local or migratory movements, they are constantly exposed to new floral resources, whose availability may vary spatially and temporally and, considering that the energetic cost of hovering demands the consumption of an enormous amount of nectar, which is scattered in hundreds of flowers (Gass et al., 1999), it is assumed that hummingbirds require certain efficiency and time investment in foraging. Thus, a hummingbird must constantly make foraging decisions about which patches, plants, and flowers to visit, partly as a function of the nectar reward (Pyke, 1978b; Gass and Montgomerie, 1981), while using the memory to avoid visit the flowers that they have emptied recently (Cole et al., 1982; Gass and Sutherland, 1985; Brown and Gass, 1993; Brown, 1994; Hurly, 1996). The uncertainty of these decisions would be reduced if a hummingbird could remember the spatial location or adequate visual cues in order to avoid revisiting flowers that had previ-

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ously been emptied, at least until the flowers re-fill their nectar content (Gass and Sutherland, 1985). However, this has been researched only in a few hummingbird species (Grant and Temeles, 1992; Healy and Hurly, 1995; Hurly, 1996; Melendez-Ackerman et al., 1997; Henderson et al., 2001; González-Gómez and Vásquez, 2006).

Most of the studies on the use of spatial memory in hummingbirds under field conditions have been carried out by using feeders or artificial flowers (Cole et al., 1982; Brown and Gass, 1993; Brown, 1994; Sutherland and Gass, 1995; Healy and Hurly, 1995, 1998; Hurly and Healy, 1996, 2002; Henderson et al., 2001; González-Gómez and Vásquez, 2006). These studies have suggested that hummingbirds (particularly *Selasphorus* species) can remember the location of the most rewarding feeders or artificial flowers, facilitating their efficient exploitation in the absence of visual cues related to nectar quantity or quality (Brown and Gass, 1993; Brown, 1994; Healy and Hurly, 1995, 1998; Henderson et al., 2001; Hurly, 1996, Hurly and Healy, 1996, 2002). Lacking are studies related to the use of spatial memory or visual cues by hummingbirds foraging on flowers that grow in their natural habitat.

In the present study, we carried out two different experiments with white-eared hummingbirds *Hylocharis leucotis*, to investigate the use of locations or visual cues as strategies when foraging in flowers growing in the study site (*Penstemon roseus*: Plantaginaceae). In experiment 1 (“between plants”) we tested the use of the two strategies simultaneously. In experiment 2 (“within plants”), we tested the use of both strategies independently.

1 Materials and Methods

1.1 Study site and species

From June to November 2008, white-eared hummingbirds were studied, after receiving ethical approval from the relevant local authorities, in the National Park “La Malinche”, Tlaxcala, México (19°14'N, 98°58'W and 3000 m elevation). This hummingbird species is resident at the study site, where it forages and competes within territories encompassing mixed assemblages of eight plant species throughout the year (Lara, 2006). The white-eared hummingbird is the most frequent visitor of *Penstemon roseus* (Cerv. ex Sweet) G. Don (hereafter *Penstemon*) a plant species with hermaphroditic and magenta tubular flowers (ca. 28 mm corolla length and 6.8 mm corolla-entrance width) with a blooming season which extends from July to December

(Lara and Ornelas, 2008). Based on nectar accumulations measures, *P. roseus* flowers produce $6.5 \pm 0.45 \mu\text{l}$ of nectar and $0.24 \pm 0.007 \text{ mg/ml}$ of sugar concentration after 24 h of accumulation (Lara, 2006).

A total of 27 adult hummingbirds (ca. 3.1–3.4 g) were mist-netted in the field for this study. All trials described below were conducted from 0800–1500 h, and birds were housed individually in collapsible field cages of plastic tubes and tulle walls (dimensions: $1 \times 1 \times 1 \text{ m}^3$), containing a wood perch. Cages were hung in the shade where the birds were captured and maintained under environmental conditions of light and temperature of study site. Prior to the trials, naive individuals were allowed to acclimate to the cages for 10 min as suggested by previous studies (Lara and Ornelas, 1998; Lara et al., 2009) but were not fed so that by the time of the experiment they were presumably hungry. During this period, perching by the birds as well as the presence of flights without trying to escape to the cage were taken as evidence that they were acclimatized to the enclosure (Lara et al., 2009). For trials in experiment 1, 50 *Penstemon* individual plants with similar size and floral display were used; one month before experiments, plants were ground extracted and transplanted in polyethylene bags and then were kept under local environmental conditions.

Because hummingbirds can attend to different cues to remember the visited flowers within a plant or between plants, we carried out two independent experiments described below.

1.2 Experiment 1: between-plant foraging

The subjects in this experiment were seven hummingbirds. To determine whether hummingbirds primarily use spatial or visual cues to return to previously visited flowers in two *Penstemon* plants, trials in this experiment consisted of two parts. In phase 1 (initial training) two plants were placed inside a cage, separated by 80 cm. The flowers from each plant were removed, except for four open flowers in the same inflorescence. The nectar was completely depleted from these flowers by means of capillary tubes. Before a trial, the flowers in one of the two plants were filled with $100 \mu\text{l}$ of 20% (by weight/weight) sucrose solution and were marked at the corolla base with bright yellow auto-adhesive paper labels ($1 \times 2 \text{ cm}$) in order to provide visual cues for the birds. Hummingbirds can remember specific visual cues associated with the flower (e.g. the flower color, color guides or shape) and return by using these as a cue. White-eared hummingbirds feed mostly from red flowers throughout the year in La Malinche, and yellow

flowers are not present in the study site (Lara, 2006). For this reason, we decided to use yellow color guides as specific novel visual cues to explore if hummingbirds can remember specific flowers by attending to these novel cues.

The flowers of the other plant were not marked and were not refilled; these were designated “irrelevant flowers”. For a trial, a naive hummingbird was introduced into the cage and their visits to the flowers were recorded for 15 minutes. We recorded for each individual (1) the number of flowers visited in both plants and, (2) the duration of the visit to each flower. At the end of phase 1, the hummingbird was moved to another cage for five minutes before initiating the next phase.

In phase 2, both plants were in the same place inside the cage, but at this stage none of the flowers had nectar reward, labels were removed from flowers marked in phase 1 and new labels were placed on four flowers of the other plant, in order to determine whether the first visits were guided by the location or the visual cues in flowers. Then a hummingbird was introduced again in the cage and its visits were recorded following the protocol described above. In order to minimize the possible effects of stress due to handling, the transference of hummingbirds from a cage to another was done without direct manipulation; for this a second cage was placed on one side with an open mesh net, so hummingbirds passed freely between the cages.

After second phase, each bird was marked by clipping the 5th rectrice feather (to avoid inadvertently recapturing the individual for use future experiments) and then was released.

1.3 Experiment 2: foraging within plants

To assess spatial memory and use of visual cues by hummingbirds foraging in flowers within a single plant, we used floral arrays; these consisted of four *Penstemon* flowers mounted on syringe needles which were inserted equidistant on a 50 cm wooden stake. In order to control the possible natural differences between the selected flowers, we selected them so that they were as similar as possible with respect to size, color tonality, age and number of anthers.

In order to dissociate visual cues and location, we divided the experiment into two independent treatments. In treatment 1 (rewarded visual cue), we determined if hummingbirds could associate visual cues with nectar reward and if they were able to modify their visits accordingly. Ten hummingbirds were tested in this treatment. A wooden stake was placed inside a cage and only a single flower was filled with 300 μ l of 20% sucrose

solution. This flower was also marked at the corolla base with the visual cues described in experiment 1. An excessive amount of nectar was aimed to ensure the constant presence of reward as reinforcement of the visual signal provided on the flower. However, we made sure that the nectar was not visible, so that the nectar itself did not function as a visual cue. The remaining irrelevant flowers were depleted and not marked. Each bird was given four trials (trial 1 was taken as an initial training in both treatments and was not included in the statistical analysis), in which an individual naïve hummingbird was introduced to the cage and the bird's visits to the array were recorded for five minutes. In each trial, the position of the rewarded and marked flower was changed to evaluate a possible enhancement in the hummingbird's performance through the use of the visual cue. During the 5 min inter-trial interval, the position of the rewarded flower was changed and it was refilled if necessary; this manipulation was done outside the cage, away from hummingbird's sight inside the cage.

In treatment 2 (rewarded location), ten different hummingbirds were used. In order to test if location is used in the choice of flowers by *Hylocharis*, we administered to each of the birds four trials using the array described above, following the same protocol. In this treatment the rewarded flower in the array was not marked, the position of nectar reward was the same in four trials (which varied for each hummingbird) but the flowers in the array were changed between trials to exclude the use of individual features of rewarded flower as visual cues. Following the exposure to the floral arrays of each treatment, birds were marked and released.

It was not our intention in these experiments to determine whether the birds had a tendency to learn a win-shift or win-stay strategy as Cole et al. (1982) have done previously.

Rather, we used a non-depleting focal flower to act as an incentive to return. We wanted to know whether the birds could remember either the color pattern or the location of this focal flower.

1.4 Data analysis

Differences in the percentage of hummingbird visits to the correct location or visual cue in both experiments were analyzed using repeated-measure ANOVA (Zar, 1999). In the model, type of flower visited (location, visual cue or irrelevant flower) was treated as fixed effect, and the percentage of visits over the phases (Exp.1) or treatments (Exp.2) were the repeated measures. Data

were arcsine transformed before statistical analysis to achieve normality.

We assessed performance in both experiments as the number of individual flowers visited before locating the reward or cue site. If subjects searched for the reward with no memory or cue to guide them, until eight (Exp.1), or four (Exp.2) visits might be needed to discover the reward. Thus, under both random and systematic searching, the expected number of visits due to chance in both experiments is 3 and 2.5 (i.e. arithmetic means), respectively. Deviations from this expected value were assessed using one-sample *t*-tests. The tests were two-tailed because there are two alternative hypotheses. The subjects may remember spatial locations and hence find the reward with less than 3 or 2.5 visits; alternatively, they may remember visual cues and thus be drawn to the switch location. Birds searching with visual cues would make more than 3 (Exp.1) and 2.5 visits (Exp.2).

To evaluate the first choices in experiment 1, phase 2, and experiment 2 (both treatments) we used chi square analysis. We analyzed three earlier visits against a null model assuming random preferences (Zar, 1999).

We used Survival Analysis (“time failure analysis”) to analyze if the presence of visual cues or rewards (or both) in a specific location reduces the time until a hummingbird visits a flower in both phases in Experiment 1. One of the advantages of using survival analysis is that allows for use of censored data. Censored data points are those in which an event is observed because the study ended before the event could have happened to some individuals under observation. This feature is apt to be useful in field biology, where the observation period may be too brief for all possible events to occur (Muenchow, 1986). Thus, we recorded the beginning of our observations as time zero and subsequent foraging events as minutes from start time. If an event occurred for a given flower, then it became uncensored data, and if it never occurred, then it became censored data. We used the Kaplan-Meier product-limit non-parametric method for the computation of the probability that hummingbirds had not yet visited a flower in the arrangement 15 minutes after the start of observation, and the logrank (Mantel-Cox) statistic to test for differences between flower types (Muenchow, 1986). All statistical analyses were done using Stat-View (Abacus Concepts, Inc. 1996). In our case, “survivorship” was the probability that a hummingbird had not yet visited flower a given number of minutes after the start of observation.

2 Results

2.1 Between plant foraging

Overall, in phase 1 hummingbirds more often visited the flowers that displayed the visual cue (and were also rewarded) ($76.05\% \pm 0.09\%$) than irrelevant flowers ($23.95\% \pm 0.05\%$), and in phase 2 they most often returned to the location of the previously rewarded flowers ($74.16\% \pm 0.03\%$) rather to the visual cue ($25.84\% \pm 0.05\%$) ($F_{1,24} = 27.68$, $P = 0.002$; Fig. 1). However, the intensity of visitation (number of visits) was similar in both phases ($F_{1,24} = 0.13$, $P = 0.91$). In this way, the percentage of visits made to each array (containing a visual cue or irrelevant flowers) in both phases was not statistically different ($F_{3,24} = 0.09$, $P = 0.76$). This suggests the use of location and spatial cues when foraging among two plants, rather than the use of visual cues associated with individual flowers. In phase 1, the flowers were never emptied during the course of the experiment. That is, birds keep returning to the rewarded plant and keep getting nectar. However, in phase 2, in which the nectar was removed from all flowers, the birds kept returning to the location of the flowers that had been rewarded in phase 1, despite visiting other flowers in the arrangement. The performance of all seven birds in phase 2 was significantly better than chance (Mean \pm SE: 1.1 ± 0.03 visits; $t = 11.53$, $df = 6$, $P < 0.05$).

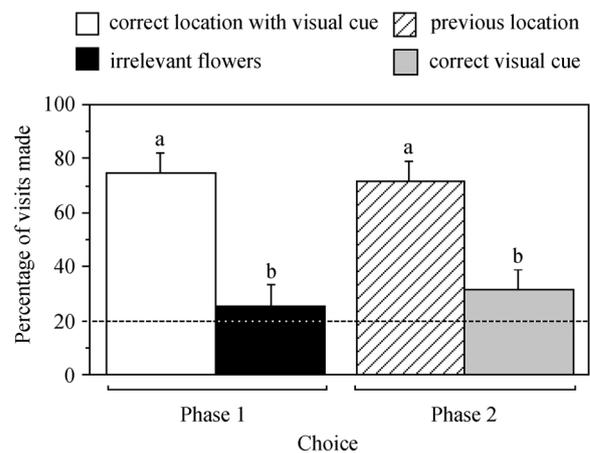


Fig. 1 The percentage of visits made by seven white-eared hummingbirds (Experiment 1: between plant foraging) when the flowers in one of the two plants provided visual cues (phase 1), and when labels were changed to another plant (phase 2).

Values are means and standard error (maximum = 100%). Data with the same superscript letters are not significantly different between groups (Repeated-measures ANOVA; $P > 0.05$). The expectation of random choice is indicated by the dotted line.

We analyzed the first three visits to flowers in both plants during phase 2 (Fig. 2). All first choices were made to the location associated with the originally rewarded flower ($n = 7$, $\chi^2 = 7.77$, $df = 2$, $P = 0.02$). Visits made in the second choice showed no differences between flowers in the location previously visited and newly flowers carrying visual cues ($n = 7$, $\chi^2 = 1.11$, $df = 2$, $P = 0.57$). However, birds return again to visit previously flower location in their third choice ($n = 7$, $\chi^2 = 5.06$, $df = 2$, $P = 0.04$). The trend of visits suggests that hummingbird's foraging was predominantly driven by the memory of the location of flowers with nectar.

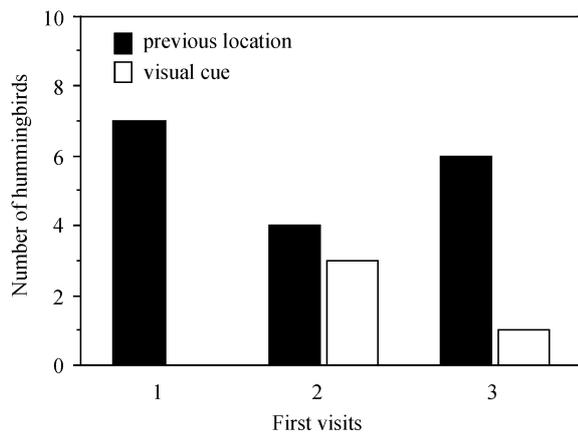


Fig. 2 The first three choices made in phase 2 (Experiment 1: between plant foraging) by seven white-eared hummingbirds to flowers carrying visual cues or flowers placed in the previously visited location (phase 1)

We found significant differences between the probability curves of hummingbirds visiting plants in the two experimental phases (Fig. 3). In phase 1, rewarded flowers marked with visual cues were not more likely to be visited first by the hummingbirds than irrelevant flowers (Mantel-Cox Logrank: $\chi^2 = 0.32$, $df = 1$, $P = 0.56$). However, in phase 2 when birds were tested on arrangements where the visual cues were changed to another plant, the arrival times to flowers with visual cues were shorter (although they were less visited as shown in Figure 1) than those in flowers without them (Logrank-Mantel Cox: $\chi^2 = 8.44$, $df = 1$, $P = 0.003$).

2.2 Within plant foraging

Treatment 1: Rewarded visual cue. By using the number of choices made before the bird finally finds the reward flower, we assessed the performance of birds foraging within a single array of flowers where only one of those flowers was rewarded and marked with a visual cue (Exp. 2: treatment 1). This analysis showed that the birds' performance was significantly worse than expected

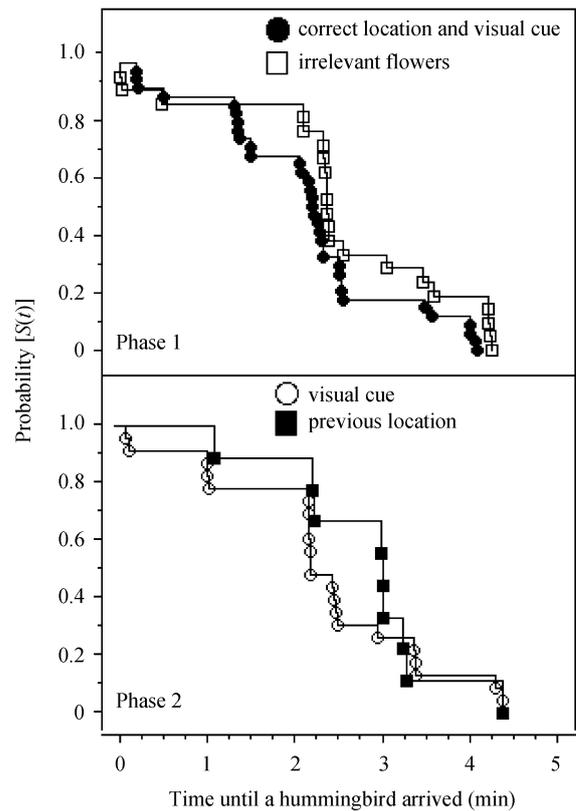


Fig. 3 Comparison using survival analysis of the probability that a hummingbird has not yet visited a flower in both phases during a 5-min observation period (Experiment 1: between plant foraging)

"Time until a hummingbird arrived" refers to the time elapsed since the start of the observation period.

by chance (Mean \pm SE: 3.6 ± 0.06 visits; $t = 15.80$, $df = 9$, $P < 0.05$). However, when we analyzed the percentage of total visits, we found a significant effect of flower type (marked vs. irrelevant, $F_{1, 54} = 29.49$, $P = 0.001$), no effect of trial (trials 1 through 4, $F_{3, 54} = 0.002$, $P = 0.99$) and a significant interaction between flower type and trial number ($F_{3, 54} = 3.35$, $P = 0.025$; Fig. 4a).

Again, when we analyzed first three choices, we found no differences in the first choices made to flowers marked with the visual cue and the irrelevant flowers through trials ($n = 10$, $\chi^2 = 2.72$, $df = 4$, $P = 0.436$). This behavior suggests that at the beginning of each treatment the birds can visit all the flowers in the plant, but subsequently they can use the visual cues to do most of their visits towards these flowers (Fig. 5a).

Treatment 2: Rewarded location. Interestingly, in treatment 2 where only a single flower was rewarded (unchanged nectar position), the performance of birds (by using the first visits) was significantly better than

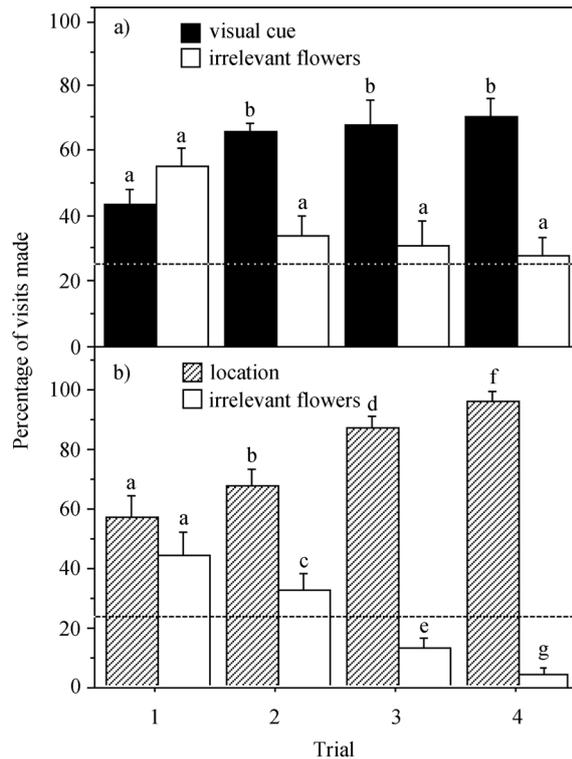


Fig. 4 The percentage of visits made by ten white-eared hummingbirds to arrays containing flowers with (a) and without visual cue (b) (Experiment 2: within plant foraging). Values are means and standard error (maximum = 100%). Data with the same superscript letters are not significantly different between groups (Repeated-measures ANOVA; $P > 0.05$). The expectation of random choice is indicated by the dotted line.

expected by chance (Mean \pm SE: 1.8 ± 0.01 visits; $t = 12.33$, $df = 9$, $P < 0.01$). Furthermore, using the percentage of total visits, we found that birds mostly return to visit flowers on the original location than irrelevant flowers (location vs. irrelevant, $F_{1,54} = 113.20$, $P = 0.001$); no effect of trial (trials 1 through 4, $F_{3,54} = 0.014$, $P = 0.99$) and a significant interaction between flower type (flower in the correct location or irrelevant flower) and trial number ($F_{3,54} = 37.79$, $P = 0.001$). Thus, birds consistently returned significantly more often to the location of the rewarded flower than irrelevant flowers. It seems from our analyses that the birds relied heavily on the difference of nectar presence between the focal and irrelevant flowers to choose the focal flower in the subsequent trials, suggesting that birds were able to remember the location of the focal flower without an additional cue (Fig. 4b).

However, yet again if we consider only first visits, the analysis showed no differences in the probability to visit a flower location or an irrelevant flowers at the beginning of each trial ($n = 10$, $\chi^2 = 5.91$, $df = 4$, $P = 0.11$;

Fig. 5b). Thus, a hummingbird foraging within a plant needs to probe several flowers to increase its performance in remembering the correct location of the rewarded flower through trials.

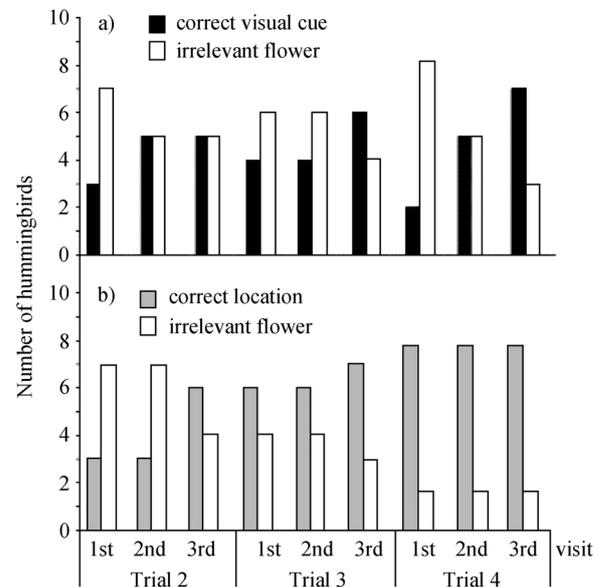


Fig. 5 The first three choices per trial made by ten white-eared hummingbirds (Experiment 2: within plant foraging), when they were confronted to an array with flowers with (a) and without a visual cue (b)

3 Discussion

The results of our study showed that, during foraging, white-eared hummingbirds use both location and visual cues to remember previously rewarded flowers. Thus in Experiment 1, where location and visual cues were tested simultaneously, we found evidence of a hierarchical use of location over visual cues (location > visual cues) as well as the use only of location. However, the results of Experiment 2 showed that the birds could also use both cues independently.

In Experiment 1, the proportion of visits made at the location of nectar (phase 1) suggests that foraging was guided by reinforcement or incentive of reward, but in phase 2 we evidenced the memory abilities of hummingbirds to recall and use newly acquired information about the location of nectar. Thus, we have demonstrated that white eared hummingbirds can use both location and visual cues.

Our finding that hummingbirds pay much more attention to the spatial location of sources of nectar rather than to visual cues associated with these sources when they are foraging among two plants (Experiment 1), has

been previously demonstrated in field and laboratory tests of spatial memory, especially in *Selasphorus* species and the Green-backed firecrown hummingbird *Sephanoides sephaniodes* (Cole et al., 1982; Hurly and Healy, 1996; Healy and Hurly, 1998; Henderson et al., 2001, Healy and Hurly, 2003; González-Gómez and Vázquez, 2006). These studies have suggested that preference for responding to spatial over color cues when hummingbirds are foraging in more than one plant (artificial array or feeder) is likely to be an expression of normal cue use during foraging behavior. This ability to precisely relocate a spatial position based on visual landmarks has been also reported in food-storing birds such as corvids and parids (Bossema, 1979; Balda and Kamil, 1989; Sherry, 1989; Gould-Beierle and Kamil, 1998); revealing that hummingbirds can recall information about the spatial location as well as the content (quality) of foraging sites.

However, the results obtained in Experiment 2 of the present study suggest that hummingbirds may also behave as non-storing birds, in the sense that they can guide their foraging according to visual cues rather than location of landmarks. Our contrasting results suggest that hummingbirds are capable of learning and employing this flexibility depending on environmental conditions. Thus, as Pyke suggested in its pioneering work (1978a), hummingbirds can use information gathered during previous visits, rather than the automatic use of a visual attractor, the use of a general directionality during a foraging bout, or a simple decision rule, such as has been described for honeybees, bumblebees and wasps (Pyke, 1978b; Corbet et al., 1981; Heinrich, 1983).

To nectivores that feed at flowers in inflorescences or on plants, these clusters of flowers represent patches (Pyke, 1984). Thus, as such an animal moves among the flowers within a patch, it may be increasingly likely to revisit a flower (e.g. Pyke, 1981; 1982), it may visit flowers that tend to contain less nectar than others (e.g. Hodges, 1981; Pyke 1981), or it may obtain information about the otherwise unknown quality of its present patch (Pyke 1978a). Moreover, as suggested by Hurly and Healy (1996), preference for attending to or remembering the location of flowers, rather than the color of them, does not seem surprising as a hummingbird's territory contains many flowers of the same species. Thus, it would be advantageous for a bird to remember which flowers of that species it had emptied recently and avoid them until they were replenished. However, this can be completely different in more complex environments such as the Neotropics, where numerous hummingbird

plants species bloom synchronously, and it is highly probable that a territory contains species of plants with flowers of different colors. Also, many flowers change of color with age, a visual change often associated with decreased nectar volume. Thus, the selection pressure on hummingbirds to remember the locations or visual cues of many flowers—can be attributed to differences in the ecological circumstances that they face. However, whether or not the birds learn motor vectors that they simply follow, and/or orient according to general environmental cues is not yet known.

Pollinators just beginning a foraging activity must learn which flowers are likely to provide the most pollen or nectar, and which flowers are not profitable to visit (Waddington, 2001). Thus, foragers must decide where and when to look for food, and which food to search out, pollen or nectar. Although the ability to build representations of many spatial locations from familiar environments, referred to as “spatial memory”, has been documented in diverse pollinator taxa, such as some species of bumblebees (Burns and Thomson, 2006; Ohashi and Thomson, 2009), honeybees (Collett et al., 1993; Menzel et al., 2000; Collett, 2009), hummingbirds (Brown and Gass, 1993; Healy and Hurly, 1995; Hurly, 1996; Sutherland and Gass, 1995) and bats (Winter and Von Helversen, 2001), it is necessary to assess these cognitive abilities in a larger number of species to assess generality.

In summary, our results showed that white-eared hummingbirds prefer to use location when foraging in two plants, but they efficiently use visual cues to locate rewarded flowers when foraging in a single plant. However, in absence of visual cues, in both experiments birds mainly used the location of previously visited flowers to make subsequent visits. Our data suggest that hummingbirds foraging behavior is influenced by environmental information (related to food resources) acquired in previous visits.

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