Colour cues facilitate learning flower refill schedules in wild hummingbirds

Michael Samuels [a,*], T. Andrew Hurly [b], Susan D. Healy [c]

[a] School of Psychology and Neuroscience, University of St. Andrews, St. Mary's College, South Street, St. Andrews KY16 9JP, UK
[b] Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada
[c] School of Biology, University of St. Andrews, St. Andrews KY16 9TH, UK

ARTICLE INFO

ABSTRACT

Free-living hummingbirds can learn the refill schedules of individual experimental flowers but little is known about what information they use to do this. Colour cues, in particular, may be important to hummingbirds when learning about rewarded flower properties. We investigated, therefore, whether colour cues facilitated the learning of flower refill schedules in wild, free-living rufous hummingbirds (*Selasphorus rufus*). In the Cued condition, we presented birds with an array of six flowers, three of one colour, each of which were refilled 10 min after being emptied by the bird and three of a different colour, which were refilled 20 min after being emptied. In the Uncued condition we presented birds with six flowers of the same colour, three of which were refilled after 10 min and three of which were refilled after 20 min as for the birds in the Cued condition. In the second part of the experiment, we moved the array 2 m and changed the shape of the array. Across both phases, birds in the Cued condition learned to discriminate between 10 and 20-min flowers more quickly than did the birds in the Uncued condition. The Cued birds were also better at discriminating between the two distinct refill intervals. Colour cues can, therefore, facilitate learning the refill schedules of experimental flowers in these birds.

This article is part of a Special Issue entitled: Cognition in the wild.

1. Introduction

For many animals, the ability to learn and discriminate between temporal regularities forms an important part of successful interactions with their environment (Buhusi and Meck, 2005; Gallistel, 1990). Interval timing, the ability to accurately time and discriminate between short intervals in the range of seconds-to-minutes, has been studied extensively in laboratory settings. This work has revealed that mechanisms for timing short intervals are phylogenetically widespread, with examples across a range of taxa including humans (Rakitin et al., 1998), mice (Buhusi et al., 2009; Lejeune and Wearden, 1991), rats (Lowe et al., 1979), pigeons (Cheng and Westwood, 1993; Dews, 1962), rhesus monkeys (Dews, 1978), cats (Lejeune and Wearden, 1991), goldfish *Carassius auratus* (Talton et al., 1999), Siamese fighting fish *Betta splendens* (Higa

and Simm, 2004) and bees *Bombus impatiens* (Boisvert and Sherry, 2006). To our knowledge, however, little effort has been made to examine interval timing outside a controlled laboratory environment, which means that little is known currently about the value or specific functions of such timing mechanisms in the wild. Still less is known about the types of cues that animals might use when learning about temporal regularities in their environment (Bateson, 2003).

A useful model for investigating the specific ecological challenges and cognitive mechanisms for timing intervals in a naturalistic setting, along with the relevant cues that might be used when acquiring this temporal information, is the free-living, foraging hummingbird. This is because hummingbirds forage from a large number of flowers within their territories containing nectar that, once depleted, replenish at predictable rates in time over the course of several hours (Henderson et al., 2006; Marshall et al., 2012). In addition to location and refill rate, the flowers from which hummingbirds naturally forage also vary in features such as colour, shape, size and content (Healy and Hurly, 2003; McDade and Weeks, 2004). It might, then, be useful for hummingbirds to learn and remember specific flower features in order to increase foraging efficiency and avoid costly energy-use in foraging flights

* Corresponding author at: School of Natural Sciences and Psychology, Liverpool John Moores University, Tom Reilly Building, Byrom Street, Liverpool L3 3AF, UK. Tel.: +44 754 803 7009.

E-mail addresses: michaelsamuels001@gmail.com, m.samuels@2013.ljmu.ac.uk (M. Samuels).

to recently emptied flowers (Cole et al., 1982). This supposition has some support: hummingbirds can remember the locations of recently emptied flowers and are more likely to avoid these flowers in preference for flowers they have not previously visited (Healy and Hurly, 1995; Hurly, 1996). There is also good evidence that hummingbirds can learn the specific refill schedules of individual experimental flowers, and that they can combine this temporal information with spatial information and adjust their visit rates to flowers depending on when those flowers were last visited (Gill, 1988; González-Gómez et al., 2011; Henderson et al., 2006; Marshall et al., 2012).

Although hummingbirds learn to time the refill schedules of experimental flowers, it is not clear what cues they use to do so. One conspicuous cue they might use is flower colour. For nectarivorous species like hummingbirds, the ability to use colour cues when learning about the traits of flower species that vary in aspects such as colour and refill rate may be beneficial for two reasons. Firstly, such a hummingbird could reduce the amount of information it needs to learn and remember when foraging from a large number of flowers (Dukas and Waser, 1994; Zentall et al., 2008). Secondly, a hummingbird might save time by both using this information when foraging from new flowers and when in unfamiliar surroundings such as on a migration route (Dukas and Waser, 1994; Grant, 1966; Hurly and Healy, 2002).

Hummingbirds possess colour vision that ranges through the visible light spectrum into the near ultraviolet (Goldsmith, 1980; Goldsmith et al., 1981) and although it is often assumed that hummingbirds prefer red, largely due to the prevalence of red hummingbird-pollinated flowers (Grant, 1966), hummingbirds feed from flowers of a variety of colours (Bené, 1941, 1945). The degree to which they might use colour as a cue to learning and remembering different flower characteristics such as refill rate is not, however, clear as hummingbirds often prefer to use spatial cues over colour cues and are more likely to return to a rewarded flower's location irrespective of the colour cues that are available or the changes made to a rewarded flower's colour (Lyerly et al., 1950; Hurly and Healy, 1996, 2002; Miller and Miller, 1971; Miller et al., 1985).

In some circumstances hummingbirds will learn the specific colour-reward associations of flowers when colour signals different types of reward (Collias and Collias, 1968; Melendez-Ackerman et al., 1997; Miller et al., 1985; Sandlin, 2000) and such colour-reward associations can be learnt readily, within as few as 2–3 experiences (Hurly and Healy, 1996). Hummingbirds can also learn and remember colour cues that are independent of any spatial cues when both relative and absolute spatial cues have been removed by moving the array of flowers from which the bird feeds a distance of 2 m from its original position and changing its shape (Hurly and Healy, 2002). Finally, colour can also facilitate the learning of rewarded spatial locations: hummingbirds learned the locations of rewarded flowers with fewer errors when those flowers each bore a distinct colour pattern than when they were all the same colour (Healy and Hurly, 1998; Miller and Miller, 1971).

It appears, then, that when colour is the most salient cue for returning to a reward location, hummingbirds will use this cue. In the only experiment to date that has examined the role of colour cues in the learning of flower refill schedules, Marshall et al. (2012) presented two groups of hummingbirds with an array of four flowers, two refilling every 10 min after being emptied by the bird and two every 20 min. For the Cued birds in this experiment, 10-min flowers were one colour and 20-min flowers were another colour, whereas for the Uncued birds all of the flowers differed in colour. Those birds provided with colour cues that signalled refill schedule did not, however, learn to discriminate between the two types of flower refill interval more quickly than did the birds without colour cues (Marshall et al., 2012). In that experiment, however,

colour cues did not provide the only reliable indicator of reward. Furthermore, by presenting flowers each of a different colour to the Uncued birds, it is possible that these birds did use colour to learn the refill schedules, but that they learned four different associations, one for each flower. This might then have led them to learn at the same rate as the Cued birds.

Here we aimed to determine whether colour cues would facilitate the learning of flower refill schedules in free-living rufous hummingbirds (*Selasphorus rufus*) by testing: firstly, whether birds provided with colour cues signalling two different flower refill schedules learned to discriminate between these two flower types more quickly than would birds provided with no colour cues and secondly, the extent to which the birds provided with colour cues could remember this colour information when both relative and absolute spatial cues to flower refill schedules were removed.

2. Methods

2.1. Subjects and study site

The experiment was conducted in the Eastern range of the Rocky Mountains, at the University of Lethbridge Field Station, Westcastle Valley, Alberta, Canada at an elevation of 1400 m (49°21′ N; 114°25′ W). The subjects were six free-living, territorial male rufous hummingbirds each defending a territory around a commercial artificial feeder containing 14% sucrose solution (weight/weight). The birds established territories from late May onwards.

To identify them individually, males were trapped a short distance from the feeder at the beginning of the field season and marked with a distinct, non-toxic, waterproof, coloured ink mark on their breast. This mark lasted for the duration of the experiment but was indistinct by the end of the breeding season.

Two of the birds tested (1 Cued, 1 Uncued) were experimentally naive, but the other four birds had all previously taken part in at least one other unrelated memory or decision-making experiment.

This research was conducted with the permission of the University of Lethbridge Animal Welfare Committee, under permit from Environment Canada and Alberta Environment and Sustainable Resource Development and was granted ethical approval by the School of Psychology Ethics Committee, University of St Andrews.

2.2. Initial experimental training with artificial flowers

All birds were initially trained to feed from artificial flowers of the kind used in the subsequent experiment. Artificial flowers consisted of a circle of cardboard that was approximately 6 cm in diameter and painted with acrylic paint (training flowers were painted yellow). In the centre of this cardboard circle, a well was formed by a blue syringe tip capable of containing up to 120 μl of 25% sucrose (weight/weight). This 'flower' was positioned on top of a 60 cm high wooden stake that could be placed into the ground and from which the bird could then feed freely. Once the bird had fed several times from a flower that had been moved short distances between feeding bouts (increments of approximately 1 m) within its territory, he was considered to be trained to feed from artificial flowers and the experiment could then begin. Flower training usually took 2–3 h to complete.

2.3. Experimental procedure

Birds were pseudorandomly assigned to either the Cued or Uncued Condition (3 Cued birds, 3 Uncued birds). Each condition contained two experimental phases: in both phases of each condition, the birds were presented with an array of six flowers, each containing 20 μl of 25% sucrose (weight/weight) and with a nearest neighbour distance of 60 cm between flowers, centre to centre.
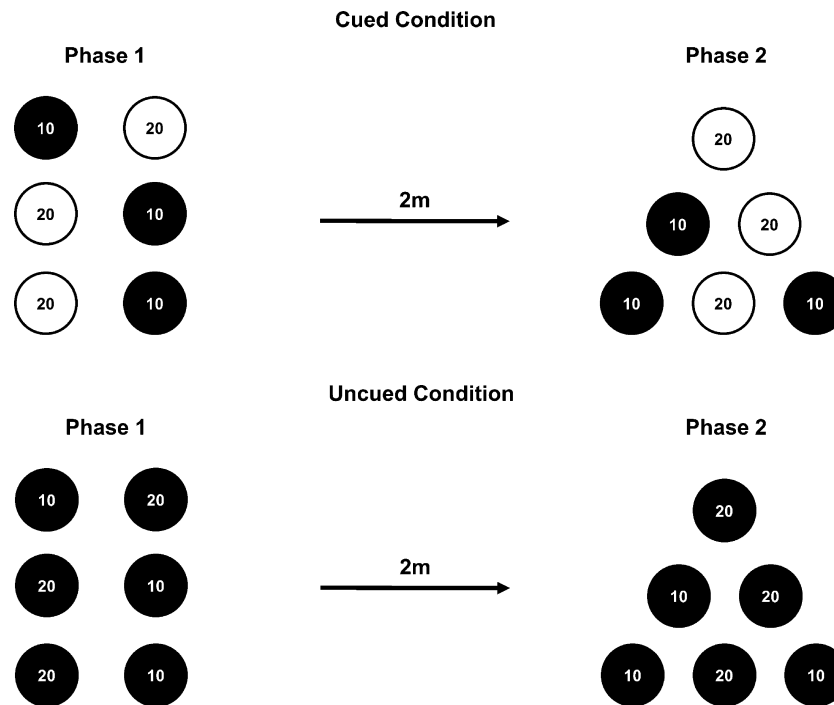
Cued Condition

Phase 1   **Phase 2**

2m

Uncued Condition

Phase 1   **Phase 2**

2m

Fig. 1. A schematic of the experimental design. Each circle represents a flower with either a 10 or 20-min refill schedule. For Cued birds 10 and 20-min flowers were associated with a different flower colour (represented by black and white circles here) whereas for Uncued birds all flowers were of the same colour. Flowers' nearest neighbours were at a distance of 60 cm, centre to centre. The positions of 10 and 20-min flowers were pseudorandomised for each phase for each bird in both conditions. The order of array shape presented first between equilateral triangle and rectangle was also varied across birds.

In the Cued condition, three flowers were of one colour and three were of a different colour. Each colour represented a flower refill schedule, which was either 10 or 20 min. The colours used (neon green, blue and pink) were pseudorandomised across birds. In the Uncued condition, birds were presented with six flowers, all of the same colour (blue, pink or neon green), which was also pseudorandomised across birds (Fig. 1).

In phase 1 of both conditions, we pseudorandomly assigned each of the six flower positions within the array a refill schedule of either 10 or 20 min, which remained the same for the duration of that phase. We assigned three of the flowers to be refilled 10 min after being emptied by a bird and three flowers for refilling 20 min after being emptied. The experimenter manually refilled the flowers.

At the beginning of an experimental session, the bird's feeder was removed and the array of six flowers was placed within the bird's territory. The shape of the flower array was either in the form of a rectangle or equilateral triangle (Fig. 1), with the order of array shape presented in phase 1 of the experiment being pseudorandomised across birds to reduce the possibility that birds might use the spatial position of rewarded flowers in phase 1 to learn which flowers were rewarded in phase 2.

Once the flower array was presented and the experimenter had refilled all six flowers, the session began and the bird could then visit and feed from all six flowers in the array freely throughout the day. From the first visit onwards, we recorded each time that the bird visited the flower array, the time of the visit, the flowers that he probed, the order of flowers probed and whether that flower was rewarded or unrewarded at the time of the visit. The rewarded flowers visited were then emptied and refilled manually by the experimenter either 10 or 20 min later, depending on the designated refill schedule. Although it is unlikely that the birds would use this manual refilling schedule as a visual cue to flower refill intervals, each time that one or more flowers were refilled, all other flowers were 'sham' refilled at the same time so as not to provide to the birds extra visual cues as to which flowers contained

reward. For all the birds, during an experimental session, the experimenter sat on a chair at a distance of approximately 10 m from the flower array at all times, except for when emptying/refilling flowers. Testing sessions lasted for a maximum of 10 h per day. At the end of a day's session, the array was removed and the bird's feeder was returned to its usual place.

At the end of each session we calculated the time taken to revisit a flower after the last visit to that flower (inter-visit interval) for each individual flower across the whole of the session. We then organised these data chronologically into groups of 50 flower visits. The corresponding inter-visit intervals to both 10 and 20-min flowers for each block of 50 visits were then compared using two-tailed Mann–Whitney U tests. Birds were deemed to have learned to differentiate between the two refill schedules when these return intervals differed significantly ($p < 0.05$) from each other, such that a bird revisited 10-min flowers significantly sooner than he revisited 20-min flowers. Phase 1 of the experiment ended when the bird either reached this criterion for learning to differentiate between 10 and 20-min flowers, or when the bird had experienced a total of 500 flower visits across flowers of both refill intervals. Once either point was reached, phase 2 of the experiment could then begin.

For all birds, we left at least 2 h between the end of phase 1 and the beginning of phase 2 of the experiment, during which time the bird's feeder was available in its normal location. In phase 2, the aim was to determine whether the colour-time association learned in phase 1 in the Cued condition enabled those birds to more quickly learn the intervals at which the flowers refilled in phase 2 relative to the rate at which the Uncued birds learned the intervals at which flowers refilled in phase 2. Therefore, we moved the array to a new location and placed the flowers in the alternate spatial layout. For the Cued birds, the same two colours as in phase 1 represented the flower refill schedules, while the Uncued birds faced flowers of the same colour they saw in phase 1.

Because the purpose of phase 2 was to determine whether the influence of colour cues on the learning of refill schedules that

developed in phase 1 would extend to new flowers, we had to eliminate other associations with refill schedule. We eliminated associations with spatial locations by moving the array by 2 m and we eliminated associations with position in the array by configuring the array in a new shape (rectangle to triangle, or vice versa). Finally, we eliminated associations with subtle visual features on the flowers by using new cardboard disks painted the same colours. Thus, if the birds learned the flowers' reward schedules sooner in phase 2 than they had in phase 1 it seemed likely that they must have used colour as the cue to do so. As for phase 1, phase 2 came to an end when the birds either reached the criterion for learning to differentiate between 10 and 20-min flowers, or when the bird had experienced a total of 500 flower visits across flowers of both refill intervals.

3. Results

3.1. Learning

3.1.1. Did birds learn the task?

Of the six birds tested, five of the birds learned to revisit 10-min flowers significantly sooner than they visited 20-min flowers in phase 1 (Table 1). The bird that did not learn the discrimination was in the Uncued condition (Bird UC2). When the array was moved 2 m and both the shape and relative flower positions altered in phase 2, four of the six birds learned according to this same criterion (Table 1). Of the two that did not learn, one of these birds was the same Uncued bird that did not learn in phase 1 (Bird UC2), with the other bird failing to reach this criterion also in the Uncued group (Bird UC3).

3.2. Rate of learning

3.2.1. Did the colour cue facilitate learning?

We used a two-factor ANOVA (Treatment, between-subject, cued vs. uncued; Experimental Phase, within-subject, phase 1 vs. phase 2) to compare the number of blocks of 50 visits required by birds to discriminate between flower types. The Uncued birds that failed to discriminate between flower types (Bird UC2 in both phases and Bird UC3 in phase 2) were assigned the maximum number of 10 blocks (500 flower visits) that they had experienced for the analysis. Cued birds learned to discriminate between 10 and 20-min flowers significantly sooner than Uncued birds ($F_{1,4}$ = 19.12, p = 0.012; Fig. 2). There was, however, no difference in the birds' speed to learn between the phases ($F_{1,4}$ = 0.19, p = 0.68) and no interaction between condition and phase ($F_{1,4}$ = 2.57, p = 0.184). Furthermore, we found no effect of the type of array shape on performance (rectangle or equilateral triangle: $F_{1,4}$ = 0.01, p = 0.912).

3.3. Timing performance

3.3.1. How did the birds' choices influence their experience of the different flowers?

Across training, Cued birds made a mean (±SE) of 310 ± 54 visits to 10-min flowers and 211 ± 43 visits to 20-min flowers, whereas Uncued birds made an mean of 493 ± 22 visits to 10-min flowers and 450 ± 42 visits to 20-min flowers. A two-factor ANOVA (Treatment, between-subject, cued vs uncued; Flower Type, within-subject, 10-min vs. 20-min) revealed that across training, both Cued and Uncued birds experienced significantly more visits to 10-min flowers than to 20-min flowers ($F_{1,4}$ = 14.91, p = 0.018). Furthermore, birds in the Uncued condition experienced significantly more visits to both 10 and 20-min flowers than did the Cued birds ($F_{1,4}$ = 14.23, p = 0.02), with no interaction between condition and flower type ($F_{1,4}$ = 2.36, p = 0.199).
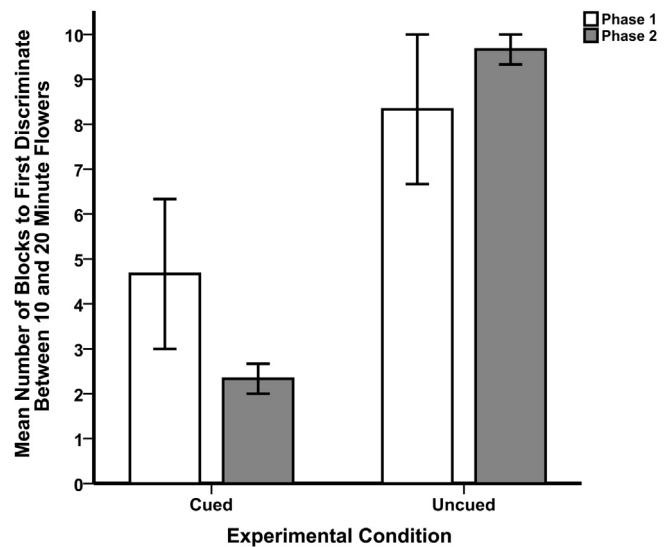

Fig. 2. Mean ± 1 SE number of training blocks taken to discriminate between 10 and 20-min flowers for each experimental group in each phase of the experiment (Cued N = 3, Uncued N = 3).

3.3.2. Did the birds discriminate between 10- and 20-min flowers?

In phase 1, their first experience of the flowers, it is clear that five of the six birds adjusted the timing of their visits to the 10-min and 20-min flowers (Table 1). Across birds, the inter-visit interval was greater for the 20-min flowers than it was for the 10-min flowers (two-factor ANOVA; Flower Type, $F_{1,4}$ = 10.1, p = 0.034, Fig. 3A).

This discrimination was not based on the birds synchronously visiting groups of 10-min and 20-min flowers. Although each day began with all flowers rewarded, the refill rates of individual flower soon lost synchrony with others of the same type. Birds visited a mean (±SE) of 3.57 ± 0.20 flowers per foraging bout at the array. On only 2% ± 0.01 of foraging bouts did birds visit only the three 10-min flowers and on 70% ± 6 of foraging bouts the birds did not visit all three of the 10-min flowers.

3.3.3. Did birds learn the specific intervals of 10 and 20 min?

The inter-visit intervals across all of training for two of the birds in the Cued condition did not significantly differ from 10 min (two-tailed, one-sample Wilcoxon Signed Rank Tests Bird C1: n = 399, z = 0.761, p = 0.45, Bird C2: n = 285, z = 1.95, p = 0.06) but were significantly longer than 10-min for the other four birds (Bird C3: n = 207, z = 9.55, p = 0.001, Bird UC1: n = 444, z = 12.24, p = 0.001, Bird UC2: n = 471, z = 13.06, p = 0.001, Bird UC3: n = 496, z = 10.34, p = 0.001; Fig. 3B). For the 20-min flowers, all of the birds' inter-visit intervals differed significantly from 20 min: for five of the birds the intervals were significantly shorter than 20 min (Bird C1: n = 282, z = 7.39, p = 0.001, Bird C2: n = 189, z = 5.31, p = 0.001, Bird UC1: n = 356, z = 5.21, p = 0.001, Bird UC2: n = 493, z = 7.82, p = 0.001, Bird UC3: n = 462, z = 8.68, p = 0.001) and for one bird the intervals were significantly longer than 20 min (Bird C3: n = 125, z = 2.35, p = 0.019).

None of the birds visited 10-min flowers twice as often as they visited 20-min flowers (ratio of visits to 10:20 min flowers), although the average ratio for Cued birds (1.5 ± 0.1) was greater (and closer to 2:1) than for Uncued birds (1.1 ± 0.1) (t_4 = 4.02, p = 0.016).

3.3.4. Did colour cues influence the timing of visits to flowers' 10- and 20-min refill intervals?

Although five of the six birds individually discriminated between flower type (above), flower type influenced inter-visit interval (above), and colour cue influenced rate of learning of

Table 1
Summary statistics for each bird in each phase of the experiment for the test block in which he first significantly discriminated between 10 and 20-min flowers (or for the last block of training for Bird UC2 in both phases and Bird UC3 in phase 2). $N = 6$.

Bird	Test block	Mean inter-visit interval ± SD		Mann–Whitney U	Z	p
		10 min flowers	20 min flowers			
Phase 1						
Bird C1	8	12.69 ± 10.24, $N = 29$	17.05 ± 9.76, $N = 21$	409.50	2.08	0.038
Bird C2	3	7.84 ± 2.72, $N = 31$	12.47 ± 7.14, $N = 19$	399.00	2.11	0.035
Bird C3	3	18.56 ± 13.28, $N = 32$	34.66 ± 21.09, $N = 18$	407.50	2.43	0.015
Bird UC1	5	15.32 ± 7.71, $N = 34$	25.25 ± 20.50, $N = 16$	368.50	2.01	0.044
Bird UC2	10	18.12 ± 12.40, $N = 26$	19.17 ± 11.09, $N = 24$	345.50	0.65	0.514
Bird UC3	10	14.33 ± 8.74, $N = 27$	25.00 ± 15.67, $N = 17$	340.00	2.67	0.007
Phase 2						
Bird C1	2	8.79 ± 3.47, $N = 34$	16.69 ± 8.32, $N = 16$	431.00	3.36	0.001
Bird C2	2	11.45 ± 7.02, $N = 31$	15.37 ± 6.83, $N = 19$	403.50	2.19	0.029
Bird C3	3	18.29 ± 10.67, $N = 31$	46.54 ± 37.74, $N = 13$	327.50	3.25	0.001
Bird UC1	9	16.24 ± 8.57, $N = 25$	24.47 ± 14.49, $N = 19$	336.00	2.34	0.019
Bird UC2	10	22.20 ± 16.39, $N = 23$	20.52 ± 13.02, $N = 27$	323.50	0.25	0.800
Bird UC3	10	14.52 ± 6.88, $N = 23$	12.07 ± 7.13, $N = 27$	229.00	−1.59	0.111

Note: C denotes birds in the Cued condition and UC denotes birds in the Uncued condition.
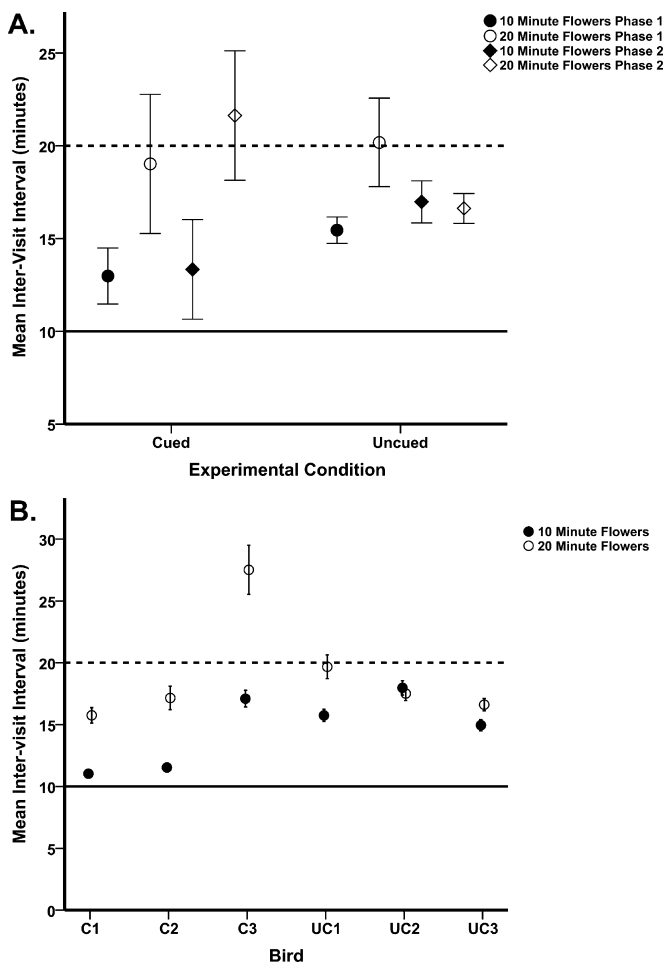

Fig. 3. Mean ± 1 SE inter-visit intervals to 10 and 20-min flowers across all of training for (A) Cued and Uncued experimental conditions in each phase of the experiment (Cued $N = 3$, Uncued $N = 3$) and (B) each individual bird across both experimental phases. C denotes Cued and UC denotes Uncued ($N =$ Bird C1: 681, Bird C2: 474, Bird C3: 332, Bird UC1: 800, Bird UC2: 964, Bird UC3: 958).

discrimination (above), colour cue did not have an overall significant influence on inter-visit intervals (Colour Cue, $F_{1,4} = 0.22$, $p = 0.656$; Fig. 3A and B). Nor was there an interaction between flower type and colour cue ($F_{1,4} = 0.05$, $p = 0.830$).

3.3.5. Did colour cues influence the re-learning of intervals in phase 2?

Although colour cues influenced the rate of learning to discriminate between flowers, and it influenced the inter-visit interval in phase 1, it did not influence the inter-visit interval in phase 2 (Colour Cue, $F_{1,4} = 0.03$, $p = 0.871$; Flower Type, $F_{1,4} = 3.17$, $p = 0.150$, interaction, $F_{1,4} = 2.08$, $p = 0.223$; Fig. 3A).

4. Discussion

Of the six hummingbirds we tested, five learned to revisit flowers that were refilled every 10 min significantly sooner than they revisited the flowers that were refilled every 20 min. Across both experimental phases, birds that were presented with flowers for which refill schedules were associated with a different colour (Cued condition) learned to discriminate between 10 and 20-min flowers more quickly than did birds that were presented with flowers that were all of the same colour. Furthermore, despite the fact that birds in the Cued condition had approximately half the amount of experience of visiting 10 and 20-min flowers than did birds in the Uncued condition, Cued birds also tended to be better at discriminating between 10 and 20-min flowers compared to Uncued birds. Although the colour cues facilitated birds' learning to discriminate between 10 and 20-min flowers, they did not lead to the Cued birds learning the discrimination more readily in phase 2 than they had in phase 1.

These data provide evidence that colour cues can facilitate the acquisition of the discrimination between flower refill schedules in these birds. They also confirm previous findings that hummingbirds can discriminate between flowers that differ in refill intervals (Henderson et al., 2006; Marshall et al., 2012). These data appear, however, to be inconsistent with the findings of Marshall et al. (2012), in which Cued and Uncued birds did not differ in the number of trials they took to discriminate between flower refill schedules. We think it likely that this is explained by the birds in our Uncued condition having no colour information for use when learning the flower refill schedules whereas in the Marshall et al. (2012) experiment, Uncued birds were presented with flowers of different colours. Marshall et al.'s (2012) Uncued birds, then, had colour cues available although those cues were specific to each flower and could not be used to generalise across the flowers with the same colour and refill schedule (as in the Cued condition in both our work and that of Marshall et al.). It is possible that the provision of colour cues specific to each flower enabled enhanced discrimination among the flowers and thus made it difficult to detect an effect of colour between the Cued and Uncued experimental conditions

in that experiment. Our data, on the other hand, are consistent with evidence that colour can facilitate the learning of rewarded flower locations in these birds (Healy and Hurly, 1998; Hurly and Healy, 2002; Miller and Miller, 1971). To our knowledge, however, this is the first instance of a colour cue facilitating the discrimination of temporal components of rewarded flower locations.

It is less clear whether this colour facilitation of learning to discriminate between temporal features also applies to learning the refill intervals themselves. The birds' inter-visit intervals to 10-min flowers across training did not significantly differ from the target value of 10 for two of the three birds in the Cued condition, showing that these birds learned to time the 10-min intervals well. This was not the case, however, for any of the birds in the Uncued condition. For 20-min flowers, the inter-visit intervals differed significantly from the target value of 20 for all of the birds in each condition, which matches previous findings showing that these birds generally take longer to learn to time 20-min intervals (Henderson et al., 2006; Marshall et al., 2012). Given our experimental design it is possible that the birds could have adopted a strategy of learning the 10-min refill schedule and then simply visiting 20-min flowers every second visit. We think that this is highly unlikely, however, since the pattern of visits made by individual birds did not show any evidence of such a grouping strategy. Furthermore, such as strategy should have lead the birds to time their visits to 20-min flowers with an accuracy similar to that with which they visited the 10-min flowers, which was not case. Additionally, although the birds in the Cued condition did get closer to the 2:1 ratio of visits to 10-min flowers than 20-min flowers that would be expected if birds visited according to the matching law (Herrnstein, 1970; Miller and Loveland, 1974 but see Henderson et al., 2006) than did birds in the Uncued condition, we found no overall effect of the colour cue on the inter-visit intervals for birds in the two groups.

It was a little surprising that the effect of colour did not enhance the Cued birds' performances in phase 2 of the experiment where the colour and refill schedules were matched as in phase 1 as hummingbirds can remember and will use colour cues when presented with a new array of flowers when a flower array is moved and spatial cues are no longer a reliable indicator of rewarded flowers (Hurly and Healy, 2002). It seems likely that this is, at least in part, due to the small sample size and therefore low level of statistical power in our analyses for detecting effects when comparing the performance of birds within and across groups: two of the three birds in the Cued condition did learn to discriminate between 10 and 20-min flowers sooner in phase 2 of the experiment than in phase 1, whereas none of the 3 Uncued birds learned more quickly in phase 2 (indeed, two of these birds appeared to learn the discrimination more slowly in phase 2). It is also important to note that although the results of our group-level statistical analyses are consistent with our overall predictions based on data from previous studies with these birds (Marshall et al., 2012; Henderson et al., 2006), generally speaking, caution needs to be exercised in interpreting the results of these group-level analyses. These were limited given the very small sample size and low level of statistical power, which could not be avoided.

One question that remains unanswered is the specific mechanism by which birds were able to use the colour cues available when learning about flower refill schedules. It is possible that the birds presented with colour cues used the category information provided by the colour cues to form distinct categories of 10 and 20-min flowers based on colour, making it easier for birds to learn and remember the refill schedules of flowers by forming groups based on these shared attributes (Dukas and Waser, 1994). Alternatively, the effect of facilitation observed may be due to the birds using the colour cues to help 'chunk' information about flower refill schedules into memory, making it easier for them to recall which flowers should be visited and when (Dallal and Meck,

1990). Assessing the relevance of these mechanisms requires more experimentation.

Acknowledgements

References

Bateson, M., 2003. Interval timing and optimal foraging. In: Meck, W.H. (Ed.), Functional and Neural Mechanisms of Interval Timing. CRC Press, New York, pp. 113–141.

Bené, F., 1941. Experiments on the color preference of black-chinned hummingbirds. Condor 43, 237–242.

Bené, F., 1945. The role of learning in the feeding behavior of black-chinned hummingbirds. Condor 47, 3–22.

Boisvert, M.J., Sherry, D.F., 2006. Interval timing by an invertebrate, the bumble bee *Bombus impatiens*. Curr. Biol. 16, 1636–1640.

Buhusi, C.V., Aziz, D., Winslow, D., Carter, R.E., Swearingen, J.E., Buhusi, M.C., 2009. Interval timing accuracy and scalar timing in C57BL/6 mice. Behav. Neurosci. 123, 1102–1113.

Buhusi, C.V., Meck, W.H., 2005. What makes us tick? Functional and neural mechanisms of interval timing. Nat. Rev. Neurosci. 6, 755–765.

Cheng, K., Westwood, R., 1993. Analysis of single trials in pigeons timing performance. J. Exp. Psychol. Anim. Behav. Process 19, 56–67.

Cole, S., Hainsworth, F.R., Kamil, A.C., Mercier, T., Wolf, L.L., 1982. Spatial learning as an adaptation in hummingbirds. Science 217, 655–657.

Collias, N.E., Collias, E.C., 1968. Anna's hummingbirds trained to select different colors in feeding. Condor 70, 273–274.

Dallal, N.L., Meck, W.H., 1990. Hierarchical structures: chunking by food type facilitates spatial memory. J. Exp. Psychol. Anim. Behav. Process 16, 69–84.

Dews, P.B., 1962. The effect of multiple S delta periods on responding on a fixed-interval schedule. J. Exp. Anal. Behav. 5, 369–374.

Dews, P.B., 1978. Studies on responding under fixed-interval schedules of reinforcement: 2. Scalloped pattern of cumulative record. J. Exp. Anal. Behav. 29, 67–75.

Dukas, R., Waser, N.M., 1994. Categorization of food types enhances foraging performance of bumblebees. Anim. Behav. 48, 1001–1006.

Gallistel, C.R., 1990. The Organization of Learning. MIT Press, Cambridge, MA.

Gill, F.B., 1988. Trapline foraging by hermit hummingbirds: competition for an undefended, renewable resource. Ecology 69, 1933–1942.

Goldsmith, T.H., 1980. Hummingbirds see near ultraviolet light. Science 207, 786–788.

Goldsmith, T.H., Collins, J.S., Perlman, D.L., 1981. A wavelength discrimination function for the hummingbird. J. Comp. Physiol. 143, 103–110.

González-Gómez, P.L., Bozinovic, F., Vásquez, R.A., 2011. Elements of episodic-like memory in free-living hummingbirds, energetic consequences. Anim. Behav. 81, 1257–1262.

Grant, K.A., 1966. A hypothesis concerning the prevalence of red coloration in California hummingbird flowers. Am. Nat. 100, 85–97.

Healy, S.D., Hurly, T.A., 1995. Spatial memory in rufous hummingbirds (*Selasphorus rufus*): a field test. Anim. Learn. Behav. 23, 63–68.

Healy, S.D., Hurly, T.A., 1998. Rufous hummingbirds' (*Selasphorus rufus*) memory for flowers: patterns or actual spatial locations? J. Exp. Psychol. Anim. Behav. Process 24, 396–404.

Healy, S.D., Hurly, T.A., 2003. Cognitive ecology: foraging in hummingbirds as a model system. Adv. Study Behav. 32, 325–359.

Henderson, J., Hurly, T.A., Bateson, M., Healy, S.D., 2006. Timing in free-living rufous hummingbirds, *Selasphorus rufus*. Curr. Biol. 16, 512–515.

Herrnstein, R.J., 1970. On the law of effect. J. Exp. Anal. Behav. 13, 243–266.

Higa, J.J., Simm, L.A., 2004. Interval timing in Siamese fighting fish (*Betta splendens*). Behav. Process 67, 501–509.

Hurly, T.A., 1996. Spatial memory in rufous hummingbirds: memory for rewarded and non-rewarded sites. Anim. Behav. 51, 177–183.

Hurly, T.A., Healy, S.D., 1996. Memory for flowers in rufous hummingbirds: location or local visual cues? Anim. Behav. 51, 1149–1157.

Hurly, T.A., Healy, S.D., 2002. Cue learning by rufous hummingbirds (*Selasphorus rufus*). J. Exp. Psychol. Anim. Behav. Process 28, 209–223.

Lejeune, H., Wearden, J.H., 1991. The comparative psychology of fixed-interval responding: some quantitative analyses. Learn. Motiv. 22, 84–111.

Lowe, C.F., Harzem, P., Spencer, P.T., 1979. Temporal control of behaviour and the power law. J. Exp. Anal. Behav. 31, 333–343.

Lyerly, S.B., Riess, B.F., Ross, S., 1950. Color preference in the Mexican violet-eared hummingbird, *Calibri T. thalassinus* (Swainson). Behaviour 2, 237–248.

Marshall, R.E.S., Hurly, T.A., Healy, S.D., 2012. Do a flower's features help humming-birds to learn its contents and refill rate? Anim. Behav. 83, 1163–1169.

McDade, L.A., Weeks, J.A., 2004. Nectar in hummingbird-pollinated neotropical plants I: patterns of production and variability in 12 species. Biotropica 36, 196–215.

Melendez-Ackerman, E., Campbell, D.R., Waser, N.M., 1997. Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. Ecology 78, 2532–2541.

Miller, H.L., Loveland, D.H., 1974. Matching when the number of response alternatives is large. Anim. Learn. Behav. 2, 106–110.

Miller, R.S., Miller, R.E., 1971. Feeding activity and color preference of ruby-throated hummingbirds. Condor 73, 309–313.

Miller, R.S., Tamm, S., Sutherland, G.D., Gass, C.L., 1985. Cues for orientation in hum-mingbird foraging: color and position. Can. J. Zool. 63, 18–21.

Rakitin, B.C., Gibbon, J., Penney, T.B., Malapani, C., Hinton, S.C., Meck, W.H., 1998. Scalar expectancy theory and peak-interval timing in humans. J. Exp. Psychol. Anim. Behav. Process 24, 15–33.

Sandlin, E.A., 2000. Cue use affects resource subdivision among three coexisting hummingbird species. Behav. Ecol. 11, 550–559.

Talton, L.E., Higa, J.J., Staddon, J.E.R., 1999. Interval schedule performance in the goldfish *Carassius auratus*. Behav. Process. 45, 193–206.

Zentall, T.R., Wasserman, E.A., Lazareva, O.F., Thompson, R.K., Rattermann, M.J., 2008. Concept learning in animals. Comp. Cogn. Behav. Rev. 3, 13–45.