Do a flower's features help hummingbirds to learn its contents and refill rate?

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ARTICLE INFO

Colour is a cue that animals can use to categorize rewards and may be particularly important to nectarivores, which forage on flowers that vary in hue. Here, we investigated whether colour facilitated the learning of the properties of artificial flowers in free-living rufous hummingbirds, *Selasphorus rufus*. Whereas refill rates and sucrose concentrations of flowers were readily learned, we could not detect in the birds' performance an effect of colour on the learning of either floral property. As these results seem unlikely to have been the result of a ceiling effect or an inability to perceive colour variation, we suggest they are due to overshadowing. This apparently counterintuitive result, where birds do not attend to what appears to be a very prominent cue, is consistent with evidence that hummingbirds pay more attention to space than to colour.
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Many animals can use an object's colour to categorize rewards (e.g. pigeons, *Columba livia*: Logue 1980; mantis shrimps, *Odontodactylus scyllarus*: Marshall et al. 1996; chicks, *Gallus gallus*: Vallortigara 1996; squirrel monkeys, *Saimiri sciureus* and common marmosets, *Callithrix jacchus*: Laska & Metzker 1998; dogs, *Canis familiaris*: Elgier et al. 2009) including a range of nectarivores (e.g. bumble bees, *Bombus terrestris*: Raine & Chittka 2008; various butterfly species: Kandori et al. 2009). The ability to make use of colour as a categorizing variable by nectarivorous species may be a useful way of discriminating between resources in nature, as the floral resources they exploit can vary considerably with respect to colour.

Red has traditionally been thought of as an important colour in the feeding of nectarivorous hummingbirds, owing to the predominance of red coloration in hummingbird-pollinated flowers (e.g. Grant 1966). However, tests of colour preference in hummingbirds have been less compelling. Birds may sometimes prefer red (Collias & Collias 1968; Meléndez-Ackerman et al. 1997) but often they do not (Bené 1941; Wagner 1946; Lyerly et al. 1950; Grant 1966). They will also shift their preference from red quickly if another colour becomes associated with a more valuable

reward (Meléndez-Ackerman et al. 1997). The red coloration of hummingbird-pollinated flowers may be a floral mechanism for decreasing visits by other nectar feeders as although hummingbirds can see in colour throughout the visible light spectrum and into the near ultraviolet (Goldsmith 1980; Goldsmith et al. 1981), insect vision is generally poorer at the red end of the visible light spectrum (Raven 1972; Briscoe & Chittka 2001; Altshuler 2003).

Not only are birds able to see colour, they are also able to use it to identify rewards. Hummingbirds can form associations between various colours and the presence or absence of a reward (Goldsmith & Goldsmith 1979; Goldsmith et al. 1981; Hurly & Healy 1996), as well as a reward's magnitude (Bateson et al. 2002, 2003) and its variability (Hurly & Oseen 1999). They also appear to be able to gain these colour associations through observation of conspecifics (Lara et al. 2009). But although hummingbirds can learn colour–reward associations, in most cases where colour and location provide the same information, hummingbirds appear to prefer to rely on spatial cues, both with regard to making a choice between options of equal value, where they will tend to pick options in a particular place rather than of a particular colour (Lyerly et al. 1950; Miller & Miller 1971; Miller et al. 1985), and in terms of learning whether an object is or is not rewarded (Goldsmith & Goldsmith 1979; Miller et al. 1985; Hurly & Healy 1996).

Despite hummingbirds' preference for spatial over colour cues, however, colour does, in some circumstances, facilitate learning. For example, hummingbirds learn the location of a reward set among

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nonrewarding options more quickly when all options are different colours than when they are all identical (Miller & Miller 1971; Healy & Hurly 1998), potentially because variation in colour enhances discrimination of similar options. The purpose of the current investigation was to discover whether rufous hummingbirds, *Selasphorus rufus*, might also use colour in a different fashion, this time as a common cue to distinguish between flowers of different reward value. In previous work, where space and colour have been investigated together, colour has either provided the same information provided by space or has served as an aid to increase individual flowers' discriminability. Here, we wished to look at whether birds paid attention to colour when it provided information on top of that provided by location, by allowing flowers to be classified into different 'types'. These birds can learn the refill rates of individual flowers, demonstrating a form of episodic-like memory (Henderson et al. 2006; as can green-backed firecrown hummingbirds, *Sephanoides sephaniodes*: Gonzalez-Gomez et al. 2011), but it is not clear whether colour plays a role in this acquisition. We also asked whether colour could enhance the learning of reward concentration.

In these experiments, birds were presented with arrays of four flowers. Each of these flowers had one of two possible refill rates or concentrations of sucrose solution. In the Refill Treatment, for one group of birds each of the two refill rates of the flowers was associated with a floral colour, while for the other group each flower was a different colour. In the Concentration Treatment, individual birds' performance was compared when fed from flowers that were either colour-cued to concentration or individually distinctive. We predicted that birds would learn the refill rates and the contents of the flowers more rapidly when these were distinguishable by their colour label.

METHODS

This study was conducted at the University of Lethbridge Westcastle Field Station, Alberta, Canada, at 1400 m elevation in the Eastern Rocky Mountains (49°35′N, 114°41′W). The subjects were 12 territory-holding, free-living, adult male rufous hummingbirds, each defending an established territory around an artificial feeder containing 14% sucrose solution (weight by weight, as are all other sucrose concentrations in this experiment). At the beginning of the field season, birds were trapped by a qualified hummingbird bander using a trap surrounding the feeder and marked on the breast with nontoxic, waterproof coloured ink so that they could be distinguished without recapture. Birds were also banded to allow individuals to be identified between years. Trials were run from 0700 to 2000 hours Mountain Standard Time in May and June 2009 and in July 2010. All work was approved by the University of St Andrews Ethical Committee, conducted according to the requirements of the Canadian Council on Animal Care and was carried out under permits from Alberta Sustainable Resource Development and Environment Canada.

Initial Training

All subjects were initially trained to feed from artificial flowers. A 'flower' consisted of a syringe tip forming the 'nectar' well, containing up to 600 μl of 25% sucrose, surrounded by a circle of card painted orange using acrylic paint, of roughly 6 cm diameter, all of which was mounted horizontally on a 60 cm high wooden stake (for a diagram, see Appendix Fig. A1). Flowers were refilled manually by the experimenter between visits.

Concentration Treatment

This experiment was conducted in July 2010. The subjects in this treatment were five male territorial rufous hummingbirds. Each

bird was presented with four artificial coloured flowers in a 1 m square array. Two birds were initially presented with four flowers of different colours (dark green, dark blue, light blue, yellow, pink or purple), two containing 40 μl of 20% sucrose, and two containing 40 μl of 30% sucrose. The remaining three birds were presented with two flowers of one colour, which contained 40 μl of 20% sucrose and two of another colour containing 40 μl of 30% sucrose (i.e. flower colour signalled sucrose concentration, the Cued condition, see Fig. 1). The flowers were coloured using acrylic paint. The reward–colour association was varied between birds (see Appendix Table A1). The positions of flowers of each type were pseudorandomly assigned so that for some birds like concentrations were adjacent and for some they were diagonally opposite. After each feeding bout, all flowers visited were refilled manually by the experimenter. As this took less than 1 min, and typical return times for birds feeding from this kind of apparatus are in the region of 10 min, we considered that it was unlikely that this would have affected the birds' behaviour. We assumed birds were able to discriminate between the flower colours we provided, both because hummingbird colour vision is good across the visible light spectrum (Goldsmith & Goldsmith 1979; Goldsmith et al. 1981) and because rufous hummingbirds have successfully discriminated similar flower colours in other unrelated experiments (Healy & Hurly 1998; Hurly & Oseen 1999; Bateson et al. 2002, 2003; Bacon et al. 2010; Morgan et al. 2011). Birds in each treatment group were allowed to make 60 flower visits.

All birds experienced both conditions. After completion of the first condition, the flowers were removed for at least 1 h. This corresponds to five or six feeding bouts at hummingbirds' typical feeding rates. Each bird was then presented with the alternative condition (Cued condition followed by the Uncued or vice versa) using novel flower colours in a different location within their territory. Birds were again allowed to make 60 flower visits.

For each condition, we recorded all visits to the array made by the bird, the flowers he probed, the time of the visit and the order in which flowers were visited. We did not measure visit duration, as this may be a somewhat unreliable measure of amount of sucrose solution taken across different concentrations owing to differences in viscosity (Bacon et al. 2011).

We analysed the proportion of choices birds made to higher and lower concentration flowers in both conditions using one-tailed Wilcoxon signed-ranks tests. One-tailed tests were used as previous work strongly suggested that, if birds were to show a preference it would be towards the more highly rewarded flower, as hummingbirds will prefer the most concentrated of two sucrose solutions up to 40–45% (e.g. Tamm & Gass 1986; Roberts 1996).
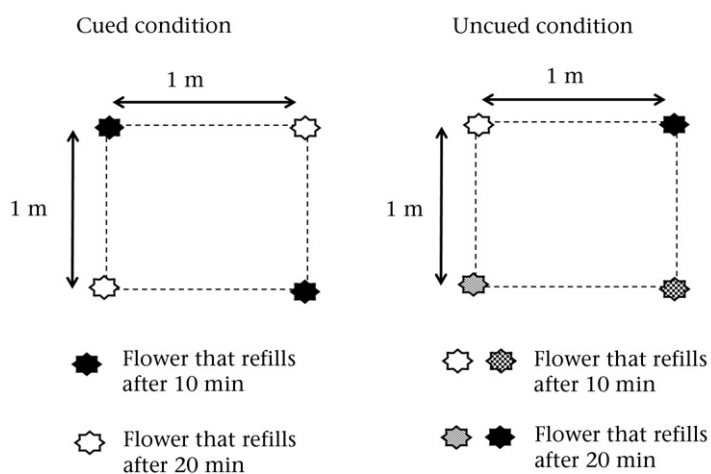
Refill Treatment

This experiment was conducted from May to July 2009. The subjects in this treatment were eight territorial male rufous hummingbirds, one of which was tested in the Concentration Treatment the following year. The birds were pseudorandomly assigned to one of two experimental conditions, either the Cued or Uncued condition. The birds assigned to the Cued condition were presented with an array consisting of four flowers, two purple and two yellow. The birds in the Uncued condition were presented with four flowers, each a different colour (pink, purple, blue and yellow). Each flower colour for each bird was pseudorandomly assigned a refill time of either 10 or 20 min, such that two flowers for each bird were manually refilled 10 min after being emptied and two were refilled 20 min after being emptied. As for the Concentration Treatment, these flowers were positioned at the corners of a 1 m square array. For half the birds in each condition adjacent flowers shared a refill schedule, while for the remaining

Concentration Treatment
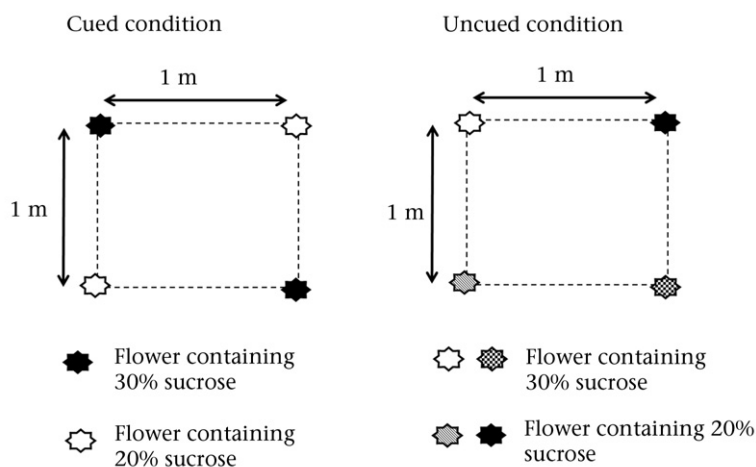
Refill Treatment

Figure 1. Schematic of the experimental layouts for the Concentration and Refill treatments. The position of flowers of each type was varied within treatments and conditions.

subjects adjacent flowers were refilled on the alternative refill schedule (Fig. 1).

When filled, each flower contained 25% sucrose. Owing to large variation in birds' rates of feeding, the volume of sucrose in flowers was varied within and across days. It was increased in increments of 5 µl if birds were consistently returning to the array before any flowers had refilled and decreased if birds were consistently returning less often than every 10 min, to allow them an opportunity to learn about the refill schedules of 10 min flowers. Volume was varied simultaneously across flowers, so changes in volume should not have interfered with relative revisit rates between flowers, which was the response used in later analyses.

When a bird visited the array, the flowers it probed, the time of the visit and the order in which flowers were approached were recorded. A 20 µl capillary tube was then used to measure and remove any remaining sucrose in all of the visited flowers. The visited flowers were then refilled either 10 or 20 min later. Birds were tested for a maximum of 10 h per day.

The time taken to revisit a flower after the last reinforced visit (the postreinforcement pause) was calculated for each bird visiting each flower across all training periods. If a bird did not visit the array for more than 1 h during training, he was judged not to be

participating in the experiment and the corresponding interval between visits was removed from subsequent analysis. This was to prevent long intervisit intervals, where we judged it unlikely that birds were attending to either refill rate, from skewing the data. The data were then divided chronologically into groups of 50 postreinforcement pauses (with the exception of the final training block for bird C2, which consisted of 24 visits and the final block for bird UC2, which consisted of 37 visits), and birds' postreinforcement pauses when visiting 10 and 20 min flowers were compared using one-tailed Mann–Whitney U tests. One-tailed tests were used as we saw no theoretical reason why birds would revisit 20 min flowers sooner than 10 min flowers, and past research strongly suggested that if we were to observe any effect of refill time it would be in the direction expected (Henderson et al. 2006). A bird was taken to have learned the appropriate association when these return times were significantly different from each other. This block size of 50 visits was selected as a compromise between test reliability and power and for the grain of the scale over which it would allow comparisons. As a result the data were divided into up to seven blocks of 50, each containing 30.5 ± 3.5 (mean $\pm$ SD) visits to 10 min flowers and 18.4 ± 2.7 visits to 20 min flowers, respectively.

Testing ended for seven of the eight birds when $25 - 30$ h of training had elapsed. The $25 - 30$ h training cap corresponded to an average of 317 postreinforcement pauses, and ranged from 261 to 350. One further bird discriminated between times within the first 50 postreinforcement pauses and only contributed 79 postreinforcement pauses to the subsequent analysis.

RESULTS

Concentration Treatment

In both conditions, birds selected the more concentrated option significantly more often than would be expected by chance (Cued condition: 0.65 ± 0.15 proportion of choices to the more highly rewarded flower; one sample, one-tailed Wilcoxon signed-ranks test: $W = 1.75$, $N = 5$, $P = 0.04$; Uncued condition: 0.63 ± 0.10 proportion of choices to the rewarded flower; one sample, one-tailed Wilcoxon signed-ranks test: $W = 2.06$, $N = 5$, $P = 0.02$).

As can be seen from Fig. 2, there appeared to be little to no effect of condition on birds' choices. To investigate whether this was in fact the case, we developed a model using Bayesian methods to compare learning in the two groups, Bayesian methods being more suitable than traditional hypothesis testing for situations in which the data seem to support the null hypothesis (Gallistel 2009). We investigated whether birds learned differently about floral concentrations in the presence of colour cues by constructing a generalized linear mixed model with a Bernoulli error structure and logit link function, modelling birds' choices of low- or high-reward flowers as a function of standardized visit number, trial type and condition order. We controlled for the effects of individual birds on baseline preference, learning rate and difference between conditions. We used Markov Chain Monte Carlo methods to develop the model in WinBUGS 1.4 (Lunn et al. 2000). The model
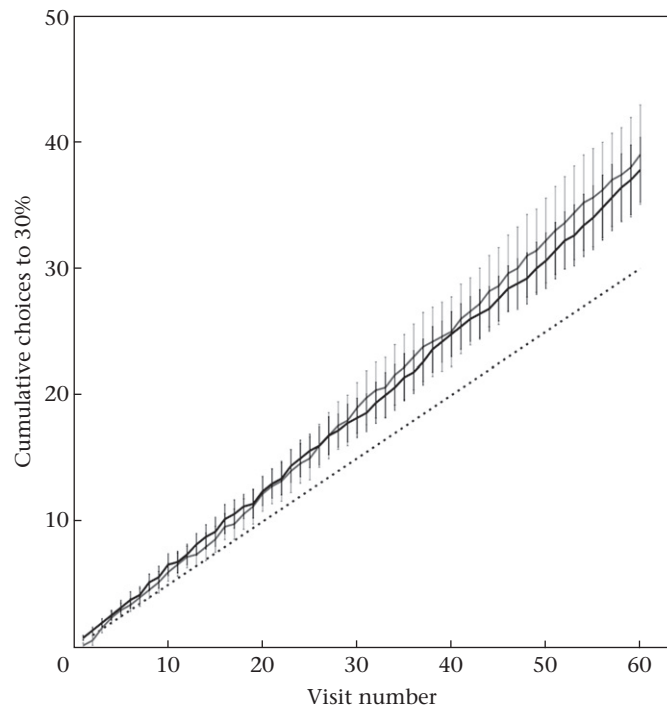
was allowed to run for 10 000 iterations with three independent chains for each parameter, with a suitable burn-in period to allow the three chains to converge. The chains were thinned to remove autocorrelations resulting in 1237 independent samples for each parameter. The distributions of these samples were then used to estimate parameters. This family of analysis is not based upon significance testing, but rather on parameter estimation; therefore we have provided median estimates and their 95% central credible intervals (CI), as is conventional for this type of analysis. A value of 0 indicates that a parameter had no effect on the data, whereas positive and negative values reflect positive and negative effects, respectively.

We carried out the analysis with and without the bird that had previously participated in the Refill Treatment. As there was no qualitative difference between the results for the two tests the data presented here include all five birds. There was no evidence of an effect of condition on the way in which a bird's preference for flowers containing 30% sucrose changed over time (median: 0.033; 95% CI: -0.394, 0.438) and no evidence of an effect of whether or not birds were in the Cued condition on the strength of their preference for the 30% flowers (median: 0.019; CI: -0.322, 0.375). There was also no effect of condition order on how preferences changed with experience (median: -0.010; CI: -0.498, 0.307), although there was some evidence that bird's preferences may have been slightly weaker on their second trial (median: -0.326; CI: -0.674, 0.008). Birds' preferences for flowers containing 30% sucrose appear to have increased slightly with visit number, although if so the effect was not strong (median: 0.156; CI: -0.236, 0.589).

Refill Treatment

Seven of the eight birds tested learned to revisit 20 min flowers significantly later than they visited the 10 min flowers (Table 1). For those birds that did learn according to this criterion, at the point of first making the discrimination, across birds, the mean return time to 10 min flowers was 11 ± 2 min and that to 20 min flowers was 16 ± 3 min. The difference between these is significant (Wilcoxon signed-ranks test, one-tailed: $W = 2.37$, $N = 7$, $P = 0.009$). The bird that failed to learn was a subject in the Uncued group (UC2; Table 1).

Figure 3 shows running average return times to each type of flower across training for the Cued and Uncued conditions. As with the Concentration Treatment, these data appear to suggest that condition had no effect on learning, so we developed a generalized linear mixed model with a normal error structure, modelling the log of birds' postreinforcement pauses as a function of flower type (10 or 20 min), condition (Cued or Uncued), standardized volume

Figure 2. Mean cumulative visits to flowers containing 30% sucrose in the Concentration Treatment. The grey line represents the Cued condition and the black line represents the Uncued condition. The dotted line gives chance performance. Error bars: $\pm$ SEM. $N = 5$ in each condition.

Table 1
Summary statistics for each bird in the Refill Treatment for the test block at which that bird first discriminated significantly between 10 and 20 min flowers or for the last block of training (for Bird UC2)

Bird	Test block number	Mean return time$\pm$SD		Mann–Whitney U	Z	P
		10 min flowers	20 min flowers			
C1	1	10 ± 5, $N=32$	16 ± 8, $N=18$	148.00	2.84	0.002
C2	7	11 ± 4, $N=16$	18 ± 7, $N=8$	18.00	2.87	0.001
C3	3	11 ± 4, $N=32$	15 ± 8, $N=18$	202.50	1.74	0.042
C4	6	13 ± 7, $N=29$	16 ± 5, $N=21$	173.00	2.59	0.004
UC1	1	8 ± 15, $N=32$	10 ± 4, $N=18$	189.50	2.00	0.023
UC2	7	11 ± 5, $N=23$	11 ± 6, $N=14$	160.00	0.32	0.491
UC3	3	10 ± 6, $N=31$	18 ± 10, $N=19$	149.50	2.91	0.002
UC4	6	14 ± 5, $N=33$	19 ± 11, $N=17$	192.00	1.82	0.034

C birds experienced the Cued condition; UC birds experienced the Uncued condition.
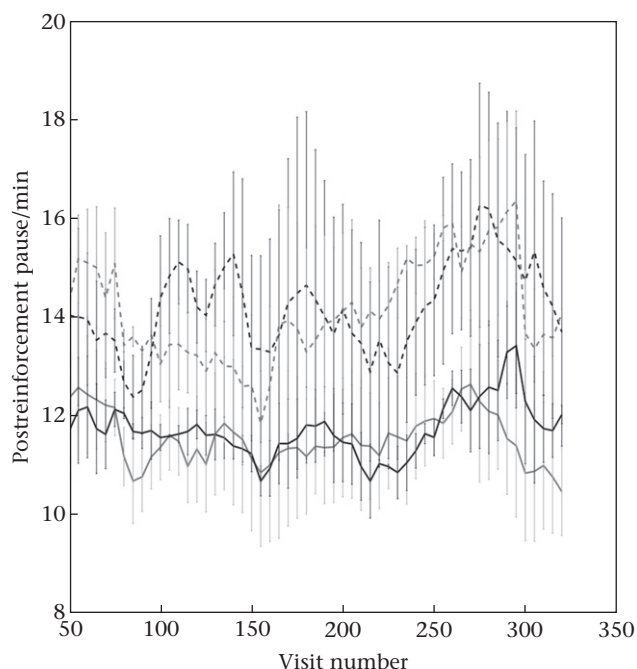
Figure 3. A plot of rolling average postreinforcement pauses for 10 and 20 min flowers across training for both groups in the Refill Treatment. Averages were calculated every 10 floral visits, across blocks of 50 visits. The grey lines denote the Cued condition and the black lines the Uncued condition. Dotted lines represent 20 min flowers and solid lines represent 10 min flowers. Error bars: ± SEM. $N = 4$ in each condition.

and standardized visit number, including random bird effects on visit rate and rate of learning. We used Markov Chain Monte Carlo methods to develop the model in WinBUGS 1.4. The model was allowed to run for 20 000 iterations across three chains, with a suitable burn-in period. The chains were thinned to remove autocorrelations to generate 975 independent samples for each parameter. The difference in number of iterations between this simulation and that for the concentration data reflected differences in the time taken for the chains to converge between the two models and differences in the level of thinning necessary to remove autocorrelations. Both, however, gave a large number of samples on which parameter estimates are based (1237 for the concentration model and 975 for the refill rate model).

The principal node of interest in this model was the difference between return times to 10 and 20 min flowers across training in the Cued condition compared with the Uncued condition. This was not detectably different from 0 (median: 5.35×10^{-4}; 95% CI: -0.004, 0.002), giving a strong indication that condition had no effect on how birds' discrimination between the two flower types changed with time. There was a small effect of volume, with higher volumes corresponding to a slight increase in visit rate to both 10 and 20 min flowers. This increase was, however, small (median: 0.097; CI: 0.069, 0.128), and indicates that this technique of varying flower volume had the desired effect of keeping birds' feeding rates near constant.

DISCUSSION

Over a very short training period (up to 30 h), seven of the eight birds tested learned to discriminate between flowers that refilled 10 min after they were last visited and flowers that refilled after 20 min. This confirms the findings of Henderson et al. (2006) that hummingbirds can indeed differentiate between intervals of this magnitude. Although birds here did not learn to the levels of

accuracy seen in Henderson et al.'s experiment, as they tended to return to 20 min flowers before they had refilled, the test period here was considerably shorter (a maximum of 3 days rather than 10). Some birds were also able to learn to discriminate between the flower types very quickly: one bird in each of the treatment groups had learned to discriminate between 10 and 20 min flowers within the first 50 floral visits.

Birds did not learn the flowers' refill rates or their contents more rapidly, however, if those flowers' conditions were cued by colour. This result is consistent with evidence that hummingbirds prefer spatial to colour cues: if given the option of revisiting a flower in a location that was previously rewarded or of a colour that was previously rewarded, birds tend to return to the correct location (Goldsmith & Goldsmith 1979; Miller et al. 1985; Hurly & Healy 1996). Similarly, if presented with an array of flowers in a novel part of their territory, having experienced an array of the same shape elsewhere, birds are more likely to visit flowers in the new array that occupy the same relative position as rewarded flowers in the previous array, as opposed to flowers of the same colour (Hurly & Healy 2002). Our results are, however, novel, in that they illustrate that, even where colour provides extra information to that provided by location, it appears that the birds do not attend to it. This is inconsistent with findings where colour appears to enhance learning of reward location. In both Miller & Miller (1971) and Healy & Hurly (1998), colour facilitated the learning of reward location by hummingbirds compared to a condition in which all of the available options were of identical colour. It may be that colour is used by birds under circumstances like those of Miller & Miller (1971) and Healy & Hurly (1998), where colour helps birds to learn locations by helping birds discriminate otherwise identical flowers from each other, but is not used to generate categories or types, which was the extra information colour provided here.

One explanation for the lack of an effect of colour on learning is that spatial information has overshadowed the cue provided by flower colour. Overshadowing can occur when two cues provide the same information but differ in their salience and it is characterized by a reduced attendance to one of the cue types coupled with increased reliance upon the other. This is a ubiquitous effect, found in animals from bees to humans (Couvillon & Bitterman 1980; Spetch 1995) and can occur in, among other contexts, spatial landmark learning (Spetch 1995) and associative learning with auditory, visual and taste cues (Mackintosh 1976; Macedo et al. 2008). Which kind of cue overshadows another, however, appears to depend on the species or at least on the context. For chickadees, *Poecile gambeli*, and rats, *Rattus norvegicus*, it appears that unlike hummingbirds, colour can overshadow spatial information (Gray et al. 2005; Pearce et al. 2006), while for bees, *Apis mellifera*, odour can overshadow colour (Couvillon & Bitterman 1980, 1982). Overshadowing relates to cue salience: generally a salient cue is thought to overshadow a less salient one (Mackintosh & Dickinson 1979), and saliencies are likely to differ between species and circumstances. As hummingbirds will prefer spatial cues to colour cues (Goldsmith & Goldsmith 1979; Miller et al. 1985; Hurly & Healy 1996), location overshadowing colour in this experiment would be consistent with their wider behaviour.

As well as being consistent with apparent cue saliencies, the overshadowing of colour by space in hummingbirds may also have an ecologically plausible explanation. Flowering plants such as those the birds feed on naturally vary considerably in their nectar production rates and thus in the volume of nectar available at a given flower. This is true both for plants within the same species (Pleasants 1983; Hodges 1993; Boose 1997; McDade & Weeks 2004) and for different flowers on the same plant (Boose 1997; McDade & Weeks 2004). This variation may be a strategy by the plant to decrease sequential visits to flowers on the same plant by foragers

(Biernaskie et al. 2002; Biernaskie & Cartar 2004). Thus, if flowers of the same colour and shape vary in their reward value, the overshadowing of colour by location may not be as inefficient as it might appear at first glance, as learning about the contents of each flower individually may sometimes be more reliable than generalizing across them.

The lack of effect of colour is unlikely to be caused by the birds finding the tasks too easy. In the Refill Treatment, it took the seven of eight birds that learned to discriminate between flower types an average of 189 floral visits to learn the intervals over which the two groups of flowers refilled. In the Concentration Treatment, birds' learning appears to have been far more rapid than this, although learning was no slower in the Uncued condition than it was in the Cued condition. Birds in the Cued condition were no more likely than birds in the Uncued condition to choose 30% flowers at the end of training.

These results are also unlikely to be caused by perceptual constraints. Psychophysical experiments have shown that black-chinned hummingbirds, *Archilochus alexandri*, can see across most of the visual light spectrum (tapering off towards the red end of the spectrum: Goldsmith et al. 1981), and ruby-throated, *Archilochus colubris* (Miller & Miller 1971) and rufous hummingbirds (Healy & Hurly 1998) can, on occasion, learn more rapidly when supplied with useful colour cues, and they can learn about presence of reward (Hurly & Healy 1996; Lara et al. 2009), reward magnitude (Bateson et al. 2002, 2003; Bacon et al. 2010; Morgan et al. 2011) and variability (Hurly & Oseen 1999) using colours similar to those we used.

In this experiment, our flowers presented a very simplified version of those found in nature, varying only in one modality (colour) whereas the natural flowers a pollinator encounters are likely also to vary in other ways, such as shape and odour. The use of these different sources of information may well be complex (Leonard et al. 2011), which may aid learning as bees learn discriminations more quickly when stimuli vary in more than one dimension (Kulahci et al. 2008), while hawkmoths, *Manduca sexta*, use odour to locate a flower's general location but visual cues to identify where to feed (Balkenius & Dacke 2010). It may be that hummingbirds tend to make use of a different cue, for example scent, to categorize flowers in nature, or categorizing by colour is potentiated by another feature not varied here. However, despite the relative simplicity of our flowers, this experiment still provides a compelling test of these birds' use of colour, as in similar circumstances where colour is the only floral cue and space is irrelevant, birds are able to learn colour rules.

Drawing conclusions from these results to hummingbird foraging in nature is not straightforward. While, in the limited circumstances presented here, hummingbirds appear not to generalize on the basis of a type or species cue such as colour, in a more naturalistic setting, where birds have to forage from many more flowers over a wider area to meet their energy demands it is conceivable that space may become less important and that rules about type, based on traits such as colour, may assume more importance. Within this investigation however, whether hummingbirds do not make use of colour because of overshadowing, floral variability or some other cause, this experiment highlights a case where an animal does not make use of what would appear to us to be one of a resource's most obvious attributes, according to how we see the world. This apparent lack of use of colour is not due to an inability of birds to learn the type of categorical rules under investigation but rather seems to be because they do not always attend to some features of that resource. This is an important methodological point regarding testing of hummingbirds and research with animals more generally. For example, the colour of flowers in the Refill Treatment could represent the 'what' dimension of a 'what, where, when' test of episodic-like memory in

hummingbirds. Failure by a rufous hummingbird in such an experiment to learn about colour and refill rate might then be interpreted as a failure in birds to exhibit episodic-like memory, whereas our results suggest that such an outcome would be caused by the birds attending only to the relevant cues (where the birds determine relevance, not the human experimenter). In general, then, we need to be careful to ensure that we test our assumptions about the features of a resource to which an animal attends before we draw conclusions about their cognitive abilities.

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Appendix

Table A1
Table of flower colour combinations used in the Concentration Treatment

Bird	Cued		Uncued	
	20%	30%	20%	30%
1	Light blue	Yellow	**Green, purple**	**Pink, navy**
2*	**Pink**	**Navy**	Green, yellow	Light blue, purple
3	**Green**	**Pink**	Light blue, yellow	Navy, purple
4	**Light blue**	**Purple**	Yellow, navy	Green, pink
5	Green	Navy	**Pink, purple**	**Yellow, blue**

Bold text denotes the condition each bird received first.

* This bird had participated in the Refill Treatment the previous year.

Table A2
Table of flower colour combinations used in the Refill Treatment

Bird	10 min	20 min
UC1*	Blue, purple	Pink, yellow
UC2	Purple, yellow	Blue, pink
UC3	Blue, pink	Purple, yellow
UC4	Purple, blue	Pink, yellow
C1	Purple	Yellow
C2	Yellow	Purple
C3	Purple	Yellow
C4	Yellow	Purple

UC birds experienced the Uncued condition; C birds experienced the Cued condition.

* This bird participated in the Concentration Treatment the following year.
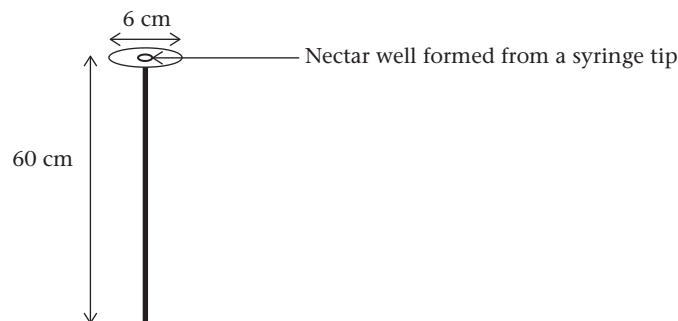
Figure A1. Diagram of the 'flowers' used in the Refill and Concentration Treatments.