Sunbirds increase foraging success by using color as a cue for nectar quality

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Short title: Color cues increase sunbird foraging success

Nectar concentration and composition varies widely between plant species. Nectarivorous birds that associate floral characteristics with nectar quality may be able to avoid less rewarding flowers and therefore forage more efficiently. We assessed the abilities of amethyst sunbirds (*Chalcomitra amethystina*) to utilize color cues to discriminate between concentrated (1 M) and dilute (0.25 M) sucrose solutions. In an outdoor aviary, birds were presented with three rich feeders among six poor feeders on a feeding board. Following the assessment of baseline performance with optically identical feeders, color cues were added to the feeders for a 6 h training period. To assess the retention of learnt visual cues, birds were tested 1 and 7 days following the training. Observations lasted for 3 h, with feeders being re-arranged every 30 min to minimize spatial learning. In the absence of color cues, birds selected feeders randomly, but when color cues were available visited more rich than poor feeders. This more rapid identification of rich feeders resulted in a decrease in feeding duration and feeding frequency, compared to the baseline performance. Energy uptake from rich feeders, and therefore the rate of energy gain, increased when birds foraged with color cues. No differences were found between days 1 and 7 after training. Total energy intake decreased with visual cues, which may indicate a reduction in foraging costs when cues allow for location of rewarding feeders. Our findings demonstrate that sunbirds forage more efficiently with cues, which may lead to increased fitness.

Keywords: nectar quality, amethyst sunbirds, *Chalcomitra amethystina*, associative learning, color cues, foraging efficiency, selectivity

INTRODUCTION

Food resources are often patchily distributed in the natural environment, and in addition vary over time. It would be an advantage for foragers to identify profitable food patches, retain the spatial information and then re-visit multiple patches to meet their nutritional requirements. Foraging in a dynamic environment requires a balance between exploitation of known resources and exploratory sampling of new resources (Dall and Johnstone 2002). In addition to spatial memory, learnt associations between cues and a food reward can increase the rate of encountering food patches, thus facilitating the exploitation of spatial heterogeneity in food resources (Edwards et al. 1996). Examples of increased foraging efficiency brought about by learning are widespread in the animal kingdom, ranging from insects (Johnson 1991; Durier and Rivault 2000) to vertebrates (Hurly and Healy 1996; Warburton 2003). Animals learn to identify food sources using individual cues or a combination of cues; these include visual, olfactory, auditory, tactile and gustatory cues (Montgomerie and Weatherhead 1997; Hill et al. 2001; Croney et al. 2003; Goyret and Raguso 2006; Ishii and Shimada 2010; Arenas and Farina 2012). The use of cues other than spatial cues may be particularly important for foragers in an environment with temporal and spatial variation in food availability.

Pollinators forage on patchily distributed plants, and nectar availability varies over time, depending on flowering times of individual plant species available in the environment. As well as varying between species, nectar reward varies between flowers on the same plant, depending on flower age (Nicolson and Nepi 2005) and with the time of day and environmental conditions (Corbet et al. 1979). Nectar volumes may also become depleted, and pollinators have been shown to memorize the location of recently sampled flowers (Lehrer 1994; Hurly 1996; Winter and Stich 2005). It is essential for nectarivores to locate and remember profitable flowers, and they use spatial and visual cues, amongst others, to direct their foraging (e.g. Irwin 2000; Toelch et al. 2008; Hsu and Yang 2012).

Bird-pollinated flowers commonly advertise their nectar reward with brightly colored, typically red, flowers (Rodríguez-Gironés and Santamaría 2004). Recently, it has been shown that angiosperm flowers have evolved chromatic cues that suit the color discrimination abilities of their respective bird or insect pollinators, most likely to reinforce visits by efficient pollinators (Shrestha et al. 2013). Despite their innate preference for red flowers, hummingbirds can be trained to select other floral colors if they are more rewarding (Meléndez-Ackerman et al. 1997). Similarly, insect pollinators can learn to associate different color cues with a nectar reward, irrespective of their initial color preferences (Weiss 1997; Raine and Chittka 2008).

The foraging behavior of free-living rufous hummingbirds (*Selasphorus rufus*) has been studied extensively, as their territorial behavior allows for individual birds to be trained and tested using artificially manipulated feeders in the field (see Healy and Hurly 2013 for a review). These hummingbirds retain information on the location of high-quality nectar feeders, learn nectar refill rates, and also learn and retain color cues (Hurly and Healy 1996; Hurly and Healy 2002; Henderson et al. 2006). Hummingbirds predominantly use spatial information when returning to flowers (Hurly and Healy 1996; Marshall et al. 2012), possibly because floral color may not be a reliable cue for a nectar reward during repetitive sampling. However, color cues are likely to be important during foraging in unfamiliar places and exploratory sampling of new flowers. Sandlin (2000) demonstrated that three coexisting species of hummingbirds foraging with color cues identified and almost exclusively selected high quality nectar sources, thereby ignoring poor quality feeders. Apart from hummingbirds, other avian nectarivores, both specialist and generalist, have been shown to learn spatial and color cues to identify rewarding flowers or feeders, such as bananaquits (Wunderle and Martinez 1987) and rainbow lorikeets (Sulikowski and Burke 2011). Honeyeaters (*Acanthorhynchus tenuirostris*) have been shown to use the bright yellow pollen of mountain correa flowers to find large nectar rewards (Scoble and Clarke 2006). Furthermore, the colored nectar of *Aloe vryheidensis* has a signaling function: it attracts short-billed facultative avian nectarivores (*Pycnonotus tricolor*) that are effective pollinators of these flowers, while long-billed sunbirds (*Chalcomitra amethystina*) are repelled by this phenolic-containing nectar (Johnson et al. 2006).

Studies on the foraging behavior of free-living sunbirds are scarce. Unlike territorial hummingbirds, the nomadic lifestyle of sunbirds does not facilitate the study of cognitive abilities in the wild. Irregular visits of individuals to a particular feeding site, as well as multiple individuals visiting the same feeders, would complicate training and repeated testing in the field. We therefore assessed the color learning and cue use ability of captive amethyst sunbirds (*Chalcomitra amethystina*) in a semi-natural aviary setting. We predicted that the sunbirds would learn to associate colors on feeders with the nectar concentration they contain, and would subsequently select more high-reward feeders. The novel contribution of our study is to test whether the higher selectivity for rewarding feeders translates to a higher foraging efficiency. To assess foraging efficiency, we recorded energy intake, feeding duration and feeding frequency of the sunbirds foraging with and without cues. We hypothesized that birds would forage more efficiently once they had learned to associate certain color cues with a high reward. In addition, few studies have determined longer-term retention of cue information and we therefore tested the birds again seven days after learning, with the expectation that the memory for color cues would be retained and utilized to improve foraging success.

METHODS

Sunbird maintenance

Four male and four female non-reproductive amethyst sunbirds (*C. amethystina*) were mist-netted in Jan Cilliers Park and the National Botanical Gardens in Pretoria in June 2011 (South African autumn). Birds were weighed and ringed at time of capture, and were housed together in an outdoor aviary (12 x 6 x 2.5 m) at the University of Pretoria Experimental Farm. During the first 14 days, birds became accustomed to captivity and artificial feeders, which consisted of inverted, stoppered 25 ml syringes. Their maintenance diet was a 0.63 M sucrose solution with a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa) to provide dietary nitrogen. Water was available from water feeders and water baths. The aviary consisted of four inter-leading compartments, with natural branches for perching, allowing for individual birds to be separated from the group during the experiment. All birds were released at the site of capture in October 2011 following completion of the study.

Experimental design

To assess whether learnt color cues increase the foraging efficiency of sunbirds, we built a feeding board with nine feeders (25 ml inverted syringes) mounted in an oval

(Fig. 1). Feeders were 20 cm apart and each had its own wooden perch for the bird to sit on during feeding. The bottom of the feeding board was 1 m above the ground so that predator avoidance would not prevent the birds from visiting the bottom feeders (Sandlin 2000). A container with liquid paraffin was attached to the board below each feeder to catch any spilt sucrose solution and prevent its evaporation. Sunbirds show a preference for more concentrated sucrose solutions up to 1 M (Leseigneur and Nicolson 2009), enabling them to gain more energy per time feeding, compared to dilute sucrose solutions. On our feeding board, we placed three feeders with 1 M sucrose-only solution (30% w/w, rich feeders) among six feeders with a dilute sucrose-only solution of 0.25 M (8.5% w/w, poor feeders). A rich:poor feeder ratio of 1:3 was chosen to allow us to better discriminate learned performance from chance performance. Water was available to the birds at all times to ensure that they did not drink from poor feeders to avoid dehydration. The feeding board was introduced during the 14-day acclimation period and was readily accepted by the birds.

The sunbirds were tested individually in the following sequence: A) assessment of ***baseline performance*** prior to cue training; followed by ***training*** with visual cues; and then testing for the ***retention of visual cues*** B) 1 day and C) 7 days after training. The total observation period for each part of the experiment (A–C) consisted of six 30-min replicates per bird, *i.e.* 3 h of observation. Feeders were randomly arranged on the feeding board and re-positioned at the start of each replicate to minimize spatial learning and side biases (Jackson et al. 1998). After preliminary observations, we chose 30-min periods as a compromise between minimizing disturbance and avoiding spatial learning by the birds. We recorded the number of visits to each feeder as well as feeding duration (using a stopwatch). Multiple short feeding events were merged into one event when a bird remained perched at a given feeder position and fed intermittently at this feeder. Feeders and paraffin containers were weighed before and after each 30 min replicate to determine food intake (corrected for spillage), then re-positioned, resulting in 10 min breaks between replicates. The observer had to walk into the aviary compartment to change the feeders, but birds were observed to return to the feeding board rapidly after the observer left the compartment.

Following the assessment of baseline performance without visual cues, color cues in the shape of a flower were painted around the feeding holes of the syringes using non-metallic, colored nail polish in yellow, orange, blue or purple (Fig. 1). Randomly chosen colors represented either rich or poor feeders, and each of the eight birds was presented with a different color pair. Each bird was allowed a 6 h training period with its own color pair, with feeders being re-positioned hourly to minimize the effect of spatial learning. On the following day, feeder visits and intake were recorded during six 30-min replicates as described above. Birds were presented with the same colors seven days after the training period to test for longer-term retention of color cues. To ensure that the birds fed in each 30 min replicate, observations began at 09h30 as sunbirds feed more frequently in the morning than in the afternoon (Köhler et al. 2006). After observations were complete, the bird was released back into the group and returned to the maintenance diet.

Data handling

For each replicate and each bird, we recorded whether a rich or a poor feeder was visited first, and calculated the proportion of ***correct first visits to rich feeders***, *i.e.* first visits to rich feeders in two out of six replicates equals 33.3% correct first visits to rich feeders. If color cues allow the birds to better locate a rich feeder on the first attempt, then the proportion of correct first visits to rich feeders should be higher with cues compared to the baseline performance without cues. In addition, we recorded the total number of visits to rich and poor feeders within each 30 min replicate, and calculated overall ***rich feeder selectivity*** as the number of correct visits to rich feeders as a percentage of total visits.

The change in feeder masses yielded consumption (g) at rich and poor feeders, and total consumption of sucrose solution. From this, we calculated sucrose consumption (g) using the densities of 0.25 M and 1.0 M sucrose solutions and ***energy intake (kJ) from rich and poor feeders*** assuming 17 kJ/g sucrose. To estimate the birds' investment in foraging, we determined ***feeding duration*** (s / 30 min) and ***feeding frequency*** (number of feeding events / 30 min). We then calculated the ***rate of energy gain (J/s)*** by dividing total energy intake by feeding duration; this was further divided by the number of feeding events to give the ***rate of energy gain per meal***.

Statistical procedure

Proportions of correct first visits to rich feeders and rich feeder selectivity over multiple visits were tested against chance (33.3%) using one-sample signed rank tests. Selectivity and consumption data were arcsin or log transformed when not normally distributed or homogenous. Repeated measures (RM) two-way ANOVAs were performed to compare foraging parameters between baseline foraging (without cues) and foraging with cues (1 and 7 d after training). Both experimental series (A–C) and 30 min replicates (N=6) were included as factors. Feeding data are shown as means for each replicate in the supplementary Table S1. When no difference between replicates was found, we present the results of RM one-way ANOVAs on the averages of the six replicates. The ANOVAs were followed by Holm-Sidak *post hoc* tests. Ambient temperature affects the energy intake of sunbirds (Köhler et al. 2010). Therefore, ambient temperature data for each test day were obtained from the nearest weather station on Weather Underground (www.wunderground.com) and were included as a covariate in the Generalized Linear Mixed Model used to compare total energy intake between the treatments. Statistical analysis was done in R 2.14.0 and SigmaStat integrated with SigmaPlot 12.5. Significance level was $P \leq 0.05$; all data are presented as means and SE.

RESULTS

Sunbirds select more rewarding feeders when color cues are present

In the absence of color cues, sunbirds started foraging randomly. Three out of nine feeders on the feeding board contained a concentrated sucrose solution of 1 M, and the proportion of ***correct first visits to rich feeders*** was 27% when all feeders looked identical. This first choice did not differ significantly from random selection (33.3%; one-sample signed rank test: $Z_7 = -0.43$, $P = 0.74$; Fig. 2A). Following the training to color cues representing rich and poor feeders, the birds more often located a rich feeder on the first attempt: on the day after training, the proportion of correct first visits to rich feeders was 63% (almost twice as high as expected from random selection; $Z_7 = 2.59$, $P < 0.01$), and remained high seven days after training (75%; $Z_7 = 2.56$, $P < 0.01$). Comparing the different experimental series, birds located more rich feeders for the first feeding bout when foraging with color cues, than during baseline foraging (RM one-way ANOVA: $F_{2,23} = 21.20$, $P < 0.001$; Holm-Sidak: $P < 0.001$), and first visits to rich feeders did not differ significantly between day 1 and 7 after training ($P = 0.12$; Fig. 2A).

Looking at all feeder visits, overall ***rich feeder selectivity*** tended to be higher than chance in the absence of color cues ($Z_7 = 1.96$, $P = 0.06$; Fig. 2B), indicating that the birds returned to rich feeders once they had located them. Rich feeder selectivity was significantly higher than chance when birds foraged with color cues ($Z_7 \geq 2.52$, $P < 0.01$). Similar to first visits, rich feeder selectivity was higher with color cues than without ($F_{2,23} = 33.92$, $P < 0.001$; Holm-Sidak: $P < 0.001$), and did not differ between the first and the seventh day after training ($P = 0.56$; Fig. 2B). When feeding without cues, birds gained 69.0 ± 4.5% of their total energy intake from rich feeders. With color cues, energy obtained from rich feeders increased to 89.5 ± 2.0% (Day 1) and 82.7 ± 2.7% (Day 7), being significantly higher than the baseline value ($F_{2,23} = 14.66$, $P < 0.001$; Holm-Sidak: $P \leq 0.01$).

Foraging effort decreases when color cues are present

Feeding frequency differed between treatments ($F_{2,23} = 5.08$, $P = 0.02$; Fig. 3A), with birds feeding more often without color cues than when cues were present ($P \leq 0.04$). The number of feeding events was similar on Day 1 and Day 7 after training ($P = 0.72$). ***Feeding duration*** was also affected by the presence of color cues (RM two-way ANOVA: $F_{2,143} = 3.99$, $P = 0.04$; Fig. 3B). Without cues, birds spent more time feeding than with cues 7 days after training ($P = 0.04$). Feeding duration on the day after training was intermediate and did not differ significantly from Day 7 and from feeding without cues ($P \geq 0.16$). A significant difference in feeding duration was also found between replicates ($F_{5,143}$ = 6.73, $P < 0.001$), with birds feeding for longer in the first replicate than in other replicates ($P < 0.01$; Table S1).

Sunbirds forage more efficiently using color cues

Rate of energy gain did not differ significantly between the presence and absence of color cues (RM one-way ANOVA: $F_{2,23}$ = 0.45, P = 0.65), although on average it was lower without cues (86.7 ± 8.5 J/s) than with them (Day 1: 98.6 ± 11.6 J/s; Day 7: 100.1 ± 9.1 J/s). However, due to differences in feeding frequency, ***rate of energy gain per meal*** increased significantly in the presence of color cues ($F_{2,23}$ = 6.16, P = 0.01; Holm-Sidak: $P < 0.03$; Fig. 4), with no difference between Day 1 and 7 with cues (P = 0.67).

Total energy intake differed between experimental series ($F_{2,143}$ = 6.14, P = 0.01), being higher during the baseline foraging (5.9 ± 1.7 kJ/30 min) than with cues on Day 7 after training (4.3 ± 1.6 kJ/30 min; P = 0.01). Energy intake on the day after training was intermediate (5.3 ± 2.1 kJ/30 min), and did not differ significantly from the others ($P \geq 0.11$). There was a significant difference between replicates (RM two-way ANOVA: $F_{5,143}$ = 3.95, $P < 0.01$). Energy intake was higher in the first than in other replicates ($P < 0.05$). The interaction between experimental series and replicate was only marginally significant ($F_{10,143}$ = 1.94, P = 0.05), and *post hoc* analysis revealed significant differences between the first replicate and others when birds were foraging with cues on the day after training ($P < 0.04$; Table S1). Ambient temperature did not have an effect on energy intake (GLMM: χ^2_2 = 0.98, P = 0.30). The decrease in total energy intake in the presence of visual cues may therefore indicate that sunbirds spend less energy foraging when color aids allow them to locate rewarding feeders.

DISCUSSION

The presence of visual cues associated with nectar sources has a marked effect on the foraging behavior of sunbirds. Following training to color cues, amethyst sunbirds selected more rich than poor feeders at the first attempt and throughout the feeding period, thus decreasing the total time spent feeding and the number of feeder visits, and increasing their rate of energy gain per meal. Our findings of non-random feeding under semi-natural aviary conditions are in agreement with a study on foraging behavior of four sunbird species under natural conditions (Gill and Wolf 1977): birds increased their foraging efficiency by utilizing the initial flowers probed as a predictor for the remaining flowers on the inflorescence, and by preferentially feeding at unvisited inflorescences.

Many animals, including nectarivores, can form associations between colors and a reward (e.g. Vallortigara 1996; Kelber 2005; Raine and Chittka 2008). Hummingbirds have been shown in multiple studies to learn rapidly to associate color cues with the presence or absence of a reward (Goldsmith and Goldsmith 1979; Meléndez-Ackerman et al. 1997; Hurly and Healy 2002). Rufous hummingbirds can associate different colors with either nectar volume or concentration (Bateson et al. 2003; Bacon et al. 2010), and color cues enhance their ability to return to focal flowers in the presence of distracting flowers (Hurly and Healy 1996). Besides color cue use, spatial learning may allow birds to visit more flowers per unit foraging time, and may thus increase foraging efficiency. Hummingbirds have been shown to preferentially use spatial memory to return to rewarding nectar sources, while color did not increase their learning capabilities for nectar concentrations and flower refill rates (Marshall et al. 2012). Nectarivorous bats

(*Glossophaga commissarisi*) similarly relied on spatial memory to return to rewarding feeders, but they also utilized object cues (Thiele and Winter 2005). In a natural foraging environment, it is probable that nectarivores employ spatial memory to return to rewarding food sources, but may use object or visual cues to aid in finer-scale spatial discrimination and to discover novel nectar sources.

Sunbirds select more rewarding feeders when color cues are present

During the search for new nectar sources, nectarivores may not be able to predict the quality of a nectar reward, and therefore sample flowers randomly, as they did for first feeder visits in the baseline phase of our experiment. During their first visit to the feeding board, when all feeders looked identical, sunbirds correctly approached rich feeders in 27% of cases: this success rate did not differ from random foraging (three rich among six poor feeders, *i.e.* 33.3%). However, after training to color cues, birds were able to identify rich feeders on the first attempt, and 63–75% of first visits were to rich feeders. This confirms that these sunbirds, like other nectarivores, are capable of learning to associate color cues with nectar concentration, independent of spatial cues.

Looking at overall feeder visits, birds tended to visit rich feeders more often (38%) than predicted from random selection, indicating that they memorized the position of rich feeders and returned to them in subsequent feeding flights. Switching feeder positions every 30 min was therefore not sufficient to avoid spatial learning, but disturbing the birds more often was not desirable. Spatial memory may be an invaluable tool in natural foraging situations, where birds travel larger distances between plants, and nectar sources are renewable and re-visitation is therefore profitable (Castellanos et al. 2002).

The higher number of visits to rich feeders in the presence of color cues (73–76% of total visits) demonstrates that color cues are used in addition to spatial memory and facilitate the return of sunbirds to rich feeders. Our results for rich feeder selectivity are comparable to the selectivity of >70% found by Scoble and Clarke (2006) in eastern spinebills in a natural environment. However, exclusive use of rich feeders, as observed by Sandlin (2000), was uncommon. This could reflect the low costs of exploratory sampling behavior in a situation where nectar sources are in close proximity, as well as the absence of any negative effects incurred when feeding on poor feeders (Hurly and Healy 1996).

Because we used the same color for six out of nine feeders, it can be argued that the sunbirds only needed to avoid the common color (or approach the rare color) to gain high concentration rewards. A better design would perhaps have included three different colors, one for rich feeders and two others for poor feeders. However, our results were consistent even though individual birds received different color pairs. Moreover, sunbirds forage on many plant species over a wide area; many of these plants have red or orange flowers, such as *Aloe* and *Erythrina* species (Johnson and Nicolson 2008), and avoidance of these common colors would not make ecological sense.

Foraging effort decreases when color cues are present

Sunbirds encountering variable nectar concentrations adjust their feeding patterns rapidly to maintain energy balance (Köhler et al. 2008). This compensatory feeding response means that, in order to defend a constant energy intake, birds must increase their feeding frequency and feeding duration when ingesting dilute nectar. In our experiment, sunbirds foraging without cues were likely to sample from more poor than rich feeders, compared to birds with cues, and consequently visited feeders more often and spent more time feeding. When foraging with color cues, on the other hand, we found a decrease in feeding frequency and total feeding duration. Birds that are not equipped with the knowledge of where their next high quality meal is located must spend more time searching and sampling, and therefore incur higher search costs (Sandlin 2000). Nectarivorous birds have high energy needs and use recently ingested sugar to fuel flight (Welch et al. 2006). To remain in positive energy balance, the birds are therefore likely to increase their energy intake rapidly when foraging costs are higher. The higher total energy intake of our sunbirds during foraging without color cues, compared to foraging when color cues were present on Day 7 after training, indicates that they spent more energy visiting more feeders, despite being close to the feeding board. This is further supported by our findings that feeding duration and total energy intake decreased during the three hours of observation. Birds consumed less energy in later 30 min replicates than in the first replicate when foraging with color cues on the day after training, but not when foraging without visual cues.

Sunbirds forage more efficiently using color cues

Easier identification of rich feeders with color cues, and therefore lower foraging effort, resulted in a higher rate of energy gain per meal. We argue that by combining the energy gained through foraging (which was measured directly), and the energy spent foraging (which was estimated using feeding frequency and duration), we could obtain a general idea of how the ability to predict resource quality through cue usage affected the overall foraging success of sunbirds. The improved foraging performance not only conserves energy through a decrease in flight costs, but may also allow more time to be allocated to other activities, such as resting, grooming and territory defense.

After learning, it is not surprising that sunbirds retained the association between color cues and reward value over the studied period of one week. Correct first visits to rich feeders, overall feeder selectivity and rate of energy gain did not differ between Day 1 and 7 post training, despite the absence of color cues on the feeders for the six day period between the two tests. Nectar sources are often clumped in patches of different species assemblages, and a nomadic foraging sunbird revisits certain areas at seasonally prosperous times when nectar is abundant. It is therefore possible that a sunbird's foraging success would be increased if it were able to firstly recall the area's location, and subsequently recall the color cues associated with its particular plant species assemblage. On the other hand, our birds may have simply re-learnt the color cues that they had been exposed to the previous week, as Goldsmith and Goldsmith (1979) demonstrated that hummingbirds could learn to associate color with nectar reward within six to 22 visits. However, there was no trend for selectivity to start low and increase with each 30-minute replicate, which would be expected if re-learning was playing an important role.

Conclusion

This study demonstrates that a nectarivorous passerine, the amethyst sunbird, is able to utilize color cues through associative learning to improve overall foraging success and decrease the costs associated with random foraging. The learning and recalling of visual cue information was demonstrated in an aviary experiment in which spatial cues were minimized by frequent switching of feeder positions. It remains to be tested whether the use of visual cues by these birds is equally important under natural foraging conditions. Increased foraging efficiency following cue use will more likely result in a positive daily energy budget, enabling an animal to undertake energetically costly activities such as territorial defense, predator avoidance, seasonal or migratory movements, and reproduction, all of which increase individual fitness. By discriminating between food resources differing in color, morphology, food quality, and spatial and temporal distribution, sunbirds are equipped to face the challenges of a dynamic foraging environment.

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REFERENCES

Arenas A, Farina WM. 2012. Learned olfactory cues affect pollen-foraging preferences in honeybees, *Apis mellifera*. Anim Behav. 83:1023-1033.

Bacon IE, Hurly TA, Healy S. 2010. Both the past and the present affect risk-sensitive decisions of foraging rufous hummingbirds. Behav Ecol. 21:626-632.

Bateson M, Healy S, Hurly TA. 2003. Context-dependent foraging decisions in rufous hummingbirds. Proc Roy Soc B. 270:1271-1276.

Castellanos MC, Wilson P, Thomson JD. 2002. Dynamic nectar replenishment in flowers of *Penstemon* (Scrophulariaceae). Am J Bot. 89:111-118.

Corbet SA, Unwin DM, Prys-Jones OE. 1979. Humidity, nectar and insect visits to flowers, with special reference to *Crataegus*, *Tilia* and *Echium*. Ecol Entomol. 4:9-22.

Croney CC, Adams KM, Washington CG, Stricklin WR. 2003. A note on visual, olfactory and spatial cue use in foraging behavior of pigs: indirectly assessing cognitive abilities. Appl Anim Behav Scie. 83:303-308.

Dall S, Johnstone R. 2002. Managing uncertainty: information and insurance under the risk of starvation. Phil Trans Roy Soc Lond B. 357:1519-1526.

Durier V, Rivault C. 2000. Learning and foraging efficiency in German cockroaches *Blatella germanica* (L.) (Insecta: Dictyoptera). Anim Cogn. 3:139-145.

Edwards GR, Newman JA, Parsons AJ, Krebs JR. 1996. The use of spatial memory by grazing animals to locate food patches in spatially heterogeneous environments: an example with sheep. Appl Anim Behav Scie. 50:147-160.

Gill F, Wolf L. 1977. Nonrandom foraging by sunbirds in a patchy environment. Ecology. 58:1284-1296.

Goldsmith T, Goldsmith K. 1979. Discrimination of colors by the black-chinned hummingbird, *Archilochus alexandri*. J Comp Physiol. 130:209-220.

Goyret J, Raguso RA. 2006. The role of mechanosensory input in flower handling efficiency and learning by *Manduca sexta*. J Exp Biol. 209:1585-1593.

Healy SD, Hurly TA. 2013. What hummingbirds can tell us about cognition in the wild. Comp Cogn Behav Rev. 8:13-28.

Henderson J, Hurly TA, Bateson M, Healy SD. 2006. Timing in free-living rufous hummingbirds *Selasphorus rufus*. Curr Biol. 16:512-515.

Hill PSM, Hollis J, Wells H. 2001. Foraging decisions in nectarivores: unexpected interactions between flower constancy and energetic rewards. Anim Behav. 62:729-737.

Hsu P-S, Yang E-C. 2012. The critical cue in pattern discrimination for the honey bee: Color or form? J Insect Physiol. 58:934-940.

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

Hurly TA, Healy SD. 1996. Memory for flowers in rufous hummingbirds: location or local visual cues? Anim Behav. 51:1149-1157.

Hurly TA, Healy SD. 2002. Cue learning by rufous hummingbirds (*Selasphorus rufus*). J Exp Psych - Anim Behav Proc. 28:209-223.

Irwin RE. 2000. Hummingbird avoidance of nectar-robbed plants: spatial location or visual cues. Oikos. 91:499-506.

Ishii Y, Shimada M. 2010. The effect of learning and search images on predator–prey interactions. Popul Ecol. 52:27-35.

Jackson S, Nicolson SW, Lotz C. 1998. Sugar preferences and “side bias” in cape sugarbirds and lesser double-collared sunbirds. Auk. 115:156-165.

Johnson R. 1991. Learning, memory, and foraging efficiency in two species of desert seed-harvester ants. Ecology. 72:1408-1419.

Johnson SD, Nicolson SW. 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. Biol Lett. 4:49-52.

Johnson SD, Hargreaves AL, Brown M. 2006. Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. Ecology. 87:2709-2716.

Kelber A. 2005. Alternative use of chromatic and achromatic cues in a hawkmoth. Proc Roy Soc B. 272:2143-2147.

Köhler A, Verburgt L, Nicolson SW. 2006. Short-term energy regulation of whitebellied sunbirds (*Nectarinia talatala*): effects of food concentration on feeding frequency and duration. J Exp Biol. 209:2880-2887.

Köhler A, Verburgt L, Fleming PA, Nicolson SW. 2008. Changes in nectar concentration: how quickly do whitebellied sunbirds (*Cinnyris talatala*) adjust feeding patterns and food intake? J Comp Physiol B. 178:785-793.

Köhler A, Verburgt L, McWhorter TJ, Nicolson SW. 2010. Energy management on a nectar diet: can sunbirds meet the challenges of low temperature and dilute food? Funct Ecol. 24:1241-1251.

Lehrer M. 1994. Spatial vision in the honeybee: the use of different cues in different tasks. Vision Res. 34:2363-2385.

Leseigneur C, Nicolson SW. 2009. Nectar concentration preferences and sugar intake in the white-bellied sunbird, *Cinnyris talatala* (Nectariniidae). J Comp Physiol B. 179:673-679.

Marshall RES, Hurly A, Healy S. 2012. Do a flower's features help hummingbirds to learn its contents and refill rate? Anim Behav. 83:1163-1169.

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

Montgomerie R, Weatherhead PJ. 1997. How robins find worms. Anim Behav. 54:143-151.

Nicolson SW, Nepi M. 2005. Dilute nectar in dry atmospheres: Nectar secretion patterns in *Aloe castanea* (Asphodelaceae). Int J Plant Scie. 166:227–233.

Raine NE, Chittka L. 2008. The correlation of learning speed and natural foraging success in bumble-bees. Proc Roy Soc B. 275:803-808.

Rodríguez-Gironés M, Santamaría L. 2004. Why are so many bird flowers red? PLoS Biol. 2:1515-1519.

Sandlin E. 2000. Cue use affects resource subdivision among three coexisting hummingbird species. Behav Ecol. 11:550-559.

Scoble J, Clarke M. 2006. Nectar availability and flower choice by eastern spinebills foraging on mountain correa. Anim Behav. 72:1387-1394.

Shrestha M, Dyer AG, Boyd-Gerny S, Wong BBM, Burd M. 2013. Shades of red: bird-pollinated flowers target the specific color discrimination abilities of avian vision. New Phytol. 198:301-310.

Sulikowski D, Burke D. 2011. Win-shift and win-stay learning in the rainbow lorikeet (*Trichoglossus haematodus*). J Comp Psychol. 125:143-149.

Thiele J, Winter Y. 2005. Hierarchical strategy for relocating food targets in flower bats: spatial memory versus cue-directed search. Anim Behav. 69:315-327.

Toelch U, Stich KP, Gass CL, Winter Y. 2008. Effect of local spatial cues in small-scale orientation of flower bats. Anim Behav. 75:913-920.

Vallortigara G. 1996. Learning of color and position cues in domestic chicks: males are better at position, females at color. Behav Proc. 36:289-296.

Warburton K. 2003. Learning of foraging skills by fish. Fish Fisheries. 4:203-215.

Weiss M. 1997. Innate color preferences and flexible color learning in the pipevine swallowtail. Anim Behav. 53:1043-1052.

Welch Jr. KC, Bakken BH, Martínez del Rio C, Suarez RK. 2006. Hummingbirds fuel hovering flight with newly ingested sugar. Physiol Biochem Zool. 79:1082-1087.

Winter Y, Stich KP. 2005. Foraging in a complex naturalistic environment: capacity of spatial working memory in flower bats. J Exp Biol. 208:539-548.

Wunderle JM, Martinez JS. 1987. Spatial learning in the nectarivorous bananaquit: juveniles versus adults. Anim Behav. 35:652-658.

Table S1: Feeding parameters of eight amethyst sunbirds (*Chalcomitra amethystina*) presented with three rich (1 M sucrose) and six poor (0.25 M) feeders. Data were collected for six consecutive 30-min replicates for each treatment: (1) without cues, (2) with color cues (1 day after training), and (3) with color cues (7 days after training).

Treatment	Replicate	Number of birds that visited a rich feeder first	Rich feeder selectivity (proportion)	Feeding frequency (events/30 min)	Total feeding duration (s/30 min)	Total energy intake (kJ/30 min)	Energy intake from rich feeders (kJ/30 min)	Rate of energy gain (J/s/meal)
			Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Foraging **without** color cues	1	2	0.35 ± 0.07	8.00 ± 1.32	114.50 ± 24.10	6.24 ± 0.86	4.19 ± 0.97	9.88 ± 2.13
	2	5	0.51 ± 0.11	7.50 ± 1.44	70.21 ± 8.15	5.19 ± 0.42	3.89 ± 0.57	13.30 ± 2.51
	3	0	0.36 ± 0.10	12.38 ± 3.59	85.63 ± 18.30	5.81 ± 0.43	4.01 ± 0.74	11.16 ± 3.55
	4	3	0.35 ± 0.08	11.13 ± 2.84	68.83 ± 5.22	6.41 ± 0.60	4.73 ± 0.53	12.33 ± 2.58
	5	1	0.25 ± 0.08	9.75 ± 2.10	79.94 ± 17.01	5.69 ± 0.61	3.34 ± 0.83	10.89 ± 2.50
	6	3	0.45 ± 0.09	8.00 ± 1.69	62.88 ± 4.12	6.23 ± 0.68	5.03 ± 0.58	19.59 ± 6.27
Foraging **with** color cues (Day 1)	1	5	0.67 ± 0.08	6.38 ± 1.88	95.71 ± 16.36	7.62 ± 0.98	6.86 ± 1.00	21.34 ± 5.44
	2	5	0.79 ± 0.09	5.25 ± 1.47	58.41 ± 8.26	4.90 ± 0.57	4.55 ± 0.54	36.69 ± 12.19
	3	4	0.73 ± 0.10	5.13 ± 1.52	53.30 ± 5.37	4.66 ± 0.47	3.84 ± 0.48	30.88 ± 7.71
	4	6	0.70 ± 0.10	4.63 ± 0.80	55.19 ± 7.19	5.39 ± 0.60	4.83 ± 0.70	36.61 ± 14.24
	5	5	0.80 ± 0.10	6.75 ± 2.22	58.97 ± 8.83	5.49 ± 0.66	5.00 ± 0.57	27.11 ± 7.20
	6	5	0.87 ± 0.06	5.88 ± 1.59	38.37 ± 6.41	3.56 ± 0.31	3.33 ± 0.35	32.07 ± 10.61
Foraging **with** color cues (Day 7)	1	4	0.59 ± 0.11	4.75 ± 0.94	64.09 ± 7.45	5.12 ± 0.63	4.35 ± 0.65	24.97 ± 6.91
	2	8	0.80 ± 0.11	5.63 ± 1.55	58.56 ± 14.03	4.28 ± 0.62	3.83 ± 0.72	23.96 ± 5.97
	3	7	0.78 ± 0.10	5.00 ± 1.46	49.25 ± 11.83	4.25 ± 0.65	3.70 ± 0.74	44.19 ± 13.40
	4	7	0.70 ± 0.10	6.50 ± 1.78	43.83 ± 6.48	4.14 ± 0.65	3.43 ± 0.65	34.01 ± 15.18
	5	7	0.86 ± 0.06	4.38 ± 0.91	36.57 ± 4.91	3.86 ± 0.46	3.33 ± 0.56	39.17 ± 13.42
	6	3	0.64 ± 0.10	4.75 ± 1.06	46.72 ± 6.95	4.32 ± 0.48	2.86 ± 0.27	35.13 ± 13.07

FIGURE LEGENDS

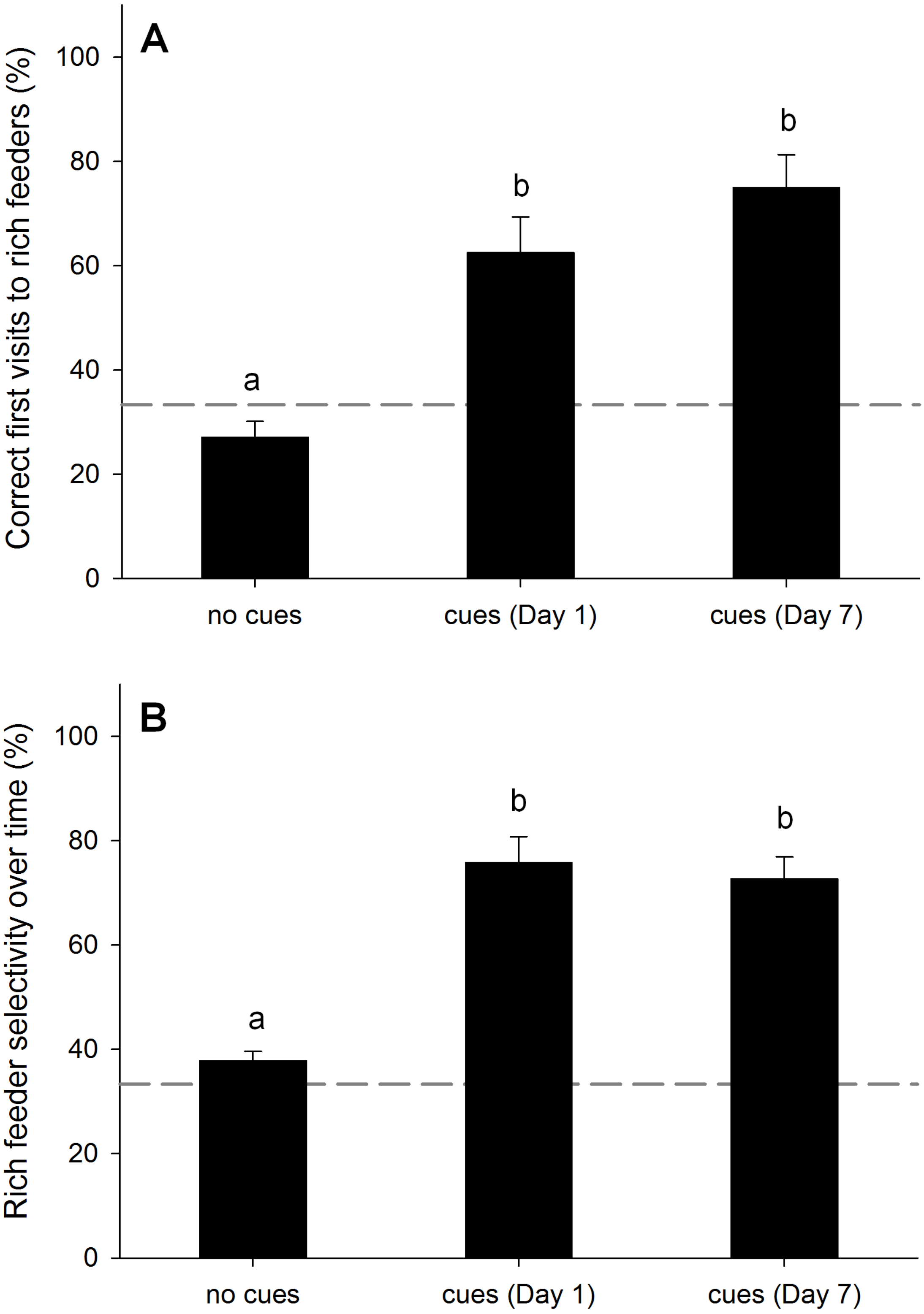
Figure 1. The feeding board used to assess whether learnt color cues increase sunbird foraging efficiency. Nine syringe feeders were mounted in an oval, each with its own wooden perch and a container with liquid paraffin underneath to catch any spilt sucrose solution. Three ‘rich’ feeders (1 M sucrose solution) were randomly placed among six ‘poor’ feeders (0.25 M). One randomly selected spot remained empty at all times. Color cues in the shape of a flower were painted around the feeding holes with nail polish (see small images). One color represented rich and one represented poor feeders; each bird was assigned to a different color pair.

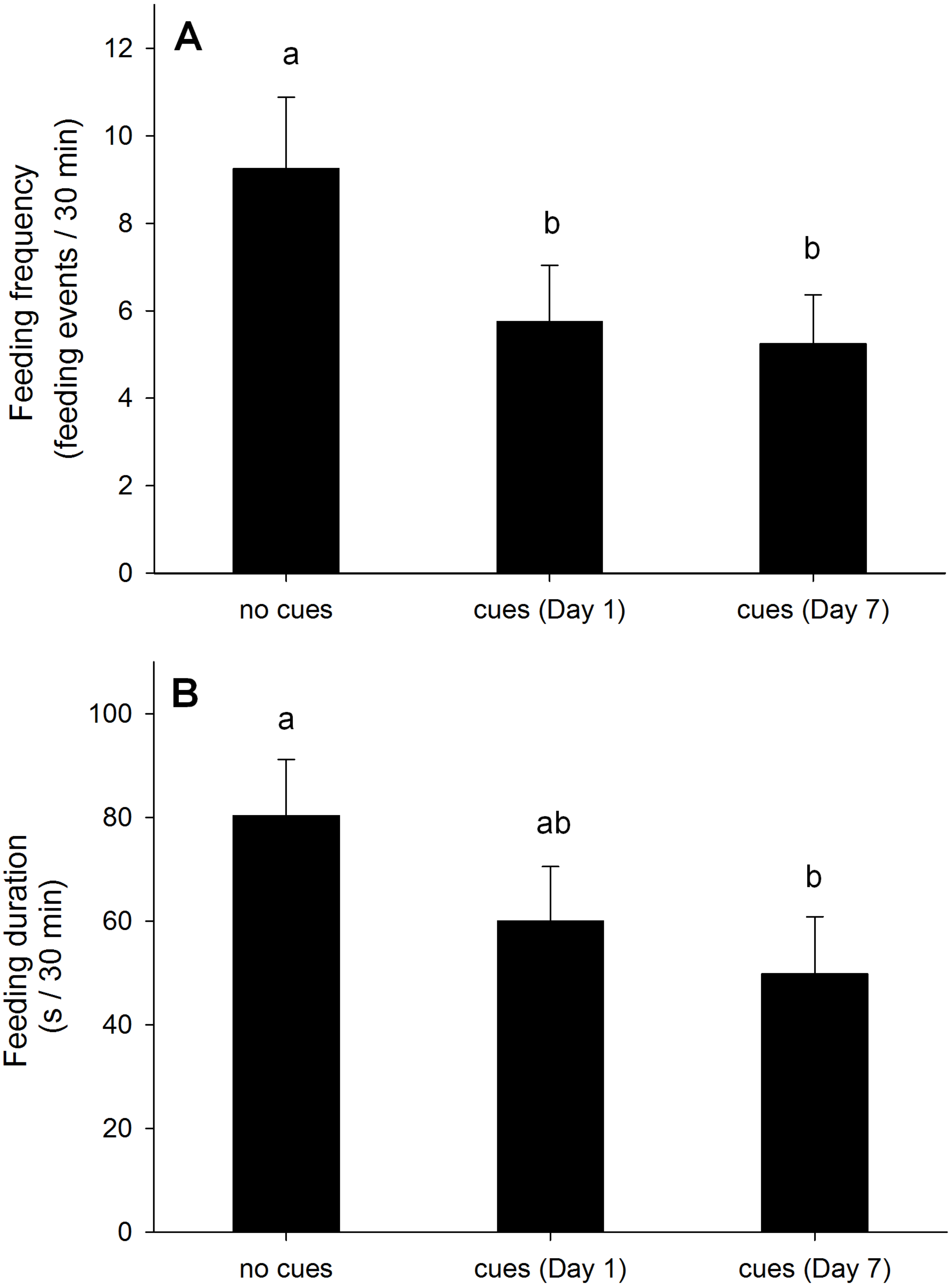
Figure 2. Feeding choices of eight amethyst sunbirds (*Chalcomitra amethystina*) presented with three rich (1 M sucrose) and six poor (0.25 M) feeders. Birds fed without and with color cues (1 and 7 days after cue training). **A)** Correct first visits to rich feeders (%). **B)** Overall rich feeder selectivity (%). The grey dashed line represents the proportion of correct first visits and rich feeder selectivity expected from random foraging (33.3%). Data are presented as means+SE; different letters indicate significant differences between experiments.

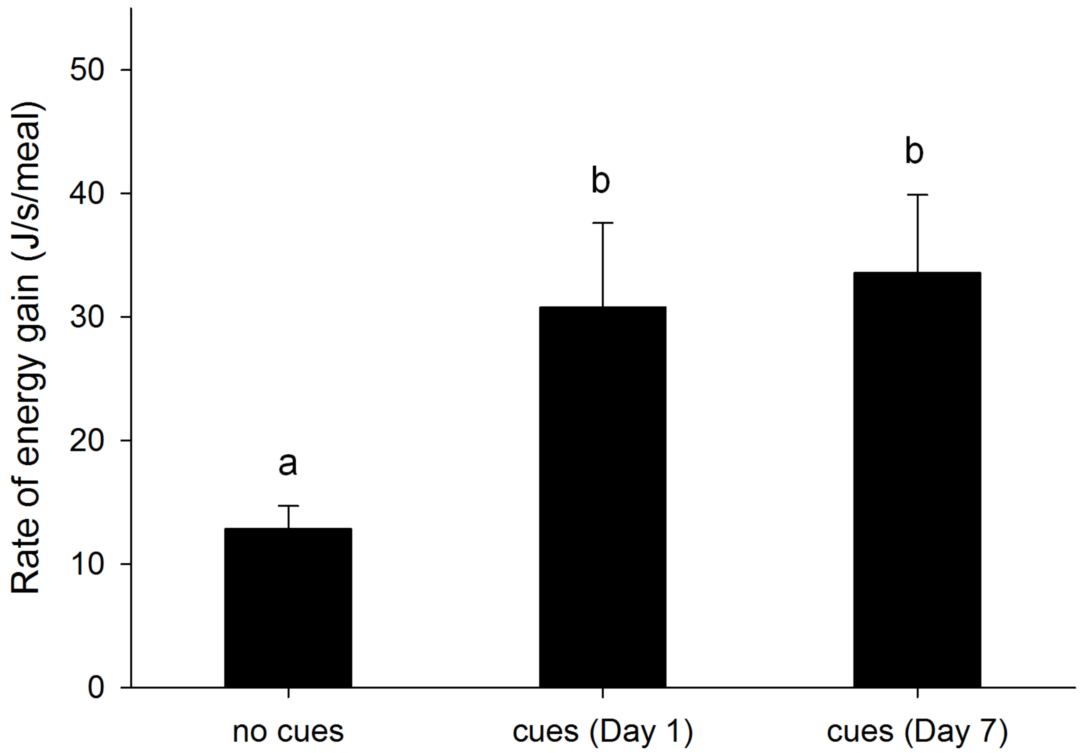
Figure 3. Feeding frequency (**A**) and feeding duration (**B**) of eight amethyst sunbirds presented with three rich (1 M sucrose) and six poor (0.25 M) feeders. Birds fed without and with color cues (1 and 7 days after cue training). Data are presented as means+SE; different letters indicate significant differences between experiments.

Figure 4. Rate of energy gain of eight amethyst sunbirds presented with three rich (1 M sucrose) and six poor (0.25 M) feeders. Birds fed without and with color cues (1 and 7 days after cue training). Data are presented as means+SE; different letters indicate significant differences between experiments.


1
2
3
4
5
6
7
8
9
10
Y1
Y3
O3
O2
O1
Y2


A
Correct first visits to rich feeders (%)
0
20
40
60
80
100
a
b
b
no cues
cues (Day 1)
cues (Day 7)
B
Rich feeder selectivity over time (%)
0
20
40
60
80
100
a
b
b
no cues
cues (Day 1)
cues (Day 7)


A
Feeding frequency
(feeding events / 30 min)
0
2
4
6
8
10
12
a
b
b
no cues
cues (Day 1)
cues (Day 7)
B
Feeding duration
(s / 30 min)
0
20
40
60
80
100
a
ab
b
no cues
cues (Day 1)
cues (Day 7)


Rate of energy gain (J/s/meal)
0
10
20
30
40
50
a
b
b
no cues
cues (Day 1)
cues (Day 7)