

Hummingbird color preference within a natural hybrid population of *Mimulus aurantiacus* (Phrymaceae)

CORINNE HANDELMAN and JOSHUA R. KOHN

Section of Ecology, Behavior, and Evolution, Division of Biological Sciences, University of California San Diego, La Jolla, California, USA

Abstract

Hummingbird flowers are typically red in color but the reasons for this are not well understood. Relatively few studies have examined hummingbird flower color preferences under natural conditions in which flower color varies within a species. We recorded hummingbird visitation rates to flowers that vary in color from yellow to red in a natural hybrid population between red- and yellow-flowered *Mimulus aurantiacus* subspecies. We also examined whether there were any correlations between color and flower size or nectar content. Finally, we reviewed the literature on hummingbird color choice tests using feeders and flowers. There were no correlations in this population between flower color and flower size, nectar volume, or sugar concentration. Nevertheless, hummingbirds undervisited the two most yellow color classes, overvisited orange flowers, and visited the two most red color classes in proportion to their frequency in the population. While Hummingbirds preferred flowers expressing red pigments to those that did not, the flowers with the most red hue were not the most attractive, as has been observed in similar studies with other species of *Mimulus*. While feeder studies generally fail to show hummingbird preference for red, all studies using flowers, including those that control all floral traits other than color, find consistent preference for red. Experiments are suggested that might help disentangle hypotheses for why hummingbirds exhibit this preference.

Keywords: color preference, hummingbird, *Mimulus*, monkeyflower, pollination.

Received 18 April 2012; revision received 13 July 2012; accepted 7 August 2012

The great diversity of floral forms found in nature is thought to be caused, at least in part, by the association of different species of plants with different types of pollinators (Grant & Grant 1965; Faegri & van der Pijl 1979; Johnson & Steiner 2000; Fenster *et al.* 2004). Hummingbirds, for instance, generally visit species with red-pigmented flowers (Grant & Grant 1968) but the causes of this association are not well understood. At least three hypotheses have been put forward to explain the association of hummingbirds with red flowers. First, hummingbirds may have an innate preference for or ability to perceive red over other colors, at least against the background of natural vegetation (Chittka & Waser 1997). Second, insects may detect red more poorly, thereby tending to remove resources from non-red flowers more efficiently (Raven 1972). This may cause hummingbirds to

prefer to visit red flowers to obtain the higher nectar rewards (Rodríguez-Gironés & Santamaría 2004). Third, hummingbird flowers are associated with large quantities of relatively dilute nectar (Baker & Baker 1975). Hummingbirds may come to associate red flowers with copious nectar creating a positive feedback loop at the community level (Grant 1966) where red-flowered species may form a mimicry complex for attracting hummingbirds for pollination (Brown & Kodric-Brown 1979). This last hypothesis is not entirely separable from the first two as, while hummingbird-pollinated plants may benefit collectively from sending a similar attraction signal, the reason this signal is red rather than some other color is hypothesized to be because red is either more conspicuous to birds or less conspicuous to insects (Grant 1966). Despite the intense focus on pollination biology over the past century or more, we still have no clear understanding of one of the strongest observed patterns, the association of red flowers with bird pollinators.

Correspondence: Joshua R. Kohn
Email: jkohn@ucsd.edu

In San Diego County, the native perennial bush monkeyflower (*Mimulus aurantiacus*) is represented by two forms. In western San Diego County, red-flowered *M. aurantiacus puniceus* occurs, while yellow-flowered *M. aurantiacus australis* is found further east (Streisfeld & Kohn 2005). Flower color breeds true in greenhouse and garden studies and appears to be controlled by a major gene and several modifiers (Streisfeld 2005; Streisfeld & Rausher 2009). Hummingbirds have been observed visiting pure populations of the red and yellow forms at similar rates, while hawkmoths, the major alternative pollinator (Grant 1993), have only been observed visiting the yellow form (Streisfeld & Kohn 2007). Hummingbirds, even those that have been visiting natural yellow-flowered populations, strongly prefer the red form, probing red flowers 95% of the time when given the choice between plants of the red- and yellow-flowered subspecies (Streisfeld & Kohn 2007). However, red flowers, on average, are shorter, narrower, and produce somewhat more nectar than yellow flowers (Streisfeld & Kohn 2005). So whether hummingbirds prefer red-flowered *Mimulus* because they are red or because of these correlated characters is not known. The two subspecies of monkeyflower are separated by a narrow hybrid zone. Hybrid and greenhouse-grown F2 populations display a continuous range of flower color from yellow to red (Streisfeld 2005; Streisfeld & Kohn 2005). The behavior of hummingbirds in relation to flower color in populations displaying continuous variation has not been recorded.

The present study takes advantage of a large natural hybrid population between the red and yellow forms of bush monkeyflower to ask whether hummingbirds preferentially visit red flowers when continuous variation in flower color is present in the population. In order to dissect the causes of any observed preference, we also measured the association of flower color with variation in corolla size and nectar content. If the hybrid zone represents advanced generations of hybrid forms, and if genes controlling flower color, shape, and nectar content are independent and not tightly linked, there might be no correlation between flower color and nectar content or

flower size. Our goal was to examine the role of hummingbird color preference in a natural hybrid zone and to determine whether any preferences seen could be influenced by correlated floral traits. We also review the literature on color choice tests using hummingbirds to summarize evidence on what role hummingbird color preference plays in the association of red-flowers with hummingbird pollination.

Materials and methods

Bird observations

The large hybrid monkeyflower study population occurs in the Blue Sky Ecological Reserve, Poway California (33°00'51.98"N, 117°01'00.48"W, elevation 227 m). Focal plants occurred within a 6 m × 20 m area nested within the larger population. On April 19, May 3, and May 18, 2010 all plants and flowers within the focal area were censused, the number of open flowers counted, and each plant was grouped by eye into one of five color categories: yellow, yellow-orange, orange, red-orange and red (Fig. 1). This one-month period corresponded to the peak of the blooming season at this site in this year. Hummingbird visits were observed for 1–3 hours per day for several days following each floral census (April 23–30, May 3–13, May 20 to June 1). Observations were made from a location where the entire focal area could be viewed. Hummingbird visits were recorded during a total of 22.5 hours of observation.

When a hummingbird foraged, the flower color of each plant and number of flowers probed were recorded sequentially for the duration of the foraging bout within the focal area. Hummingbirds often could not be identified to species. Anna's hummingbirds (*Calypte anna*) are by far the most common hummingbird species in this area, but Allen's (*Selasphorus sasin*), Costa's (*Calypte costae*), black-chinned (*Archilochus alexandri*), and Rufous (*Selasphorus rufus*) hummingbirds are also occasional visitors. Birds could not be identified individually, so the number of different pollinators recorded in this study is

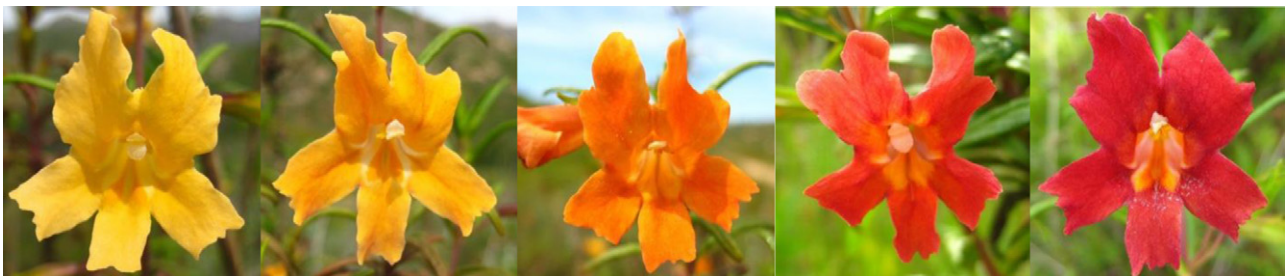


Fig. 1 Flower color variation in a natural population of bush monkeyflower *Mimulus aurantiacus*, Blue Sky Ecological Reserve, Poway CA. Flowers are representative of the five color categories used: (left to right) yellow, yellow-orange, orange, red-orange, and red.

not known. However, several hummingbirds were likely involved as displacements were observed and birds are not highly territorial at this site. Other floral visitors were rare, consisting of small pollen-collecting bees that did not probe the corolla tube to remove nectar and usually failed to contact the stigma while foraging. Honeybees and bumblebees were active in the area but foraged almost entirely on other species, ignoring *Mimulus* flowers. Hawkmoths were not observed. We calculated the total, pooled, and heterogeneity χ^2 values across the three census periods comparing census frequencies of plants or flowers of each color category to observed numbers of visits by hummingbirds.

Floral traits

We evaluated the accuracy of our visual classification of flower color (Fig. 1) by measuring the hue of a sample of classified flowers using an Ocean Optics (Dunedin, FL) USB2000 spectrophotometer following the protocol found in Streisfeld and Kohn (2005). Flowers from 33 plants, each classified by eye into one of the five color categories, were selected from those growing outside the focal area. Flowers were selected to provide roughly equal representation across the color categories. Flowers of *M. aurantiacus* last about 8 days (Fetscher & Kohn 1999), and can fade slightly in color as they age. Flowers that have been open for less than 24 hours (first-day flowers) are identifiable by their indehiscent anthers. We measured the hue of two first-day flowers from each inflorescence and analyzed hue by color class using ANOVA. In addition, we looked for associations between flower color and corolla length, width, and nectar content. Length and width were measured on three (sometimes two) flowers per plant for 40 plants using dial calipers. Standing nectar volumes were measured between 08:00 and 12:00 hours both on open first-day flowers (open flowers) and on flowers that were closed, but going to open the day of measurement (closed flowers). These were identifiable as having fully elongated corollas but with corolla lobes that, instead of being reflexed, were folded across the end of the corolla tube preventing entry by a hummingbird bill. These two classes of flowers provide estimates of standing nectar content (open flowers) and nectar production just prior to anthesis (closed flowers), respectively. Nectar volume was measured by inserting a 5-uL micropipette down the floral tube to the base of the style and measuring the length to which the micropipette filled with nectar using dial calipers. These lengths were then calibrated against known amounts of water (1–5 uL) drawn into the micropipettes in the laboratory to convert linear measures to volumes. Nectar sugar content was measured using a Bellingham and Stanley Eclipse® model 45-03 refractometer. This instrument measures the refractive index of the

nectar on the Brix scale, the concentration of sucrose that would produce the observed refractive index.

Results

A total of 73 hummingbird visits to plants and 125 probes of flowers were recorded during the 22.5 hours of observation reflecting relatively low rates of visitation common for this plant (Fetscher & Kohn 1999; Streisfeld & Kohn 2007). Hummingbird visits differed significantly from that expected based on the frequencies of flower colors present in the focal population (plants visited: $\chi^2_{\text{pooled}} = 11.51$, d.f. = 4, $P < 0.025$, $\chi^2_{\text{heterogeneity}} = 2.02$, d.f. = 2, ns; flowers visited: $\chi^2_{\text{pooled}} = 16.00$, d.f. = 4, $P < 0.005$, $\chi^2_{\text{heterogeneity}} = 5.05$, d.f. = 2, ns). Because the heterogeneity among census periods is not significant, we present the pooled data for visitation in Figure 2. Although the expected numbers of visits are relatively small, hummingbirds were never observed visiting the yellow color class. The yellow-orange class was visited about half as often as predicted by its frequency. The orange class was overvisited and hummingbirds visited the two most red color classes approximately in proportion to their frequency in the population.

Classification of plants into color classes reflected real differences in hue as measured spectrophotometrically. Strong, monotonically decreasing differences in hue are seen across the color classes arrayed from yellow to red ($F_{4,30} = 177.8$, $P < 0.001$; Fig. 3). However, neither flower size (Fig. 4) nor nectar content (Fig. 5) differed significantly across color classes (corolla length: $F_{4,35} = 0.31$, ns; corolla width: $F_{4,35} = 0.62$, ns; nectar volume: closed flowers $F_{4,90} = 0.68$, ns; open flowers $F_{4,120} = 0.023$, ns; nectar sugar concentration: closed flowers $F_{4,78} = 1.84$, ns; open flowers $F_{4,87} = 1.72$, ns; Fig. 4).

Discussion

The hybrid study population provided a natural setting where the effect of color variation on hummingbird visitation could be evaluated in the absence of correlations between flower color and flower size or nectar content, which differ between red- and yellow-flowered subspecies (Streisfeld & Kohn 2005). This may indicate that advanced generation hybrids are present in this population as a reduced but still significant correlation between flower color and size occurs among greenhouse-grown F2 plants (Streisfeld 2005). In the absence of correlations between flower color and these other traits that might affect visitation, hummingbirds showed significant floral preference in this study by avoiding visits to yellow flowers in favor of flowers that ranged from orange to red. Hummingbirds appear capable of exerting considerable selection on genes that control flower color in this popu-

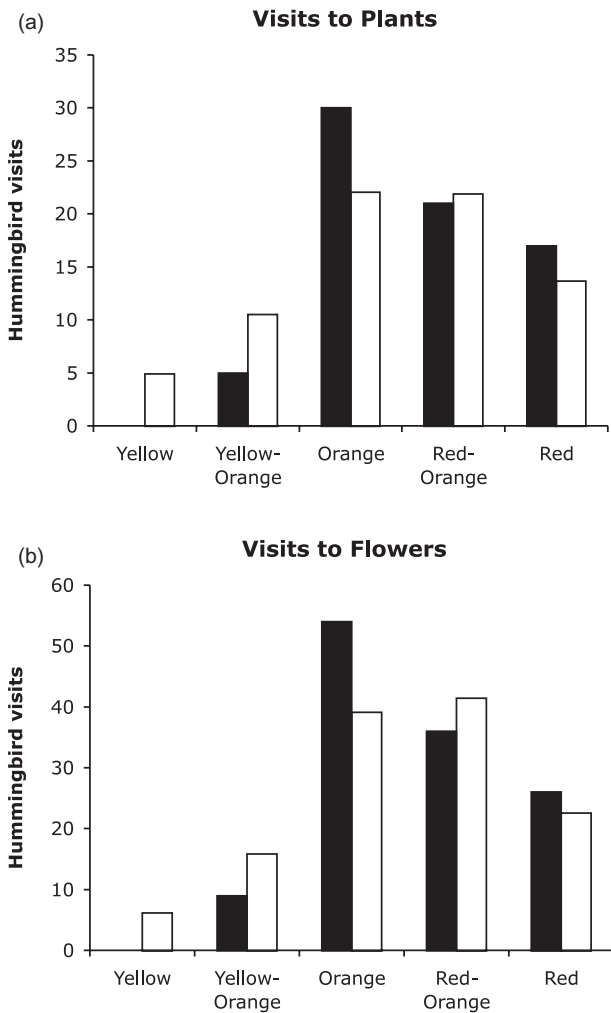


Fig. 2 Observed and expected visits by hummingbirds to (a) plants and (b) flowers of the five color classes of *Mimulus aurantiacus*. Expected values were obtained by multiplying the frequency of the different flower colors censused by the total number of visits. ■, observed visits; □, expected visits.

lation. However, while hummingbirds disfavored yellow, they did not prefer the most red color classes. Instead, the orange color class appeared to be overvisited while the two most red color classes were visited roughly in proportion to their frequency.

Vickery (1992, 1995) studied free ranging hummingbirds visiting experimental arrays containing red, yellow, and orange (F1 hybrid) forms of two additional species of *Mimulus*, *M. cardinalis* and *M. verbenaceus*. Similar to our results, in both species hummingbirds disfavored the yellow morph, overvisited the orange form, and visited the red form in proportion to its frequency (Vickery 1992, 1995). So while expressing some level of red pigments appears to increase visitation by hummingbirds to *Mimulus* flowers, the data suggest that the levels of antho-

cyanins present in natural hummingbird-pollinated *Mimulus* populations may be higher than optimal for attracting birds. Choice tests using red and orange flowered plants in the absence of yellow forms would be useful in confirming the most attractive color for bird visitation in this system and whether birds directly select for the deep red colors seen in *Mimulus aurantiacus* and several other species of bird-pollinated *Mimulus*.

Several authors have advanced the hypothesis that red coloration in bird-pollinated flowers may serve not only to attract hummingbirds but also to discourage visitation by insects (Castellanos *et al.* 2004; Rodríguez-Gironés & Santamaría 2004; Lunau *et al.* 2011 and references therein). Although insects can discern red (Chittka & Waser 1997) their ability to do so, particularly against green background vegetation may be reduced relative to birds (Rodríguez-Gironés & Santamaría 2004; Forrest & Thomson 2009). The charge of poor red vision has been particularly leveled against bees, which are very infrequent visitors to *M. aurantiacus* flowers, whereas various Lepidoptera are said to have good ability to discern red. In the case of these two subspecies of *Mimulus*, the insect pollinator of the yellow morph is the hawkmoth *Hyles lineata* (Streisfeld & Kohn 2007). In San Diego County, this moth forages at dusk. Low light levels at this time of day may make the red morph hard to pick out while the yellow morph might be more easily perceived because of higher contrast with background colors (Kelber *et al.* 2003). Ultraviolet reflectance by the yellow, but not the red morph (Streisfeld & Kohn 2005) may also influence the yellow-morph's visibility to hawkmoths. Lunau *et al.* (2011) found that hummingbirds did not differ in their response to UV absorbing or UV reflecting artificial red or white flowers. Euglossine bees, on the other hand, preferred artificial red flowers that reflected, and white flowers that absorbed, UV. No similar tests with hawkmoths have been performed. Coupled with choice tests using birds, testing the effectiveness of a yellow to red flower color shift in reducing insect visitation would provide evidence on whether the deep red color seen in natural populations near the coast results primarily from selection for visitation by birds, selection to discourage insect visitation, or some other selective force.

In this hybrid population there was no correlation of flower color with other measured traits thought likely to affect hummingbird visitation. Neither flower size, nectar volume nor nectar concentration differed among the color classes. Therefore, hummingbirds should not have expected yellow flowers to contain less nectar unless this expectation was based upon experience gained elsewhere, or from visitation to other species. Physiologically, hummingbirds should have good color vision throughout the range visible to humans and extending somewhat into the UV (Hart & Hunt 2007; Ödeen & Håstad 2010). Thus they



Fig. 3 Hue (mean ± SE) of flowers from each color class.

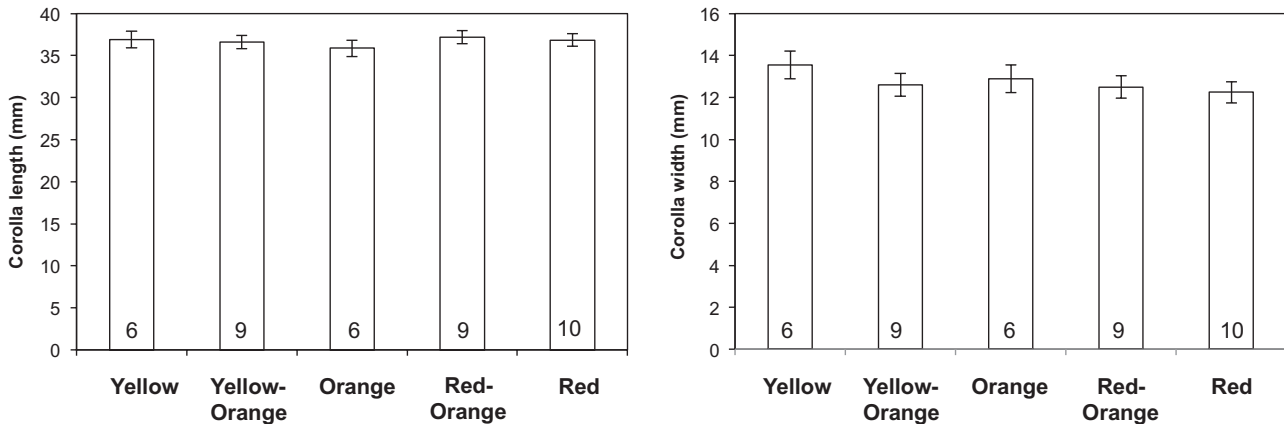


Fig. 4 Lengths and widths (mean ± SE) of corollas of flowers in each color class. Usually three (sometimes two) flowers per plant were measured. Numbers in bars are the number of plants measured in each color class.

should discern red well, but any preference for red is probably not driven by increased visual acuity in the red range, or limited ability to discern other colors visible to humans. Visitation by hummingbirds to natural yellow-flowered populations of *M. aurantiacus australis*, at rates similar to those recorded in red-flowered *M. aurantiacus puniceus* populations (Streisfeld & Kohn 2007), shows that birds are able to detect and visit yellow flowers. They nevertheless prefer red-pigmented flowers when present, as shown here and, much more strongly, in the choice tests using only red- and yellow- flowered plants (Streisfeld & Kohn 2007).

Hummingbirds showed very strong preference for the red form when given a choice only between pure forms of

each subspecies (Streisfeld & Kohn 2007) but visitation by hummingbirds in this hybrid population with continuous flower color variation would promote maintenance of intermediate forms and continued segregation of flower color variants. Similar results have been found in other studies where choice tests between pure forms of two species or subspecies are compared to those which include intermediate (usually F1) forms. In several cases (Vickery 1992, 1995; Schemske & Bradshaw 1999; Ippolito *et al.* 2004) presence of intermediate forms reduced the apparent strength of pollinator mediated reproductive isolation in comparison to tests in the absence of intermediates. This means the context under which selection on color is measured will strongly affect the results and that

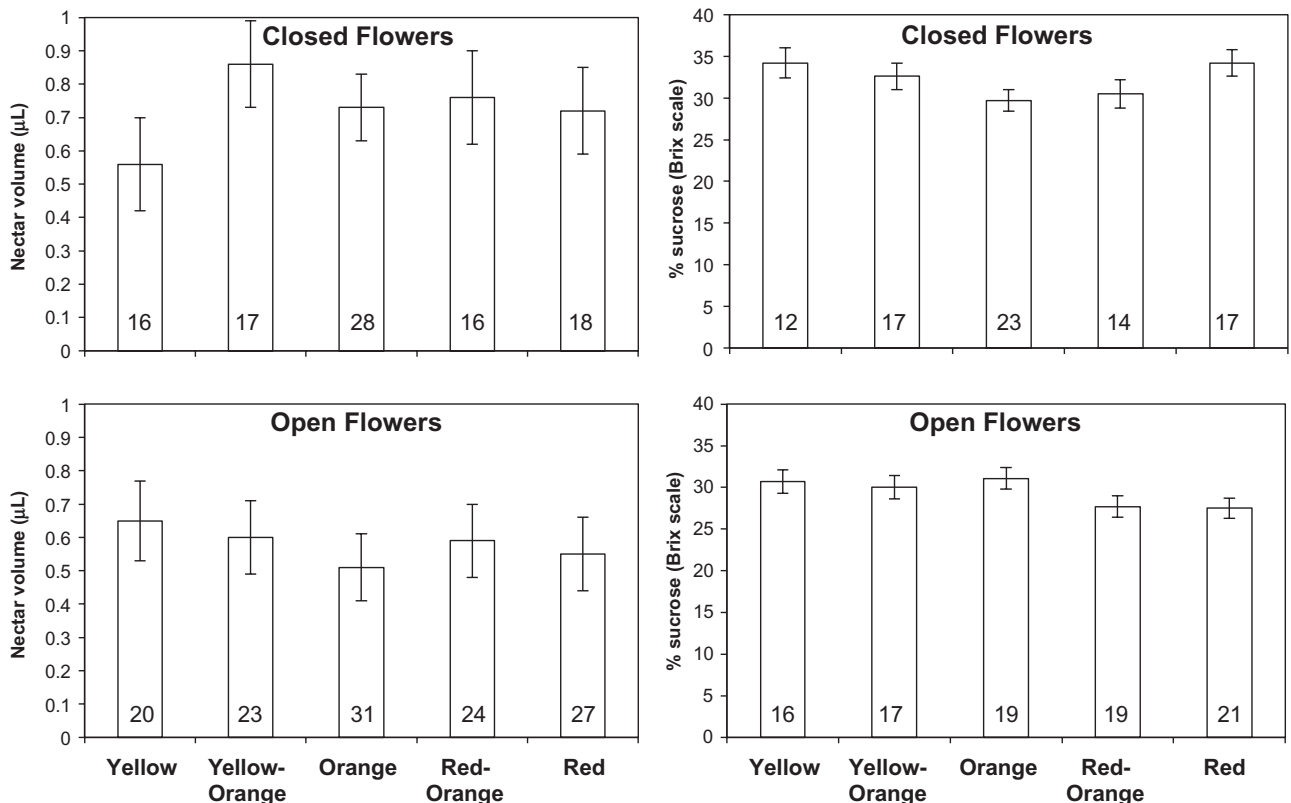


Fig. 5 Nectar volumes (mean \pm SE) and sugar concentrations in flowers of each color class. Closed flowers are flowers measured just prior to anthesis (see text). Open flowers have indehiscent anthers indicating dehiscence occurred < 24 hours before nectar was measured. Numbers in bars are the numbers of flowers measured. At most one flower of each type was measured per plant.

studies where only a limited number (often two) of qualitative choices are available (e.g. Bradshaw & Schemske 2003) may not reflect how pollinators will select on color in a natural situation where hybrid forms are present and continuous variation in color is displayed.

Reviewing color-based choice tests using hummingbirds reveals several trends. Most feeder studies fail to show a consistent preference for red (Table S1; Sherman 1913; Bené 1941; Pickens 1941; Lyerly *et al.* 1950; Grant 1966; Collias & Collias 1968; Miller & Miller 1971; Welker 1984). The exceptions (Stiles 1976; Goldsmith & Goldsmith 1979) are when birds have been trained to red or previously visited red-flowered species. For instance, Stiles (1976) found strong preference for red feeders in hummingbirds caught adjacent to (and presumably feeding on) red-flowered *Ribes speciosum*, but preference (though not as strong) for yellow feeders when hummingbirds were caught while feeding on yellow flowered *Nicotiana glauca*. In several feeder studies (Wagner 1946; Collias & Collias 1968; Goldsmith & Goldsmith 1979), hummingbirds could be trained to whatever color was experimentally associated with the highest sugar reward, proving not only that hummingbirds possess keen color

vision but also that they can learn to associate a color with a reward.

One feeder study, however, did show a clear preference for red. Wheeler (1980) found that when feeders were put in previously unused locations, hummingbirds were more likely to probe feeders filled with red than with other colors (yellow, blue, green, clear) of sugar solution. Therefore red coloration may provide an advantage when hummingbirds are looking for new food sources even if, once the location of a source is known, color makes no difference to visitation (Wheeler 1980). In summary, feeder studies show little if any evidence for innate preference by hummingbirds for red. Instead they provide support for the idea that experience plays a role in hummingbird color preference and that hummingbirds swiftly learn which color is associated with increased caloric reward. Also, even when trained to a particular feeder position or color, birds regularly explore alternative food sources (feeders) and are apparently capable of switching if these are found to be more rewarding.

In contrast to feeder studies, choice tests using real or artificial flowers that vary in color commonly show hummingbirds prefer forms expressing red pigments to those

that do not (Table S1; Vickery 1992, 1995; Melindez-Ackerman *et al.* 1997; Melindez-Ackerman & Campbell 1998; Bradshaw & Schemske 2003; Ippolito *et al.* 2004; Gegeer & Burns 2007), even when no other floral characters differ (Melindez-Ackerman *et al.* 1997; Melindez-Ackerman & Campbell 1998; Bradshaw & Schemske 2003; Gegeer & Burns 2007). In all of those studies, free-living or recently captured hummingbirds were used. These may have experienced an association between red-colored flowers and higher nectar rewards, which could explain their preference for red-pigmented flowers. Alternatively, red may be more conspicuous to hummingbirds when viewed against the background of natural vegetation (Chittka & Waser 1997).

While feeder studies have shown that hummingbirds can be trained to visit the most rewarding color, only one study using flowers has shown evidence that hummingbirds learn to modify their initial preference for red. Melindez-Ackerman *et al.* (1997) observed hummingbird visitation to arrays in which flowers of *Ipomopsis aggregata* were painted either red or white and did not differ in morphology. In this case the white flowers were provisioned with 2 μ L of nectar and the red flowers had none. Hummingbirds initially strongly preferred red flowers but shifted to preferring white flowers during the course of 125 visits. Using feeders, Goldsmith and Goldsmith (1979) estimated that the rewarding color of feeders could be learned in 6–22 visits.

In the current study, floral morphology and rewards did not differ among flower color classes. Nevertheless, over the month of the observations, hummingbirds continuously preferred flowers with substantial red pigmentation to those that lacked it and did not show any increase in visitation to non-red flowers as evidenced by a lack of heterogeneity in visitation rates among census periods. In contrast to the experimental studies with flowers or feeders where reward was manipulated to be zero in all but the rewarding color class, the natural setting provided equal rewards across colors. This may make learning (or unlearning) an association between color and reward more difficult. More experimentation on the time-course of learning and preference switching are warranted.

Flower color differences producing shifts in pollinators have sometimes been thought of as potential means of achieving reproductive isolation and speciation in plants (reviewed in Kay & Sargent 2009). This view has been challenged (e.g. Waser 2001), partially because, in experiments where mixtures of flower color morphs are presented to pollinators, preferences are seldom if ever strong enough to prevent substantial gene flow between the forms. The observations described here are no exception, with, in particular, high visitation to intermediate (orange) forms with hue values similar to F1 hybrids (Streisfeld 2005). However, experiments where pollina-

tors are exposed to mixtures of flower types are usually performed with even or nearly even representation of floral forms. It remains to be seen whether substantial mixing of floral color genes would continue as one color morph became rare due to selection against it by pollinators. The rate of visitation per flower to the non-preferred form may decline with its frequency, producing a positive feedback towards fixing the preferred color. Further experiments testing whether pollinators will maintain variation in color as frequencies of forms change are clearly needed.

References

- Baker H. G. & Baker I. (1975) Studies of nectar constitution and plant–pollinator coevolution. In: Gilbert L. E. & Raven P. H. (eds). *Animal–Plant Coevolution*. University of Texas Press, Austin, TX, pp. 100–140.
- Bené F. (1941) Experiments on the color preference of black-chinned hummingbirds. *The Condor* **43**: 237–242.
- Bradshaw H. D. & Schemske D. W. (2003) Allele substitution at a flower color locus produces a pollinator shift in monkeyflowers. *Nature* **426**: 176–178.
- Brown J. H. & Kodric-Brown A. (1979) Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology* **60**: 1022–1035.
- Castellanos M. C., Wilson P. & Thomson J. D. (2004) ‘Anti-bee’ and ‘pro-bird’ changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology* **17**: 876–885.
- Chittka L. & Waser N. M. (1997) Why red flowers are not invisible to bees. *Oikos* **45**: 169–183.
- Collias N. E. & Collias E. C. (1968) Anna’s Hummingbirds trained to select different colors in feeding. *The Condor* **70**: 273–274.
- Faegri K. & van der Pijl L. (1979) *The Principles of Pollination Ecology*, 3rd edn. Pergamon Press, Oxford.
- Fenster C. B., Armbruster W. S., Wilson P., Dudash M. & Thomson J. D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics* **35**: 375–403.
- Fetscher A. E. & Kohn J. R. (1999) Stigma behavior in *Mimulus aurantiacus* (Scrophulariaceae). *American Journal of Botany* **86**: 1130–1135.
- Forrest J. & Thomson J. D. (2009) Background complexity affects colour preference in bumblebees. *Naturewissenschaften* **96**: 921–925.
- Gegeer R. J. & Burns J. G. (2007) The birds, the bees, and virtual flowers: can pollinator behavior drive ecological speciation in flowering plants? *The American Naturalist* **170**: 551–566.
- Goldsmith T. H. & Goldsmith K. M. (1979) Discrimination of colors by the black-chinned hummingbird, *Archilochus alexandri*. *Journal of Comparative Physiology A* **130**: 209–220.
- Grant K. A. (1966) A hypothesis concerning the prevalence of red coloration in California hummingbird flowers. *The American Naturalist* **100**: 85–97.
- Grant K. A. & Grant V. (1968) *Hummingbirds and Their Flowers*. Columbia University Press, New York.
- Grant V. (1993) Origin of floral isolation between ornithophilous and sphingophilous plant species. *Proceedings of the National*

- Academy of Sciences of the United States of America* **90**: 7729–7733.
- Grant V. & Grant K. A. (1965) *Flower Pollination in the Phlox Family*. Columbia University Press, New York.
- Hart N. S. & Hunt D. M. (2007) Avian visual pigments: characteristics, spectral tuning, and evolution. *The American Naturalist* **169** (Suppl): S7–S26.
- Ippolito A., Fernandes G. W. & Holtsford T. P. (2004) Pollinator preferences for *Nicotiana glauca*, *N. glauca* and their F1 hybrids. *Evolution* **58**: 2634–2644.
- Johnson S. D. & Steiner K. E. (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* **15**: 140–143.
- Kay K. M. & Sargent R. D. (2009) The role of animal pollination in plant speciation: integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution and Systematics* **40**: 637–656.
- Kelber A., Balkenius A. & Warrant E. J. (2003) Colour vision in diurnal and nocturnal hawkmoths. *Integrative and Comparative Biology* **43**: 571–579.
- Lunau K., Papiorek S., Eltz T. & Szajma M. (2011) Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *Journal of Experimental Biology* **214**: 1607–1612.
- Lyerly S. B., Riess B. F. & Ross V. (1950) Color preference in the Mexican violet-eared hummingbird, *Calibri T. thalassinus* (Swainson). *Behaviour* **2**: 237–248.
- Melindez-Ackerman E. & Campbell D. R. (1998) Adaptive significance of flower color and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution* **52**: 1293–1303.
- Melindez-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 R. S. & Miller R. E. (1971) Feeding activity and color preference of ruby-throated Hummingbirds. *The Condor* **73**: 309–313.
- Ödeen A. & Håstad O. (2010) Pollinating birds differ in spectral sensitivity. *Journal of Comparative Physiology A* **196**: 91–96.
- Pickens A. L. (1941) A red figwort as the ideal nearctic bird-flower. *The Condor* **43**: 100–102.
- Raven P. H. (1972) Why are bird-visited flowers predominantly red? *Evolution* **26**: 674.
- Rodríguez-Gironés M. A. & Santamaría L. (2004) Why are so many bird flowers red? *PLoS Biology* **2**: 1515–1519.
- Schemske D. W. & Bradshaw H. D. (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences of the United States of America* **96**: 11910–11915.
- Sherman A. R. (1913) Experiments in feeding hummingbirds during seven summers. *The Wilson Bulletin* **25**: 153–166.
- Stiles G. F. (1976) Taste preferences, color preferences and flower choice in hummingbirds. *The Condor* **78**: 10–26.
- Streisfeld M. A. (2005) Ecological genetics of a flower color polymorphism in southern California bush monkeyflowers (Ph.D. dissertation). University of California, San Diego.
- Streisfeld M. A. & Kohn J. R. (2005) Contrasting patterns of floral and molecular variation across a cline in *Mimulus aurantiacus*. *Evolution* **59**: 2548–2559.
- Streisfeld M. A. & Kohn J. R. (2007) Environment and pollinator-mediated selection on parapatric floral races of *Mimulus aurantiacus*. *Journal of Evolutionary Biology* **20**: 122–132.
- Streisfeld M. A. & Rausher M. D. (2009) Altered *trans*-regulatory control of gene expression in multiple anthocyanin genes contributes to adaptive flower color evolution in *Mimulus aurantiacus*. *Molecular Biology and Evolution* **26**: 433–444.
- Vickery R. K. (1992) Pollinator preferences for yellow, orange, and red flowers of *Mimulus verbenaceus* and *M. cardinalis*. *Great Basin Naturalist* **52**: 145–148.
- Vickery R. K. (1995) Speciation in *Mimulus*, or, can a simple flower color mutant lead to species divergence? *Great Basin Naturalist* **55**: 177–180.
- Wagner H. O. (1946) Food and feeding habits of Mexican hummingbirds. *The Wilson Bulletin* **58**: 69–93.
- Waser N. M. (2001) Pollinator behavior and plant speciation: looking beyond the ‘ethological isolation’ paradigm. In: Chittka L. & Thomson J. D. (eds). *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution*. Cambridge University Press, Cambridge, pp. 318–336.
- Welker H. J. (1984) Food color preference in the Anna’s hummingbird. *Western Birds* **15**: 23–27.
- Wheeler T. G. (1980) Experiments in feeding behavior of the Anna Hummingbird. *The Wilson Bulletin* **92**: 53–62.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 (a) Feeder and (b) flower studies of hummingbird color preference.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.