

RESEARCH ARTICLE

Color vision and learning in the monarch butterfly, *Danaus plexippus* (Nymphalidae)

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SUMMARY

The monarch butterfly, *Danaus plexippus*, is well known for its intimate association with milkweed plants and its incredible multi-generational trans-continental migrations. However, little is known about monarch butterflies' color perception or learning ability, despite the importance of visual information to butterfly behavior in the contexts of nectar foraging, host-plant location and mate recognition. We used both theoretical and experimental approaches to address basic questions about monarch color vision and learning ability. Color space modeling based on the three known spectral classes of photoreceptors present in the eye suggests that monarchs should not be able to discriminate between long wavelength colors without making use of a dark orange lateral filtering pigment distributed heterogeneously in the eye. In the context of nectar foraging, monarchs show strong innate preferences, rapidly learn to associate colors with sugar rewards and learn non-innately preferred colors as quickly and proficiently as they do innately preferred colors. Butterflies also demonstrate asymmetric confusion between specific pairs of colors, which is likely a function of stimulus brightness. Monarchs readily learn to associate a second color with reward, and in general, learning parameters do not vary with temporal sequence of training. In addition, monarchs have true color vision; that is, they can discriminate colors on the basis of wavelength, independent of intensity. Finally, behavioral trials confirm that monarchs do make use of lateral filtering pigments to enhance long-wavelength discrimination. Our results demonstrate that monarchs are proficient and flexible color learners; these capabilities should allow them to respond rapidly to changing nectar availabilities as they travel over migratory routes, across both space and time.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/214/3/509/DC1>

Key words: monarch, Lepidoptera, learning, vision, innate color preference.

INTRODUCTION

Most animals make use of color information, including wavelength, spectral purity and intensity, as they explore their environments. Butterflies in particular rely on light in a variety of behavioral contexts, and the range of their light perception, which in some taxa extends from ultraviolet through red (300 to 700 nm), is among the broadest known in the animal kingdom (Briscoe and Chittka, 2001; Silberglied, 1984). True color vision, the ability to discriminate visual stimuli based on wavelength, independent of intensity, has been demonstrated explicitly for several butterfly and moth species (Kelber and Henique, 1999; Kelber and Pfaff, 1999; Kinoshita et al., 1999; Sison-Mangus et al., 2008).

Recent research has demonstrated that butterfly eyes are remarkably varied and complex; families and even species differ significantly with respect to the number, absorbance spectrum and spatial distribution of photoreceptor pigments, as well as the type and distribution of intracellular lateral filtering pigments in the compound eye (Arikawa et al., 2009; Arikawa et al., 1999; Briscoe, 2008; Frentiu et al., 2007a; Frentiu et al., 2007b; Zaccardi et al., 2006). Diversity in butterfly eyes translates into diversity in butterfly vision at two different levels. At one level, the anatomical and physiological diversity of eyes means that different species will collect different spectral information; at the next level, the processing and weighting of this spectral information in the brain is likely to

result in perceptual differences between taxa (Briscoe and Chittka, 2001; Kelber et al., 2003). Thus, color perception and discrimination ability cannot be determined by knowledge of a butterfly's 'hardware' alone. It is necessary to conduct behavioral assays in order to determine what butterflies of a given species can actually see (Kelber et al., 2003; Kinoshita et al., 1999).

The particulars of a butterfly's ability to perceive and discriminate colors will be manifested in its innate color preferences, its ability to learn to associate colors with salient cues in the environment and the extent to which it generalizes between or confuses colors. Although it is common for flower-visiting insects in general to have two preference peaks – one in the violet–blue region and one in the long-wavelength region – reported color preferences differ within a family or even a genus; e.g. pipevine swallowtails (*Battus philenor*, Papilionidae) have a strong innate preference for yellow, with weaker preferences for blue and purple (Weiss, 1997), whereas Japanese swallowtails (*Papilio xuthus*, Papilionidae) strongly prefer yellow and red models when offered a choice of red, yellow, green and blue, and foraging citrus swallowtails (*Papilio demoleus*, Papilionidae) prefer purple and blue flower models from amongst a broad array of colors (Ilse and Vaidya, 1955). The diversity of preferred colors reported in the literature is a function of a number of features, including the visual system of the butterfly, the choice of colors

offered by the investigator, the background color and the nature of the ambient illumination.

Innate color preferences are thought to provide behavioral biases that aid in the initial location or recognition of flowers (Goyret et al., 2008; Gumbert, 2000). Once at the flower, associative learning comes into play; moths and butterflies can readily associate colors (Crane, 1955; Goulson and Cory, 1993; Goyret et al., 2008; Kinoshita et al., 1999; Swihart, 1971; Weiss, 1997) or patterns (Kelber et al., 2002) (M. C. Wadlington and M.R.W., unpublished data) with sugar rewards, and avoid colors that lack reward (Kelber, 1996) or provide aversive stimuli (Rodrigues et al., 2010).

The extent to which learning modifies an insect's innate color preference is variable: in some cases preference for a trained color entirely replaces innate color preference (Goyret et al., 2008; Kelber, 1996) whereas in others the two exist in parallel (Swihart, 1971; Weiss, 1997). Conversely, innate color preference can also affect the rate of color learning. In some lepidopteran species, innately preferred flower colors are learned more rapidly than non-preferred colors (Kinoshita et al., 1999; Weiss, 1997), whereas in other species, moths or butterflies reach a criterion of proficiency in visiting non-preferred colors as rapidly as they do preferred colors (Kelber, 1996). Moths can also learn to associate a second color with a reward as rapidly as they do the first (Kelber, 1996).

The monarch butterfly, *Danaus plexippus* (Linnaeus 1758), is arguably the most widely recognized and popular butterfly species in the US. A frequent visitor to elementary school classrooms across the country, it is also the focus of innovative citizen-science programs designed to involve students and the general public in activities related to monarch biology and conservation (Monarch Watch, Lawrence, KS, USA, monarchwatch.org; Journey North, www.learner.org/jnorth). Many studies have focused on the species' incredible multi-generational migrations. Individuals may live six months or more, and make use of a time-compensated sun-compass navigational system to travel thousands of kilometers to overwintering sites in California and Mexico (Altizer et al., 2000; Froy et al., 2003; Malcolm, 1987; Mouritsen and Frost, 2002; Perez et al., 1997). Monarchs are known to use polarized light for oriented flight (Reppert et al., 2004), specifically by making use of specialized UV- and polarization-sensitive photoreceptors in the dorsal rim area of the compound eye (Labhart et al., 2009; Sauman et al., 2005). However, little is known about the color perception or learning ability of monarchs, despite the importance of visual information to butterfly behavior in the contexts of nectar foraging, host-plant location and mate recognition (Silberglie, 1984).

The 'hardware' associated with monarch color perception has recently been elucidated: monarch compound eyes contain three visual pigments, encoded by genes in the UV, blue and long-wavelength opsin clades, and at least three spectral classes of photoreceptor cell, with wavelengths of peak sensitivity corresponding to 340, 435 and 545 nm, respectively (Fig. 1A) (Frentiu et al., 2007a; Stalleicken et al., 2006). Filtering pigments, which in some species have been shown to facilitate color discrimination in the long-wavelength portion of the spectrum (Zaccardi et al., 2006), have been reported in the monarch eye (Fig. 1B and C) (Briscoe, 2008; Miller and Bernard, 1968; Sauman et al., 2005), suggesting that a fourth physiologically undescribed photoreceptor with sensitivity in the long-wavelength range also likely exists.

We used both theoretical and experimental approaches to address basic questions about monarch color vision and learning ability. We modeled the location of visual stimuli in the monarchs' perceptual color space using peak sensitivities of their three known types of

photoreceptors, and we examined the conditions under which the butterflies would, in principle, be able to discriminate amongst the colored papers used in our trials or to perceive narrow-band long-wavelength lights. We also calculated the brightness of each of the colored papers to determine whether this played a role in the choices being made by the butterflies. We then conducted a series of behavioral assays, using paper model flowers, in order to: (1) determine whether the butterflies could in fact distinguish amongst the colored models, (2) assess various aspects of their innate and learned color preferences and (3) verify that monarchs do indeed have true color vision. Lastly, we used intensity-controlled lights in order to validate the results of our modeling and determine whether the dark orange filtering pigments actually contribute to color discrimination in the long-wavelength range, as well as to corroborate our experiments using paper flowers.

MATERIALS AND METHODS

Study taxa

For experiments using model flowers, monarch pupae were obtained from Greathouse Butterfly Farm, Brooker, FL, or Monarch Watch, Lawrence, KS. Pupae eclosed in an experimental cage 30 cm wide, 50 cm deep and 30 cm high. Adults were maintained under a 14h:10h light:dark regime, and housing, training and testing were performed at 24–27°C under 60% relative humidity. For investigations of color perception independent of intensity using lights of specified wavelengths, pupae of *D. plexippus* were obtained from Shady Oak Nursery, Brooker, FL, and allowed to eclose in an experimental cage 55 cm wide, 70 cm deep and 88 cm high. The cage was illuminated from above by two to six 18W General Electric WarmWhite tubes, under a 14h:10h light:dark regime. The temperature in the cage was 29–31°C during training and testing.

Monarch color space calculations

The receptor-noise-limited color opponent model of Vorobyev and Osorio (Vorobyev and Osorio, 1998) as implemented in the program SPEC (for details, see Briscoe et al., 2010) was used to estimate the discriminability (ΔS) of pairs of colored papers and intensity-controlled colored lights used in the behavioral tests (see below). This model has been used extensively to explore the visual systems of animals as diverse as birds (Goldsmith and Butler, 2003), honeybees (Vorobyev et al., 2001) and butterflies (Briscoe et al., 2010; Koshitaka et al., 2008). Briefly, inputs include spectral sensitivity curves for the three known *D. plexippus* photoreceptors, approximations of which were generated using rhodopsin templates (Palacios et al., 1996) based on experimentally determined λ_{\max} values (Fig. 1A) (Frentiu et al., 2007a; Stalleicken et al., 2006), the irradiance spectra of the lights used to illuminate the paper flowers during behavioral testing, and the reflectance spectra data for the colored papers used to make the flowers. The relative number of receptor types in the monarch eye, n_i , was determined by counting the number of photoreceptor cells in adjacent sections of the same 102 ommatidia probed for *UVRh*, *BRh* and *LWRh* expression shown in Sauman et al. (Sauman et al., 2005). We note that this represents the number of each photoreceptor subtype over the entire sample of 102 ommatidia and not the actual number of photoreceptor subtypes in trichromatic ommatidia. Besides trichromatic ommatidia, two types of dichromatic ommatidia are present in the monarch retina, ommatidia expressing *UVRh* and *LWRh* only or *BRh* and *LWRh* only. The number of each photoreceptor type out of the 816 photoreceptors in our 102 scored ommatidia was 98, 106 and 612 for UV, blue and long wavelength, respectively, which approximately corresponds to a receptor ratio of 1UV:1B:6LW.

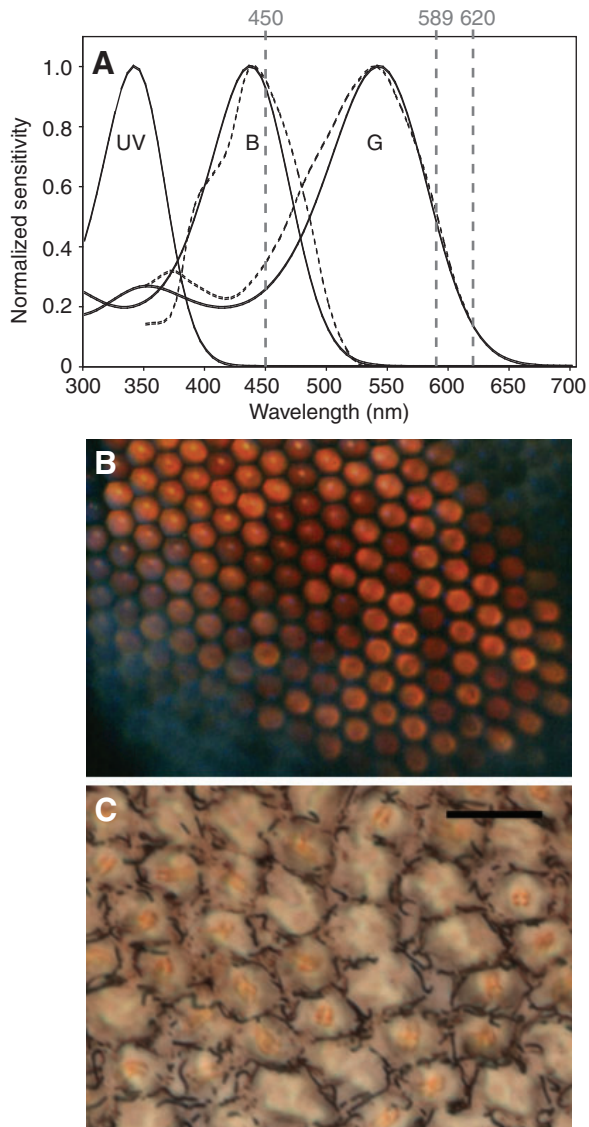


Fig. 1. Normalized sensitivities of three types of photoreceptor and eyeshine in the adult compound eye of *Danaus plexippus*. (A) Solid lines represent rhodopsin absorbance spectra based on the Bernard rhodopsin template in Palacios et al. (Palacios et al., 1996). Dotted lines represent spectral sensitivities of the blue [B] and long wavelength [LW] photoreceptors measured intracellularly by Stalleicken et al. (Stalleicken et al., 2006). Vertical lines represent emission maxima for lights used in choice assays. (B) Monarch dorsal eye showing red- and orange-reflecting ommatidia. Courtesy of Dr Gary Bernard. (C) Monarch cryostat sectioned eyeshine showing ommatidia that contain an orange filtering pigment adjacent to ommatidia lacking this filtering pigment.

Threshold values of $\Delta S \geq 1, 2$ and 3 in units of just noticeable differences (JNDs) were chosen to account for the difficulty in estimating true photoreceptor noise values in the butterfly and their potential impact on modeling results (Lind and Kelber, 2009). Under the receptor noise model, compared colors whose ΔS values exceed the threshold values are more likely to be perceived by monarchs as distinct colors, whereas colors that fall below these threshold values are likely to be indistinguishable.

To estimate the perceptual color distance between the paper flowers and the Styrofoam background, the color triangle model was modified for the monarch visual system. We used the sensitivity

functions of the three known photoreceptors described above, although as noted in the Introduction, it is likely that monarch eyes contain a fourth long-wavelength-sensitive receptor due to the presence of a heterogeneously expressed red filter pigment. Color loci were calculated using standard equations (Chittka and Kevan, 2005; Kelber et al., 2003), assuming photoreceptor adaptation to the background as a function of ambient illumination. Brightness was calculated as the sum of all three photoreceptor excitations.

Model flowers

To investigate innate color preferences and color learning, model flowers were constructed of papers in six saturated hues: red, orange, yellow, blue, purple and green (Color-aid Corp., Hudson Fall, NY, USA; papers catalogued as Rw-, O-, Yw-, B-, V- and Gw-hue, respectively). Reflectance spectra for all colored papers were obtained using an Ocean Optics USB2000 spectrophotometer using a deuterium tungsten halogen light source (DH2000) calibrated with a white Spectralon standard (Labsphere, North Sutton, NH, USA). Model flowers for color choice assays were created by inserting 200 μ l pipette tips into the centers of 4 cm diameter paper circles creased along two perpendicular diameters.

Innate color preferences, color training and testing were carried out using 24 paper flowers, four each of six colors, arranged in a 4 \times 6 grid, 6 cm apart (center to center) in one direction and 7 cm apart in the other, against a Styrofoam background. During trials, the array was placed inside a 30 cm wide, 50 cm deep and 30 cm high mesh cage, illuminated by two 250 W halogen lamps suspended at a height of 25 cm above the flower array on either side of the cage. The intensity of each lamp was 6.27×10^3 lux ($=4.47 \times 10^{-15}$ quanta $s^{-1} sr^{-1} cm^{-2}$) at a distance of 35 cm. The entire training array was rotated 180 deg between trials. None of the flowers in the training array contained a sucrose reward, and the position of four model flowers for each color occupied novel locations for each rotation position.

Innate color preferences

To determine the innate color preferences of monarchs and evaluate whether relative color preferences differed depending on the array of colors offered, we conducted two experiments. The first experiment utilized model flowers in six colors (red, yellow, blue, orange, green and violet), the second in three colors (yellow, red and blue). The six colors for our tests were chosen in order to offer the monarchs a range of wavelengths that were spread across the part of their perceptual color space that predominantly excited the blue and long-wavelength photoreceptors (see color triangle, Fig. 2B). The three-color array represents a subset of the six-color array.

Different butterflies were used in each experiment and food was withheld from all butterflies for 2 days following eclosion. Prior to an innate color preference trial, each individual's proboscis was gently unrolled with a dissecting pin and guided into a black paper model flower containing 20% sucrose solution in order to both expose the butterfly to the three-dimensional model and to stimulate interest in foraging. The black flower was illuminated by a 75 W soft white light at a distance of 20 cm, and the butterflies were allowed to feed for 5 s. Monarchs were then released individually into the array and their behaviors were recorded for 5 min. None of the models contained sugar water. We recorded the color and location of each flower that the butterfly probed; alights without probing were not counted. Location data were used to ascertain whether butterflies visited different individual flowers, rather than a particular area of the array. For butterflies in the six-color array, we also recorded time spent probing each model. Upon completion

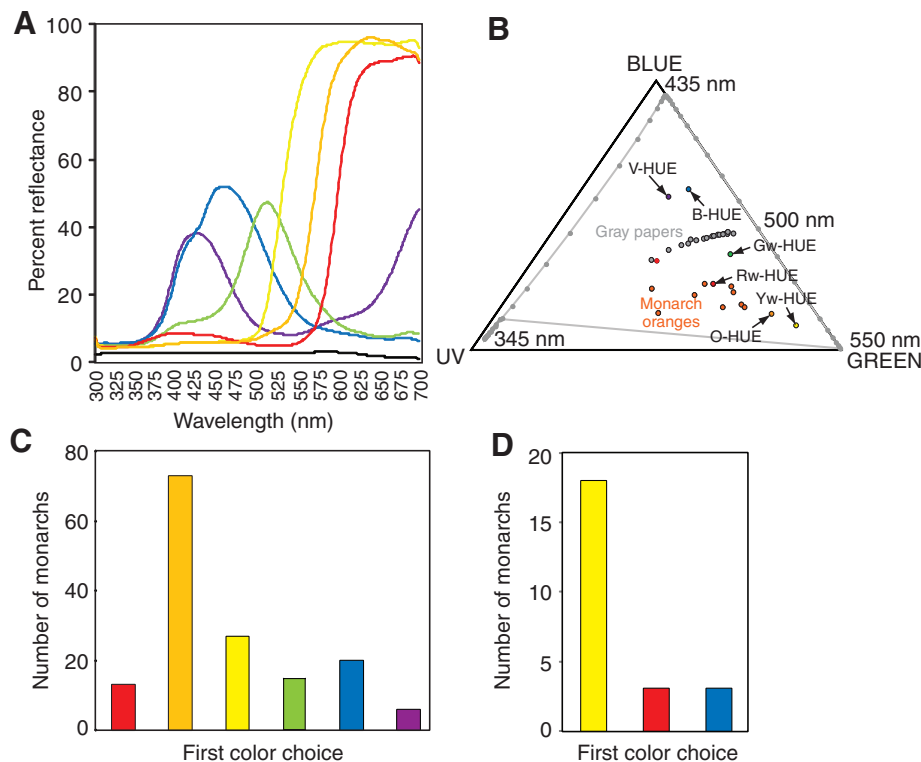


Fig. 2. Colored papers, color space triangle and innate color preferences of the monarch butterfly. (A) Spectral reflectance profiles for each colored paper model used in training and testing, and black model used in innate trials, obtained using an Ocean Optics USB2000 spectrometer. (B) The loci of colored and gray papers, and monarch orange wing pigments, represented in the color triangle model. Color distances are calculated as the Euclidean distance between two loci, assuming a trichromatic visual system, with Styrofoam used in the behavioral experiments as the adapting background and the halogen light as the illuminating light source. The orange circles represent monarch orange wing pigments sampled from the dorsal and ventral surfaces of the forewing and hindwing. Dark gray circles represent the black, white, and 17 gray papers. Arrows indicate colored papers. Red circle indicates the Styrofoam background. The gray line indicates the loci of pure spectral lights at background intensity. Brightnesses of the colored papers are: Rw-HUE, 0.4; O-HUE, 0.52; Yw-HUE, 0.68; Gw-HUE, 0.63; B-HUE, 0.75; V-HUE, 0.57. Since the filtered lights experiments (see text) demonstrate that monarchs can discriminate between colors (589 nm and 620 nm) that fall on the same color locus, independent of intensity, the color space shown is missing one dimension, that of a fourth (LW-sensitive) photoreceptor. (C) The initial color probed by naïve monarch butterflies offered six unrewarding paper models showed significant deviations from random choice, with orange being the most strongly preferred and purple the least preferred color. (D) When offered three unrewarding colored paper models, naïve monarch butterflies showed an overwhelming preference for yellow.

of the 5 min trial, butterflies were fed sugar water for an additional 55 s on the black model. Individuals that did not probe any models were given a second trial later in the day.

For both experiments, the first colored model probed by each individual was tallied across all butterflies and compared with an expected even distribution using a chi-square goodness-of-fit test. Differences between sexes in color choice were evaluated using a contingency table.

Parameters of learning

On the day following their six-color innate preference trials, we began to train butterflies to associate colors with food rewards. Different cohorts of butterflies were trained to each of the six colors. As in the innate preference trials, individuals were fed for 5 s before each trial to stimulate feeding behavior; however, this time we used a colored training flower identical in shape and size to the models in the array rather than a black model flower. We then released butterflies one at a time into the testing arena, which contained empty flower models of all six colors; we recorded both the location and time spent probing each flower. Following the 5 min trial, we fed each butterfly for an additional 55 s, so that each individual was rewarded for a total of 60 s day⁻¹ on its training color. All butterflies were tested and trained once a day for 8 days.

For the cohort of butterflies trained to each color, we examined the proportion of time that the butterflies spent probing their own training color as well as all of the other colors. Data for every 2 days of testing were combined (days 1 and 2, days 3 and 4, etc.) in order to reduce missing data points if an individual did not probe any models on a given day. Individual butterflies that spent less than a combined total of 20 s probing models over a 2 day testing sequence were not included in the analysis for those days, to avoid biasing the data set with butterflies that were not motivated to forage.

We examined three measures of learning: rate, proficiency and confusion with other colors. To assess rate of learning, we used a repeated-measures ANOVA to determine the first day on which the percentage of time spent on the training color differed significantly from the innate preference value for that color; arcsine transformations were applied where necessary to equalize variance. We defined and measured proficiency as the proportion of time each butterfly spent visiting the model flowers that matched its training color, and compared this value between cohorts of butterflies trained to each color for 8 days. Essentially this is a measure of 'success' for each training group. Finally, to assess the extent to which the butterflies confused a given trained color with other colors, we determined whether the percentage of time spent on the trained color

differed significantly from that spent on each of the five other colors on day four, halfway through the full training schedule (ANOVA followed by Tukey's *post hoc* comparisons).

Following their 8 days of color training, a subset of the monarchs was 'double-trained' – that is, they were subsequently rewarded on a different color for an additional 8 days. Specifically, blue-trained butterflies were rewarded on purple, purple-trained butterflies were rewarded on blue, and red-trained butterflies were rewarded on yellow. As with the single-trained butterflies, individuals that failed to probe flowers for a total of 20 s over two consecutive days of their second training sequence were excluded from the analysis for those days. We examined the color choices of these double-trained butterflies in order to determine whether having learned to associate one color with a food reward affected parameters of learning (rate, proficiency and confusion) for a second color. We used a two-way ANOVA to examine whether rates of learning for purple, blue or yellow trained as a second color were similar to those of butterflies trained to those colors singly. We used a Student's *t*-test to assess whether butterflies became as proficient on the second training color as they did on the single color by examining the time spent on the training color after the second 8 days of training. In addition, we compared the extent of confusion on day four of each training sequence for single-trained and double-trained butterflies.

True color vision

To determine whether monarchs could discriminate amongst colors in the training array by wavelength and not brightness, model flowers in 19 even-intensity steps from black to white were created as above (Color-aid Corp., Grayset g921). All of the gray flower models and one flower model of a trained color were inserted into a white Styrofoam base and arranged randomly in a 4×5 grid, 7 cm apart (center to center) in both directions. During trials, the array was placed inside a 30 cm wide, 50 cm deep and 30 cm high mesh cage, illuminated by two 250 W halogen lamps suspended at a height of 25 cm above the flower array on either side of the cage. The entire training array was rotated 180 deg between trials.

Butterflies trained to red, purple, blue or yellow for 8 days (described above) were tested on the ninth day using an array that consisted of the 19 gray flower models and one model of the butterfly's training color. The colored training models closely matched one or two of the grays in terms of average reflectance computed across the 300–700 nm range. None of the models contained sugar water. Each test butterfly was fed for 5 s on its colored model before it was released individually into the array; probing location and duration were recorded for 5 min. We compared time spent probing the single colored model with time spent probing all other gray models combined (because of very low visitation to the gray models) using a one-sample *t*-test.

Long-wavelength discrimination

The monarch compound eye expresses three distinct opsin-encoding mRNAs encoding ultraviolet-, blue- and long-wavelength-absorbing (LW) rhodopsins (Sauman et al., 2005). The adult eye also contains dark orange lateral filtering pigments coating the rhabdom of some ommatidia but not others, resulting in a heterogeneous orange and yellow eyeshine under brightfield illumination (Fig. 1B). In principle, this lateral dark orange filtering pigment may produce two kinds of long-wavelength-sensitive photoreceptor cell, one whose sensitivity is the result of the LW rhodopsin alone and one whose sensitivity is the result of the LW rhodopsin together with the dark orange filtering pigment. Intracellular recordings reported so far indicate that the adult monarch compound eye contains at least three spectral

classes of photoreceptor cells with wavelengths of peak sensitivity corresponding to 340, 435 and 545 nm (Stalleicken et al., 2006); on the basis of the pattern of eyeshine described above, it is likely there are more than three classes. For example, it is not yet clear whether the peak sensitivity of the 545 nm photoreceptor corresponds to the LW rhodopsin alone or to the LW rhodopsin plus the lateral dark orange filtering pigment, as the anatomical locations of the recordings were not noted in relation to the filtering pigment. Results of discriminability modeling suggest that monarch butterflies must indeed have a fourth, LW-sensitive receptor class, in order to distinguish the long-wavelength lights tested below. We provided monarchs with a honey solution in association with two different pairs of narrow-band colored lights, with the specific wavelengths chosen such that the butterflies would need to make use of information from the known blue and long-wavelength photoreceptors together, or from the long-wavelength and long-wavelength plus dark orange lateral filters together, to be able to perceive and discriminate the colors.

A pair of filters [450 nm (blue) vs 589 nm (yellow)] was chosen as a positive control to demonstrate that the butterfly uses its blue and green receptors together for color discrimination, on the basis of the overlap between the spectral position of the wavelength of maximal transmission of the 10 nm narrow band-pass filters and the spectral sensitivity curves of the blue and green photoreceptors (Fig. 1A, gray dotted lines). To demonstrate that the long-wavelength receptors and the heterogeneously arrayed dark orange filter pigments must work together for color vision in the long-wavelength range, a second set of narrow band-pass filters [589 nm (yellow) vs 620 nm (orange)] was chosen because their wavelengths of maximal transmission are outside the range of sensitivity of the blue photoreceptor but still within the range of sensitivity of the long-wavelength photoreceptor (Fig. 1A, gray dotted lines).

We used a feeder consisting of two 50.8 mm², 10 nm narrow band-pass filters (Edmund Optics) through which light, first diffused with a piece of waxed paper, was emitted from a KL2500 Schott cold light source (Elmsford, NY, USA). The light exiting the filter passed through a protective Plexiglas layer that could be removed for cleaning of the apparatus after each round of testing. The Plexiglas layer was cleaned using warm soapy water followed by ethanol. Beneath each feeder (approximately 2.5 cm below the spot of projected light) was a horizontal reservoir 3 mm wide for 20% honey solution.

Individual butterflies were allowed to feed on a training color, either yellow (589 nm) or orange (620 nm), and then offered a choice between blue (450 nm) and yellow or yellow and orange, depending on what color they had been trained on. The butterflies were trained on the feeders by holding their forewings together, 40 cm away from the feeders, such that the dorso-frontal region of the eye could view the testing apparatus (proboscis facing forward) but not the honey solution. The animals were gently and slowly waved back and forth in front of both the rewarded and unrewarded feeders, and allowed to probe the feeders. This was repeated until the animals could be released mid-air, such that they would fly to the rewarded feeder (see supplementary material Movie 1). Only instances in which animals flew to the correct feeder and unrolled their proboscis or landed on the rewarded light and probed with their proboscis were counted as correct choices. The animals learned to fly to the feeders and extend their proboscides after 3–4 days of training in which the animals were fed twice a day at the rewarded light.

We trained and performed dual choice tests with both yellow and blue filters illuminated, or orange and yellow filters illuminated. In the yellow vs blue test, only the yellow feeder contained honey solution whereas the blue feeder contained water. In the orange vs

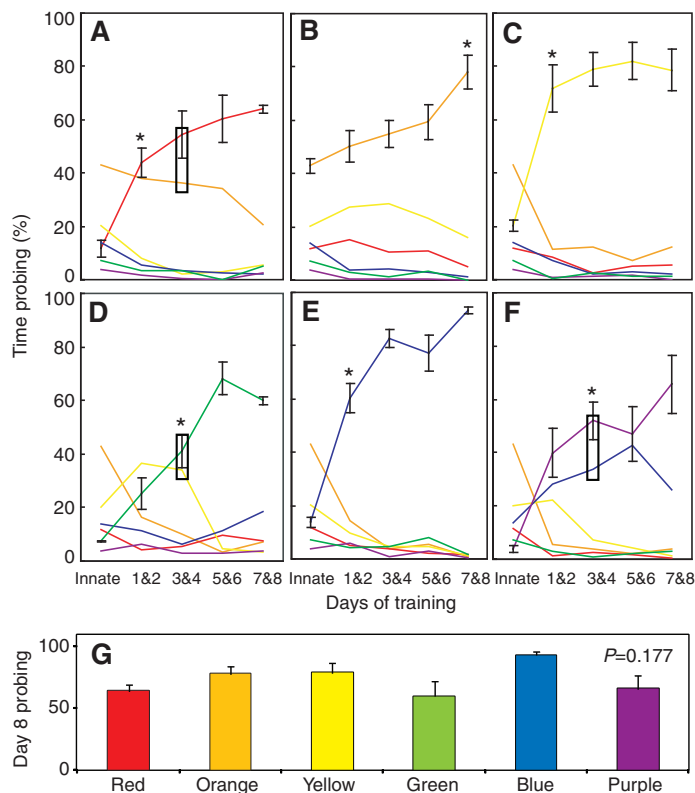


Fig. 3. Monarchs learned to associate colored model flowers with a sucrose reward. Following an assay for innate color preference, different cohorts of butterflies were trained to red (A; $N=22$), orange (B; $N=18$), yellow (C; $N=13$), green (D; $N=20$), blue (E; $N=23$) and purple (F; $N=11$) respectively, by feeding the monarchs on their assigned color for eight days. Asterisks (*) indicate the first day of training that differs significantly from innate preference for the trained color, by repeated measure ANOVA followed by Tukey *post hoc* comparisons. Boxes in A, D and F indicate non-significant differences (confusion) between the training color and another color at day 4. (G) Comparison of the time spent probing each training color after eight days of training did not differ across training color, indicating a comparable “success rate” following the training regime. Values are means \pm 1 s.e.m.

yellow test, only the orange feeder contained honey solution whereas the yellow feeder contained water.

In order to assess the relative importance of wavelength vs intensity, the intensity of the rewarded color (yellow or orange) and the unrewarded color (blue or yellow) was adjusted by changing either the electrical output of the lamps or the diaphragm such that the ratio of the intensities of rewarded and unrewarded color ranged from 0.1 to 4 (yellow vs blue) or 0.1 to 10 (orange vs yellow).

The radiant light from the 620, 589 and 450 nm filters was measured using an Ocean Optics USB2000 spectrometer (200–850 nm bandwidth, 25 μ m slit width) with a cosine corrector, and analyzed using the SpectraSuite software over a 20 nm bandwidth around each filter peak. The range of intensities produced by changing the lamp settings was 1.3×10^{14} to 6.78×10^{15} quanta $s^{-1} sr^{-1} cm^{-2}$. Butterflies were tested for ca. 2–3 h day^{-1} over a 12 h time frame, and different tests were performed in a pseudorandom order where intensity ratio and filter position were varied between choices.

RESULTS

Predictions from model calculations

As mentioned above, monarch eyes contain at least three spectral types of photoreceptor cell with λ_{max} values corresponding to ~340, 435 and 545 nm, respectively (Fig. 1A). Thus, the simplest assumption we could make about the monarch visual system is that it is trichromatic, like that of bees. The first set of modeling results discussed below is therefore based on the assumption that monarchs are using the three types of rhodopsin, in the absence of filtering pigments, to perceive colors. The discriminability of two colors, ΔS , is modeled in units of JND, where a threshold value of one has been proposed as the minimum value to discriminate between two color stimuli (Vorobyev and Osorio, 1998). Because of the inherent difficulty in determining actual

receptor noise values in butterfly eyes, and the demonstrated sensitivity of the model to this parameter (Lind and Kelber, 2009), we choose ΔS values of one, two and three JNDs for comparison. Comparing all colored papers used in the choice experiments in a hypothetical pairwise manner, and under the bright illumination used in training and testing, all 15 pairs of colors exceeded the ΔS thresholds of one and two JNDs, and 12 of 15 (all but blue vs violet, yellow vs orange and green vs red) exceeded the threshold of three JNDs under high halogen light intensity ($T=10,000$), making it likely that the colors can be discriminated using the three known photoreceptor types mentioned above. Comparing the discriminability of the narrow band-pass filters under bright illumination, 450 vs 589 nm exceeded the threshold of one and two JNDs and almost exceeded the threshold of three JNDs with only three photoreceptor classes, whereas the 589 vs 620 nm comparison did not even exceed the threshold of one JND. Therefore, in order to explain the ability of monarchs to discriminate these narrow-band colors as we show below, we must postulate the presence of a fourth photoreceptor cell class.

The spectral reflectance curves of the colored papers used in behavioral testing show a difference in shape (Fig. 2A); however, to examine whether the colored papers also differ in their appearance to the monarch’s visual system, we plotted their colors in a monarch-specific color triangle (Fig. 2B), which again assumes that monarchs are using the three types of rhodopsins, in the absence of filtering pigments, to perceive colors. The colored papers vary in their color contrast to the background (distance from the center of the plot), as well as in their hue. In this chromaticity plot, the pairs of colored papers that are closest in the perceptual space of the monarch are blue and violet, green and red, and orange and yellow. Brightnesses of the colored papers, defined as the sum of the photoreceptor excitations (Chittka and Kevan, 2005), from dimmest to brightest, were 0.4,

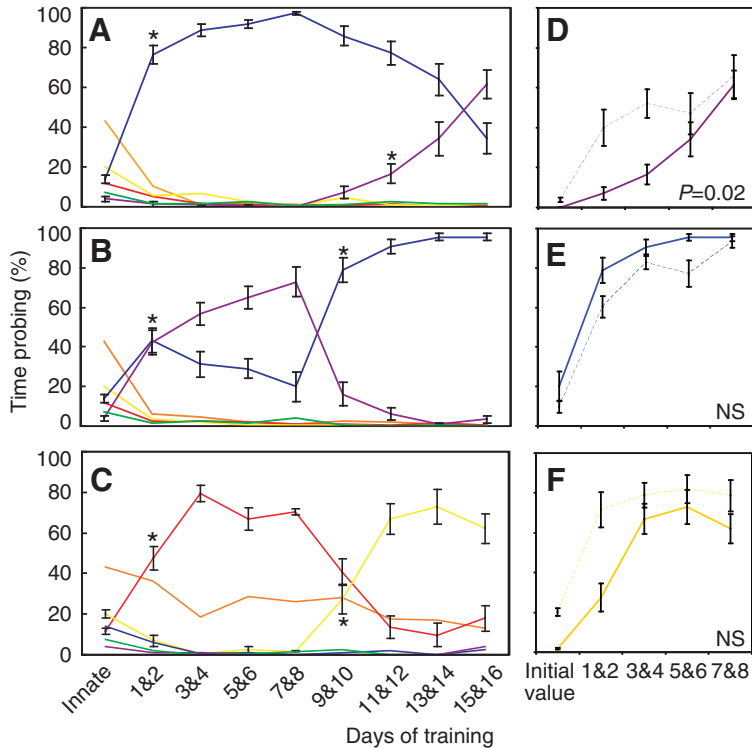


Fig. 4. Monarchs change preferences quickly when a new color becomes rewarding. Different cohorts of butterflies were trained to a single color for 8 days, at which time the color of the training model was changed. Monarchs were trained to blue followed by purple (A), purple followed by blue (B) or red followed by yellow (C). Asterisks (*) indicate values that differ significantly from innate preferences for each of the two training colors, by repeated measure ANOVA followed by Tukey *post hoc* comparisons. Values are means \pm 1 s.e.m.; $N=17$, 17 , and 16 , respectively. (D–F) Comparing learning rates for colors trained singly (dashed line) with those for the same color trained as a second color (solid line), blue and yellow were learned at the same rate whether they were the first or second color trained; however, butterflies showed a slower rate of learning for purple when it was trained as the second color.

0.52, 0.57, 0.68, 0.63 and 0.75 for red, orange, violet, yellow, green and blue, respectively.

Innate color preferences

Monarch innate preferences, measured as initial visits to a color, were assayed by offering naïve butterflies a choice of either six or three colors. Choices differed significantly from random in both the six-color and three-color arrays (six colors, $\chi^2=114.39$, d.f.=5, $P<0.001$, $N=154$, Fig. 2C; three colors, $\chi^2=18.75$, d.f.=2, $P<0.001$, $N=21$, Fig. 2D). In the six-color array, monarchs showed the strongest preference for orange (72 visits, 47%) and the weakest for purple (six visits, 4%). In the three-color array, the butterflies showed a strong preference for yellow (18 of 24 initial visits, 75%) and equally weak preferences for red and blue (three visits each, 12.5%). Male and female monarchs did not differ in their pattern of visitation in either trial (six colors, $\chi^2=6.93$, d.f.=5, $P=0.14$; three colors, $\chi^2=3.41$, d.f.=2, $P=0.18$).

Parameters of learning

Single-trained butterflies

Rate

Single-trained monarchs learned to associate all colors in the array with a sugar reward, but did so at different rates (Fig. 3A–F). Visitation to red, yellow and blue models increased significantly above innate values within the first 2 days of training (repeated-measures ANOVA, red, $F_{4,20}=28.508$, $P<0.001$, $N=22$; yellow, $F_{4,46}=7.97$, $P=0.005$, $N=13$; blue, $F_{4,17}=83.86$, $P<0.001$, $N=23$) whereas visits to purple and green models did so within 4 days and orange within 8 days (purple, $F_{4,8}=5.18$, $P=0.002$, $N=11$; green, $F_{4,22}=22.35$, $P<0.001$, $N=20$; orange, $F_{4,11}=5.19$, $P=0.006$, $N=18$).

Proficiency

Despite differences in the rate of learning, by day eight butterflies in all of the color training groups showed similar proficiencies for their trained colors (ANOVA, $F_{5,48}=1.60$, $P=0.177$; Fig. 3G).

Confusion

The extent to which single-trained butterflies were ‘confused’, or visited non-trained colors, varied with training color. After day four, butterflies trained to orange, blue or yellow did not confuse their trained colors with any other; that is, the percentage of time spent probing the trained color was significantly different from that on all other colors. In contrast, after the same time interval, red-trained butterflies also visited orange models, purple-trained butterflies also visited blue models and green-trained butterflies also visited yellow models (boxes, ANOVA followed by pairwise comparisons, $P>0.05$ in all cases; Fig. 3A,D,F). This confusion was asymmetrical; that is, yellow-trained butterflies did not make mistakes on green, blue-trained butterflies did not make mistakes on purple and orange-trained butterflies did not make mistakes on red (Fig. 3A–F).

Double-trained butterflies

Rate

When butterflies that had been trained to an initial color were subsequently offered sucrose associated with a second color, they chose the novel color once it became rewarding, but the rate at which the second color was learned depended on the color (Fig. 4A–C). In the purple I–blue II training regime (Fig. 4A), butterflies showed a significant increase in preference for blue within two days of switching to a blue reward (repeated-measures ANOVA, $F_{8,30}=52.30$, $P<0.001$, $N=17$), but in the blue I–purple II training regime (Fig. 4B) they did not significantly favor purple until 4 days after switching (repeated-measures ANOVA, $F_{7,22}=4.34$, $P=0.003$, $N=17$). In the red I–yellow II regime (Fig. 4C), the butterflies favored yellow after 4 days of training to yellow (repeated-measures ANOVA, $F_{8,24}=32.87$, $P<0.001$, $N=16$). Blue and yellow were learned at the same rate whether they were the first or second color trained (two-way ANOVA, blue, $F_{4,158}=0.86$, $P=0.49$; yellow, $F_{4,112}=2.05$, $P=0.09$); however, butterflies showed a slower rate of learning for purple when it was trained as the second color (two-way ANOVA, $F_{4,127}=2.98$, $P=0.02$, $N=28$) (Fig. 4D–F).

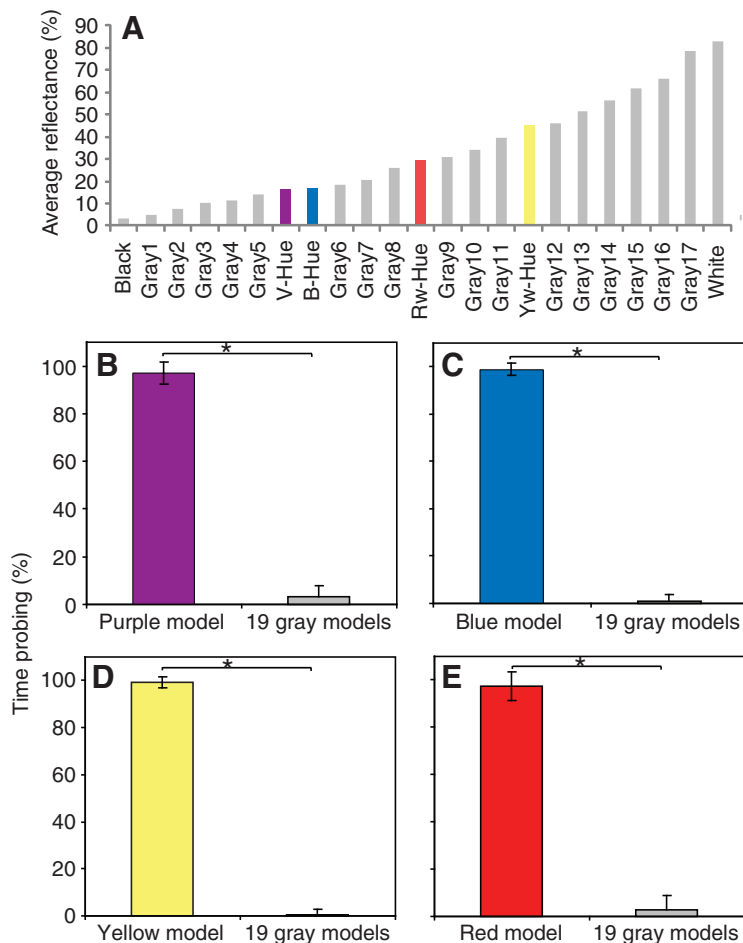


Fig. 5. Monarchs discriminate colors based on wavelength and not intensity. After being fed on a colored model flower for 8 days, monarchs were offered a choice between their colored training model and 19 gray models of varying brightness in an array where none of the artificial flowers contained nectar. (A) Intensity of each model offered in the choice array. Cohorts of butterflies trained to different colors, purple (B), blue (C), yellow (D) or red (E) were able to accurately discriminate between the single colored model and all the gray models in the arena. Significance was assessed by single sample *t*-test. Values are means \pm 1 s.e.m.

Proficiency

Monarchs reached the same level of success after 8 days of training to a second color as they did during single color training (*t*-test, $P > 0.05$ in all cases).

Confusion

Confusion on the second training colors mirrored that seen in single-color training: no confusion was evident when butterflies were trained to yellow or blue as the second color, but butterflies trained to purple as a second color continued to visit blue models.

True color vision

Although the average reflectance of training color closely matched that of one or two models in the grayscale array for all four colors tested (Fig. 5A), monarchs spent significantly more time probing the single model of their trained color than they did all 19 of the gray models combined, in every case (yellow, $t_{12} = 144.12$, $P < 0.001$, $N = 13$; blue, $t_4 = 84.50$, $P < 0.001$, $N = 5$; purple, $t_9 = 62.18$, $P < 0.001$, $N = 10$; red, $t_{14} = 57.99$, $P < 0.001$, $N = 15$; Fig. 5B–E). Individuals generally spent more than 95% of their time probing the colored model, and in the rare instances in which gray models were probed, the overwhelming number of visits was to the gray models in closest physical proximity to the colored model, rather than to those that matched it in intensity. No individual gray model was visited by more than one monarch.

Long-wavelength discrimination

For each of the nine monarchs, a total of 28–41 choices were registered (Table 1), and choices were summed over three intensities (0.1, 1 and 4) for the yellow (589 nm) vs blue (450 nm) experiments and over three intensities (0.1, 1 and 10) for the orange (620 nm) vs yellow (589 nm) experiments (Fig. 6).

At each of the three relative intensities tested, the correct number of choices was compared against the expected proportion of 0.5 using a one-tailed binomial test, as it is hypothesized that monarchs can discriminate between the tested colors (i.e. their correct proportion of choices should be significantly higher than 0.5). All nine animals tested chose the correct color at a statistically significantly higher frequency ($P < 0.05$) than chance at all three relative intensities, except for monarch 18, which died before enough choices could be registered, and whose correct number of choices was only significant ($P < 0.05$) at the relative intensities of 0.1 and 1 (see Table 1 for exact *P*-values). Our results from the tests with colored lights ranging from 450 to 620 nm indicate that *D. plexippus* has color vision in the 450–620 nm range; we did not test the response of butterflies to light in the UV range.

DISCUSSION

Surprisingly, until now, little has been known about what colors monarch butterflies can see or how well they can learn (Reppert et al., 2004; Rodrigues et al., 2010; Sauman et al., 2005). Our results paint a comprehensive picture of monarchs' capacities with respect to color vision and color learning. We have shown that monarchs

Table 1. *P*-values of dual-choice tests using monarch butterflies

Monarch	<i>P</i> -value as a function of intensity rewarded/intensity unrewarded			<i>N</i>
	0.01	1	4	
589 nm (+) vs 450 nm (-)				
14	0.0002	0.0016	0.0039	36
17	0.0001	0.0010	0.0039	32
18	0.0002	0.0439	0.0938	28
25	0.0085	0.0095	0.0010	39
620 nm (+) vs 589 nm (-)				
31	0.0010	0.0002	0.0161	34
33	0.0490	0.0386	0.0386	41
35	0.0020	0.0010	0.0078	29
39	0.0063	0.0034	0.0352	40
40	0.0215	0.0063	0.0386	34

P-values are the results of one-tailed binomial tests.
N, number of choices registered.

have a broad visual spectrum, true color vision, strong but malleable innate color preferences and the ability to learn colors rapidly and reversibly.

Color vision

Results from our behavioral studies corroborate the predictions of our color-space modeling. Monarchs were indeed able to discriminate amongst the six colored paper models, and though it may be possible for them to do so using only three spectral classes of photoreceptor, discrimination between the orange and red models is likely improved through the use of their lateral filtering pigments. Furthermore, we have established that the monarchs can make use of their LW rhodopsin, together with the dark orange lateral filtering pigments, to perceive colors in the long-wavelength range.

Not all butterflies have eyes that contain orange or red filtering pigments (Briscoe and Bernard, 2005) but for those that do, filtering pigments have been shown to narrow the shape of spectral sensitivity curves (e.g. *Papilio*) (Arikawa et al., 1999) by filtering short-wavelength light, and also red-shifting the sensitivity peak of the photoreceptor cell expressing the green rhodopsin, although the overall wavelength range of sensitivity does not change. Together with ommatidia that express only the unfiltered *LWRh* opsin, color vision in the long-wavelength range with only one LW rhodopsin has been demonstrated in *Heliconius erato* (Nymphalidae) (Zaccardi et al., 2006).

We suggest that monarchs are similar to *H. erato* in having filter-pigment-mediated color vision in the long-wavelength range, as this is the only explanation of the results of our dual-choice experiment using 589 vs 620 nm narrow-band filters (Fig. 6B). This theoretical and behavioral result provides a compelling reason to search for and characterize the spectral sensitivity of the fourth (long wavelength) photoreceptor class in future studies. Monarch color space may, therefore, be better represented by a tetrahedron rather than the color triangle shown in Fig. 2B. Although it is probable that monarchs, like other butterflies with a UV-sensitive rhodopsin, can perceive UV light, we did not explicitly test their color vision abilities in the 340–440 nm range such that we could rule out through testing and modeling the contribution of other photoreceptor classes. Thus, although we assumed in our discriminability (Vorobyev and Osorio, 1998) and color-space modeling (Chittka et al., 1992; Kelber et al., 2003) that they are using their UV photoreceptor, we cannot say definitively that they are tetrachromats. Tetrachromacy has recently been demonstrated in a papilionid butterfly with five opsins expressed in the eye and eight varieties of photoreceptor because

of the presence of several kinds of lateral filter pigments in combination with the opsins (Kinoshita et al., 1999).

Results from both the grayscale and light-intensity experiments (Kelber et al., 2003) confirm that monarchs have true color vision, as they are able to use wavelength, independent of intensity, to discriminate amongst colors in a testing array. True color vision has been demonstrated explicitly for several butterfly and moth

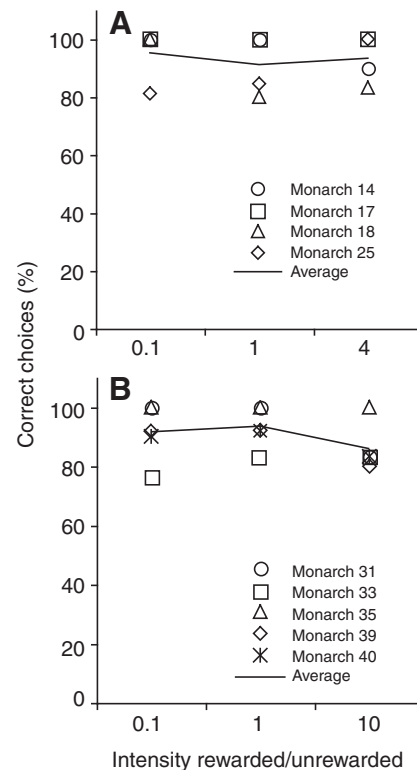


Fig. 6. Choice frequencies of *Danaus plexippus* for three colors after training, as a function of the ratio between the intensities of the rewarded color and the unrewarded color. The symbols represent the individual performance and the line the average. (A) Four *D. plexippus* trained to 589 nm as the rewarded color and 450 nm as the unrewarded. All correct choices are significantly higher than chance (*P*<0.05) except for Monarch 18 at the intensity rewarded/unrewarded ratio 4. (B) Five *D. plexippus* trained to 620 nm as the rewarded color and 589 nm as the unrewarded. The correct choices of every animal are significantly higher than chance (*P*<0.05).

species (Kelber and Henique, 1999; Kelber and Pfaff, 1999; Kinoshita et al., 1999; Sison-Mangus et al., 2008), and is likely to be common across Lepidoptera.

Innate color preferences

Monarchs' relative innate color preferences differ depending on the choice of colors offered. When presented with a six-color array, both male and female butterflies showed a strong innate preference for the color orange, choosing it in close to 50% of initial visits. Yellow, the next most preferred color, was chosen less than half as often as orange; blue was chosen less often than yellow, and red still less. Relative innate preferences differed markedly, however, in the three-color array: yellow was favored fourfold over red and blue, each of which was visited equally often. Relative preference hierarchies can differ further in binary choice tests. For example, although monarchs showed similar innate preferences for red and green in our six-color array, they strongly preferred red when given the choice of only red and green models (mean±s.e.m., visits to red, 6.8±1.36, visits to green, 1.0±0.45, $N=10$ butterflies; D. Rodrigues, unpublished data). Thus it would be possible to conclude that monarchs have strong innate preferences for orange, yellow or red, depending on the assay used. Clearly it is difficult (or even misleading) to compare innate preferences across species, or even between studies within a species, as the results will depend on the constellation of choices offered.

Although the monarchs' strong innate preference for orange (as seen in the six-color array) may bear no direct relationship to the butterflies' orange coloration, it is possible that the insects, based on the need to recognize conspecifics, might have developed a 'sensory bias' for orange that is also expressed in the context of nectar foraging. Indeed, comparison of the orange pigments sampled from the dorsal and ventral surfaces of the monarch forewing and hindwing indicates that the orange paper is somewhat similar in hue, as defined in the color triangle of Chittka and Kevan (Chittka and Kevan, 2005), to some of the sampled wing pigments, though not identical in color contrast to any particular orange wing patch (Fig. 2B). Alternatively, a pre-existing bias in the monarchs' sensory system may have selected for the orange coloration of the insects. At this point, there is no evidence, one way or the other, for either scenario. For some taxa, it has been suggested that innate color preferences related to dietary needs can be manifested in other behavioral contexts. In zebrafish, for example, a female preference for red coloration on males may reflect a dietary preference for red that is based on a need for carotenoids in the diet (Spence and Smith, 2008). Similarly, many flowers have bright yellow nectar guides or other floral structures that seem to mimic anthers (Heuschen et al., 2005; Lunau et al., 2006; Lunau and Knüttel, 1995). These yellow guides may exploit bees' innate preference for yellow (Pohl et al., 2008), which is correlated with their dependence on pollen in the diet; further, the presence of yellow nectar guides on flowers may prevent bees from learning to discriminate against unrewarding floral morphs (Pohl et al., 2008).

Color learning

Monarchs readily learned to associate all six of their training colors with a sucrose reward. After 8 days of training (or, in some cases, considerably less), involving only 60 s of reinforcement per day, all groups of butterflies spent at least 60% (and up to >90%) of their probing time on their trained color, when only 16.7% would be expected by chance. Proficiency (measured as time spent probing the correct color after 8 days of training) did not differ significantly across training colors, including green, a color not ordinarily

characteristic of butterfly-pollinated flowers. Studies of other lepidopteran species, including hawkmoths (Goyret et al., 2008; Kelber and Henique, 1999) and papilionids (Kinoshita et al., 1999; Weiss and Papaj, 2003), show similarly high levels of discrimination in favor of the trained color.

Although the monarchs became equally proficient at learning all six colors, they did so at apparently different rates and with different degrees of confusion. Interestingly, non-innately preferred colors (again, in the six-color array) were learned rapidly; time spent probing on red, yellow and blue models increased significantly above the innate value for each color within the first 2 days of training (and may have reached that level after only a single day), i.e. after only 60–120 s. In contrast, time spent probing orange increased significantly above the innate value for orange only after 8 days of training; this long interval most likely reflects the difficulty in reaching a level of visitation significantly higher than the already high innate value rather than a propensity to learn orange more slowly. Indeed, if we were to define the rate of learning using the number of days required for each cohort of butterflies to visit a color significantly more than the 16.7% of time expected by chance, rather than by days to surpass innate preference for the color, then we would see that visitation to orange starts above chance (ANOVA, $P=0.008$, $N=18$), and all other colors reach significance within the first 2 days of training. Like the monarchs, day-flying hawkmoths (*Macroglossum stellatarum*) (Kelber and Henique, 1999) learn most colors rapidly, irrespective of whether they are innately preferred. However, two papilionid taxa, *Battus philenor* (Weiss, 1997) and *Papilio xuthus* (Kinoshita et al., 1999), seem to learn innately preferred colors more rapidly than non-preferred colors. Honey bees also are thought to learn innately preferred colors more rapidly than non-preferred colors (Giurfa et al., 1995; Menzel, 1985). As with the current data, it is likely that in all cases, interpretations of learning parameters reflect both the choices offered and definition of learning rate used.

Confusion, measured as the extent to which butterflies visited non-trained colors, varied with training color. After day four, butterflies trained to orange, blue or yellow did not confuse their trained colors with any other, but red-trained butterflies also visited orange models, purple-trained butterflies also visited blue models and green-trained butterflies also visited yellow models. Moreover, for butterflies trained to purple and green, time spent on blue and yellow, respectively, actually increased in parallel with the increase in time spent probing the trained colors, providing further evidence for confusion as opposed to maintenance of innate preference.

Interestingly, confusion between colors was asymmetrical; that is, yellow-trained butterflies did not make mistakes on green, blue-trained butterflies did not make mistakes on purple and orange-trained butterflies did not make mistakes on red. It is notable that for each pair of colors in which the monarchs showed confusion (red/orange, purple/blue, green/yellow), the asymmetry is directly linked to the brightness of the stimulus, as defined by the sum of excitation of all three photoreceptor classes. Butterflies trained to the dimmer color always 'made mistakes' on (i.e. also visited) the brighter color, whereas the reverse was not true. In the cabbage white butterfly, *Pieris brassicae*, feeding responses depend on both wavelength and intensity, with butterflies preferentially choosing brighter colors at a given wavelength (Scherer and Kolb, 1987); a similar response may also occur in the monarch. Along the same lines, the hummingbird hawkmoth, *Macroglossum stellatarum*, is able to distinguish similar wavelengths by both intensity and wavelength, but gives more weight to chromatic aspects of decisions when both are present (Kelber, 2005; Kelber and Henique, 1999).

In our experiments using the paper models, intensity and wavelength are presented as simultaneous cues and individuals appear to focus on the brighter color of the pair.

Apart from perceptual differences, asymmetries in similarity judgments, including color confusion, seem to be widespread in animals (e.g. Nosofsky, 1991; Tascini et al., 2006). For example, in experiments with chicks simultaneously trained to one rewarded color and one unrewarded color, and given a choice of several alternative colors designed to be an equal distance from the trained color in bird color space, the alternatives were not treated equally, for reasons that are not entirely clear (Osorio et al., 1999).

Asymmetrical color learning may have implications for flowers with respect to butterflies as pollinators. Butterflies that nectar at purple flowers, for example, might show less fidelity to those flowers than would butterflies nectaring at blue flowers, potentially leading to selection for flower colors that are less prone to confusion. Extension of these experiments to field studies may help explain why butterflies in natural settings do not automatically forage preferentially at flowers with the highest nectar standing crop (Neumayer and Spaethe, 2007), but are constrained by tradeoffs in the detection and processing of chromatic vs achromatic flower cues (Spaethe et al., 2001).

High fidelity to the trained color, and a low level of confusion with other colors, can result when an innately preferred color is either reinforced or countered by learned associations. Insects may visit an innately preferred color almost exclusively when it also provides a reward (Hill et al., 1997; Weiss, 1997). As is seen with the monarchs, visitation to an innately preferred color can also diminish or disappear with increased experience on an alternative rewarded color (Goyret et al., 2008; Kelber, 1996). However, in some other species, innate color preferences persist in parallel with learned associations, regardless of duration of experience with an alternative color (Swihart, 1971; Weiss, 1997), yielding lower levels of proficiency at the trained color and higher levels of confusion with the innate color.

Results of our double-training experiments demonstrate that monarchs can learn new colors readily, as has been shown for some other lepidopterans (Kelber, 1996; Weiss, 1997). Learning parameters following training to a second color show many similarities to those evident after single-color training; monarchs reach the same level of proficiency after 8 days of training on the second color as they did for the first color and show the same asymmetric confusion between pairs of colors (e.g. blue and purple).

In summary, monarch butterflies are proficient and flexible color learners. With a broad visual spectrum and an ability to learn colors rapidly and reversibly, monarchs are likely to be able to respond quickly to changing nectar availabilities as they explore their environments in both space and time. Our results, which integrate animal behavior, physiology and modeling, provide useful insights that will aid in our understanding of the ecological dynamics of flower and host choice in this well-known species, both in their summer ranges and as they travel to and from their winter habitats.

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