Reward Tracking and Memory Decay in the Monarch Butterfly, *Danaus plexippus* L. (Lepidoptera: Nymphalidae)

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Abstract

Due to their long-distance migration routes and high longevity, monarch butterflies (*Danaus plexippus)* are likely to benefit from learning how to discriminate and remember suitable feeding resources. In this study, we assessed monarchs' abilities to track changing nectar sources over time and to retain learned information presented in two conditioning schedules. Non-preferred (blue and red) and preferred (yellow) artificial flowers were concomitantly offered to monarchs in a three-phase experiment. In each phase, flowers of only one color contained sucrose solution, while the others contained water. The rewarding color was changed in each phase. Instantaneous observations were made to assess butterfly visits to each color during each phase; continuous observations over the first 90 min of a new phase allowed us to look in more detail at the transition process. Overall, monarchs tracked sucrose availability, visiting the rewarding flowers more often than the unrewarding ones, regardless of innate preferences. However, butterflies reverted to innate color preferences when the newly rewarding color was different from the initial trained color. In a second experiment, memory decay was compared for butterflies trained according to two schedules: 'single training' (sucrose solution in red vs. water in blue artificial flowers in one 15-min session per day) or 'intermittent training' (as above, but in two 7.5-min sessions per day). Afterwards, butterflies were tested on alternate days for a week in arrays containing unrewarding models of both colors. Following either training schedule, memory persisted for at least 3 d after reinforcement ceased. Our findings reveal that monarchs are able to change their feeding responses according to the flowers' reward status despite innate preferences, as well as to retain flower information for about half a week regardless of the conditioning dynamics.

Introduction

Memory, defined as the retention of newly acquired information over time (see Dukas 2008), has been a subject of recent interest in cognitive studies. Memory of learned information may allow organisms to forage more efficiently, improve escape from natural enemies, and locate high-fitness mates and suitable habitats, all of which may lead to increased fitness (Dukas 2008). Within insects, although memory dynamics have been investigated in a broad taxonomic range of insects, it has been studied more intensely in social

insect species than in solitary groups. Honeybees have been shown to retain olfactory information for at least 4 d, but are able to remember information regarding flower color for about 2 wks after contacting rewarding flowers for the last time (Menzel & Erber 1978; Menzel & Greggers 1992; Hammer & Menzel 1995). Seed-harvester ants fail to recall information regarding feeding resources in a matter of days, and the decay of memory is slower than its acquisition (Johnson 1991). Workers of *Odontomachus troglodytes,* the trap-jaw ant, retain information about prey possessing secretory reflexive defenses for almost a

month after last contact (Dejean 1988). With respect to solitary insects, immature crickets can recall learned olfactory information for weeks in the absence of reinforcement, and such information can be easily reversed in case the training odor changes (Matsumoto & Mizunami 2002a). Retention of information across complete metamorphosis has recently been demonstrated in the moths *Manduca sexta* (Blackiston et al. 2008) and *Trichoplusia ni* (Shikano & Isman 2009), where adults retain olfactory and gustatory information learned in the larval stage. Adults of the moth *Macroglossum stellatarum* have been also shown to be able to remember flower information over a span of 3 wks, when they emerge from a period of hibernation (Kelber 2010).

Differences in persistence of insect memory have been shown to depend on temporal dynamics of conditioning (Menzel 1999; Matsumoto & Mizunami 2002b; reviewed in Hoedjes et al. 2011) and can be found even among closely related species whose environments (i.e., hosts) differ (Bleeker et al. 2006). With respect to flower-visiting insects, the time flowers are available (i.e., daily flowering periods) plays a role in the visitors' activity (e.g., Barp et al. 2011). In turn, both the frequency and the timing of pollen and nectar foraging may influence how long the learned information will persist in the central nervous system (see Menzel 1999). Bees, moths, and butterflies have been shown to be able to switch rapidly as the color of the rewarding flower changes (Goulson & Cory 1993; Kelber 1996; Weiss 1997; Gumbert 2000), resulting in a synergistic association between flower constancy (i.e., memory) and ability to learn novel suitable nectar sources.

In butterflies, the most well-studied phenomenon involving the persistence of memory (that is, the time that information is retained in the nervous system) is flower constancy (Goulson & Cory 1993; Goulson et al. 1997), where butterflies continue to visit a rewarding type of flower, despite the availability of other rewarding flowers. In addition, memory constraints were first demonstrated in *Pieris rapae,* where flower-handling abilities vary according to previous experience (Lewis 1986). Studies of *P. rapae* have also shown that memory of a formerly rewarding flower color persists for at least 3 d (Kandori & Ohsaki 1996). However, longevity of memory in the absence of reinforcement, as well as duration of flower color information under different training schedules, has not to date been investigated in butterflies.

The monarch butterfly (*Danaus plexippus*) is a long-lived nymphalid that has been extensively examined with respect to its interaction with milkweeds (Malcolm 1994; Zalucki et al. 2001; Helmus & Dussourd 2005), role as a Batesian model (Brower 1988; Ritland & Brower 1991), and remarkable long-distance, multi-generational migration (Calvert & Brower 1986; Mouritsen & Frost 2002). Because monarchs are likely to encounter a large variety of nectar sources over the course of yearly migrations that can extend from southern Canada to central Mexico (Ackery & Vane-Wright 1984), an ability to associate floral features with nectar quantity and/or quality is likely to be of great importance. Surprisingly, however, until recently very little attention has been paid to monarch learning abilities (Rodrigues et al. 2010; Blackiston et al. 2011). Recent studies have shown that in the context of nectar foraging, monarchs show strong innate color 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 (Blackiston et al. 2011). In addition, monarchs can be conditioned to avoid aversive stimuli, prefer to visit flowers with constant rather than variable nectar volume (Rodrigues et al. 2010), and can learn to make use of floral nectar guide patterns (M. R. Weiss, M. C. Wadlington & D. Rodrigues, unpubl. data).

Although monarchs clearly learn to associate color with rewarding or aversive stimuli, the dynamics of their memory retention have not been explored in any detail. Blackiston et al. (2011) found that monarchs will switch to a newly rewarding flower color within a period of 2 d; however, it is likely that they will do so much more rapidly – perhaps on a scale of minutes. In addition, it is possible that butterflies, when confronted with a novel rewarding color that does not resemble the initially rewarding color, will revert to innate color preferences, as Gumbert (2000) found for bumblebees. Furthermore, it is not known how long monarchs will continue to visit flower colors after they have ceased to offer a reward, or whether timing of the initial training schedule affects duration of memory.

In this study, we explore the dynamics of learning and memory over short-term time scales and examine memory decay in monarchs subjected to different conditioning schedules. Specifically, we asked: (1) How long does it take for monarchs to shift to newly rewarding resources? (2) Over a short-time scale, how do monarch butterflies react when a previously rewarding color no longer offers a reward? (3) Do monarchs revert to innate preferences in the face of an unfamiliar reward? (4) How long does memory last in the absence of reinforcement? (5) Does the conditioning schedule influence the rate of monarchs' memory decay?

Methods

Insects and General Procedures

Monarch pupae were purchased from the following suppliers: Monarch Watch (University of Kansas – Kansas), Live Monarch Foundation (Florida) and Great House Butterfly Farm (Florida) and inspected daily until emergence. Newly emerged adults were separated by sex, marked individually, and placed in green mesh 0.3 m³ cages (maximum of five butterflies per cage). Cages were maintained under controlled conditions (25 ± 2°C; 14L: 10D). Twenty-four h after emergence, butterflies were fed for one min with 20% sucrose solution (hereafter 'nectar') in order to standardize hunger levels; the proboscis was gently unrolled with a dissecting pin and inserted into the central well of a black artificial flower containing the sucrose solution under a 75-watt soft white incandescent lamp (see Rodrigues et al. 2010; Blackiston et al. 2011).

All adults were 2 d past emergence and had no experience with colored artificial flowers when the experiments began. Experiments were conducted under controlled conditions, as described above, and number of visits to each colored artificial flower was recorded. Visits included all behaviors performed on a given artificial flower, as follows: landing (arriving on a given flower through flying or walking), exploring (nectar searching through proboscis extension), probing (consuming nectar through feeding bouts of 1-s duration), drinking (consuming nectar through feeding bouts longer than 1 s), and resting (no locomotion or other behaviors described above).

Reward Tracking

Artificial flowers

Paired artificial flowers were constructed out of two-part plastic Petri dishes (8.5 cm diameter × 1 cm high) with two holes drilled in the upper dishes, one forming the center of each 'flower.' Holes were 2 mm diameter and located 4.2 cm apart from each other. Two smaller (3.5 cm diameter) plastic Petri dishes were glued to the inside of the bottom dish, one below each of the two holes, to contain nectar or water, depending on the situation. One four-cm-diameter circle of matte-finish Coloraid™ colored paper (red, Rw-Hue; blue, B-Hue; yellow, Yw-Hue) was centered on and glued around each hole, so that every Petri dish had one of the following two-color combinations: blue and yellow, blue and red, or red and yellow (Fig. 1). Two Petri dishes having each

two-color combination (total = six Petri dishes; 12 artificial flowers) were offered per training occasion. Artificial flowers were placed pseudo-randomly (i.e., flowers having the same colors were not placed next each other) on the bottom of the cage over a 0.6 m² white sheet of cardboard and rotated daily because location is a spatial cue that improves learning performance in some insect species (e.g. Ney-Nifle et al. 2001).

Instantaneous observations of phases

To examine the dynamics of flower choice when the rewarding color changes, adults were placed indoors into 0.6 m³ green mesh cages (five of one sex at a time for a total of 20 butterflies; 10 males:10 females), under controlled temperature (25 ± 2°C) and sunlight, and observed according to the following schedule: training on blue artificial flowers from day three to six (phase 1, or blue phase); training on red artificial flowers from day eight to eleven (phase 2, or red phase), and training on yellow artificial flowers from day 13 to 16 (phase 3 or yellow phase) (see Fig. 1 for color reflectance). Between each phase (that is, on days 7 and 12), butterflies were not trained and were fed with nectar on a black artificial flower for one min. Blue, red, and yellow were chosen as test colors because in an innate preference test using a three-color array, monarchs showed a strong preference for yellow (75% of visits), and equally weak preferences for red and blue (12.5% of visits to each color) (Blackiston et al. 2011). The experiment ended with nectar present in the yellow flowers, as we wanted to avoid reinforcing the innately preferred color at the beginning of the experiment (see Kelber 1996). We
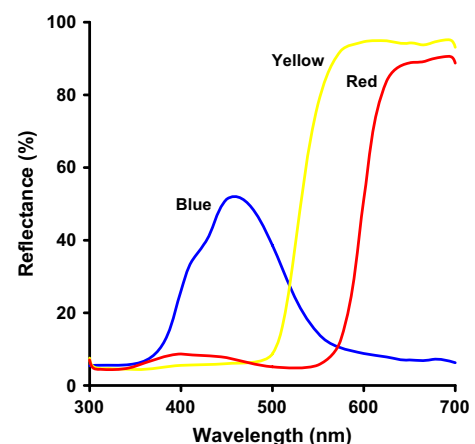

Fig. 1: Reflectance spectra of Color-Aid™ papers used for constructing artificial flowers.

recognize that choice of other colors or orders of presentation might have yielded different results; however, our focus was on the specific dynamics of transitions between rewarding colors, rather than a comprehensive examination of learning across a range of colors, as Blackiston et al. (2011) has explored.

Training consisted of placing nectar *ad libitum* into one of the three artificial flower colors and water into the remaining two, depending on the schedule. Every training session lasted 4 h (total time = 16 h per phase), and instantaneous observations of the butterflies' location and activity (*sensu* Altmann 1974) were made every 30 min. After 4 h, artificial flowers were removed from the cage and each butterfly was allowed to feed on a black artificial color for 1 min to standardize hunger levels (see above).

Continuous observations of transitions

While periodic instantaneous observations were made to assess butterfly visits to each color/phase, continuous observations (*sensu* Altmann 1974) lasting 90 min at the beginning of phases two and three allowed us to look in more detail at the dynamics of the transition from one rewarding color to another. Following transition to a newly rewarding color, we asked whether the butterflies (1) continue to visit the previously rewarding color, (2) switch to a new rewarding color once they experience it, or (3) revert to their innate preference. All butterflies were observed in groups of five of one sex at a time (as above).

In both instantaneous and continuous observations, we recorded number of visits made by each butterfly to each flower color/phase and calculated the corresponding percentages.

Memory Decay

Artificial flowers

Two colors were used in this experiment: red (rewarding) and blue (non-rewarding) (Fig. 1). Artificial flowers were constructed from 3.5-cm-diameter Petri dishes, but differed from those in the reward tracking experiment, in that each dish contained just one flower. A 2-mm-diameter hole was made in the center of the dish's upper side, and a 4-cm circle of matte-finish colored paper (either blue or red) with creased margins (2-mm high) was glued on this side. Artificial flowers were placed alternately and equidistantly (total of 16 flowers; eight flowers/color) on the bottom of the testing cage, over a 0.6 m² white sheet of cardboard. Butterflies were both trained and tested

individually in a 0.6 m³ green mesh cage illuminated by two 250 watt halogen lamps suspended approximately 3 cm from the sides of the cage. In order to stimulate butterflies to forage, monarchs were allowed to feed at a black artificial flower for 5 s before training and testing. After training and testing, adults were fed again at the black flower for 55 s to standardize hunger levels (see above).

Single vs. intermittent training

Two treatments (n = 20 butterflies/treatment; 10 males:10 females) were conducted to test whether foraging dynamics influence rates of memory decay: both groups trained for the same total amount of time (15 min/d for 4 d), either in one training session per day, or two. In treatment one (single training), inexperienced butterflies were released in the cage with the flowers above and allowed to forage once a day for 15 min over 4 d (total time: 1 h). In treatment two (intermittent training), monarchs were allowed to forage in the above setup twice a day (once in the morning and once in the afternoon), and each training session lasted 7.5 min. Training also lasted 4 d in this treatment (total time: 1 h). Twenty-four hours after training, butterflies from both treatments were tested on alternate days from day one to seven, for a total of four testing sessions. Each testing session lasted 10 min, in which empty (unrewarding) blue and red flowers were presented to individual butterflies exactly as in the training.

During each testing session, we recorded number of monarch visits to each artificial colored flower, as well as time spent visiting flowers. The corresponding percentages of visits and time spent on each flower color were then calculated and used for analysis (see below).

Statistical Analysis

Reward tracking

In the instantaneous observations, data were not normally distributed and variances were heteroscedastic (Shapiro–Wilk and Bartlett tests, respectively). The proportion of monarchs' visits to each flower color within phases was then arcsine transformed and compared using a repeated measures ANOVA followed by Tukey's *post hoc* tests using GLM procedures. In order to consider the effect of group on individual response, cages were treated as blocks. Frequency of monarch visits on a given flower color across the phases was also compared using the same test tests as above. But-

terflies that were not observed visiting flowers were excluded from the analysis.

In the continuous observations, different butterflies were observed visiting flowers in phases two and three, so that observations constitute independent events. Data were normally distributed and variances were homoscedastic (Shapiro–Wilk and Bartlett tests, respectively), so that proportion of monarchs' visits to each flower color was compared within phases through one-way ANOVA followed by Turkey's multiple comparison tests (Sokal & Rohlf 1995). Butterflies that were not observed making choices in a given phase were excluded from the corresponding analysis.

Memory decay

Non-parametric tests were performed in both treatments, as data were not normally distributed and variances were heteroscedastic (Shapiro–Wilk and Bartlett tests, respectively). For each testing day, frequency of visits to, as well as proportion of time spent on red vs. blue artificial flowers was compared through Wilcoxon signed-rank tests. Monarchs that did not make choices in the testing sections were excluded from the analysis.

Results

Reward Tracking

Instantaneous observations of phases
Of the twenty butterflies, 17 were observed visiting the artificial flowers in phase 1, 19 in phase 2, and 12 in phase 3. One butterfly died during phase 2 and 3 died during phase 3. Minimum, maximum, and total number of monarch visits to each flower color/phase are provided in Table 1. Cage (block) and flower color alone did not affect butterfly visits (Table 2). There was a significant effect of phase, as well as a significant interaction between phase and color on monarch visits. In phase 1 (blue phase), butterflies visited the rewarding color significantly more than the non-rewarding colors (red and yellow) (Fig. 2a). In phase 2 (red phase), frequency of visits to the three colors was not significantly different (Fig. 2b). In phase 3 (yellow phase), monarchs visited yellow flower models significantly more than they did red colored flowers (Fig. 2c).

When the same flower color is analyzed across phases, both blue and red flowers were visited significantly less often when these colors no longer offered rewards [Tukey's *post hoc* tests, blue flowers:

Table 1: Total number of monarch visits to each flower color/phase in the reward tracking experiment

Observation	Phase	Blue	Red	Yellow
Instantaneous	One (blue)	48 (0–12)	10 (0–3)	20 (0–3)
	Two (red)	15 (0–3)	39 (0–9)	22 (0–8)
	Three (yellow)	8 (0–3)	3 (0–2)	47 (0–18)
Continuous	Beginning of phase 2 (blue to red)	23 (0–5)	22 (0–5)	48 (0–19)
	Beginning of phase 3 (red to yellow)	6 (0–3)	21 (0–12)	23 (1–5)

Minimum and maximum numbers of visits are given in parentheses.

Table 2: Reward tracking experiment, instantaneous observations: repeated measures ANOVA using GLM procedures of the effects of cage (block), phase and color on monarch visits to artificial colored flowers

Parameter/interaction	Sum of squares	df	x̄ square	F	p
Cage (block)	0.28	3	0.09	0.91	0.44
Phase	0.70	2	0.35	3.33	0.04*
Error	5.63	54	0.10		
Color	0.94	2	0.47	1.60	0.21
Color × Cage	1.23	6	0.20	0.69	0.65
Color × Phase	11.64	4	2.91	9.88	<0.01*
Error	31.81	108	0.29		

*significant effects.

phase 1 (blue) to 2 (red), p = 0.005; phase 1 (blue) to three (yellow), p = 0.0001 and phase 2 (red) to three (yellow), p = 0.99; red flowers: phase 1 (blue) to two (red), p = 0.007; phase 1 (blue) to three (yellow), p = 0.99 and phase 2 (red) to three (yellow), p = 0.001]. In contrast, yellow flowers did not suffer significant changes in the proportion of monarchs' visits over time [Tukey's *post hoc* tests, phase 1 (blue) to two (red), p = 1.0; phase 1 (blue) to three (yellow), p = 0.09 and phase 2 (red) to three (yellow), p = 0.49].

Continuous observations of transitions

Twelve butterflies were observed visiting flowers during the first 90 min of phase 2, and seven during the same period of phase 3. At the beginning of phase 2, when the color of the artificial flower providing nectar was switched from blue to red, butterflies visited all the three flower colors at similar frequencies (one-way ANOVA, F = 1.17, p = 0.32; n = 12) (Fig. 3a). At the beginning of phase 3, monarchs visited the preferred, currently rewarding color (yellow), followed
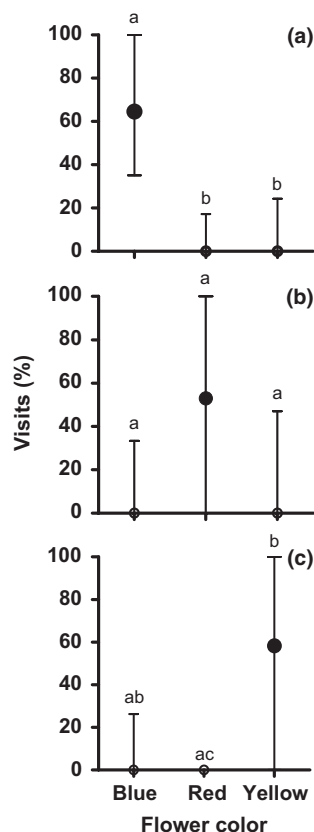
Fig. 2: Relative frequency of monarch visits (median ± interquartile range) to artificial flowers while trained to a specific color (black dots). (a), blue phase (N = 17); (b), red phase (N = 19); (c), yellow phase (N = 12). In each panel, different letters above bars indicate significant differences in the proportion of visits in a given phase (Dunn's multiple comparison tests, α = 0.05).

by the former rewarding color (red) (one-way, ANOVA, $F = 4.30$, p = 0.03; n = 7); blue artificial flowers were significantly less visited compared with yellow ones (Fig. 3b) (Tukey's multiple comparison tests, $q = 4.1$, p < 0.05).

Memory Decay

Single vs. intermittent training

In the single training, 16, 16, 17, and 16 of the twenty monarchs visited flowers on testing days 1, 3, 5, and 7, respectively. One butterfly died after testing day 3, and another after testing day 5. For intermittent-trained monarchs, 17, 14, 19, and 19 of the original butterflies made choices on testing days 1, 3, 5, and 7, respectively; no deaths were observed. Total, as well as minimum and maximum number of monarch visits to each flower color and training condition are provided in Table 3.
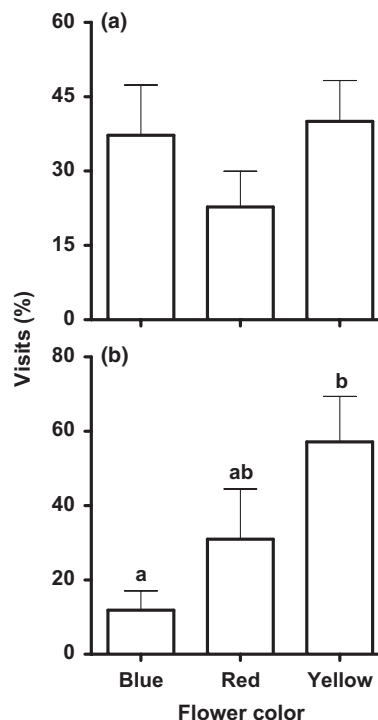

Fig. 3: Relative frequency of monarch visits ($\bar{x}$ ± SE) to artificial flowers in the first 90 min of the red (a; N = 12) and yellow (b; N = 7) phases. In each panel, different letters above bars indicate significant differences in the number of visits in a given phase (Tukey's multiple comparison tests, α = 0.05).

Butterflies from both the single- and intermittent-training conditions visited the rewarding color significantly more often than the non-rewarding color one and 3 d after cessation of reward training; by days 5 and 7 visitation to the colors no longer differed significantly (Wilcoxon signed-rank test, two-tailed: Single training: p = 0.031, 0.0045, 0.57 and 0.73 on testing days 1, 3, 5, and 7, respectively (Fig. 4a); Intermittent training: p = 0.01, 0.05, 0.17, and 0.91 on testing days 1, 3, 5, and 7, respectively) (Fig. 4b). With respect to duration of visits, single-trained butterflies spent significantly more time visiting the red flowers than the blue ones on testing days 1 and 3 and but not so on days 5 and 7 (Wilcoxon signed-rank test, two-tailed, p = 0.0035, 0.0045, 0.62, and 0.96 on testing days 1, 3, 5, and 7, respectively; data not shown). For intermittently-trained butterflies, time spent visiting red and blue flowers differed significantly only on day 1, and not thereafter (Wilcoxon signed-rank test, two-tailed, p = 0.007, 0.12, 0.17, and 0.91 on testing days 1, 3, 5, and 7, respectively; data not shown).

Table 3: Total number of monarch visits to each flower color/phase in the memory decay experiment

Treatment	Testing day	Blue (unrewarding color)	Red (rewarding color)
Single training	1	174 (0–38)	3509 (0–572)
	3	355 (0–154)	2484 (0–454)
	5	504 (0–143)	1992 (0–372)
	7	762 (0–202)	1155 (0–224)
Intermittent training	1	601 (0–295)	3847 (0–531)
	3	819 (0–197)	2597 (0–513)
	5	1431 (0–398)	1853 (0–241)
	7	1893 (0–478)	1438 (0–281)

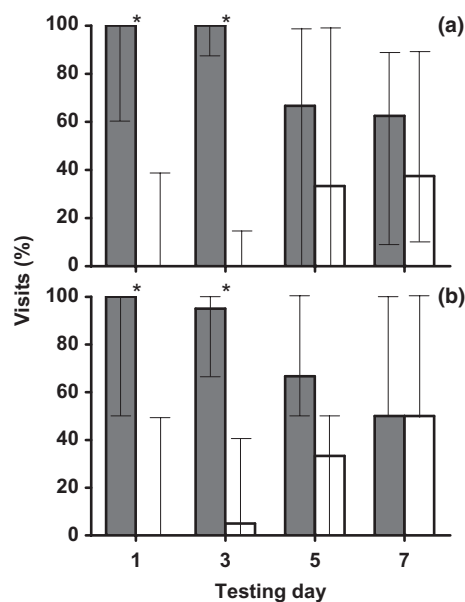Minimum and maximum numbers of visits are given in parentheses.

Fig. 4: Relative frequency of monarch visits to empty red (former rewarding; gray bars) and empty blue (former unrewarding; white bars) artificial flowers on different testing days (median ± interquartile range). (a): single training; (b): intermittent training. In each panel, asterisks indicate significant differences in proportion of visits on each colored artificial flower on a given testing section (Wilcoxon signed-rank tests, two-tailed, α = 0.05). Single training: N = 16, 16, 17, and 16; intermittent training: N = 17, 14, 19, and 19 on days 1, 3, 5, and 7, respectively.

Discussion

Monarchs tracked the rewarding artificial flowers throughout the first experiment, as seen by data from instantaneous observations. In phase 1, they showed a preference for the rewarding blue flowers despite innate preference for yellow, as was seen by Blackiston et al. (2011). Interestingly, in phase 2, when red flowers became rewarding, the butterflies were able to find the reward. However, they continued to visit the formerly rewarding learned color (blue in this case), and also started to visit the innately preferred, non-rewarding color (yellow). These results are in agreement with those of Gumbert (2000), who found that bumblebees trained to a given color will revert to visiting an innately preferred color if they are offered a novel color that differs from the trained color. In addition, Kelber (1996) found that the diurnal, long lived, and migratory hawkmoth *Macroglossum stellatarum* is able to learn rewarding colors and avoid unrewarding colors, and that innate preferences also interfered with learning novel colors. As monarchs are day-flying, can undertake long migrations, and have a long life span, rapid adjustments to novel food sources are probably adaptive; this ability is especially important in long-lived organisms that use ephemeral resources across large geographical areas (see Kelber 1996). Indeed, long-lived and large-sized butterfly species have been shown to present higher color learning rates than smaller, short-lived counterparts (Kandori et al. 2009).

Red artificial flowers were not the preferred ones in the corresponding phase possibly for two reasons. First, monarch learning rates for the red color are somewhat lower than those for blue and yellow (Blackiston et al. 2011), suggesting that the rate at which every color is learned varies. Second, the order at which rewarding colors are presented to butterflies might be an issue; contrary to butterflies in phase 2, naïve monarchs have been shown to prefer rewarding red flowers over unrewarding ones (Blackiston et al. 2011; this study).

At the beginning of phase 2, when red flowers offered a reward, butterflies continued to visit blue flowers (the former rewarding color), started visiting yellow (the innately preferred but still unrewarding one) and were positively reinforced when visiting red (current rewarding). Consequently, no differences among the three colors were observed in the first 90 min of reward change. Thus, our findings suggest that the butterflies' choices at the transition period reflect an interaction between memory of the formerly rewarding flowers (blue), reversion to innate preferences (yellow) and rapid learning of the new rewarding color (red). At the beginning of phase 3 (yellow), the significant decline in blue relative to yellow visits, as well as the lack of difference between red and yellow visits, reflects an ability to learn and remember novel rewarding colors (red) despite previous experience (that is, blue being rewarding at the beginning), as well as reinforcement in visiting flower colors that are both preferred and rewarding.

It is interesting to note that the changes in monarchs' response to novel rewarding colors vary according to the time scale: monarchs visited red as often as yellow and more often than blue at the beginning of phase 3, showing a short-term memory effect due to the reward in red in the previous phase. As days progressed, they abandoned red and kept visiting yellow followed by blue, showing a tendency to use blue flowers more intensely than red when both are unrewarding. Thus, when considering both instantaneous and continuous observations, our results show that monarchs' responses in the transitional period differ from the whole phase, reflecting legacies of prior rewards and choices.

Our memory experiment showed that the formerly rewarding red flowers were visited significantly more than the non-rewarding blue flowers in both single and intermittent treatments. Our results suggest that in the absence of other reinforcement, monarchs are able to retain information for several days regardless of the foraging dynamics. We have no information about the timing of monarchs' nectar foraging activities in nature, although they are likely to vary according to the seasons (i.e., day length and/or phenology of resources), innate color/odor preferences and even for individual butterflies (age, sex, or butterfly 'personality').

Studies on memory of floral visitors have been mainly focused on bees (see Menzel 1999 and references therein). However, as the methods employed to assess memory dynamics in bees strongly vary with that of the present study, comparisons are difficult in this regard. To our knowledge, the only other study of butterfly memory has shown retention of flower information in the absence of reinforcement for a similar period of time, that is, 3 d (Kandori & Ohsaki 1996). Retention of flower information may also last for a shorter period of time in nature, where butterflies need to deal with stimuli in different behavioral contexts, including oviposition as well as nectar foraging, and as a consequence keep in mind several kinds of information concomitantly (Weiss & Papaj 2003). Otherwise, monarchs might be able to retain such information for the same period of time as they do under laboratory conditions (i.e., with no additional stimuli), but with some potential costs (see Dukas 1999). For example, a broad diet may incur costs as processing information, and thus, time taken for decision making in a polyphagous grasshopper species (Bernays 1998).

Flowers present multimodal cues that permit learning in different ways (Raguso 2004). Apart from flower color, odor cues have been shown to play an important role in learning by flower visitors and pollinators (see Andersson & Dobson 2003 for an example on butterflies). Nevertheless, the physiological and behavioral bases for memory in butterflies are still unknown. In honeybees, long-term memory is protein dependent, while medium-term memory is protein independent (Wüstenberg et al. 1998). Also, mushroom bodies are involved in the retention of olfactory information in several insect groups (see Fahrbach 2006), but there is no information about the role of this structure in color memory in butterflies (see Kroutov et al. 2002).

Because butterflies are considered important – but neglected – pollinators (see Weiss 2001), more studies are necessary for understanding their cognitive abilities and their implications for pollination ecology, including the role of olfactory cues and decision-making strategies as already examined in bees (Hammer & Menzel 1995; Chittka & Spaethe 2007). From the plant's perspective, learning, and remembering nectar sources by visitors is important for assuring floral constancy and thus pollination (Barth 1991). In butterflies, it is still unclear how concomitant use of multiple rewarding flowers may affect memory and thus floral constancy. Finally, as stated above, memory decay can vary according to innate preferences, in which preferred colors or odors could be more remembered than non-preferred ones. In the case of monarchs, memory may last longer when the rewarding colors are orange or yellow, a matter that deserves further investigation.

Acknowledgements

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