CrossMark

Use of Flower Color-Cue Memory by Honey Bee Foragers Continues when Rewards No Longer Differ between Flower Colors

Marisol Amaya-Márquez[1] · Charles I. Abramson[2] ·
Harrington Wells[3]

Abstract Honey bees (Hymenoptera: Apidae) were used as a model insect system to explore forager use of a learned color-cue memory over several subsequent days. Experiments used artificial flower patches of blue and white flowers. Two experiments were performed, each beginning with a learning experience where 2 M sucrose was present in one flower color and 1 M sucrose in the alternative flower color. The first experiment followed flower color fidelity over a series of sequential days when rewards no longer differed between flowers of different color. The second examined the effect of intervening days without the forager visiting the flower patch. Results showed that color-cue memory decline was not a passive time-decay process and that information update in honey bees does not occur readily without new experiences of difference in rewarding flowers. Further, although the color cue learned was associated with nectar reward in long term memory, it did not seem to be specifically associated with the 2 M sucrose nectar reward when intervening nights occurred between learning and revisiting the flower patch.

Keywords *Apis mellifera* · honey bee · foraging experience · flower color-cue memory · behavioral plasticity · memory

---

✉ Marisol Amaya-Márquez
    mamayam@unal.edu.co

[1] Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia

[2] Laboratory of Comparative Psychology and Behavioral Biology, Oklahoma State University, Stillwater, OK, USA

[3] Department of Biological Science, University of Tulsa, Tulsa, OK, USA

🖄 Springer

Introduction

Learning is central to forager energy-maximization models (Houston 1997; Stephens and Krebs 1986). In theory, foragers will exploit food resources efficiently to maximize net reward and respond to changes in the environment in order to do so. Energy maximization theory is predictive of nectivore foraging in many natural landscapes and artificial flower patches (e.g., Chittka and Thomson 1997; Heinrich 1979; Laverty 1994; Laverty and Plowright 1988; Percival 1965; Waddington and Holden 1979; Wells and Wells 1986). Nevertheless, memory is a 'double edge sword' when foraging in a complex environment.

On one hand, the result of learning is that bees are able to use memory to maximize net energy gain by maximizing calorie intake (e.g. Waddington et al. 1981; Wells and Wells 1986; Greggers and Menzel 1993; Greggers and Mauelshagen 1997; Dukas 1998; Cnaani et al. 2006), reducing traveling time (Marden and Waddington 1981; Hill et al. 2001), or reducing handling time (Heinrich 1979; Waddington and Gottlieb 1990; Sanderson et al. 2006). Energy maximization expectations have even been observed in honey bee decisions where different sugars (glucose, fructose, sucrose) are involved in the alternative rewards (Wells et al. 1992). However, reward and/or cost perception can cause consistent deviations from energy maximization expectations when several variables (e.g. reward quality, quantity and rate) in alternative rewards exist (Chittka et al. 1999). This is evident when cost (e.g. ability to reach the reward) and reward molarity or volume both vary between flower types, and bees systematically overestimate costs (Cnaani et al. 2006; Sanderson et al. 2006). Further, flower constancy in bees can also result from factors such as memory limitations, inefficiency involved in changing flower handling technique, flower visualization against various backgrounds, and spatial arrangement and densities of alternative flowers (Chittka et al. 1999).

On the other hand, learned information is useful only if it can be applied under the appropriate circumstances to a future problem (Rescorla 1988). While this seems likely when making repeated visits to the same flower patch, the relevance of the learned information would be highly questionable when entering a new floral landscape at a different geographical position. Changes in nectar resource distribution, even over the period of a day, impose the need for changes in flower fidelity to maximize reward collected. This ability to change is known as behavioral flexibility. Serial reversal tasks show that bees do exhibit behavioral flexibility when dealing with flower fidelity (Chittka 1998; Strang and Sherry 2014), but apparently not under all contexts (e.g. Menzel 1969; Couvillon and Biterman 1986). Improved performance across successive reversals of reward association in a discrimination task was mostly due to a reduction in perseverant errors, but not nearly to the degree seen in some vertebrates (Strang and Sherry 2014).

Thus, new situations warrant a brief initial random-sampling period to assure foraging efficiency (e.g. Stephens and Krebs 1986; Amaya-Márquez et al. 2014). However, in a previous study, we found that the lasting memory of a previously learned color led honey bee foragers to an anachronism in the information used to exploit a new floral landscape in morning versus afternoon flower patches at different geographic locations (Amaya-Márquez et al. 2014). When visiting the afternoon flower patch foragers showed high fidelity to the flower color offering the higher caloric reward in the morning patch even though nectar rewards no longer differed. This was in spite of

the fact that flower morphology, scent, time of day, and flower patch location were different from the morning flower patch. These results extended those of earlier work which showed that memory decay was not significant when bees were prevented from returning for just one hour to a single site (Cheng and Wignall 2006).

Presented is a study of multimodal floral cues in which one component (color) is only initially informative about a difference in rewards. Here we ask how long it takes for the memory of a color-cue preference to be rewritten. Two experiments were performed. The first followed flower color fidelity over a series of sequential days when rewards no longer differed between flowers of different color. The second examined the effect of intervening days without the forager visiting the flower patch before revisiting it. Overnight memory has been shown to fade in bumblebees (Keasar et al. 1996), and predicts what is expected in the second experiment reported here.

Materials and Methods

All experiments used honey bees, *Apis mellifera ligustica*, (Hymenoptera: Apidae) foraging on artificial flower patches of 36 flowers spaced 75 mm apart in rows and columns of a 6×6 Cartesian coordinate system on a brown pegboard. Bees used were from a single honey bee colony. Flower patches consisted of 18 blue and 18 white flowers arranged with respect to color. Bees freely made foraging trips from the one-frame colony, and navigated to and from the flower patch on their own. When experiments were not being performed, the colony had an unscented 1 M sucrose feeder inserted at the entrance. Recruitment of bees for experiments consisted simply of moving the feeder from the hive entrance to the flower patch site and using the first 2 to 3 bees that visited the artificial flower patch, and those bees were each uniquely marked upon their first flower visited. All other bees that visited the flower patch were removed permanently from the system (i.e. the colony and surrounding foraging environment). Since bees spent much longer in the hive than on the flower patch, generally one bee was on the flower patch at a time. No interaction between bees on the flower patch was ever observed.

Artificial Flowers

Each flower consisted of a 28 mm × 28 mm Plexiglas square, 6 mm thick with an 8 mm diameter (ID) well in one corner. The well was a 15 mm deep Plexiglas tube, which extended below the Plexiglas square and contained the nectar (sucrose) reward. Each flower was mounted on a 90 mm pedicel of 5 mm doweling. Flowers were either blue or white. Flowers of different colors were created by painting the lower surface of square and the outer surface of the Plexiglas tube with enamel paint (Testors™ paint Nos. 1208 blue, or 1245 white). The reflectance spectra for the paints, and a color hexagon depicting how these colors are perceived by the honey bee, can be found in Hill et al. (1997). Flowers were washed in unscented detergent, then triple rinsed and allowed to dry after each use. A new set of flowers was used for each treatment, washing all flowers before use again.

Experiments

Foragers were each uniquely marked with a dot of Testors® paint on the thorax upon their first flower visited, and flower choices recorded for each bee. A flower visit by a forager was recorded when a bee completely entered the nectar well and drunk the reward. A flower was refilled when a bee consumed the nectar and made the next flower choice. Two experiments were performed. A new set of bees was used in each experiment. Unmarked bees were removed from the system.

Experiment I

The purpose of Experiment I was to investigate flower color fidelity over a series of sequential days when rewards no longer differed between flowers of different color. Experiment I was performed outdoors and uncaged, with bees free to visit natural floral resources in the environment as well as the artificial flower patch. The artificial flower patch was located 20 m from the hive. The first experiment had three treatments the goal of which was to look at bee flower-color fidelity over time after it learned to associate the better reward with one of the flower colors. The treatments represent a time series of conditions each bee received, hence sequential in nature (Fig. 1).
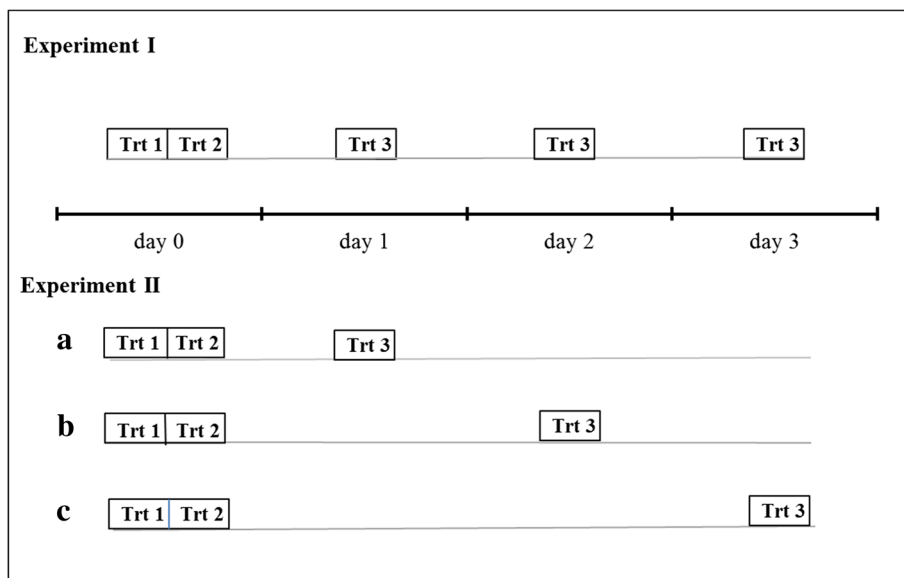
Fig. 1 Experimental Designs. Above the time axis (x-axis) is depicted Experiment I, and below the time axis is depicted Experiment II. Treatment 1 offered bees 1 M sucrose in both blue and in white flowers. Treatment 2 offered half of the bees 2 M sucrose in blue and 1 M sucrose in white flowers, and the other half of the bees 2 M sucrose in white and 1 M sucrose in blue flowers. Treatment 3 offered bees 1 M sucrose in both blue and white flowers. It differed from Treatment 1 only in timing, being after the learning phase (Treatment 2). **Experiment I** had Treatment 3 repeated on 3 successive days, and data was recorded on each bee for all three days of Treatment 3 as well as for Treatments 1 and 2. **Experiment II** had Treatment 3 on only one day, but different bees experienced Treatment 3 after differing time spans from the end of Treatment 2 (a, b and c scenarios). Data was recorded on each bee for all three treatments, **Control bees** were given the same experimental designs as shown above, but 1 M sucrose was present in both blue and white flowers in all three treatments

Treatment 1 offered foragers 4ul of 1 M sucrose scented with 5ul/L clove oil in both blue and white flowers. Its purpose was to gather baseline data on color choice before bees were given a situation where rewards differed between flower colors. Treatment 2 presented bees with an energy maximization learning experience. It offered foragers the choice of 4ul of 2 M versus 4ul of 1 M sucrose each scented with 5ul/L clove oil. Approximately half of the bees experienced blue flowers offering the greater reward, and the other half had white flowers offering the greater reward. Treatment 3 tested for continued use of information about flower color associated reward now when the two flower colors again offered bees the same reward (4ul 1 M sucrose with 5ul/L clove oil). The rationale behind using scented sucrose is that it made it easier to get bees to return to the flower patch on subsequent days, and it made it more likely that they would visit both flower colors extensively. Further, it follows using scent in Amaya-Márquez et al. (2014) and thus makes results easier to compare with that study.

Treatments 1 and 2 were performed sequentially without interruption on the first day (day 0). On the following 3 days (days 1, 2 and 3) only Treatment 3 was performed. Each treatment was performed for 1 h.

A separate set of bees acted as a control. These bees experienced the same treatment regime (day 0, 1, 2, 3) but rewards did not differ between flower color in Treatment 2. Control bees received 4ul of 1 M sucrose with 5ul/L clove oil in both blue and white flowers during Treatment 2. Thus, the control bees did not learn to associate one flower color with a higher value of nectar reward. There were 11 experimental bees that collectively visited 3577 flowers and 11 control bees that collectively visited 2452 flowers in Experiment I.

The experiment was repeated several times with a cohort of two or three naïve bees each time. Experimental and control bees were not run at the same time, but were interspersed with one another over the course of the study in a random manner.

Experiment II

The purpose of experiment II was to observe the effect of intervening days without the forager visiting the flower patch on its original flower color fidelity (Fig. 1).

The experimental design was the same as Experiment I on day 0, but each bee only received Treatment 3 one day. Thus, a set of bees was given Treatment 3 one day after training (but not day 2 or 3), a second set two days after training (but not day 1 or 3), and a third set three days after training (but neither day 1 nor 2). Experiment II was performed with the hive and artificial flower patch in a cage (2.5 m on each side) to prevent the bees from abandoning the experimental site over several non-rewarding days, and to control the number of days after the bees have formed the flower color-cue memory at Treatment 2, before exposing them again to Treatment 3. The bees were free to fly in the outdoor cage at will. The artificial flower patch was 3.3 m diagonally opposite of the hive.

As in Experiment I, a group of control bees was given 4ul of 1 M sucrose scented with 5ul/L clove oil in both blue and white flowers as a sham-training Treatment 2. There were 34 experimental bees that collectively visited 3687 flowers and 17 control bees that collectively visited 1851 flowers in Experiment II.

The experiment was repeated several times with a cohort of two or three naïve bees each time. Experimental and control bees were not run at the same time, nor were

cohorts experiencing different number of days before receiving Treatment 3. Control and experimental cohorts were interspersed with each other over the course of the study in a random manner.

Data Analysis

A repeated-measures MANOVA (Sall and Lehman 1996) was used to test for changes in flower fidelity of experimental bees among treatments in Experiment I and II separately. Effects tested were training (blue vs. white flowers in Treatment 2 contained the high molar reward), treatment (Treatment 1, Trt. 2, Trt.3_{day1}, Trt. $3_{day2,}$ and Trt. 3_{day3}), and interaction effects were analyzed for experimental and control bees. The treatment effect tested for change in flower fidelity among treatments. All analyses used arcsine square-root transformation of the frequency of visits (following, Sokal and Rohlf 2012). The same statistical test was used for control bees in Experiment I but without training effect, and thus there was also no interaction effect. The same statistical test was used for control bees in Experiment II but with the training effect replaced by the day Treatment 3 was performed. Thus, there was a Treatment 3 day × treatment interaction.

Results

Experiment I

Experimental bees learned to associate flower color with reward quality, which was the training effect ($F_{1,9} = 18.00$; $p = 0.0022$). Bees given the better reward in blue flowers during Treatment 2 showed increased fidelity to blue flowers, and the reverse was true; bees given the better reward in white flowers during Treatment 2 showed increased fidelity to white flowers (interaction effect: $F_{4,6} = 15.82$; $p = 0.0024$). The lack of a treatment effect alone ($F_{4,6} = 0.95$; $p = 0.50$) was because training to blue flowers has the opposite effect from training to white flowers in terms of percentage of blue flowers visited (Fig. 2).

Based on these results we went on to test (MANOVA) whether there was a change in flower color fidelity using only the data from Treatment 3 (Trt. 3_{day1}, Trt. 3_{day2} and Trt. 3_{day3}). Both for bees trained to white and for those trained to blue, forager fidelity for the training color diminished over days, so that by day 3 each bee extensively visited both blue and white flowers (training $F_{1,9} = 14.24$; $p = 0.0044$; interaction $F_{2,8} = 17.50$; $p = 0.0012$). In the MANOVA an interaction effect occurred because training to blue flowers has the opposite effect from training to white flowers in terms of percentage of blue flowers visited (treatment $F_{2,8} = 0.70$; $p = 0.52$).

Control bees showed no preferences for blue or white flower color morphs throughout the set of treatments ($F_{4,7} = 2.07$; $p = 0.19$), as seen in Fig. 2.
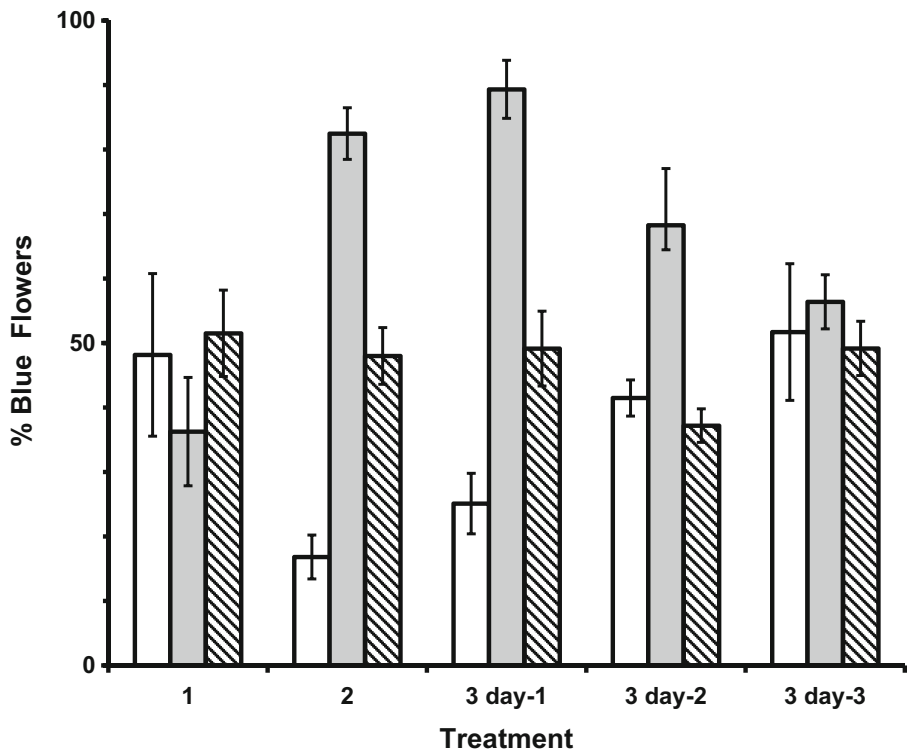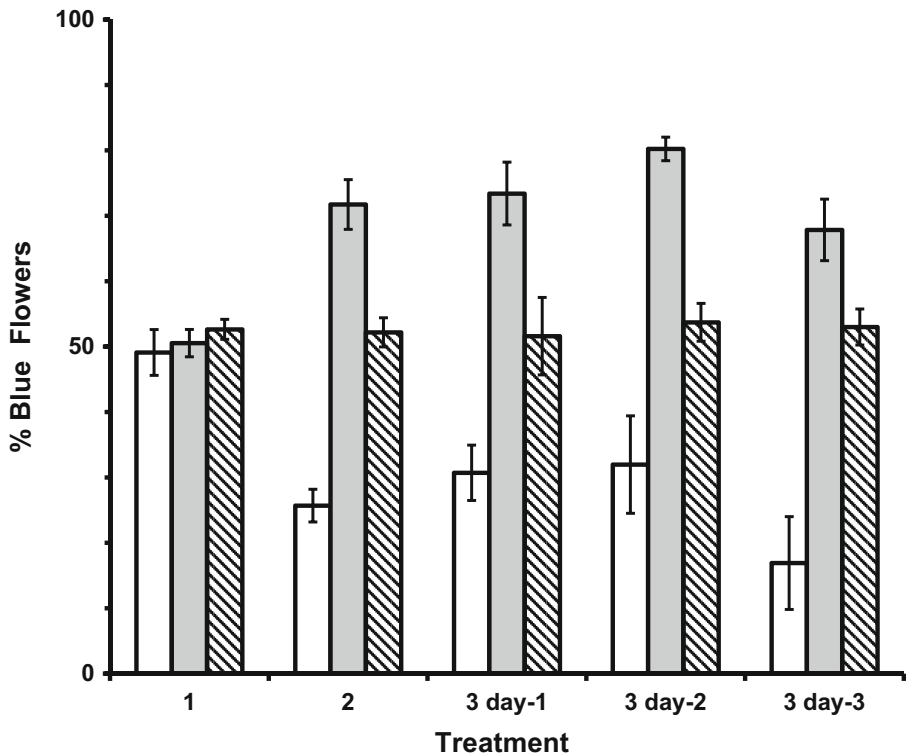
Fig. 2 Percentage visitation to blue flowers (mean ± SE) by experimental and control bees in Experiment I. Treatment 1 offered *experimental* bees 1 M sucrose in both blue and white flowers. Treatment 2 offered approximately half the *experimental* bees 2 M sucrose in white flowers and 1 M sucrose in blue flowers (white bars). The remaining *experimental* bees were offered 2 M sucrose in blue flowers and 1 M sucrose in white flowers (gray bars). Treatment 3 on each subsequent day offered experimental bees 1 M sucrose in both blue and white flowers. Each experimental bee experienced all three days of Treatment 3 as well as Treatments 1 and 2. In contrast, each treatment offered *control* bees 1 M sucrose in both blue and white flowers (hatched bars). Each *control* bee experienced all three days of Treatment 3 as well as Treatments 1 and 2

Experiment II

Experimental bees readily associated flower color with reward quality and showed high fidelity to the flower color with the higher quality reward (training $F_{1,32} = 80.83$; $p < 0.0001$; interaction $F_{2,31} = 30.68$; $p < 0.0001$). Like Experiment I, the lack of a training effect alone ($F_{1,31} = 0.06$; $p = 0.94$) was because training to blue versus white flowers had the opposite effect on fidelity to blue flowers (Fig. 3).

Based on those statistical results we went on to test for difference among Treatment 3 results based on the day Treatment 3 was performed (ANOVA). Thus, there were a training, day, and training × day effects in the test. The time between training (Treatment 2) and again visiting the flower patch (Treatment 3) did not alter flower color fidelity (training $F_{1,28} = 73.831$; $p < 0.0001$; day $F_{2,28} = 3.08$; $p = 0.062$; interaction $F_{2,28} = 0.53$; $p = 0.59$).

Fig. 3 Percentage visitation to blue flowers (mean ± SE) by experimental and control bees in Experiment II. Treatment 1 offered *experimental* bees 1 M sucrose in both blue and white flowers. Treatment 2 offered approximately half the *experimental* bees 2 M sucrose in white flowers and 1 M sucrose in blue flowers (white bars). The remaining *experimental* bees were offered 2 M sucrose in blue flowers and 1 M sucrose in white flowers (gray bars). Each experimental bee experienced Treatments 1 and 2, but only experienced Treatment 3 on one day, some on day 1, some on day 2 and some on day 3. Treatment 3 offered *experimental* bees 1 M sucrose in both blue and white flowers. In contrast, each treatment offered *control* bees 1 M sucrose in both blue and white flowers (hatched bars). Each control bee experienced Treatments 1 and 2, but only experienced Treatment 3 on one day, some on day 1, some on day 2 and some on day 3

Control bees did not change their flower color fidelity over time (treatment $F_{2,13} = 0.03$; $p = 0.9696$; day ($F_{2,14} = 0.40$; $p = 0.67$; interaction $F_{4,26} = 0.78$; $p = 0.55$), extensively visiting both blue and white flowers (Fig. 3).

Discussion

Foragers given a choice between a 1 M and 2 M sucrose reward learned to associate the greater reward with a flower color. Further, these trained bees continued to use this learned association even when rewards no longer differed between flower colors the next day (Exps. I and II). This behavior, however, was not irreversible. By the second day a forager's fidelity for the training flower color had decayed, and by the third day both flower colors were visited extensively (Exp. I). The decline in fidelity for the flower color associated with a greater reward was not simply a process of memory

decay over days. Honey bees that were prevented from foraging after Treatment 2 continued to favor the flower color associated with the greater reward for at least three days after training (Exp. II), which supports the memory model proposed by Menzel in that he attributed memory to flower-type fidelity (Menzel 1999, 2001). However, it is contrary to the behavior reported for bumble bees when overnight intervening intervals existed (Keasar et al. 1996). The fact that bees exposed to new foraging experiences decrease the use of a previously acquired flower color-cue memory indicates that the flower decision of honey bees is based on behavioral and cognitive flexibility. However, a phase lag between information previously acquired and information actually gathered leads to a temporal anachronism in the use of information as we observed (also see: Amaya-Márquez et al. 2014), which leads to a behavioral cost represented in suboptimal flower exploitation.

Honey bees have been shown to have many cognitive abilities originally thought to be limited to vertebrates (reviewed in: Giurfa et al. 2001; Abramson et al. 2010), which is truly remarkable in itself. However, even though the behavioral action may be the same, there is evidence that honey bees reach this point by very different cognitive processes. A case in point is the honey bee's inability to use the removal of a cue in learning either to expect a reward or punishment (e.g. Abramson 1986; Suddendorf and Corballis 2007; Abramson et al. 2010). Honey bees learn very rapidly to associate reward difference with flower color cues, as seen in Treatment 2 here, and in fact often occurs in just visitation to 3 flowers (e.g. Hill et al. 1997). However, results presented here and those of Amaya-Márquez et al. (2014) suggest that this information update by honey bees does not readily occur without a new experience involving a reward difference between flower types (Fig.1). Further, the fading of a memory is not simply a passive time-decay process (Fig. 2). This was to be expected with a delay of an hour through overnight (Chittka 1998; Cheng and Wignall 2006), or several hours between morning and afternoon (Amaya-Márquez et al. 2014), but is quite unexpected over a stretch of several nights (Keasar et al. 1996).

Honey bee extreme flower fidelity is well documented in the agricultural literature (reviewed in Wells et al. 1983) and diverse experimental designs (e.g. Wells and Wells 1983; Ohyama et al. 1995; Hill et al. 1997, 2001) when flower choices involve colors such as yellow and blue, which are spectrally well separated in the bees color-vision space (Chittka and Wells 2004). In contrast here we use a floral color context (blue vs. white) where bees are known to quickly switch flower color fidelity in response to changes in rewards (e.g. Wells and Wells 1986; Sanderson et al. 2006; Giray et al. 2015). In fact, Menzel (Menzel and Erber 1978; Menzel 1999) describes complete learning in just three flowers being visited in such situations. Further, the floral constancy under the first color-context (BY) remains true even when bees are under the influence of EtOH, but this is not true for the latter (BW) flower color-context (Abramson et al. 2005). Thus, when considering the flower colors used in experiments reported here, the flower constancy is even more surprising.

What was also of particular interest in our study is that the color cue remembered, although linked to the higher reward in treatment II (i.e. 2 M sucrose), apparently was not linked to a specific value of nectar concentration in the long term memory. That is, in previous work where sequential treatments are without-interruption and rewards associated with flower color are reversed, bees within just a few flowers visited (not trips from the hive) have switched flower color fidelity, which is reflected in flower

color fidelity by treatment (e.g. Wells and Wells 1986; Wells et al. 1992; Hill et al. 1997). Expectation of a specific reward is readily apparent in the reaction of foragers when rewards are lowered in those experiments. This reward expectation was also observed by Bitterman (1976) in experiments that are discussed further below.

These areas seem to represent fundamental aspects of the cognitive architecture of bees that differs from vertebrates and are of interest for studies investigating the effect of foraging experience on the neuroanatomical plasticity of the brain (Fahrbach et al. 1998). There have been several experimental designs demonstrating the effect of "learned expectancy" on performance. In the successive negative contrast experiment first described by Elliott (1928), two groups of rats were given preferred and non-preferred food one trial a day in a complex T maze. In the pre-shift phase rats receiving the non-preferred food made more errors than those receiving the preferred food. However, after only one experience with the non-preferred food in the post-shift phase, rats that had received the preferred food immediately made more errors. This result was confirmed by Crespi (1942) who used a runway rather than a complex T maze. Three groups of rats received one trial every 24 h. The groups were differentiated based on whether animals received 256, 64, or 16 food pellets during the pre-shift phase. In the post-shift phase, all rats received 16 pellets. Again, after only one trial the performance of the 256 and 64 pellet groups deteriorated. Similar results are found with other experimental designs including behavioral contrast (Williams 2002) and simultaneous contrast (Dwyer et al. 2011). These experiments demonstrate that vertebrate animals readily form expectancies based on the type of reward they experience.

"Learned expectancy" (Elliott 1928; Crespi 1942) from Treatment 2 is expected to produce a different behavioral response in Treatment 3. That is, the experimental group experienced a differential reward in Treatment 2, but not in Treatments 1 or 3 of either Experiment I or II. The differential reward in Treatment 2 not only presented foragers with an immediate difference in value (taste 1 M vs 2 M sucrose) but also a differential reward rate since foraging on 2 M sucrose would yield twice the sucrose of 1 M sucrose each trip from the colony. Thus, learned expectancy should have led to bee flower-color fidelity deteriorating in treatment 3 regardless of which reward-currency was used by foragers.

The uncoupling of task with reward expectation is supported by an early work of Bitterman (1976). In an experiment with free-flying honey bee foragers received a 40% sucrose reward for landing on a target, but that reward was then shifted to a 20% sucrose reward for landing on the same target on return trips from the hive. The bees on discovering that the reward was now 20% sucrose exhibited distress and reluctance to drink the 20% solution. This disruption could be eliminated by increasing to 24 min the time period between the last trial with 40% sucrose and the first trial with 20% sucrose (Bitterman 1976). Bitterman interpreted the experiment as sensory adaptation rather than as a learned effect.

Our results also bear upon ameliorating the effects of pesticides on honey bee behavior (Taylor et al. 1987; Abramson et al. 2004, 2006, 2012; Balbuena et al. 2015; Karahan et al. 2015). Bees can be prevented from foraging during an application of pesticide and released when the pesticide degrades to less harmful levels without loss of previously learned foraging information. We show that if bees are confined they will remain highly crop attached. When considering that flower color fidelity remains when foragers first visit new locations (Amaya-Márquez et al. 2014) and that this crop

attachment can remain without degradation for days (our data), bees can potentially be transported considerable distances from field to field of the same plant species and have bees continue as if they were not moved.

A central result of our study was that foragers showed high fidelity for a color-cue memory associated with nectar reward even after several intervening days when the flower patch was not present. This observation suggests that new experiences involving difference in rewarding flowers are central for information updating in honey bees, and this is reflected in a prior study showing that honey bees could not learn the removal of a stimulus as a conditioning cue as seen in vertebrates such as rodents and primates (Abramson et al. 2010). Nevertheless, after days without visiting the flower patch, receiving a 1 M reward rather than the training 2 M reward did not elicit visitation to the alternative flower color as seen when rewards are changed without a delay (e.g. Sanderson et al. 2006). 'Immediacy' may also be a component of recognizing difference in honey bees, and is worth further exploration.

References

Abramson CI (1986) Aversive conditioning in honey bees (*Apis mellifera*). J Comp Psychol 100:108–116

Abramson C, Squire J, Sheridan A, Mulder PG (2004) The effect of insecticides considered harmless to honey bees (*Apis mellifera*): proboscis conditioning studies by using the insect growth regulators tebufenozide and diflubenzuron. Environ Entomol 33:378–388

Abramson CI, Sanderson C, Painter J, Barnett S, Wells H (2005) Development of an ethanol model using social insects V: honey bee foraging decisions under the influence of alcohol. Alcohol 36:187–193

Abramson CI, Singleton JB, Wilson MK, Wanderley PA, Ramalho F, Michaluk LM (2006) The effect of an organic pesticide on mortality and learning in Africanized honey bees (*Apis mellifera* L.) in Brasil. Am J Environ Sci 2:37–44

Abramson CI, Nolf SL, Mixson TA, Wells H (2010) Can honey bees learn the removal of a stimulus as a conditioning cue? Ethology 116:843–854

Abramson CI, Sokolowski MB, Brown EA, Pilard S (2012) The effect of pymetrozine (Plenum WG-50®) on proboscis extension conditioning in honey bees (*Apis mellifera*: Hybrid var. Buckfast). Ecotoxicol Environ Saf 78:287–295

Amaya-Márquez M, Hill PS, Abramson CI, Wells H (2014) Honey bee location-and time-linked memory use in novel foraging situations: floral color dependency. Insects 5:243–269

Balbuena MS, Tison L, Hahn M-L, Greggers U, Menzel R, Farina WM (2015) Effects of sublethal doses of glyphosate on honeybee navigation. J Exp Biol 218:2799–2805

Bitterman ME (1976) Incentive contrast in honey bees. Science 192:380–382

Cheng K, Wignall AE (2006) Honeybees (*Apis mellifera*) holding on to memories: response competition causes retroactive interference effects. Anim Cogn 9:141–150

Chittka L (1998) Sensorimotor learning in bumblebees: long-term retention and reversal training. J Exp Biol 201:515–524

Chittka L, Thomson JD (1997) Sensori-motor learning and its relevance for task specialization in bumble bees. Behav Ecol Sociobiol 41:385–398

Chittka L, Wells H (2004) Color vision in bees: mechanisms, ecology and evolution. In: Prete F (ed) Complex worlds from simpler nervous systems. MIT Press, Cambridge, pp 165–191

Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. Naturwissenschaften 86:361–377

Cnaani J, Thomson JD, Papaj DR (2006) Flower choice and learning in foraging bumblebees: effects of variation in nectar volume and concentration. Ethology 112:278–285

Crespi LP (1942) Quantitative variations of incentive and performance in the white rat. Am J Psychol 55:467–517

Couvillon PA, Bitterman ME (1986) Performance of honeybees in reversal and ambiguous-cue problems: tests of a choice model. Anim Learn Behav 14 (3):225–231

Dukas R (1998) Cognitive ecology: the evolutionary ecology of information processing and decision making. University of Chicago Press, Chicago

Dwyer DM, Lydall ES, Hayward AJ (2011) Simultaneous contrast: evidence from licking microstructure and cross-solution comparisons. J Exp Psychol Anim Behav 37:200–210

Elliott MH (1928) The effect of change of reward on the maze performance of rats. Univ CA Pub Psychol 4:19–30

Fahrbach SE, Moore D, Capaldi EA, Farris SM, Robinson GE (1998) Experience-expectant plasticity in the mushroom bodies of the honeybee. Learn Mem 5:115–123

Giray T, Abramson CI, Chicas-Mosier A, Brewster T, Hayes C, Rivera-Vega K, Williams M, Wells H (2015) Effect of octopamine manipulation on honeybee decision making: reward and cost differences associated with foraging. Anim Behav 100:144–150

Giurfa M, Zhang S, Jenett A, Menzel R, Srinivasan MV (2001) The concept of 'sameness' and 'difference' in an insect. Nature 410:930–933

Greggers U, Mauelshagen J (1997) Matching behavior of honeybees in a multiple-choice situation: the differential effect of environmental stimuli on the choice process. Anim Learn Behav 25:458–472

Greggers U, Menzel R (1993) Memory dynamics and foraging strategies of honeybees. Behav Ecol Sociobiol 32:17–29

Heinrich B (1979) "Majoring" and "minoring" by foraging bumblebees, *Bombus vagans*: an experimental analysis. Ecology 60:245–255

Hill PS, Wells PH, Wells H (1997) Spontaneous flower constancy and learning in honey bees as a function of colour. Anim Behav 54:615–627

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

Houston AI (1997) Natural selection and context-dependent values. Proc R Soc Lond B 264:1539–1541

Karahan A, Çakmak I, Hranitz JM, Karaca I, Wells H (2015) Sublethal imidacloprid effects on honey bee flower choices when foraging. Ecotoxicology 24:2017–2025

Keasar T, Motro UZI, Shur Y, Shmida AVI (1996) Overnight memory retention of foraging skills by bumblebees is imperfect. Anim Behav 52:95–104

Laverty TM (1994) Bumble bee learning and flower morphology. Anim Behav 47:531–545

Laverty TM, Plowright RC (1988) Flower handling by bumblebees: a comparison of specialists and generalists. Anim Behav 36:733–740

Marden JH, Waddington KD (1981) Floral choices by honeybees in relation to the relative distances to flowers. Physiol Entomol 6:431–435

Menzel R (1969) Das gedächtnis der honigbiene für spektralfarben II umlernen und mehrfachlernen. Z Vergl Physiol 63:290–309

Menzel R (1999) Memory dynamics in the honeybee. J Comp Physiol A 185:323–340

Menzel R (2001) Behavioral and neural mechanisms of learning and memory as determinants of flower constancy. In: Chittka L, Thomson JD (eds) Cognitive ecology of pollination. Cambridge University Press, Cambridge, pp 21–40

Menzel R, Erber J (1978) Learning and memory in bees. Sci Am 239:102–110

Ohyama T, Couvillon PA, Bitterman ME (1995) Perseveration in the color choices of honeybees. J Insect Behav 8:409–415

Percival MS (1965) Floral biology. Pergamon Press, New York

Rescorla RA (1988) Behavioral studies of Pavlovian conditioning. Annu Rev Neurosci 11:329–352

Sall F, Lehman A (1996) JMP IN, SAS Institute, Inc. Ducksberry Press, Belmont

Sanderson CE, Orozco BS, Hill PS, Wells H (2006) Honeybee (*Apis mellifera ligustica*) response to differences in handling time, rewards and flower colours. Ethology 112:937–946

Sokal R, Rohlf F (2012) Biometry, 4th edn. WH Freeman & Co, New York

Stephens DW, Krebs JR (1986) Foraging theory. Princeton University Press, Princeton

Strang CG, Sherry DF (2014) Serial reversal learning in bumblebees (*Bombus impatiens*). Anim Cogn 17:723–734

Suddendorf T, Corballis MC (2007) The evolution of foresight: what is mental time travel, and is it unique to humans? Behav Brain Sci 30:299–351

Taylor KS, Waller GD, Crowder LA (1987) Impairment of a classical conditioned response of the honey bee (*Apis mellifera* L.) by sublethal doses of synthetic pyrethroid insecticides. Apidologie 18:243–252

Waddington KD, Gottlieb N (1990) Actual vs perceived profitability: a study of floral choice in honey bees. J Insect Behav 3:429–441

Waddington KD, Holden LR (1979) Optimal foraging: on flower selection by bees. Am Nat 114:179–196

Waddington KD, Allen T, Heinrich B (1981) Floral preferences of bumblebees (*Bombus edwardsii*) in relation to intermittent versus continuous rewards. Anim Behav 29:779–784

Wells H, Wells PH (1983) Honey bee foraging ecology: optimal diet, minimal uncertainty, or individual constancy? Anim Ecol 52:829–838

Wells H, Wells PH (1986) Optimal diet, minimal uncertainty and individual constancy in the foraging of honey bees, *Apis mellifera*. J Anim Ecol 55:881–891

Wells H, Wells PH, Smith DM (1983) Ethological isolation of plants. I. Colour selection by honeybees. J Apic Res 22:33–44

Wells H, Hill PS, Wells PH (1992) Nectarivore foraging ecology: rewards differing in sugar types. Ecol Entomol 17:280–288

Williams BA (2002) Behavioral contrast redux. Anim Learn Behav 30:1–20