**Title:** Label-based expectations affect reward perception in bumblebees

**Authors:** Claire T. Hemingway1, Felicity Muth1

1Department of Integrative Biology, University of Texas, Austin, TX, U.S.A.

**General methods**

*Subjects and experimental set-up*

We maintained colonies in colony boxes (~40cm3) on 30% (w/w) sucrose solution and honeybee-collected pollen (~0.5g/day, Koppert Biological Systems, USA). When being used in an experiment, a given colony was attached to a flight arena (122 × 61 × 61 cm, l × w × h) using a clear plastic tube with sliding doors the control the entry and exit of bees into the arena. The bottom of the flight arena was lined with green laminate and the sides and top consisted of mesh screens. The arena was lit from above by a 40-Watt LED light placed atop the arena and the room was illuminated with florescent light on a 12/12-hour light/dark schedule.

*Floral array*

The vertical floral array used for training and testing consisted of a black corrugated plastic sheet containing 12 artificial flowers randomly arranged on a 6 × 4 grid (figure S1a). We constructed the artificial flowers from 1.5 ml plastic Eppendorf tubes with small holes in the base of the tubes through which we could pipette sucrose solution (figure S1b). The “corollas” of these artificial flowers were made from laminated, coloured circular disks (5cm in diameter) placed around the opening of the tube. Various colours were used instead of grey for the training and testing. The holes in the array not occupied by artificial flowers were sealed using black rubber bungs.



**Figure S1.** a) vertical floral array used for training and testing; b) bee entering the tube to access the sucrose reward.

*Training and testing stimuli*

For experiments 1 and 2, we used a total of seven colour stimuli (two for Experiment 1 and five for Experiment 2). Stimuli were printed on an inkjet printer (HP Envy 60055e) on Cotton Fine Art Archival OBA free paper (Pacific Inkjet, USA). We used a Flame UV-VIS spectrometer (Ocean Insight, Florida, USA) to measure reflectance of each stimulus and irradiance in the foraging arena. The reflectance measurements were then mapped into bee colour space with AVICOL, a program for analysing spectrometric data [1] accounting for the spectral sensitivities of *Bombus impatiens* [2]. From these measurements, we were able to generate chromatic contrasts between stimuli (table S1).

*Pre-training*

Bees had no prior experience foraging from coloured flowers. During a pre-training phase, we gave bees access to a white-wicked feeder in the flight arena containing 30% (w/w) sucrose solution. We first placed the feeder close to where bees exited their colony from, and then incrementally moved the feeder towards the back of the arena over 1-2 days, until bees would readily fly to the end of the arena to forage. We then trained bees to visit a ‘pre-training array’ with 24 Eppendorf tubes without the coloured corollas arranged in a grid (6 × 4 flowers), provisioned with 50 µl of 30% (w/w) sucrose solution (figure S1). Foragers visiting the pre-training array were individually marked using non-toxic, water-based paint markers (POSCA USA). We selected bees that were seen foraging consistently for use in experiments.

*Training and Testing Procedure*

Bees were trained across three training trials. During training, bees visited coloured flowers paired with a high-quality nectar reward (8 µl of 50% (w/w) sucrose solution). A trial started when the bee left the colony through the connecting tube to the arena and ended either when the bee returned to the colony, or after 10 minutes had elapsed since the last flower visit. Across the three training trials, bees visited 31 ± 7.76 flowers in Experiment 1 (familiar: 37.4 ± 6.39; novel: 35.15 ± 6.89) and 36 ± 6.26 in Experiment 2 (Blue A: 27.06 ± 6.86; Blue B: 32.71 ± 6.40; Blue C: 30.94 ± 9.20; Blue D: 34.06 ± 8.61; Yellow: 32.94 ± 7.05). There was no difference in the number of flowers bees visited between treatments either in Experiment 1 (*t38* = 2.02, *p* = 0.29), or in Experiment 2 (*F1,4* = 2.16, *p* = 0.08). Between trials, all stimuli were rearranged on the array and wiped with ethanol to remove olfactory cues.

Following the three training trials, bees were given a single test trial where they were presented with a lower quality reward (30% (w/w) sucrose solution) paired either with a familiar colour received during training or a novel colour cue (figure 1). All trials were recorded using a Canon camcorder on a tripod placed near the nest box and facing the array.

**Table S1**: Chromatic contrasts between all combinations of colour stimuli for both experiments. Data are hexagon units.

|  |  |  |
| --- | --- | --- |
| **Experiment** | **Stimuli** | **Chromatic Contrast** |
| Experiment 1 | Blue 1-Yellow | 0.249 |
| Experiment 2 | Blue A – Blue B | 0.023 |
| Blue A – Blue C | 0.051 |
| Blue A – Blue D | 0.073 |
| Blue A – Yellow | 0.220 |

**Table S2.** Sample sizes of colonies and individuals across experiments and treatments.

|  |  |  |  |
| --- | --- | --- | --- |
| **Experiment** | **Colony** | **Total number of individuals** | **Number of individuals across treatments** |
| 1 | 1 | 22 | Novel: 12; Familiar: 10 |
| 2 | 9 | Novel: 4; Familiar: 5 |
| 3 | 9 | Novel: 4; Familiar: 5 |
| 2 | 4 | 20 | Blue A: 4; Blue B: 4; Blue C: 4; Blue D: 4; Yellow: 4 |
| 5 | 20 | Blue A: 4; Blue B: 4; Blue C: 4; Blue D: 4; Yellow: 4 |
| 6 | 20 | Blue A: 4; Blue B: 4; Blue C: 4; Blue D: 4; Yellow: 4 |
| 7 | 12 | Blue A: 3; Blue B: 3; Blue C: 2; Blue D: 2; Yellow: 2 |
| 8 | 13 | Blue A: 2; Blue B: 2; Blue C: 3; Blue D: 3; Yellow: 3 |

**Results**

**Table S3.** Post-hoc pairwise comparisons between the ‘familiar’ treatment (Blue A) to Blue B, Blue C, Blue D, and Yellow. The model contained acceptance as the binary response variable and tested for an interaction between ‘visit number’ and ‘stimulus’.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pairwise comparison** | estimate | std. error | z *-* value | *p -* value |
| Blue A – Blue B | -0.033 | 0.045 | -0.742 | 0.458 |
| Blue A – Blue C | 0.035 | 0.024 | 1.450 | 0.147 |
| Blue A – Blue D | -0.001 | 0.014 | -0.433 | 0.662 |
| Blue A – Yellow | 0.021 | 0.012 | 1.815 | 0.069 |



**Figure S2.** Number of bees that accepted vs. rejected downshifted rewards on their first visit across the familiar and novel treatments in Experiment 1. There was no difference in initial acceptance between treatment groups.

****

**Figure S3.** Acceptance behaviour (probability of accepting a reward) across visits for the four possible colour treatments used in Experiment 1. Bees were more likely to accept the novel colour across visits, but the absolute training colour did not affect this (*z* = -0.358, *p* = 0.720).



**Figure S4.** Number of visits to first acceptance by treatment group for Experiment 1. Data show average number of visits until acceptance with 95% bootstrapped confidence intervals. There was no difference between groups in the number of visits to first acceptance.



**Figure S5.** Number of bees that accepted vs. rejected downshifted rewards on their first visit across the test colour stimuli used in Experiment 2; there was no difference across stimuli.



**Figure S6.** Number of visits to first acceptance by test stimulus for Experiment 2. Data show average number of visits until acceptance with 95% bootstrapped confidence intervals. There were no differences between groups in the number of visits to first acceptance.

1. Gomez D. 2011 *AVICOL, a program to analyse spectrometric data*.

2. Skorupski P, Chittka L. 2010 Photoreceptor Spectral Sensitivity in the Bumblebee, Bombus impatiens (Hymenoptera: Apidae). *PLoS ONE* **5**, e12049. (doi:10.1371/journal.pone.0012049)

3. Leadbeater E, Chittka L. 2005 A new mode of information transfer in foraging bumblebees? *Current Biology* **15**, R447–R448.

4. Leadbeater E, Chittka L. 2009 Bumble-bees learn the value of social cues through experience. *Biology Letters* **5**, 310–312.