

1 Bumblebee visual search for multiple learned target types

2 Vivek Nityananda<sup>1\*</sup> & Jonathan G. Pattrick<sup>1</sup>

3 <sup>1</sup> Biological and Experimental Psychology, School of Biological and Chemical Sciences,  
4 Queen Mary University of London, Mile End Road, London E1 4NS

5 \*email: [v.nityananda@qmul.ac.uk](mailto:v.nityananda@qmul.ac.uk)

6 Summary

7 Visual search is well studied in human psychology, but we know comparatively little about  
8 similar capacities in non-human animals. It is sometimes assumed that animal visual search is  
9 restricted to a single target at a time. In bees, for example, this limitation has been evoked to  
10 explain flower constancy, the tendency of bees to specialize on a single flower type. Few  
11 studies however, have investigated bee visual search for multiple target types after extended  
12 learning and controlling for prior visual experience. We trained colour-naive bumblebees  
13 (*Bombus terrestris*) extensively in separate discrimination tasks to recognise two rewarding  
14 colours in interspersed block training sessions. We then tested them with both colours  
15 simultaneously in the presence of distracting colours to examine whether and how quickly  
16 they were able to switch between the target colours. We found that bees switched between  
17 visual targets quickly and often. The median time taken to switch between targets was shorter  
18 than known estimates of how long traces last in bees' working memory suggesting that their  
19 capacity to recall more than one learned target was not restricted by working memory  
20 limitations. Following our results, we propose a model of memory and learning that  
21 integrates our findings with previous studies investigating flower constancy.

22 Short title: Bumblebee visual search for learned targets

23 Keywords: visual search, bee, search template, flower constancy, working memory

24

## 25 **Introduction**

26 Visual search is an essential component of our lives and is an extremely well-researched field  
27 in human psychology and neuroscience (Chun and Wolfe, 2000; Verghese, 2001; Wolfe,  
28 1998; Wolfe, 2003). We know comparatively little, however, about visual search in other  
29 animals despite the fact that several animals also use visual search for vital biological  
30 functions like foraging, searching for mates or avoiding predators (Bond and Kamil, 2002;  
31 Ings and Chittka, 2008; Spaethe et al., 2006). Bees, for example, have to discriminate highly  
32 rewarding flowers from poorly rewarding flowers while foraging (Benard et al., 2006;  
33 Chittka et al., 1999). Several studies have shown that while performing this task, they tend to  
34 specialize on a single rewarding target type while occasionally sampling other flowers as  
35 well, a phenomenon referred to as flower constancy (Waser, 1986). It has been suggested  
36 that flower constancy reflects memory and cognitive limitations on the ability of bees to  
37 rapidly retrieve memories for multiple targets (Raine and Chittka, 2007; Waser, 1986). Other  
38 studies have, however, hinted that either of two targets can be recalled, albeit not  
39 simultaneously (Chittka and Thomson, 2012; Hill et al., 1997), but few control for prior  
40 experience and learning as well as odour cues. Those that do (Chittka and Thomson, 2012),  
41 have found that target recall might be better explained by training schedules than memory  
42 capacity, suggesting that prior learning experience must be controlled to allow adequate tests  
43 of visual search limitations.

44 Another consideration is the distinction between working memory capacity and an  
45 immediate search template, as has been made for humans (Olivers et al., 2011). If a bee  
46 learns a search template (Goulson, 2000) and fails to learn further targets while a 'primary'  
47 template is active in the working memory, this could be because of two non-mutually  
48 exclusive mechanisms: the primary search template might prevent new templates from being  
49 learned (Chittka et al., 1999) or it could prevent all use of other search templates – even if  
50 previously learnt – for as long as the primary template lasts in the working memory. In the  
51 latter case, bees should be unable to use or recall other learnt target templates soon after using  
52 a search template for a particular target. In order to investigate visual search capacity for  
53 learned targets, the experimental design should therefore allow bees to learn multiple targets  
54 with separate training tasks for each target such that each target can form a search template.  
55 One could then ask if the bee can switch between search templates, or if they are restricted to  
56 a single template until its memory trace decays.

57 We therefore designed an experiment with a controlled training schedule on  
58 bumblebees (*Bombus terrestris*) with limited previous visual experience. We tested the  
59 hypothesis that they can flexibly and rapidly retrieve more than one learned visual target as a  
60 search template when faced with a simultaneous choice between multiple learned target  
61 types.

## 62 **Materials and Methods**

### 63 **Animals**

64 Bees were obtained from a commercial supplier (Syngenta Bioline, Weert, Netherlands),  
65 tagged with Opalith number tags (Christian Graze KG, Weinstadt-Endersbach, Germany) to  
66 enable individual identification and colonies were transferred from the commercially supplied  
67 nest boxes, under red light, to one compartment of a two-chambered wooden nest box (28 x  
68 16 cm x 11 cm tall). The floor of the other compartment was covered with cat litter for bees  
69 to discard refuse. Bees were allowed to forage for 50% sucrose (v/v) in an arena consisting of  
70 a wooden box (100 cm x 60 cm x 29 cm tall) with a UV-transparent Plexiglas lid which they  
71 entered via a 24.5 cm long Perspex® tunnel. The arena floor was covered with green card and  
72 the arena was illuminated by lit by two twin lamps (TMS 24 F with HF-B 236 TLD (4.3 kHz)  
73 ballasts, Philips, The Netherlands) fitted with Activa daylight fluorescent tubes (Sylvania,  
74 UK) from above. Bees were supplied with pollen ad libitum directly into the colony on  
75 alternate evenings.

### 76 **Calculating stimuli spectral properties**

77 The reflectance spectra of the artificial flowers used in the experiments were analyzed using  
78 an Avantes AvaSpec 2048 spectrophotometer (Anglia Instruments Limited, Soham,  
79 Cambridgeshire, United Kingdom) with a deuterium-halogen light source, relative to a  
80 BaSO<sub>4</sub> white standard. Since the sensitivity of bee photoreceptors differ from ours, we  
81 converted the spectra of targets into a bee-specific hexagonal colour space (Fig 2; Chittka,  
82 1992) considering the spectral sensitivity of bumblebee photoreceptors (Skorupski et al  
83 2007), the spectral reflectance of the background and the spectral distribution of the  
84 illuminant. The colour hexagon has three vertices corresponding to the three bee  
85 photoreceptors tuned to ultraviolet, blue and green light with the Euclidean distance between  
86 the centre and any of the vertices being 1 and colour distances above 0.1 being  
87 distinguishable. Plotting the colour loci in this space thus allows the calculation of colour

88 distances between pairs of colours in the bee colour space which in turn indicate the  
89 perceptual discriminability of the colours. The colour distance between blue and magenta  
90 (0.31) was similar to the colour distance between magenta and yellow (0.25) and between  
91 pink and yellow (0.38). The colour distance between blue and pink was lower (0.13) but  
92 sufficient for the colours to be distinguished. To verify that these relatively similar colours  
93 could be behaviourally distinguished, the discriminability of the blue and pink flowers was  
94 also assessed in a control experiment (see below).

95

#### 96 Pretraining

97 Colour-naïve foragers were trained to forage from transparent square Perspex® chips (side:  
98 25 mm, thickness: 4 mm), carrying drops of 50% (v/v) sucrose, for 2-3 bouts. They were  
99 subsequently trained to feed from artificial flowers consisting of chips placed on glass vials  
100 (4 cm tall, 1.5 cm diameter) arranged in a 6 x 4 horizontal grid with vials placed 15 cm apart  
101 from each other. Twelve randomly chosen artificial flowers had 12 µl of 50% sucrose on  
102 them, while others remained empty. Training began after bees had foraged on this grid for 2-  
103 3 bouts.

#### 104 Training

105 Bees from three different colonies were trained in two experimental conditions each  
106 consisting of two alternating visual discrimination tasks (Fig. 1) in which bees had to  
107 discriminate target colours from distractors. In Condition 1, twelve bees were trained in the  
108 two separate tasks to discriminate blue target flowers from magenta distractor flowers and  
109 pink target flowers from yellow distractor flowers. In Condition 2, the target and distractor  
110 colours were reversed, and ten other bees were trained to discriminate magenta target flowers  
111 from blue distractor flowers and yellow target flowers from pink distractor flowers.

112 Each bee was individually trained on one of the two tasks until it reached a success  
113 criterion of 80% correct choices (probing of the flowers for reward) out of the last twenty  
114 choices made. The bee was then trained on the second task until it reached the same success  
115 criterion. The two tasks were subsequently alternated until the bee had successfully  
116 performed both tasks four times. Each task had twelve rewarding or 'target' flowers  
117 (Condition 1: blue/pink; Condition 2: magenta/yellow) holding 12 µl of 50% sucrose and  
118 twelve non-rewarding or 'distractor' flowers (Condition 1: magenta/yellow; Condition 2:  
119 blue/pink) holding 12 µl of distilled water. The target and distractor flowers in this and the



120 other experiments below were positioned according to previously decided random spatial  
121 configurations on every foraging bout. The flowers were not refilled during a bout and bees  
122 were allowed to revisit flowers in all experiments. Between bouts, the artificial flowers were  
123 cleaned with 99% ethanol to remove scent markings, and water to remove traces of ethanol.  
124 Half the bees began training with one of the two discrimination tasks and the other half with  
125 the other task and data from both regimes were combined in the analysis. Two bees did not  
126 complete training in Condition 1 and data from these bees was discarded.

#### 127 Test

128 Post training, bees were tested on their ability to flexibly choose the two target colours. Six  
129 flowers of each target and distractor colour were presented to the bee. All flowers contained  
130 12  $\mu$ l of distilled water, to prevent reinforcement of any colour during the test. The choices of  
131 the bee and the order of the choices were noted. The entire foraging bout was video recorded  
132 using a Sony DCR-SR58E Handycam. The recordings were later examined to obtain the  
133 times taken and the distances between consecutive artificial flowers chosen. The tests were  
134 carried out until five minutes were over or the bee returned to the colony after a minimum of  
135 twelve choices, whichever was sooner.

#### 136 Control

137 To determine whether the blue and pink flowers were discriminable, we conducted a control  
138 experiment. Each of ten new bees was pre-trained as above and tested on an array of 12 blue  
139 and 12 pink flowers, with flowers of one colour carrying 12  $\mu$ l 50% sucrose and flowers of  
140 the other carrying 12  $\mu$ l of distilled water. Half the bees tested encountered rewarding pink  
141 flowers and the other half encountered rewarding blue flowers. The choices of the bees were  
142 noted and analyzed to see if the bees could successfully learn one colour and meet a criterion  
143 of 80% correct choices out of the last twenty choices. Successful learning would show that  
144 bees could visually discriminate between the two colours.

#### 145 Data analysis

146 The choices made by the bees were divided into the four possible transitions between the two  
147 target colours. We compared the number of choices of each transition type using pairwise  
148 Wilcoxon signed rank tests ( $\alpha = 0.05$ ) to examine if bees were more likely to make constant  
149 transitions (Condition 1: blue-blue, pink-pink/ Condition 2: magenta-magenta, yellow-  
150 yellow) than switches (Condition 1: blue-pink, pink-blue/ Condition 2: magenta-yellow,

151 yellow-magenta). We also examined the number of constant transitions made before each  
152 switch to investigate if bees switched often or only occasionally. Finally, we calculated a  
153 sequence index (Heinrich, 1979) for each bee dividing the number of constant transitions by  
154 the total number of transitions. This gives the probability of a transition being constant rather  
155 than a switch. If the bees were flower constant, the expected index value would be close to 1,  
156 while if they visited two targets with equal probability the expected value would be close to  
157 0.5. We compared the observed number of constant choices with the expected values  
158 corresponding to indices of 1 and 0.5 using a chi-square test ( $\alpha = 0.05$ ).

159 To examine how quickly the bees made their choices, we compared the median times taken to  
160 make each of the four possible choice types using pairwise Wilcoxon signed rank tests ( $\alpha =$   
161 0.05). We investigated if the time taken to make choices correlated with the distance between  
162 the choices made by calculating the Pearson's correlation coefficient between the two  
163 measures. All statistical analyses were carried out using PASW Statistics v 18.0 (SPSS inc.,  
164 2009 Chicago, IL, [www.spss.com](http://www.spss.com)).

165

## 166 **Results**

### 167 **Training**

168 Bees learnt to discriminate both blue flowers from magenta ones and pink flowers from  
169 yellow ones but took longer to learn the blue-magenta discrimination than the pink-yellow  
170 discrimination. The mean number of choices per bout was 12.2 ( $\pm 4.8$  S. D.). In Condition 1,  
171 the mean number of choices taken to learn the rewarding colour was 33.5 ( $\pm 14.6$  S. D.) for  
172 the blue flowers and 20.3 ( $\pm 0.95$ ) for the pink flowers. In Condition 2, the mean number of  
173 choices to learn the rewarding colour was 24.3 ( $\pm 3.2$  S. D.) for yellow flowers and 27.1 ( $\pm$   
174 5.1 S. D.) for the magenta flowers. Bees also made more errors initially on the blue-magenta,  
175 magenta-blue and yellow-pink tasks with accuracies of 75% ( $\pm 9$  S.D.), 73.5% ( $\pm 4.9$  S. D.)  
176 and 73.7% ( $\pm 10.4$  S. D.) respectively in the very first training session compared to 91% ( $\pm 8$   
177 S. D.) in the pink-yellow task. On the final training session, however, the accuracy of bees in  
178 all tasks in both conditions was near perfect (Condition 1: 97%  $\pm 3$  S.D. for the blue-  
179 magenta, 100% for the pink-yellow, Condition 2: 96%  $\pm 5$  S.D. for the magenta-blue, 98.8%  
180  $\pm 2.7$  S.D. for the yellow-pink). The time taken for the entire training ranged from 1 hour and  
181 59 minutes to 6 hours and 28 minutes and the mean time was 3 hours and 39 minutes.

182 Test

183 Condition 1. Target colours: Blue and Pink

184 All ten bees tested in Condition 1 showed a high degree of accuracy with only one choice of a  
185 distractor out of all 281 choices made. Thus, they were not choosing randomly but focussing  
186 on the two target colours. The mean number of choices made by each bee was 27.3 ( $\pm$  12.0 S.  
187 D.). The mean number of pink choices made ( $16.5 \pm 8.1$  S. D.) was greater than the mean  
188 number of blue choices made ( $11.6 \pm 5.9$  S. D.). The difference between the number of pink  
189 and blue choices, however, just failed to reach significance at the 5% level (paired t-test;  $t_9 =$   
190  $-2.156$ ;  $p = 0.06$ ).

191 We divided the choices according to the four possible transitions: blue-blue, pink-pink, blue-  
192 pink and pink-blue and compared the numbers of each transition type. We found that while  
193 the numbers of constant choices (pink-pink and blue-blue) were higher, the median number  
194 of choices made did not differ significantly across transition types (Fig. 3A; Pairwise  
195 Wilcoxon signed rank tests, all  $p$ 's  $> 0.2$ ), suggesting that bees made approximately equal  
196 number of choices for each transition, whether they switched or stayed constant.

197 To further investigate whether bees stayed flower constant over several consecutive choices  
198 or switched colours often, we examined the number of choices each bee made before  
199 switching. The median number of consecutive blue choices made before switching to pink  
200 was 1 and the median number of consecutive pink choices made before switching to blue was  
201 2 (Fig. 3C). The respective means were  $1.9 (\pm 1.5$  S. D.) and  $2.8 (\pm 2.1$  S. D.). The mean  
202 sequence index across all bees was  $0.6 (\pm 0.1)$  and the proportion of constant choices made  
203 was significantly different from 1 ( $\chi^2 = 48.2$ , d. f. = 9,  $p < 0.05$ ) but not from 0.5 ( $\chi^2 = 11.4$ , d.  
204 f. = 9,  $p > 0.2$ ), indicating that bees were switching between two target types with equal  
205 probability. Bees thus switched frequently and only rarely stayed constant to a single colour.

206 An examination of the transition times showed that the median times between choices were  
207 not significantly different across the different transition types (Fig. 4A; Pairwise Wilcoxon  
208 signed rank tests, all  $p$ 's  $> 0.2$ ), staying at 5-6 seconds across all transition types. The low  
209 transition times were not well explained by the physical distances between the targets chosen,  
210 with a low but significant correlation between transition times and distances (Pearson's  
211 correlation coefficient = 0.1,  $n = 270$ ,  $p = 0.03$ ).

212 We compared the times taken by the bees when switching between colours (blue-pink or  
213 pink-blue) with estimates of bee working memory duration to examine if the bees switched  
214 before the memory trace of the previous search template could have completely decayed.  
215 Working memory completely decays before 8.7 seconds but is still robust at 6 s in the  
216 honeybee (Zhang et al., 2005). Field studies on bumblebees have indicated similar working  
217 memory dynamics (Chittka et al., 1999). We used both these estimates and examined the  
218 proportion of the choices made by bees below either. A total of 77.6 % (45 out of 58) of all  
219 blue-pink transitions and 75% of all pink-blue transitions were under 9 seconds (Fig. 4C).  
220 Furthermore, 62.1 % (36 out of 58) of all blue-pink transitions and 65.4 % of all pink-blue  
221 transitions were 6 seconds or under (Fig. 4C). The lowest transition time was 1 and 2 seconds  
222 respectively for the blue-pink and the pink-blue transitions. Bees were, therefore, able to  
223 quickly switch between target colours before their working memory traces of the previous  
224 colour decayed and did so frequently. An inspection of the distributions of the transition  
225 times did not indicate any bimodality which would have suggested that the choices were split  
226 – perhaps by some memory threshold- between shorter and longer times. There was also no  
227 qualitative difference between the transition time distributions between the transition types.

#### 228 Condition 2. Target colours: Magenta and Yellow

229 Similar to Condition 1, all ten bees tested in Condition 2 were highly accurate and chose no  
230 distractors across all 293 choices made. The mean number of choices made by each bee was  
231 28.3 ( $\pm 11.5$  S. D.). The mean number of magenta choices made ( $15.2 \pm 8.1$  S. D.) and the  
232 mean number of yellow choices made ( $13.1 \pm 4.6$  S. D.) were not significantly different  
233 (paired t-test;  $t_9 = 1.0$ ;  $p = 0.34$ ).

234 The median number of choices did not differ across the different transition types (magenta-  
235 magenta, yellow-yellow, magenta-yellow and yellow-magenta; Fig. 3B: Pairwise Wilcoxon  
236 signed rank tests, all  $p$ 's  $> 0.07$ ). The median number of consecutive magenta choices made  
237 before switching to yellow and the median number of consecutive yellow choices made  
238 before switching to magenta were both 2 (Fig. 3D). The respective means were  $2.5 (\pm 2.3$  S.  
239 D.) and  $2.0 (\pm 1.2$  S. D.). The mean sequence index across all bees was  $0.6 (\pm 0.1)$  and the  
240 proportion of constant choices made was significantly different from 1 ( $\chi^2 = 51.8$ , d. f. = 9,  $p$   
241  $< 0.05$ ) but not from 0.5 ( $\chi^2 = 6.1$ , d. f. = 9,  $p > 0.2$ ). This shows that in this condition too,  
242 bees switched between the two target types often rather than staying constant to a single  
243 colour.

244 The median times between choices in Condition 2 were not significantly different between  
245 the different transition types (Fig. 4B; Pairwise Wilcoxon signed rank tests, all  $p$ 's  $> 0.19$ ),  
246 and similar to Condition 1, were either 5 or 6 seconds across all transition types. The  
247 transition times and distances between targets were again weakly but significantly correlated  
248 (Pearson's correlation coefficient = 0.2,  $N = 273$ ,  $p = 0.001$ ), once more suggesting that the  
249 low transition times were not adequately explained by the distance between the targets.

250 A total of 68 % (40 out of 59) of all magenta-yellow transitions and 71% (41 out of 58) of all  
251 yellow-magenta transitions were under 9 seconds (Fig. 4D). Furthermore, 64 % (38 out of 59)  
252 of all magenta-yellow transitions and 55 % (32 out of 58) of all yellow-magenta transitions  
253 were 6 seconds or under (Fig. 4D). Both transitions had lowest transition times of one second.  
254 As with the first experimental condition, the distributions of the transition times were not  
255 bimodal and the different transition types did not differ in the distribution of transition times.

256 Thus the results of Condition 2 were qualitatively similar to Condition 1 and again, bees  
257 switched between target colours frequently and often before estimates of the time taken for  
258 their working memory traces to decay.

#### 259 Control

260 All ten bees learnt the rewarding colour in the control experiments. Bees took on average  
261 only 20.4 ( $\pm 4.7$  S. D.) choices since first choosing the rewarding colour to reach an accuracy  
262 of 80% correct choices out of the last twenty. Bees were therefore clearly able to distinguish  
263 the blue flowers from the pink flowers.

#### 264 Discussion

265 We tested bees with carefully controlled visual experience trained on a fixed schedule to  
266 examine if they flexibly search for multiple target types, and investigated the sequence and  
267 speed with which they switched between search templates, explicitly comparing this with  
268 estimates of how long working memory lasts in bees. We found that bees that have trained  
269 for a relatively extended period of time on two alternating targets can recall both targets  
270 flexibly and quickly. The times taken to switch between targets were below estimates of how  
271 long bee working memory traces take to decay, suggesting that bumblebees can utilise at  
272 least two target types, accessing multiple search templates within a short duration of time.

273 Visual search experiments in humans have typically found that people ignore distractors and  
274 focus on the target stimuli when there is only a single target type (Wolfe, 1994) but fail to  
275 ignore distractors when instructed to attend to multiple targets (Houtkamp and Roelfsema,  
276 2009; Menneer et al., 2009). This has led to the suggestion that while multiple items can be  
277 held in the working memory, we are unable to process different search templates concurrently  
278 (Olivers et al., 2011). Here, our results suggest that bees can activate two different search  
279 templates within a second of each other. The bees chose almost no distractors in our  
280 experiment, indicating that they were using search templates for particular targets rather than  
281 choosing colours in a non-specific manner.

282 This conclusion is especially supported by results from Condition 2 and the control  
283 experiment. In Condition 2, the target colours were both less saturated than the distractors.  
284 They were also well separated in the bee colour space and one of the target colours (magenta)  
285 was close to one of the distractors (blue) as well as the other target colour. Thus, the bees  
286 could not simply rely on the similarity of target colours during visual search and any natural  
287 preference for saturated colours would be insufficient to explain our results. The control  
288 experiment further shows that bees could distinguish between the blue and the pink flowers,  
289 reinforcing the idea that even for similar target colours they can use two different search  
290 templates rather than relying on one template that was non-specific enough to include both  
291 the pink and blue flowers. These results, and the clear concordance of our results from both  
292 Condition 1 and Condition 2, support the idea that the bees were using separate search  
293 templates for the target colours.

294 It is possible that what seems like an extension of working memory capacity might be  
295 explained by better and more rapid retrieval from the reference memory (Ericsson and  
296 Kintsch, 1995; Gobet, 2000). Here, however, we cannot distinguish whether the bees were  
297 simultaneously holding the two search templates in their working memory or rapidly  
298 retrieving the templates from their reference memory. One potential mechanism by which  
299 bees might switch between target types is suggested by human psychophysical studies that  
300 use the same target from trial to trial, across multiple trials (Schneider and Shiffrin, 1977).  
301 This is similar to the training phase of our study where bees were extensively ‘block-trained’  
302 on the same two targets, on average for over four hours. Studies like this found a quicker  
303 recall of items in memory and this seemed to be enabled by a transition from an ‘effortful’  
304 working memory to a more automated process that recognizes the target items (Rossi et al.,  
305 2007; Woodman et al., 2007). Given the potentially lower computational and storage

306 capabilities of the bee nervous system, the idea that similar processes might explain our  
307 results is appealing.

308

309 Our results have implications for flower constancy, the tendency of bees to specialize on a  
310 single flower species amongst all available flowers (Waser, 1986). Even in studies  
311 demonstrating that bees visit multiple targets, bees predominantly visited one flower type and  
312 only sampled other flower types (Heinrich, 1976; Heinrich, 1979). We, however, show that  
313 when colour-naive bees have extended training, they do not specialize on a single target but  
314 choose either of two targets more or less equally, often switching between them. The  
315 disparity between our results and previous findings could be due to several reasons. Our  
316 training schedule with alternate blocks of different tasks might have enabled bees to learn  
317 both the colours better and store them effectively in their reference memory. In addition, we  
318 used colour-naive bees for our study, which prevented previously learnt colour biases from  
319 interfering with the learning of new targets. Our experiment also excluded the possibility of  
320 learnt olfactory cues influencing the decisions of bees which could potentially play a role in  
321 field studies.

322 Several researchers have implicated memory limitations in flower constancy (Chittka et al.,  
323 1985; Lewis, 1986; Waser, 1986). Our results suggest that if flower constancy is due to  
324 cognitive constraints, this does not reflect a limitation of working memory, but rather  
325 represents a difficulty with learning novel targets immediately after learning a target (Chittka  
326 and Thomson, 1997). One should note, however, that in our experiment the flowers differed  
327 in only one attribute (colour) and in nature, flowers might differ in other attributes such as  
328 morphology and odour as well. It would be important to test whether memory or learning  
329 constraints determine flower constancy on flowers with these multiple attributes. Our results,  
330 in combination with previous work, however, support a model where newly learned targets  
331 hamper the learning of further targets not yet stored in the reference memory whereas targets  
332 that were previously learned are easily recalled (Fig. 5).

333 In our model, a newly learnt search template enters the working memory and is  
334 utilized to find targets. This template prevents further templates from being learnt during an  
335 inhibitory period that lasts for as long as the trace of the primary template lasts in the working  
336 memory (grey bars; Fig. 5A). This template could enter the reference memory after repeated  
337 use for a longer duration of time. After the inhibitory period, i.e., after ' $t_w$ ' seconds, another



338 target can be learnt and similarly stored in both working and later, reference memories. Now  
339 either of these two targets can be recalled from the reference memory. The recall of learnt  
340 targets is, however, not restricted by the inhibitory period following the use of a particular  
341 search template. Hence, multiple learnt search templates can be used within seconds of each  
342 other with the minimum time 't<sub>p</sub>' between switches being determined by the processing time  
343 taken to recall templates rather than by working memory duration (Fig. 5B). The model  
344 would therefore predict that the time taken by bees to recall and use secondary learnt  
345 templates after using a primary search template should be less than the time taken by bees to  
346 choose a completely novel target i.e., t<sub>p</sub> < t<sub>w</sub>. This could be tested in experiments where bees  
347 trained on two target colours are subsequently tested in a condition with both the targets and  
348 novel colours present. Our model would predict that the transition times between the learnt  
349 targets to be less than the transition times between targets and novel colours.

### 350 **Acknowledgements**

351 We would like to thank Prof. Lars Chittka for useful comments and logistical support and two  
352 anonymous reviewers for their helpful comments. VN was supported by a Marie Curie  
353 Incoming International Fellowship.

### 354 **Author Contributions**

355 VN conceived and designed the experiments and analyzed the results. VN and JGP  
356 performed the experiments and wrote the paper.

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424 **Figure Legends**

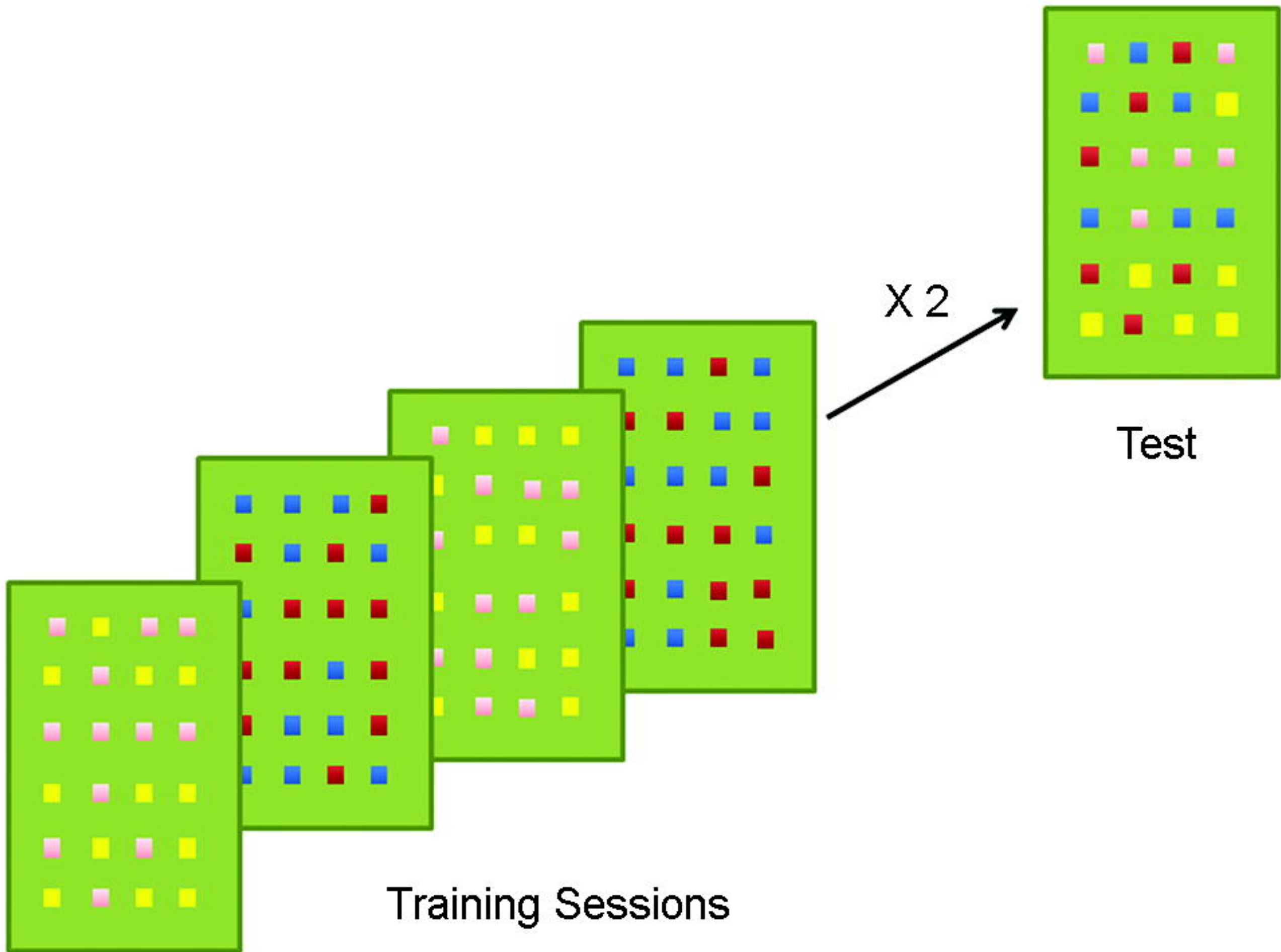
425 Fig. 1. Training tasks and test protocol used in the main experiment. Bees were trained to  
426 discriminate between a rewarding and an unrewarding colour in two alternating colour  
427 discrimination tasks. The starting colour discrimination task alternated between bees. After  
428 four training sessions on each task, bees were tested with all four colours and no reward. Two  
429 experimental conditions were conducted with different rewarding colours in each: Condition  
430 1: pink and blue; Condition 2: yellow and magenta.

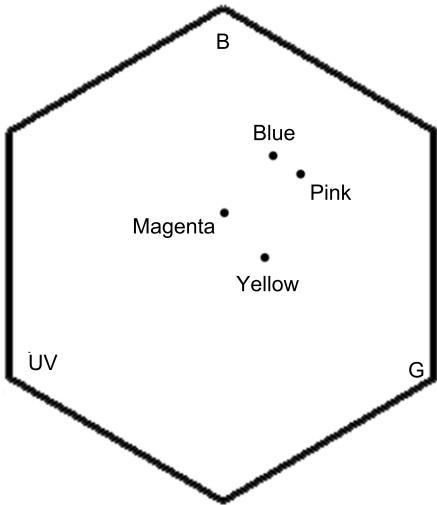
431 Fig. 2. Colour loci of the artificial flowers in the colour hexagon. The three vertices indicated  
432 correspond to the bumblebee photoreceptors sensitive to ultraviolet (UV), blue (B) and green  
433 (G). The angular distance from the centre represents the hue as perceived by the bee and  
434 distances between points indicate the discriminability of the hues. The distance between the  
435 centre and any vertex is 1 and distances above 0.1 are perceptually distinguishable. The  
436 calculated distances between colour loci plotted here based on the measured spectra show  
437 that blue-magenta (colour distance = 0.31), pink-yellow (colour distance = 0.38), blue-pink  
438 (colour distance = 0.13) and magenta-yellow (0.25) are all distinguishable.

439 Fig. 3. Median number of choices made by bees with inter-quartile range (boxes) and range  
440 (whiskers) in experimental conditions 1 (A, C) and 2 (B, D). A) & B) Median number of  
441 choices in each transition type during the test. C) & D) Median number of constant choices  
442 made by bees before switching colours. Transition types are indicated on the x-axis: P\_P:  
443 pink to pink; B\_B: blue to blue; B\_P: blue to pink; P\_B: pink to blue; M\_M: magenta to  
444 magenta; Y\_Y: yellow to yellow; M\_Y: magenta to yellow; Y\_M: yellow to magenta.

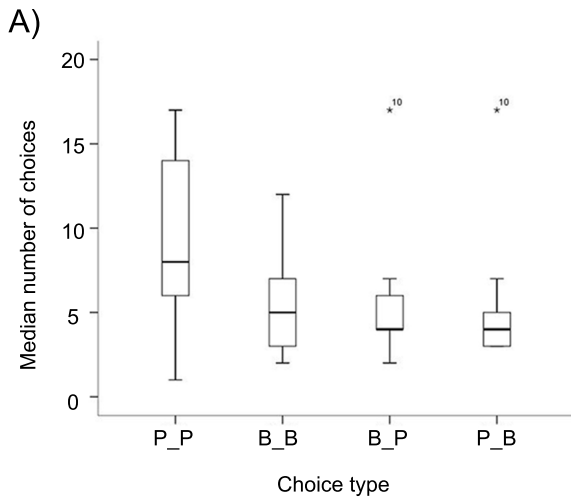
445 Fig. 4. Transition times compared to estimates of bee working memory: 9 s: Complete decay  
446 of traces in the working memory, 6s: Memory traces still robust. A) & B) Median time spent  
447 between choices split according to transition type with inter-quartile ranges (boxes) and range  
448 (whiskers) in experimental conditions 1 (A) and 2 (B). Some outliers have been excluded for  
449 ease of representation. Dotted lines represent known estimates of bee working memory. C) &  
450 D) Proportion of non-constant choices (switches) made within estimates of bee working  
451 memory in experimental conditions 1 (C) and 2 (D). Transition types are indicated on the x-  
452 axis and legends: P\_P: pink to pink; B\_B: blue to blue; B\_P: blue to pink; P\_B: pink to blue;  
453 M\_M: magenta to magenta; Y\_Y: yellow to yellow; M\_Y: magenta to yellow; Y\_M: yellow  
454 to magenta.

455 Fig. 5. Model of working memory dynamics. A) Stimuli that are visually encountered create  
456 working memory traces that decay with time. The creation of a working memory trace is  
457 followed by an inhibitory period (grey bars) during which new stimuli (first blue square)  
458 cannot enter the working memory and therefore cannot be learned. B) Stimuli encountered  
459 after this period (second blue square) can enter the working memory. Stimuli that have  
460 previously entered the reference memory (pink square) can be recalled to the working  
461 memory if encountered externally, even during the inhibitory period.

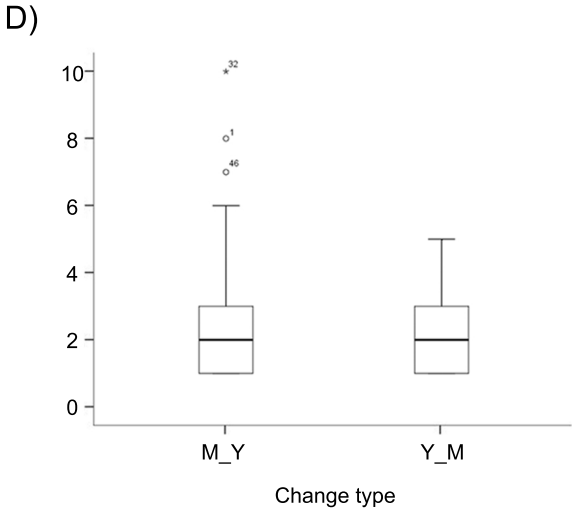
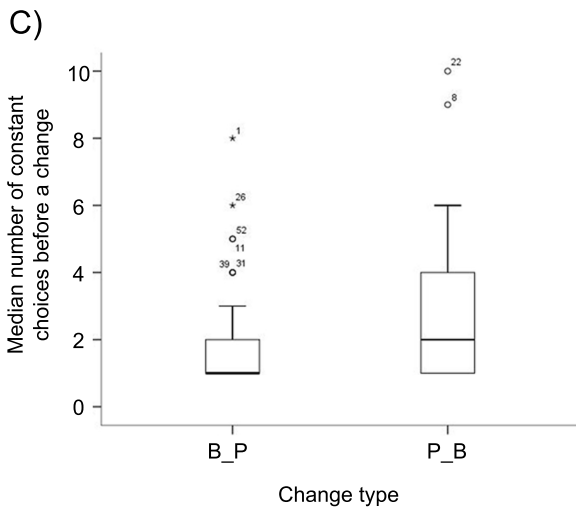
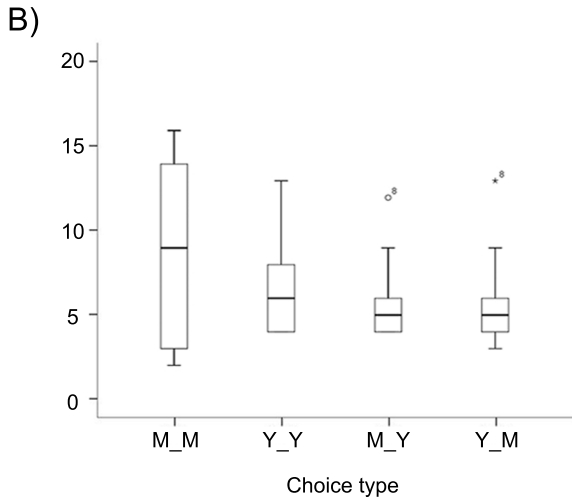




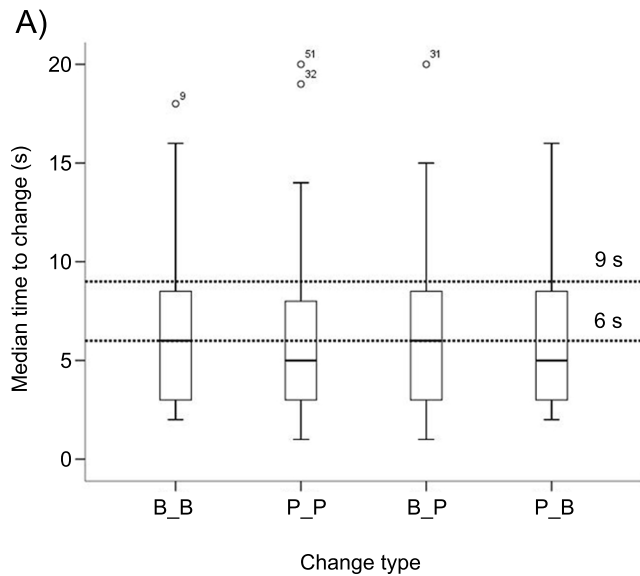
Target Colours: Pink and Blue



Target Colours: Magenta and Yellow



Target Colours: Pink and Blue



Target Colours: Magenta and Yellow

