

1 **Reward Value Is More Important Than Physical Saliency During Bumblebee Visual Search For** 2 **Multiple Rewarding Targets**

3 Vivek Nityananda^{1*} and Lars Chittka²

4 1. Behavioural Sciences and Psychology, Biosciences Institute, Newcastle University, Henry
5 Wellcome Building, Framlington Place, Newcastle Upon Tyne, NE2 4HH, UK.

6 2. School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road,
7 Mile End, London, E1 4NS, UK.

8 * Corresponding author

9 Email: vivek.nityananda@newcastle.ac.uk

10 Ph: +441912086246

11 Orcid ID: 0000-0002-2878-2425

12 **Abstract**

13 Several animals, including bees, use visual search to distinguish targets of interest and ignore
14 distractors. While bee flower choice is well studied, we know relatively little about how they choose
15 between multiple rewarding flowers in complex floral environments. Two important factors that could
16 influence bee visual search for multiple flowers are the physical saliency (colour contrast against the
17 background) of flowers and the reward value associated with them. We here investigated how these two
18 different factors contribute to bee visual search. We trained bees to independently recognize two
19 rewarding colours that in different experiments differed in either physical saliency, reward value or
20 both. We then measured their choices and attention to these colours in the presence of distractors in a
21 test without reinforcement. We found that bees preferred more salient or higher rewarding flowers and
22 ignored distractors. When the high-reward flowers were less salient than the low-reward flowers, bees
23 were nonetheless equally likely to choose high-reward flowers. Bees were more also more likely to
24 attend to these high-reward flowers, with higher inspection times around them and faster search times
25 when choosing them. When flowers differed in reward, we also found an effect of the training order

26 with low-reward targets being more likely to be chosen if they had been encountered during the more
27 immediate training session prior to the test. Our results parallel recent findings from humans
28 demonstrating that reward value can attract attention even when targets are less salient and irrelevant
29 to the current task.

30 Keywords: Visual Search, Attention, Bee, Flower Constancy, Reward, Saliency

31

32

33 **Introduction**

34 Animal foraging behaviour is very well studied, but research in this area has not often considered more
35 psychological aspects of foraging such as attention and visual search. Adapting human visual search
36 experiments to investigate visual search in other animals, including bees, has led to an increased
37 understanding of their foraging behaviour and holds promise to become a productive field of research
38 (Dukas and Kamil 2001; Bond and Kamil 2002; Spaethe et al. 2006; Morawetz and Spaethe 2012;
39 Nityananda and Patrick 2013; Ben-Tov et al. 2015; Orłowski et al. 2015, 2018; Saban et al. 2017).
40 Visual search experiments typically present individuals one target in middle of distractors. Studies have
41 also looked at how attention is deployed when more than one instance of a target type is present
42 (Horowitz and Wolfe 2001) or how attention is divided across multiple tasks (Miller 1982). Fewer
43 studies have looked at visual search for multiple object types or categories that are presented
44 simultaneously (Duncan 1980; Huang et al. 2007; Kristjánsson et al. 2014; Berggren and Eimer 2020).
45 Yet in real life we might well be searching for multiple items at a time, such as say, tomatoes and onions
46 in the supermarket.

47 In bees, studies of visual search have also focussed on how they choose single targets over others, and
48 we know less about how they search in complex floral environments. In particular, research has
49 focussed on flower constancy, the tendency of bees to specialize on one flower type (Heinrich 1979;
50 Wells and Wells 1983; Waser 1986; Hill et al. 1997). A prominent explanation of flower constancy is
51 that it is a result of a cognitive limitation (Waser 1986; Raine and Chittka 2007), suggesting that bees
52 cannot simultaneously choose multiple flower types. This view has been challenged by recent work
53 showing that if bees are given the opportunity to learn multiple rewarding flower types, they readily
54 approach both types, flexibly switching between the two (Nityananda and Patrick 2013). In fact, they
55 appear to be able to learn several different target types and this ability is reflected by changes in the
56 neural structures in their brains (Li et al. 2017). Bees can thus clearly select multiple flowers
57 simultaneously, but we still do not know the factors influencing their choices between these flowers.

58 In humans, several factors are known to influence visual search (Wolfe and Horowitz 2004; Wolfe
59 2020), but two broad processes have typically been identified as fundamental. These are often classified

60 as bottom-up and top-down visual search (Johnson and Proctor 2004). Bottom-up processes involve an
61 involuntary, rapid capture of visual attention by physically salient stimuli. Top-down processes are
62 more deliberate and guided by the goals of an immediate task. More recently, a third category of
63 processes has been proposed involving the influence of search history (Anderson et al. 2011a; Awh et
64 al. 2012; Anderson 2019; Theeuwes 2019). The most prominent examples of these processes have
65 focussed on the role of reward value (Anderson et al. 2011a, b). Target stimuli that are relevant or
66 monetarily rewarding in one task have been shown to capture visual attention even when they are
67 irrelevant to a subsequent task and not physically salient (Anderson et al. 2011b; Bourgeois et al. 2017;
68 Bucker and Theeuwes 2017). The capture of visual attention in these cases is also involuntary and
69 rapid, as is typically seen in response to physically salient stimuli. Thus, visual search and attention can
70 be influenced by three different processes dependent on physical saliency, current goals and search
71 history.

72 The physical saliency of flowers as measured by their colour contrast against the background influences
73 flower choice in bees (Lunau 1990; Lunau et al. 1996; Goulson 2000) and would also be expected to
74 influence visual search and attention. Goal-driven visual search is more difficult to study in bees given
75 the impossibility of providing verbal instructions to set goals for them. One way of specifying targets
76 for the bees is to reward certain targets compared to others and reward value (sucrose concentration)
77 does influence flower choice in bees (Benard et al. 2006; Avarguès-Weber and Giurfa 2014). However,
78 this better resembles reward-based visual search than goal-directed search. Both reward and physical
79 saliency could therefore influence visual search in bees. A bee might, however, simultaneously
80 encounter flowers with differing saliency and reward and it is not yet known how these different factors
81 could interact and influence visual search. In this study, we therefore ran a series of experiments to test
82 how saliency and reward influence bee visual search for two simultaneously rewarding target types.

83 **Methods**

84 *Bees*

85 We obtained the bees from a commercial supplier (Syngenta Bioline, Weert, The Netherlands) and

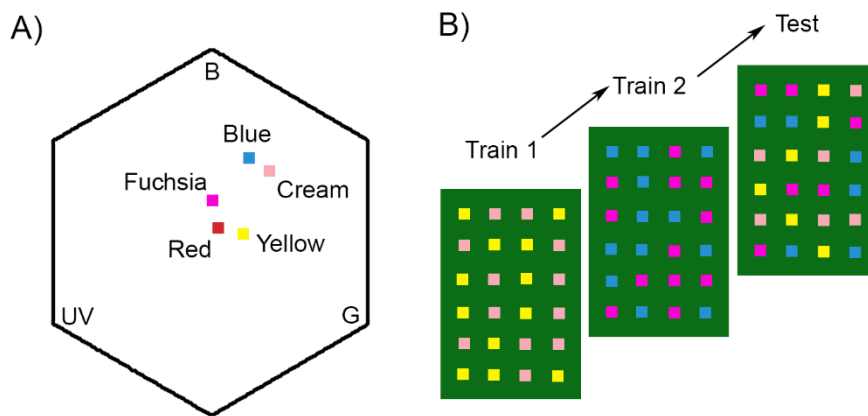
86 tagged them with Opalith number tags (Christian Graze KG, Weinstadt-Endersbach, Germany) to allow
87 for individual identification. The bee colonies were transferred under red light to one chamber of a two-
88 chambered wooden nest box (28×16×11 cm length × width × height). The floor of the other chamber
89 was covered with cat litter to give bees an area to discard refuse. The nest box was connected through
90 a 24.5 cm long transparent Perspex tunnel to an arena consisting of a wooden box (100×60×40 cm
91 length × width × height) covered with a UV-transparent Plexiglas lid. The bees could enter this arena
92 to forage for sucrose solution. The floor of the arena was covered with green card and the illumination
93 was provided from above using two twin lamps (TMS 24 F with HF-B 236 TLD (4.3 kHz) ballasts;
94 Philips, The Netherlands) fitted with Activa daylight fluorescent tubes (Sylvania, New Haven, UK).
95 Pollen was provided directly into the colony on alternate evenings.

96 *Spectral Measurements*

97 We measured the reflectance spectra of the artificial flowers using an Avantes AvaSpec 2048
98 spectrophotometer (Anglia Instruments Limited, Soham, UK) with a deuterium-halogen light source,
99 relative to a BaSO₄ white standard. To account for the difference between spectral sensitivity in humans
100 and bees, we converted the spectra of the targets into a bee-specific hexagonal colour space (Chittka
101 1992) incorporating the spectral sensitivity of bumblebee photoreceptors (Skorupski et al. 2007) , the
102 spectral reflectance of the background and the spectral distribution of the lights used. The colour
103 hexagon has three vertices corresponding to maximal excitation of each of the bee photoreceptors,
104 which are tuned to green, blue and ultraviolet (UV) light (Chittka 1992). Three further vertices
105 correspond to colour mixtures resulting from approximately equal excitation of two spectral receptors.
106 The Euclidean distance between the centre of the hexagon and each of these vertices is 1 and colour
107 distances greater than 0.1 are well distinguished by bees without special training procedures (Dyer and
108 Chittka 2004a). Once plotted in this colour space (Fig 1), the colour loci can be used to calculate the
109 distances in colour space between pairs of colours, thus indicating the perceptual discriminability of the
110 colours. All measures of colour differences between the artificial flowers used in our experiments are
111 provided in Table S1.

112 *Pretraining*

113 We trained colour-naïve foraging bees to forage from square transparent Perspex chips (side: 25 mm,
114 thickness: 5 mm) that served as artificial flowers (henceforth “flowers”). Each flower had a well in the
115 centre into which rewarding (sucrose solution) or non-rewarding (water) liquids could be placed. After
116 bees learned to approach these flowers, we placed them on glass vials (4 cm tall, 1.5 cm in diameter)
117 and trained the bees to feed from them when they were arranged in a 6 X 4 horizontal grid, with vials
118 placed 15 cm apart. In this grid, twelve randomly chosen flowers had 12 μ l of 50% sucrose on them and
119 the others remained empty. The positions of rewarding and non-rewarding flowers in all experiments
120 were determined using the random number generator function RAND() in Microsoft Excel®. Once the
121 bees had foraged on this grid for three bouts, we commenced training.



122

123 **Fig. 1.** A) Colour loci of the artificial flowers used across all experiments in the colour hexagon. Three
124 of the vertices correspond to maximum excitation of the bumblebee photoreceptors sensitive to
125 ultraviolet (UV), blue (B) and green (G). The angular distance from the centre represents the hue as
126 perceived by the bee. Distances between points indicate the hue discriminability. The distance between
127 the centre and any vertex is 1 and colours that differ by distances above 0.1 are easily distinguishable.
128 B) Example training and test protocol used in the experiments. Bees were trained on one rewarding and
129 one non-rewarding colour in each training session (Train 1 and Train 2) and tested without
130 reinforcement with all four colours in the test session (Test).

131

132 **Training**

133 In each experiment we trained bees from three different colonies on two visual discrimination tasks.
134 The tasks involved bees having to discriminate target flowers of one colour from distractor flowers of
135 another colour. The flowers consisted of coloured Perspex chips placed in a grid as described above.
136 The 12 target and 12 distractor flowers were placed in positions on the grid that were randomly chosen
137 for each bout of the training. Target flowers held 12 μ l of sucrose solution, while distractor flowers held
138 12 μ l of distilled water. Flowers were not refilled during a given training bout. Each bee was
139 individually trained on one of these tasks until it reached a success criterion of 80% correct choices out
140 of the last 20 choices made. Choices were recorded when the bee probed the flowers for reward and
141 bees could revisit flowers in all experiments. Between training bouts, we cleaned the flowers with 99%
142 ethanol to remove scent markings, and subsequently with water to remove any traces of ethanol.

143 Once a bee successfully completed one training task, it was presented with another task consisting of
144 target and distractor flowers with different colours from those in the first task. The order in which each
145 of these tasks were presented was alternated between bees. The exact details of the colours and reward
146 used are given below. Bees thus sequentially learnt two different rewarding colours.

147 *Experiment 1: How does physical saliency influence bee visual search?*

148 In this experiment, twenty bees were trained on one physically salient target and one less salient target
149 in separate discrimination tasks. For ten of these bees, one of the two tasks had Blue as the rewarding
150 colour and Cream as the non-rewarding colour. The other task had Fuchsia as the rewarding colour and
151 Red as the non-rewarding colour. Both target colours provided a reward of 50% sucrose solution (v/v).
152 The experiment was replicated with another ten bees using a different set of colours. In this replication,
153 the rewarding colours were Fuchsia and Red, while the distractors were Cream and Yellow respectively.
154 This replication ensured that Fuchsia, the less salient colour (compared to Blue) in the first combination
155 was the more salient colour (compared to Red) in the second combination of colours (Fig 1).

156 *Experiment 2: How does reward value influence bee visual search?*

157 In this experiment, 15 bees were trained on one high-reward target and one low-reward target in separate
158 discrimination tasks. One of these tasks had Blue as the rewarding colour and Fuchsia as the non-

159 rewarding colour. The other had Cream as the rewarding colour and Yellow as the non-rewarding
160 colour. These colours were chosen as the Blue and Cream colours were close in physical saliency,
161 defined as colour contrast with the background (Table S1). In the two discriminations tasks, one of the
162 target colours had a reward of 50% Sucrose solution (v/v) while the other had a reward of 30% Sucrose
163 solution (v/v). With one exception, the association of high and low rewards with each of the target
164 colours (Blue and Cream) was counterbalanced across all trials as was the order in which bees
165 experienced high and low reward in their two training tasks.

166 *Experiment 3: How does bee visual search combine reward value and physical saliency?*

167 In this experiment 16 bees were trained on two discrimination tasks. One of these had a high-reward
168 target offering a reward of 50% Sucrose solution (v/v). This target was Yellow in colour and had low
169 colour contrast (i.e. physical saliency) against the background. The distractor in this task was Cream in
170 colour. In the other task, the target offered a lower reward of 30% Sucrose solution (v/v). The target
171 was Blue in colour and had a high colour contrast against the background and the distractor was Fuchsia
172 in colour. The order in which bees encountered each of these tasks was counterbalanced.

173 *Test*

174 In all experiments, once training was completed, we tested bees on their visual search when faced with
175 multiple targets. We presented the bees with six flowers each of the two rewarding colours they were
176 trained on and six flowers of each of the distractor colours. All flowers in the test were non-rewarding
177 containing 12 μ l of distilled water. This prevented reinforcement learning during the test. We noted the
178 choices made by the bees and the order they were made in. The foraging bout of each bee during the
179 test was recorded using a Sony DCR-SR58E Handycam to enable later analysis of the times between
180 the choices. We ran the tests until five minutes were over, or the bee returned to the colony after making
181 at least 12 choices, whichever occurred sooner.

182 *Data Analysis*

183 For all experiments, we split the choices made by the bees into the different transitions between colours
184 and noted which were switches to different colours and which were constant transitions. We examined

185 the number of constant transitions made before each switch to measure how often bees had runs of
186 constant choices. We then calculated a sequence index for each bee by dividing the number of constant
187 transitions by the total number of transitions (Heinrich 1979). This index is the probability of constant
188 transitions compared to switches. An index close to 1 would indicate that the bees were flowers constant
189 while a value close to 0.5 would indicate that bees switched flowers with every new choice. We used a
190 Wilcoxon rank sum test ($\alpha=0.05$) to compare the observed number of constant choices with the index
191 values of 1 and 0.5. We also examined how quickly bees made these different choices by comparing
192 the median times taken to make constant choices and switches using Wilcoxon rank sum tests ($\alpha=0.05$).
193 Since the bees occasionally flew around the arena for extended periods of time without making a choice,
194 we ran an outlier analysis for the times within each category, and excluded data points that were greater
195 or less than 1.5 times the interquartile range prior to the second analysis. Timing data is missing for one
196 bee in Experiment 1 and five bees in Experiment 2 because of the lack of video recordings.

197 To examine how different factors influenced the proportions of choices made by the bees we ran
198 generalized linear models with the proportion of choices as a dependent variable and the different
199 factors as independent variables. For experiment 1, the independent variables were physical saliency
200 (high or low) and second variable representing the training order. This second variable was a binary
201 variable representing whether the bee first encountered the high saliency target or the low saliency target
202 during the training on visual discrimination tasks. For experiment 2, the independent variables were
203 reward value (high or low) and a second binary variable representing whether the bee first encountered
204 the high reward target or the low reward target during training. For experiment 3, we also had the two
205 independent variables as in experiment 2. In all the models, bee identity was modelled as a random
206 variable and the proportion of choices were modelled as a binomial distribution with a logit link
207 function. We ran models looking for main effects of the independent variables and interaction effects
208 between the variables as well. In this and all other analyses, models were compared using the Akaike
209 Information Criterion (AIC) and the model with the lowest criterion was chosen. The significance of
210 each variable was compared against an α of 0.05.

211 In experiment 3, we were also interested to see if higher reward could influence bee attention to a target

212 with low physical saliency. We used the positions of the bee during visual search as a proxy for
213 attention. Using the open-source program Tracker (V5.15, ©2020 Douglas Brown,
214 physlets.org/tracker), we perspective corrected each video and tracked the position of the bee in each
215 frame of the video recording during the test phase. We used this to analyse bee behaviour during the
216 first two minutes of the videos. Frames in which it was not possible to spot the bee – either because it
217 flew to the corner of the arena or due to reflections of the lighting- were labelled as missing data. Using
218 the tracked positions of the bees we obtained a map of search behaviour for each bee. We specified
219 zones on these maps corresponding to flower areas and non-flower areas. Flower areas were areas
220 within 2 cm of the flowers. All other areas were non-flower areas. We measured inspection time as the
221 time each bee spent in each of these areas by summing the number of video frames in which bees were
222 present in them and multiplying this by the frame rate (25 frames per second). We compared the
223 inspection time for the different types of targets and distractors. We used a generalized linear model to
224 model this as a binomial variable with a logit link function. As in the analysis above we used reward
225 value, physical saliency and search history as independent variables and bee identity as a random factor.
226 We ran models looking for main effects of the independent variables and for interaction effects between
227 the variables as well.

228 All statistical analyses were run in RStudio (version 1.2.5033)

229 **Results**

230 *Experiment 1: How does physical saliency influence bee visual search?*

231 The average time taken for the first and second training bouts on this experiment was 2080.7 (\pm 1418)
232 seconds and 971.9 (\pm 366.4) seconds respectively.

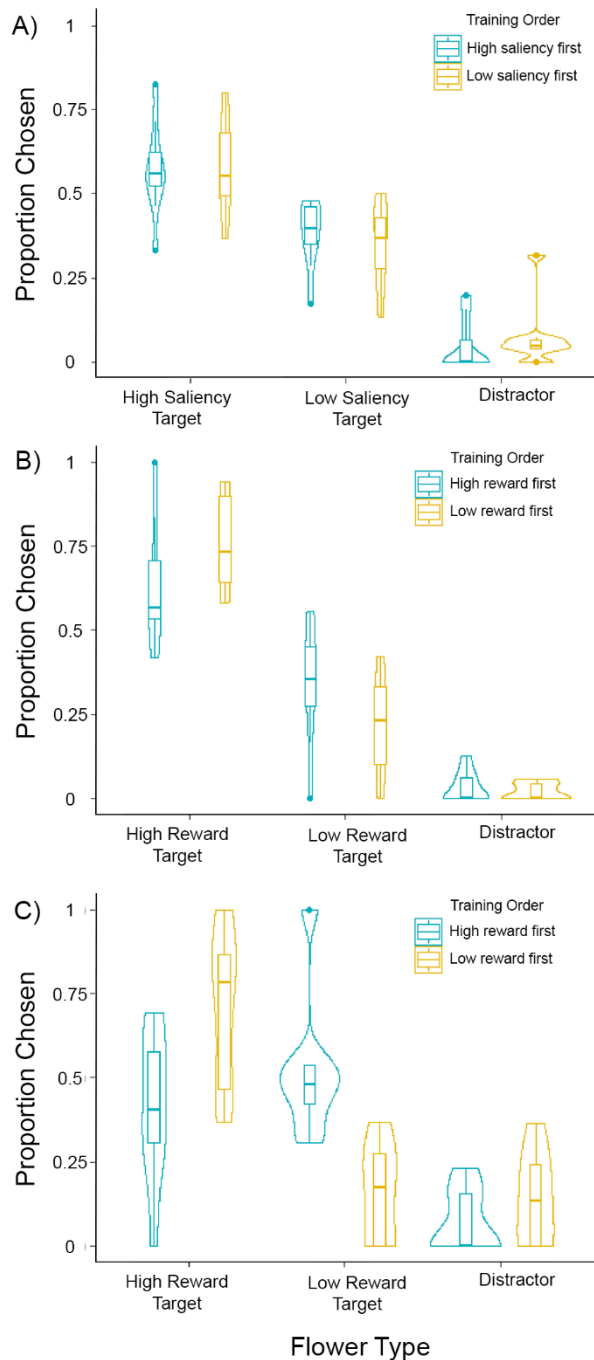
233 Combining results from both flower sets we found that the average proportion of salient target flowers
234 chosen during tests was 0.58 (\pm 0.13 S.D.) and the average proportion of equally rewarding non-salient
235 targets chosen was 0.37 (\pm 0.11 S.D.). The average proportion of distractors chosen was 0.06 (\pm 0.08
236 S.D.). If bees chose equally between the two targets without choosing any distractors, we would expect
237 an equal proportion (0.5) of both salient and non-salient targets to be chosen. Saliency had a significant

238 effect on the proportion of targets chosen; the proportion of high-saliency targets chosen was
239 significantly greater than the proportion of non-salient targets chosen (GLMM, Effect size estimate: -
240 0.84, $P = 4.3 * 10^{-9}$, Fig 2A) and the proportion of distractors chosen (GLMM, Effect size estimate = -
241 3.24, $P < 2 * 10^{-16}$). The low number of choices made to distractors demonstrates that the bees had
242 memorised both types of previously rewarding targets in the training bouts and could recall them in the
243 presence of distractors. The best model that described the data did not include the effect of training
244 order indicating that this was not an important determinant of the proportion of salient targets chosen.

245

246 The average sequence index of the bees was 0.51 (± 0.17 S.D.). An index close to 0.5 indicates equal
247 numbers of constant choices and switches, while an index close to 1 indicates complete flower
248 constancy with no switches. This index was not significantly different from 0.5 (Wilcoxon rank sum
249 test, $W = 200$, $P = 0.1$), showing that the bees were equally likely to make constant choices and switches
250 (Fig 3). The times taken for choices between like flowers and transitions between flower types were not
251 significantly different (Wilcoxon rank sum test, $W = 13036$, $P = 0.14$). The mean time taken for constant
252 choices was 7.53 (± 4.93 S.D.) seconds compared to a mean of 9.03 (± 7.05 S.D.) seconds for switches
253 (Fig 4A).

254



272

273 **Fig. 2:** Proportions of different flower types chosen in A) Experiment 1, B) Experiment 2, and C)

274 Experiment 3. Box plots depict the median and the first and third quartiles, the whiskers depict the

275 largest and smallest values that are within 1.5 times the interquartile range from the edge of the boxes.

276 Violin plots overlaid on top of the box plot depict the mirrored density plots of the data.

277

278 *Experiment 2: How does reward value influence bee visual search?*

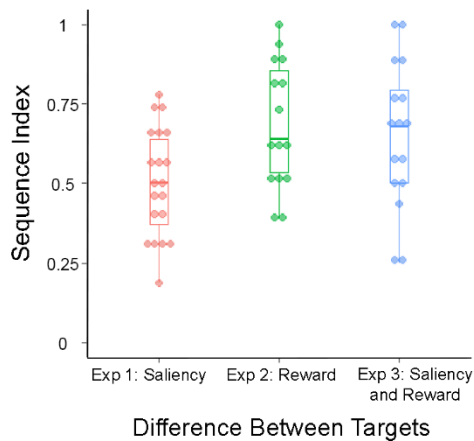
279 The average time taken for the first and second training bouts on this experiment was 1033.8 (\pm 439.8)
280 seconds and 958.7 (\pm 493.4) seconds respectively.

281 The average proportion of high-reward targets chosen by bees was 0.69 (\pm 0.18 S.D.), while the average
282 proportion of low reward targets chosen was 0.28 (\pm 0.17 S.D.). The best model for the proportion of
283 choices included an interaction between the reward value and the order of the training. Higher reward
284 value (50% Sucrose) led to significantly greater proportion of choices compared to both low reward
285 value (30% Sucrose) flowers (GLM, effect size estimate: -1.12, $P = 2.15 \times 10^{-5}$, Fig 2B) and distractors
286 (GLM, effect size estimate: -3.65, $P = 1.18 \times 10^{-13}$). Thus, bees chose high-reward targets more often
287 than low-reward targets. The average proportion of choices made to distractors was 0.02 (\pm 0.04 S.D.),
288 demonstrating that the bees were capable of simultaneously choosing between two targets even in the
289 presence of distractors.

290 Bees that were first trained on high-reward targets chose these targets significantly less than if they
291 were first trained on low-reward targets (GLMM, effect size estimate: 0.72, $P = 0.0088$). There was
292 also a significant interaction effect between training order and reward value (GLMM, effect size
293 estimate: -1.38, $P = 0.00041$). Bees were thus more likely to choose high-reward targets if they had been
294 trained on them in the bout immediately preceding the test (i.e. trained on the low-reward targets first,
295 yellow/right vs blue/left plots in Fig 2B). The training times between the end of the first bout and the
296 start of the test were, however, not significantly different when the first training bout had targets of high
297 or low reward value (Wilcoxon rank sum test, $W = 29$, $P = 0.57$). The interaction effect between training
298 order and reward value is thus not due to difference in training times.

299 The average sequence index of the bees was 0.69 (\pm 0.20 S.D.) and this was significantly different from
300 0.5 (Wilcoxon rank sum test, $W = 187.5$, $P = 0.0008$, Fig 3). This indicates that in this experiment bees
301 were more likely to have constant choices than switches. The time taken between choices was also
302 significantly different between constant choices and switches chosen (Wilcoxon rank sum test, $W =$
303 2661.5, $P = 0.01$, Fig 4B). The mean time taken for constant choices was 6.49 (\pm 3.63 S.D.) seconds
304 compared to a mean of 8.47 (\pm 4.88 S.D.) seconds for switches.

305



306

307 **Fig. 3.** Sequence indices in each of the experiments. Box plots as described for Figure 2. The actual
308 data points are overlaid on top of the box plot.

309 *Experiment 3 How does bee visual search combine reward value and physical saliency?*

310 The average time taken for the first and second training bouts on this experiment was 1884.5 (\pm 993)
311 seconds and 1681.1 (\pm 815.3) seconds respectively.

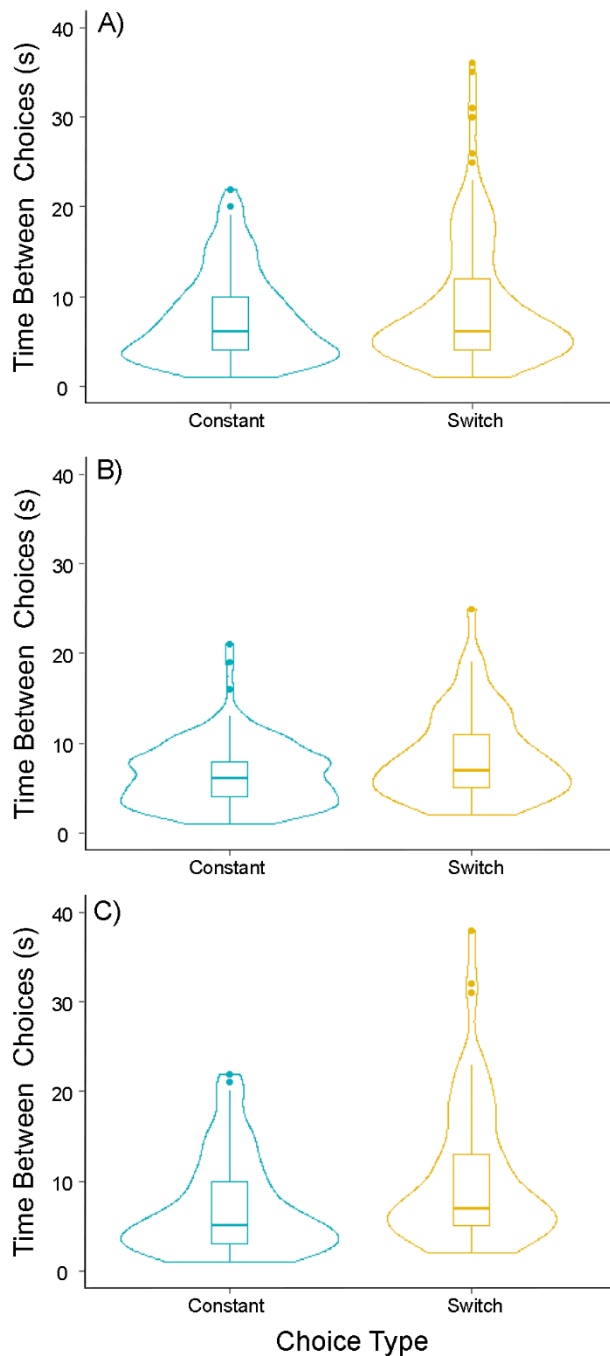
312 The average proportion of high-reward, low-saliency targets chosen by bees was 0.56 (\pm 0.27 S.D.),
313 while the average proportion of low-reward, high-saliency targets chosen was 0.34 (\pm 0.26 S.D.). There
314 was no significant main effect of reward value on the proportion of high and low reward targets chosen
315 (GLM, effect size estimate: 0.32, P = 0.23, Fig 2C) but a significantly higher proportion of high reward
316 targets were chosen compared to distractors (GLMM, effect size estimate: -2.42, P = 2.55×10^{-8}). Thus,
317 bees chose high-reward targets as often as low-reward targets, despite their lower saliency. The average
318 proportion of choices made to distractors was low at 0.10 (\pm 0.12 S.D.), demonstrating that the bees
319 were capable of simultaneously choosing between two targets even in the presence of distractors.

320 The order in which bees were trained on the high-reward and low-reward targets had a significant main
321 effect (GLM, effect size estimate: 1.1654, P = 2.01×10^{-5}). There was also a significant interaction
322 effect between reward value and the order of the training (GLM, effect size estimate: -2.8688, P = 3.39
323 $\times 10^{-12}$). Bees were thus more likely to choose high-reward targets if they were the targets in the second

324 training session (immediately prior to the test) rather than in the first training session.

325 The training times between the end of the first bout and the start of the test were however not
326 significantly different when the first training bout had targets of high or low reward value (Wilcoxon
327 rank sum test, $W = 31$, $P = 0.78$). The interaction effect between training order and reward value is thus
328 not due to difference in training times.

329 The average sequence index of the bees was $0.65 (\pm 0.25 \text{ S.D.})$ and this was significantly different from
330 0.5 (Wilcoxon rank sum test, $W = 192$, $P = 0.0084$, Fig 3). This indicates that in this experiment, bees
331 were more likely to have constant choices than switches. The duration between choosing one flower
332 and the next was also significantly different between constant choices and switches (Wilcoxon rank
333 sum test, $W = 3184$, $P = 0.00053$, Fig 4C). The mean time taken for constant choices was $7.14 (\pm 5.36$
334 $\text{S.D.})$ seconds compared to a mean of $10.51 (\pm 7.78 \text{ S.D.})$ seconds for switches.



335

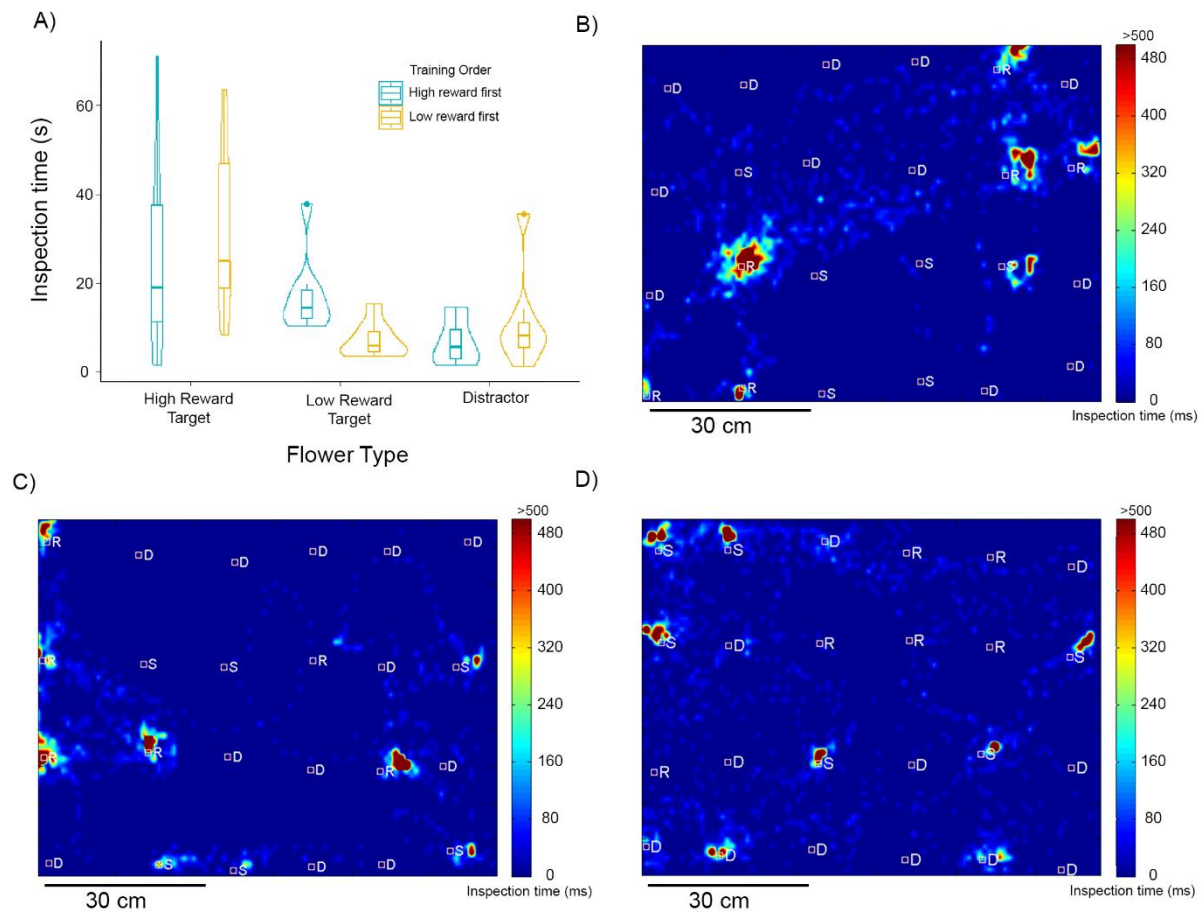
336 **Fig. 4:** Time taken to make constant choices and switches in A) Experiment 1, B) Experiment 2, and
337 C) Experiment 3. Other details as for Figure 2.

338

339 The mean search time spent before choosing a high-reward flower was $7.07 (\pm 5.15 \text{ S.D.})$ seconds while
340 the mean search time spent before choosing a low-reward flower was $9.51 (\pm 7.33 \text{ S.D.})$ seconds, and
341 these values were significantly different (GLM, Estimate = -0.009 , $P=0.009$). Thus, the bees were

342 quicker to choose high-reward targets compared to low reward targets. The model that best explained
343 the proportion of time bees spent in different zones in the arena included flower type and the order in
344 which bees were trained on high or low reward flowers as factors. Bees spent a significantly greater
345 proportion of time inspecting higher rewarding flowers than lower rewarding flowers with greater
346 physical saliency (GLMM, effect size estimate = -0.63, $P < 2 * 10^{-16}$ Fig 5A) and distractors (GLMM,
347 effect size estimate = -2.14, $P < 2 * 10^{-16}$). There was also a significant main effect of the order in which
348 bees were trained on high or low reward flowers (GLMM, effect size estimate = 0.84, $P < 2 * 10^{-16}$) as
349 well as an interaction effect between flower type and the order of training (GLMM, effect size estimate
350 = -2.28, $P < 2 * 10^{-16}$). Thus, when bees were trained on the high reward flowers first and the low-
351 reward flowers later, they were equally likely to spend time around high-reward, low saliency flowers
352 and low-reward high-saliency flowers. However, when trained on the low-reward flowers first and the
353 high-reward flowers later, they spend a greater time around high-reward low saliency flowers compared
354 to low-reward high-saliency flowers.

355



356

357 **Fig. 5.** Inspection times around different flower types. Details for A) as in Figure 2. B-D) Example
358 visual search maps for three bees depicted as a top view of the flight arena with targets and distractors.
359 Colours depict the inspection times up to a maximum of 500 ms (only 5% of all times were greater than
360 this limit). Squares depict flower positions. R = High-reward, low saliency targets; S = Low-reward,
361 high-saliency targets, D = Distractors. B and C depict examples where bees spent more time around high-
362 reward targets, D depicts an example where the bee spent more time inspecting low-reward targets.

363

364 Discussion

365 Bees trained on multiple targets can choose the targets in the presence of distractors, without staying
366 flower constant (Nityananda and Patrick 2013). When targets are matched in both saliency and reward,
367 bees are equally likely to choose either rewarding target, and switch between them often. Our results
368 here demonstrate how bees prioritize learnt rewarding targets when they differ in physical saliency,

369 reward value or both. We find that differences in saliency and reward value do not hamper the visual
370 search task and bees are still able to choose at least two target types and ignore distractors. Both saliency
371 and reward influence the proportion of targets chosen – with more salient and more rewarding targets
372 chosen in higher proportions. The order in which bees encounter the targets during training matters
373 when the targets differ in reward value and bees show a recency effect (Ebbinghaus 1885). This is
374 particularly evident when the targets differ in both saliency and reward value. While bees in this
375 condition seem to choose high reward low-saliency targets at an equal proportion as low-reward high-
376 saliency targets, a slightly different pattern is seen when training order is accounted for. Low-reward,
377 high-saliency targets are more likely to be chosen if they are encountered in the most recent training
378 bout rather than the earlier training bout. This effect is less pronounced for the high-reward, low saliency
379 targets. Our results also show that high-reward targets lead to greater flower constancy, shorter times
380 for constant choices and more time spent attending to these targets.

381 Studies have long shown that bees can differentiate between coloured targets that differ in reward value
382 (Lubbock 1881; Turner 1910; von Frisch 1914; Benard et al. 2006; Avarguès-Weber and Giurfa 2014).
383 Most studies, however, have typically used appetitive training paradigms where bees are trained to
384 distinguish targets with a reward from distractors without a reward (Avarguès-Weber and Giurfa 2014).
385 More recently, studies have focussed on aversive training paradigms where bees distinguish between
386 targets that are rewarding and distractors that contain an aversive solution like quinine (Dyer and Chittka
387 2004b; Giurfa 2004; Avarguès-Weber and Giurfa 2014). These two approaches have different effects
388 with aversive conditioning leading to more fine-grained colour discrimination (Dyer and Chittka 2004b;
389 Giurfa 2004). Studies that use two stimuli that are both rewarding but differ in reward value, as in this
390 study, are fewer but they clearly demonstrate that bees can learn to differentiate colours even in this
391 paradigm (Baude et al. 2011; Riveros and Gronenberg 2012; Avarguès-Weber et al. 2018). In one study
392 using harnessed, rather than free-flying bees, the reward differential was provided by either providing
393 the same concentration of sucrose solution to both the antenna and the proboscis (high reward condition)
394 or to only the antenna (low reward condition). This differential was sufficient for bees to distinguish
395 the colours associated with higher reward from those associated with lower rewards (Riveros and

396 Gronenberg 2012). Our results from experiment 1 demonstrates that flowers that have a higher sucrose
397 concentration are preferred by freely flying bees and bias their visual attention. The results from
398 experiment 3 further show that flowers previously associated with high reward are still chosen half an
399 hour after the training, even when they have lower saliency than low reward flowers.

400 The influence of physical saliency or colour contrast on bee visual search is less well studied than the
401 influence of reward value (but see (Spaethe et al. 2001)). However, some studies have looked at this in
402 the context of the innate preferences of bees (Lunau 1990; Giurfa et al. 1995; Lunau et al. 1996). These
403 preferences are typically biased towards the UV-blue spectral range but do not seem to reflect the colour
404 or green contrast difference from the background (Giurfa et al. 1995). Flower colours that have high
405 spectral purity against background with low spectral purity do however attract the strongest innate
406 behavioural responses from bumblebees (Lunau 1990). In addition, while bees can be trained to
407 overcome their initial biases, their preferences can remain influenced by the effect of innate preferences
408 (Gumbert 2000). In our experiment 3 we used a blue target as a low reward target to see if the high
409 reward value of the other target could overcome biases towards this target. We found this to occur if
410 the bees were trained on the blue targets further in time from the test. Higher reward also biased visual
411 attention away from the high saliency blue targets as indicated by the time spent by the bees around
412 different types of flowers.

413 Our results also show that the search history of the bees is important to consider. Bees might often
414 specialize on the first colour they find to be rewarding – regardless of saliency. This would prevent
415 them from learning multiple targets as in our study. In fact, other studies have found persistent flower
416 constancy when bees are not allowed to learn both targets independently (Wells and Wells 1983; Hill
417 et al. 1997). In nature, multiple targets might possibly be learnt when floral communities are more
418 diverse or have higher densities of flowers (Heinrich 1979; Gegear and Thomson 2004; Baude et al.
419 2011). Our results and those of previous papers show that bees can switch between flowers and do not
420 always stay flower constant. Thus, flower constancy does not stem from a cognitive limitation as has
421 been suggested before (Waser 1986; Raine and Chittka 2007). Our results further point towards the
422 importance of reward value for constancy. Bees show greater flower constancy when the targets differed

423 in reward value. In these cases, they also showed shorter times when making constant choices rather
424 than switching between colours. Bumblebees have been shown to fly shorter distances after visiting
425 rewarding flowers compared to non-rewarding flowers (Dukas and Real 1993). Our results show that
426 the experience of different reward values could also influence their foraging behaviour. Bees appear
427 more likely to switch between flowers that have equal reward value but stay constant to highly
428 rewarding flowers. Flower constancy is also affected by the density of conspecifics (Baude et al. 2011)
429 so including this along with reward value and perhaps floral diversity would make for a fuller picture
430 of the ecology of flower constancy.

431 Reward value also appears to influence the visual attention of the bees in addition to constancy and
432 choice latencies. Bees spent longer inspecting high-reward flowers compared to low-reward flowers of
433 greater saliency and were quicker to choose them. This resembles results from the human visual search
434 literature, especially experiments demonstrating that the reward value associated with a stimulus can
435 influence reaction times even if the stimulus is not task-relevant or salient (Anderson et al. 2011a, b).
436 In our experiments we cannot assign task goals to the bees. However, the training order serves as a
437 proxy for this. Half the bees in experiment 3 were initially trained on the high reward target and then
438 on the low reward target. When faced with the test, the most recent training could arguably be
439 considered the relevant task, making the previous high-reward targets irrelevant stimuli. Nonetheless
440 bees still chose and attended to these targets – paralleling results in human experiments. We might
441 potentially see different results when the reward values are lower, or the contrast of the high reward
442 target is reduced even further. When high-reward targets have very low detectability, low-reward targets
443 with high physical saliency could have lower search times. In these cases, bees might then change their
444 preference to low-reward targets rather than high-reward ones, especially if the rewards are not very
445 different. It has been argued that reward-based attentional capture in humans arises from Pavlovian
446 mechanisms, where the level of reward determines the effectiveness of attentional capture (Bucker and
447 Theeuwes 2017; Mine and Saiki 2018). Since several animals, including bees, are well known for
448 Pavlovian learning, we should therefore expect this form of attention to be widespread in several
449 animals. Our results suggest this might be true in bees and more focussed experiments showing that the

450 mechanisms of attentional capture are shared in bees and humans would be an exciting area for future
451 research.

452

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