

Temporal Variation of Floral Reward can Improve the Pollination Success of a Rare Flowering Plant

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Temporal variation of floral reward can improve the pollination success of a rare flowering plant

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¹ Abstract

² Many pollinating animals visit a variety of flowering plant species. Rare plant species
³ pollinated by such generalists may experience a low quality or quantity of pollination,
⁴ depending on the pollinators' foraging behaviour. How plants cope with this rarity disad-
⁵ vantage is not well understood. One possibility would be to offer a higher floral reward, for
⁶ example a higher nectar sugar concentration. However, since nectar production is costly,
⁷ rare plants may only be able to increase their nectar concentration for a limited time and
⁸ offer little reward afterwards. In this study we performed a laboratory experiment with
⁹ bumblebees (*Bombus terrestris*) foraging on artificial flowers of two colours to investigate
¹⁰ whether the bees' foraging behaviour produces a rarity disadvantage and if so, whether
¹¹ the rare flower type could improve its pollination success through temporal variation of its
¹² nectar sugar concentration, i.e. a temporary increase of nectar sugar followed by a period
¹³ with low concentration. We found that when both flower colours offered equal rewards,
¹⁴ the rare colour received only slightly fewer visits per flower, but had a considerably lower
¹⁵ expected pollination success based on the bumblebees' visitation sequences. Temporal
¹⁶ variation of the rare colour's sugar concentration increased both the quantity and quality
¹⁷ of visits it received. This positive effect was reduced when there were fewer rare flowers or
¹⁸ when two bumblebees foraged simultaneously. Our results suggest that temporal variation
¹⁹ of floral rewards can alleviate, but not completely eliminate the rarity disadvantage.

²⁰ **Keywords:** Nectar, bumblebee, artificial flower, frequency dependence, co-flowering.

²¹ Introduction

²² Global change causes shifts in species' relative abundances, with some populations de-
²³ clining, while others increase (e.g. Li et al. 2015; Komatsu et al. 2019). These changes in
²⁴ abundance can be further amplified or damped if the organisms' survival or reproduc-
²⁵ tive success depend on their relative abundance. While processes which reduce the fitness
²⁶ of abundant species maintain biodiversity, a fitness reduction for rare species may lead
²⁷ to their further decline or even extinction (Schreiber et al. 2019, and references therein).
²⁸ Therefore, to preserve biodiversity under rapidly changing environmental conditions it is
²⁹ important to understand the causes of rare-species disadvantages and possible mitigating
³⁰ factors.

³¹ Pollinating animals such as bees, hoverflies and hummingbirds provide crucial services
³² to many flowering plants. Most of these pollinators visit flowers of more than one plant
³³ species, often within the same foraging bout (Waser et al. 1996; Brosi 2016, and references
³⁴ therein). For plant species pollinated by such generalists, being less abundant than other
³⁵ co-flowering plants can be a disadvantage. If the pollinators visit flowers as they encounter
³⁶ them, a rare species may receive few conspecific and many heterospecific pollen grains
³⁷ because its visitors have most recently been on flowers of other more abundant species (e.g.
³⁸ Levin and Anderson 1970; Kunin 1993; Runquist and Stanton 2013). By contrast, when
³⁹ pollinators temporarily specialize on a single flower type, they deliver the same amount of
⁴⁰ conspecific pollen regardless of plant species abundance, but the visitation rate to a rare
⁴¹ plant species may be low since most animals tend to specialize on abundant flowers to
⁴² maximize their foraging efficiency (Kunin and Iwasa 1996; Smithson 2001; Schmid et al.
⁴³ 2016). Thus, depending on the pollinators' foraging behaviour, rare flowering plants may
⁴⁴ suffer from reduced quality or quantity of pollination, or a combination of both.

⁴⁵ One possible way to overcome the rare-species disadvantage could be to offer a greater
⁴⁶ floral reward than co-flowering abundant species. Many pollinating animals can learn to
⁴⁷ associate floral traits such as colours, shapes or odours with specific rewards (Goulson
⁴⁸ 1999; Jones and Agrawal 2017, and references therein) and show a preference for more

rewarding flowers, for example flowers with higher nectar sugar concentration (e.g. Roberts 1996; Cnaani et al. 2006; Amaya-Márquez and Wells 2008). A high reward therefore has the potential to increase both the quantity and quality of flower visits to rare flowering plants. However, due to the energetic cost of bypassing less rewarding, but more abundant flowers, economically foraging pollinators should only completely specialize on a more rewarding rare flower type if the high reward compensates for the increased travel cost (Stephens and Krebs 1986). Therefore, depending on the rare species' abundance and its floral reward relative to more abundant plant species, offering a higher reward may not always suffice to completely eliminate the rarity disadvantage. Moreover, since reward production can be costly for plants (Southwick 1984; Pyke 1991; Harder and Barrett 1992; Ordano and Ornelas 2005), rare plant species may only be able to increase their floral reward to a limited degree or for a limited time. If a rare plant increases its reward for a short period and offers little or no reward afterwards, it may benefit from the fact that foragers continue to sample its flowers to investigate whether the reward has increased again (e.g. Keasar et al. 2013).

In the current study, we performed a laboratory experiment with buff-tailed bumblebees (*Bombus terrestris* L) foraging on two types of artificial flowers. Our aim was to (1) establish whether the bees forage in a way which may lead to lower pollination success of rarer plant species (due to low quantity and/or quality of flower visits), and if so (2) test whether a rare flowering plant could reduce its disadvantage or even gain an advantage through a temporary increase of its nectar sugar concentration. While plants could also offer a greater reward in terms of nectar volume or replenishment rate, we chose to manipulate sugar concentration because previous studies showed that bees can learn differences in concentration faster than differences in volume (Cnaani et al. 2006; but see Amaya-Márquez and Wells 2008), and differences in replenishment rate are generally more difficult to learn because the bees' encounter rate with empty flowers also depends on their own foraging patterns.

In the experiment, we let single bumblebees forage on artificial flowers of two colours, one rare, one abundant, and varied the sugar concentration of the rare colour while keeping

the abundant colour's concentration constant. Each bee experienced two reward scenarios, a "constant reward" scenario in which both flower colours offered equal rewards (25% sucrose) and a "variable reward" scenario in which the rare colour's sucrose concentration was first increased to 40%, then decreased to 10%, while the abundant colour's concentration stayed at 25%. The "variable reward" scenario was chosen so that the average sugar concentration over both periods was equal for both flower colours (25%). In addition to these reward scenarios, we varied the rarer flower colour's relative abundance (6/36 or 3/36) and the number of simultaneously foraging bees (one or two) between experimental trials to test the influence of these factors on the rare colour's visitation rate and expected pollination success. We expected that decreasing the rare colour's abundance should exacerbate its disadvantage and possibly reduce the positive effect of a temporarily increased reward. Likewise, we expected that with two simultaneously foraging bees the positive effect of increased sugar concentration on the rare colour's visitation and pollination success might be reduced, since in this case bees more frequently encounter recently depleted and not yet refilled flowers of the rare colour, which should shift their preference back to the abundant, less rewarding colour. On the other hand, if the two foragers partition the floral resources between themselves and each one specializes on a different flower colour, the rare colour should benefit from a high quantity and quality of visits, while the more abundant colour receives fewer visits per flower.

Materials and methods

Experimental setup

We conducted the experiment in a large indoor cage ($4 \times 4 \times 2$ m). The cage was illuminated with fluorescent lights and white LED strips in addition to daylight. These light sources combined provided a light intensity well above 1000 lx on the cage floor. The cage's sides were shielded from direct light to avoid biasing the bumblebees' direction of movement. For the experiment, we used the computer-controlled robotic flower system described by Kuusela and Lämsä (2016), modified for use with two microcontrollers to

allow controlling a larger number of artificial flowers. 36 artificial flowers were placed on the floor of the cage in six rows and columns, with a distance of 50 cm between neighbouring flowers. The top of each flower was a flat circular surface of 9 cm diameter, which was covered with a disc made of rubber foam. We used white discs to train the bumblebees to forage on the artificial flowers and feed them before and after experimental trials, and blue and yellow discs for the experiment. The top of each flower had a circular opening (10 mm diameter, 15 mm deep) into which bees needed to crawl to access the reward consisting of a drop of sucrose solution in a “nectar cup” (top of a small cross-head screw) of approximately 2.5 µl. This nectar cup was connected to a motorized arm which could be lowered into a reservoir of sucrose solution to refill the reward. An infrared light barrier was integrated into the flowers’ opening to register bumblebee visits. All artificial flowers were connected via cables to a control unit with two Genuino® microcontrollers which were programmed to refill flowers and record flower visits. Throughout the experiment, flowers were refilled after each visit, with a refill period of 15 seconds. For further details on the artificial flower system, see Kuusela and Lämsä (2016).

In the experiment, we successively tested bees of two colonies of *Bombus terrestris* obtained from BioBest through Katz Biotech (Baruch, Germany). The colony’s nest box was placed on a table in a corner of the cage and connected to a gated wire mesh tunnel to control the movement of bees out of and into the nest. The bumblebee colony was fed pollen directly into the nest box every second day. Sucrose solution (25% w/w) was provided ad libitum in artificial flowers every day before and after experimental trials. Foragers of each new colony were trained to collect sucrose solution from artificial flowers in several steps. Initially, bees learned to forage on eight flowers containing an Eppendorf cup full of honey solution in the opening, with the scent of the honey acting as an olfactory cue. Once several bees had started making regular foraging trips to the flowers, the honey was replaced by sucrose. In the next step, we replaced the Eppendorf cups by a wick which hung down into a reservoir of sucrose solution. These flowers were also used to feed the colony between experimental trials. Finally, foragers were given access to the 36 electronically controlled flowers with a small, automatically refilling nectar cup at the

134 bottom of the opening. Every morning before the start of experimental trials, all bees
135 were allowed to collect sucrose solution from the electronic flowers for at least 30 minutes.
136 New foragers (bees making regular trips to the flowers and back to the nest) were caught,
137 sedated by cooling in a fridge at 4 °C and individually marked with a colour code.

138 Experimental trials were conducted between April and July 2019. Each trial consisted
139 of a series of foraging bouts (trips to the artificial flowers and back to the nest) of one
140 or two individually marked test bees. All other bees were confined to the nest during
141 experimental trials. To avoid pseudoreplication, each individual bee was only used once in
142 an experimental trial. Trials began with a training phase in which the test bee was allowed
143 to make two foraging bouts on 36 flowers of one colour (blue or yellow), then two foraging
144 bouts on 36 flowers of the other colour. The order of colours was alternated between bees.
145 We trained bees in this way to reduce possible effects of innate colour preferences on their
146 behaviour, since our aim was to test the effect of flower colour abundance, not of colour
147 per se. Directly after the training phase, the test bee was allowed to forage on the test
148 array with one rare (3 or 6 flowers) and one abundant flower colour (33 or 30 flowers,
149 respectively). The positions of the rare colour's flowers were determined by a random
150 number generator separately for each test bee. The identity of the rare colour (blue or
151 yellow) was alternated between bees. We let the test bee make three foraging bouts on
152 the test array with 25% sucrose in all flowers, then three foraging bouts on the same array
153 with increased concentration in the rare colour's flowers (40% sucrose), and finally three
154 foraging bouts with decreased concentration (10% sucrose) in the rare flower type. The
155 abundant flower colour stayed at 25% sucrose throughout each experimental trial. The
156 flowers' coloured discs were wiped with 70% ethanol between trials of different bees to
157 remove scent marks which might influence the behaviour of bees in following trials. In
158 total, we tested 67 bumblebees in 51 experimental trials (see Table 1 for sample sizes for
159 each combination of treatment, rare colour and colony).

160 We checked that the artificial flower system was working correctly each morning be-
161 fore the start of experimental trials, and exchanged any malfunctioning parts. In addition,
162 most experimental trials were recorded with a video camera installed in a corner of the

163 cage. Prior to the analysis of the data, all automatically recorded flower visitation se-
164 quences were checked for plausibility. As implausible visits we defined (1) more than three
165 visits to the same flower in a row or (2) visits of a single bee to more than one flower at
166 the same time. Most of these errors could be corrected through comparison with video
167 recordings. In the few cases with missing videos, we tried to infer the most plausible vis-
168 itation sequence based on the fact that bumblebees typically move between neighbouring
169 flowers. When in doubt, we kept all automatically recorded visits.

170 **Data analysis**

171 To assess the likely consequences of bumblebee behaviour for the rare flower type's pol-
172 lination success, we summarized the flower visitation sequences recorded by the artificial
173 flower system in several ways. First, we counted the number of visits to each flower colour.
174 In the case of trials with two bees, we included only those parts of the visitation sequence
175 when both bees were actively foraging. These times were determined by comparing the
176 visits' time stamps with video recordings. In addition, we calculated a measure of theoret-
177 ically expected pollination success using Bateman's (1947) model of pollen carryover. This
178 model is based on the assumption that pollinators take up one unit of pollen in each visit
179 and deposit a fraction λ of the pollen taken up during earlier visits (see also Montgomery
180 2009). Thus, pollen carryover was modelled as a geometric decay function, in accordance
181 with several empirical studies (e.g. Thomson and Plowright 1980; Campbell 1986; Thom-
182 son 1986). Based on the observed visitation sequence, we calculated each flower colour's
183 expected amount of conspecific pollen received for two rates of pollen deposition ($\lambda = 0.3$
184 and $\lambda = 0.8$). However, since the values of theoretically expected pollination success
185 were highly correlated (Pearson's $r > 0.98$), we only analysed the data for $\lambda = 0.3$. As
186 the artificial flowers did not record the identity of visiting bees, we could not reconstruct
187 individual visitation sequences from the data of trials with two simultaneously foraging
188 bees. Therefore, we calculated theoretically expected pollination success only for trials
189 with a single forager.

190 To be able to compare numbers of visits and theoretically expected pollination success

191 of the rare and abundant flower colour between trials with different durations and total
192 numbers of bumblebee visits, we calculated indices which quantify the rare colour's visita-
193 tion or pollination success, respectively, relative to the abundant colour, while taking into
194 account the rare colour's relative abundance. Specifically, for flower visits we calculated
195 the rare colour's relative visitation rate V as

$$V = \frac{P_{obs}}{P_{obs} + P_{exp}}, \quad (1)$$

196 where P_{obs} is the observed proportion of visits to the rare colour and P_{exp} is the ex-
197 pected proportion based on the rare colour's relative abundance (i.e. either 6/36 or 3/36).
198 This is the preference index used by Fründ et al. (2010) and Schmid et al. (2016), which is
199 based on the forage ratio of Krebs (1989). For pollination, we calculated the rare colour's
200 relative expected pollination success in an analogous way, with P_{obs} representing the theo-
201 retically expected proportion of conspecific pollen received by the rare colour. With both
202 indices, a value of 0.5 indicates that the rare colour received the same number of visits or
203 amount of conspecific pollen per flower as the abundant colour. Values below 0.5 indicate
204 a rare-colour disadvantage, values above 0.5 a rare-colour advantage. We calculated these
205 indices both for the complete visitation sequence of each individual and sucrose concen-
206 tration and for the last 1/3 of each visitation sequence, which roughly corresponds to the
207 last of the bumblebees' three foraging bouts. The behaviour of bumblebees in this last
208 foraging bout should reflect their foraging decisions after an initial learning period during
209 which they collected information about the distribution of flower types and floral rewards.

210 To investigate how the rare colour's relative visitation rate and pollination success
211 varied between experimental trials, we fitted two regression models to each data set, one
212 with relative visitation rate as response variable and one for relative expected pollination
213 success. Both models included two main categorical predictors, floral reward of the rare
214 colour (either constant or variable), treatment (combination of number of rare flowers
215 and number of simultaneously foraging bees) and their interaction. For the "variable
216 reward" scenario, we calculated the response using the sum of numbers of visits or expected

217 conspecific pollen receipt of visitation sequences with 40% and 10% sucrose in the rare
218 flowers. Treatment had three levels (6 or 3 rare flowers with one bee, 6 rare flowers with
219 two bees) in the model of relative visitation rate and two levels (6 or 3 rare flowers with
220 one bee) in the model of relative expected pollination success. In addition to the two
221 main predictors, we also included bumblebee colony (A or B) and colour of the rare flower
222 type (blue or yellow) as population-level (fixed) effects in both models. To account for
223 the non-independence of flower visitation sequences of the same bee or pair of bees, bee
224 identity was included in the models as a group-level (random) effect.

225 Statistical models were fitted in a Bayesian framework, using the “brms” package
226 (Bürkner 2017) in R (R Core Team 2021) as an interface to Stan (Stan Development
227 Team 2019). Since the response variables had continuous values between zero and one
228 and some values were exactly zero, we used a zero-inflated beta model, and modelled
229 both the beta distribution’s location parameter and the zero-inflation parameter as a
230 function of all predictors. To prevent overfitting while avoiding the drawbacks of variable
231 selection (e.g. Hastie et al. 2009; Harrell 2015), we performed penalized regression using
232 a regularized horseshoe prior with three degrees of freedom for the Student t prior of
233 the local shrinkage parameters (Piironen and Vehtari 2017; van Erp et al. 2019). We
234 used non-informative priors for all other model parameters: a non-standardized Student
235 t distribution (3, 0, 2.5) for the intercept of non-zero values and the standard deviation of
236 group-level effects, a Gamma distribution (0.01, 0.01) for the beta distribution’s precision
237 parameter and a logistic distribution (0, 1) for the intercept of the zero-inflation term. To
238 account for the different numbers of flower visits used to calculate relative visitation rate
239 or relative expected pollination success, we performed weighted regression with number
240 of visits as weights. Posterior estimates were obtained using Markov Chain Monte Carlo
241 (MCMC) sampling with four chains of 4000 iterations each. The first 2000 iterations of
242 each chain were discarded as warm-up. Convergence diagnostics indicated that the models
243 had fully converged: all \hat{R} values were smaller than 1.01, effective sample sizes were larger
244 than 1500, and no divergent transitions occurred. We checked for patterns in the residuals
245 by comparing the observed data to posterior predictive simulations from the fitted model

246 using the R package “DHARMA” (Hartig 2020). Population-level effects were considered
247 significant when their 95% credible intervals did not overlap zero.

248 To help us interpret the findings of the main analyses, for the visitation sequences
249 with 40% sucrose in the rare flower type we calculated the proportion of visits to the
250 rare colour in which the floral reward had been recently depleted and not yet refilled.
251 We modelled this proportion of visits to empty rare flowers as a function of treatment
252 (number of rare flowers and simultaneously foraging bees), rare colour and colony, using
253 a generalized linear mixed model with binomial errors and logit link. The model was
254 fitted using the function “glmer” from the R package “lme4” (Bates et al. 2015), with an
255 observation-level random effect to account for overdispersion.

256 Results

257 When both flower colours were equally rewarding (25% sucrose, “constant” reward sce-
258 nario), single bumblebees avoided the rare colour on average, i.e. they showed positive
259 frequency dependence of flower choice (Fig. 1a, 2a). This rare-colour avoidance was sig-
260 nificantly stronger when there were only three rare flowers compared to six (Table 2).
261 By contrast, two simultaneously foraging bees did not show a clear rare-colour avoid-
262 ance, although the difference to single foragers was not significant. Overall, there was
263 considerable variation between individuals, with some bees visiting the rare colour much
264 less than expected by chance, while others even showed a slight preference for the rare
265 colour. On the other hand, the rare colour’s theoretically expected pollination success in
266 the “constant” reward scenario was consistently lower than that of the abundant colour,
267 especially with only three rare flowers (Fig. 1a, 2b, Table 3).

268 As expected, the average relative visitation rate and pollination success of the rare
269 colour increased when its flowers offered 40% sucrose and decreased with 10% sucrose
270 (Fig. 1a). When analysing the visitation sequences with 40% and 10% sucrose combined
271 as one “variable” reward scenario, we found a significant increase of relative visitation
272 rate and expected pollination success compared to the “constant” reward scenario (Fig. 2,

273 Table 2 and 3). The positive effect of a variable reward on relative visitation rate was
274 slightly, but significantly weaker when there were only three rare flowers or when two
275 bees foraged simultaneously. In the model of relative expected pollination success, the
276 positive effect of variable reward markedly and significantly decreased with three rare
277 flowers (Table 3). Both relative visitation rate and expected pollination success were
278 significantly higher for yellow rare flowers compared to blue flowers. Bumblebee colony
279 only had a significant effect on relative expected pollination success, but not on relative
280 visitation rate.

281 Compared to the data set with all flower visits, when considering only the last part of
282 each flower visitation sequence there was a stronger increase and decrease of relative visi-
283 tation and pollination with 40% sucrose and 10% sucrose in the rare flowers, respectively
284 (Supplementary Material, Fig. S1). Whereas over all flower visits the weighted mean rel-
285 ative visitation rate and expected pollination success were higher at 10% sucrose than at
286 25% (Fig. 1), this relationship was reversed in the last part of the visitation sequences
287 (Fig. S1). However, when combining the data from the periods with 40% and 10% sucrose
288 into one “variable reward” scenario, the values were quite similar to the data of all flower
289 visits (Fig. S2). Consequently, the estimated effects of treatment and reward scenario
290 were also similar to those of the full data set (Tables S1 and S2). The effects of rare
291 colour and colony were no longer significant when considering only the last 1/3 of visits.
292 In the models of this last part of the visitation sequence, the zero-inflation parameter
293 (probability of an observation being exactly zero) was significantly lower in the variable
294 reward scenario, but this effect decreased with three rare flowers compared to six rare
295 flowers.

296 In the additional analysis of the proportion of visits to empty rare flowers during the
297 period with increased sucrose concentration in the rare flowers, neither treatment, nor
298 rare colour or colony had a significant effect (Table S3). Thus, we found no evidence
299 that two simultaneously foraging bumblebees or single bees foraging on three rare flowers
300 encountered empty rare flowers more frequently than single bees foraging on six rare
301 flowers.

302 **Discussion**

303 In this experiment, bumblebees indeed foraged in a way which resulted in a lower quantity
304 and quality of visits to the rarer flower type when both flower types were equally rewarding,
305 but differed in abundance. While the per-flower visitation rate of the rare colour was
306 on average only slightly lower than that of the abundant colour (relative visitation rate
307 close to 0.5), the rare colour's expected pollination success was strongly limited by the
308 fact that bumblebees often visited several flowers of the abundant colour between two
309 visits to the rare colour, and so would have lost most of the rare colour's conspecific
310 pollen according to the model of pollen carryover. Temporal variation of the rare colour's
311 sucrose concentration considerably increased both the proportion of visits it received per
312 flower and its expected pollination success. This effect is likely due to the fact that during
313 the period with 40% sucrose bumblebees concentrated their foraging effort on the few
314 available highly rewarding flowers, which each received so many visits that this more than
315 compensated for their low visitation rate during the period with 10% sucrose (see ?, for a
316 similar effect in a simulation model). In addition, during the period with low reward the
317 rare colour benefitted from the bumblebees' memory of the previous high-reward period.
318 Most bees decreased the proportion of visits they made to the rare colour only gradually
319 and continued to make a few visits to the rare colour even in their last foraging bout
320 (see Fig. S3 for an example), presumably to check whether the high sucrose concentration
321 had returned. However, despite these positive effects of temporal reward variation, the
322 rarity disadvantage with respect to expected pollination success was only reduced, but
323 not completely eliminated.

324 As expected, temporal reward variation had a weaker positive effect when there were
325 only three rare flowers or when two bumblebees foraged simultaneously. In both cases,
326 this reduction was likely caused by a higher frequency of encounters with depleted flowers
327 of the rare colour during the period with increased sucrose concentration, which prompted
328 bees to switch back to the abundant flower colour with lower sucrose concentration. While
329 we did not detect a difference in the proportion of visits to empty rare flowers between

330 treatments, that may be because bumblebees quickly responded to such unrewarding visits
331 by increasing their visitation rate to the abundant flower type, thereby allowing the rare
332 type's flowers to refill before the next visit. This is in line with previous studies which
333 showed that bees are more likely to switch to a different flower type after encountering
334 flowers with zero or low nectar (Goulson 1999, and references therein) and can even adjust
335 their visitation patterns to intra- and interspecific differences in nectar replenishment rates
336 (Klinkhamer et al. 2001; Stout and Goulson 2002).

337 The finding of a lower average visitation rate to the rarer flower type when both types
338 were equally rewarding is consistent with several previous laboratory studies which found
339 that bumblebees preferred an abundant flower colour over a rarer one, i.e. they showed
340 positive frequency dependence of colour choice (reviewed by Smithson 2001). Such posi-
341 tive frequency dependence is supposed to occur whenever it increases the flower visitors'
342 foraging efficiency by reducing the cognitive costs of switching between flowers with differ-
343 ent traits (Smithson 2001, and references therein). Thus, positive frequency dependency
344 of flower choice is a special case of flower constancy, the temporary specialization of flower
345 visitors on one flower type (e.g. Goulson 1999; Gegear and Thomson 2004; Amaya-Marquez
346 2009; Grüter and Ratnieks 2011).

347 Compared to a previous laboratory experiment with *B. terrestris* foraging on blue and
348 yellow artificial flowers (Smithson and Macnair 1996), the frequency effect in our study
349 was relatively small. Moreover, while in the present study bees on average showed a slight
350 frequency-independent preference for yellow flowers, in the experiment of Smithson and
351 Macnair (1996) bumblebees avoided yellow and preferred blue flowers. In that study, at
352 10% and 20% yellow flowers nearly all bees tested made less than 5% of their visits to
353 yellow flowers. By contrast, in our experiment the average proportion of visits to yellow
354 flowers was 6% when there were ca. 8% yellow flowers (3 of 36) and 17% when there were
355 ca. 17% yellow flowers (6 of 36). This difference between studies may be due to a difference
356 in methodology. Specifically, the difference in frequency-independent colour preferences
357 could be caused by the fact that we trained bumblebees on each colour separately, while
358 Smithson and Macnair trained bees on mixed arrays of both colours. Therefore, in their

study innate colour preferences may have had a greater influence on bumblebee behaviour. The stronger rare-colour avoidance reported by Smithson and Macnair could be due to the very short inter-flower distances in their experimental array compared to ours. Whereas in the present study neighbouring artificial flowers were spaced 0.5 m apart, Smithson and Macnair used an array with 150 flowers on an area of 0.8×0.8 m. According to foraging theory (Stephens and Krebs 1986, and references therein), a high density of floral resources should favour greater specialization of foragers due to the lower energetic cost of bypassing a rewarding flower. While Gegear and Thomson (2004) indeed found a positive effect of artificial flower density on individual specialization of bumblebees, in a follow-up study on their previous experiment Smithson and Macnair (1997) did not find an effect of flower density on the strength of rare-colour avoidance. However, the lowest flower density they tested was still much higher (248 flowers per m²) than the density of flowers in our study (9 per m²).

Whereas the evidence for a rarity disadvantage under laboratory settings with a single flower visitor is relatively clear, it is much less certain how important this disadvantage is under natural conditions. If the reproductive success of flowering plants generally increased with relative abundance, rare plant species (Levin and Anderson 1970; Benadi et al. 2012) and rare flower morphs (Smithson 2001) might become locally extinct (but diversity could still be maintained at a regional scale: Greenspoon and M'Gonigle 2013). However, while some studies of natural plant communities have found positive frequency dependence of flower visitation rates, conspecific pollen receipt or seed set (Kunin 1993; Runquist and Stanton 2013; Schmid et al. 2016), others reported no such frequency dependence (Eckhart et al. 2006) or a hump-shaped (?) or negative relationship (Bergamo et al. 2020). This inconsistency could be due to variation in frequency effects across spatial scales and in relation to plant and pollinator traits (e.g. ?Bergamo et al. 2020). In addition, exploitation competition between foraging pollinators (within and between species) could lead to a more even distribution of foragers among floral resources, thereby reducing the positive frequency dependence of flower visitation rates (Eckhart et al. 2006; Schmid et al. 2016). Our finding that the rare colour's average relative visitation rate was closer

388 to 0.5 when two bumblebees foraged simultaneously is consistent with this expectation.

389 Even if flower visitation rates are relatively independent of plant species abundance,
390 our results suggest that rare flowering plants may experience a strong disadvantage with
391 respect to the quality of flower visits, since most of their conspecific pollen is lost in visits
392 to more abundant flowering species. While factors such as spatial aggregation of conspe-
393 cific plants (Campbell 1986; de Waal et al. 2015), morphological complexity of flowers
394 (Gurevich and Hadany 2021) and differentiated pollen placement on pollinators' bodies
395 (Morales and Traveset 2008) may limit the loss of conspecific pollen, several field studies
396 found that rare plant species indeed received smaller amounts of conspecific pollen than
397 more abundant species (e.g. de Waal et al. 2015; Bergamo et al. 2020). Our experiment
398 suggests that this rarity disadvantage could be alleviated through a synchronous increase
399 in nectar sugar concentration, even if the increase is temporary and followed by a period
400 with low sugar concentration. Whether this is a feasible strategy depends on the flexibil-
401 ity of resource allocation to nectar production, which has rarely been studied until now.
402 Previous studies of floral nectar production found that the volume and concentration of
403 nectar can vary substantially both between and within plant species. While interspecific
404 differences may be adaptations to different groups of pollinators (Fenster et al. 2004), in-
405 traspecific variation in nectar amount and composition is partly heritable, but also related
406 to environmental conditions (e.g. Boose 1997; Parachnowitsch et al. 2019, and references
407 therein), time of day and flower age (e.g. Devlin et al. 1987; Witt et al. 1999). In some
408 plant species, nectar sugar (either sucrose or hexose) is directly produced in the nectaries
409 through photosynthesis, while other plants produce these sugars through hydrolysis of
410 starch stored in the parenchyma (Pacini et al. 2003). The latter are more flexible in
411 their nectar production patterns, since the stored starch allows them to produce larger
412 quantities of sugars independent of the current rate of carbon assimilation through photo-
413 synthesis. In general, the nectar sugar concentration of plant species is often considerably
414 lower than the preferred concentration of their flower visitors (e.g. Nicolson 2002) and the
415 concentration which would maximize the visitors' energy intake rate (Kim et al. 2011).
416 The reason for this discrepancy is not entirely clear, but it has been suggested that a low

417 concentration prevents the nectar from becoming too viscous over time through evapora-
418 tion (McCallum et al. 2013) or that high costs of nectar sugar production prevent most
419 plants from investing more resources into this floral reward (Parachnowitsch et al. 2019,
420 and references therein). In summary, the evidence so far suggests that there is some flex-
421 ibility in resource allocation to nectar, but that nectar production is also constrained by
422 various factors which could prevent some rare plant species from increasing their floral
423 reward. Even in our experimental “variable reward” scenario, where the average sugar
424 concentration over the two periods was the same for the rare and abundant plant species,
425 the average cost of a flower visit in terms of nectar sugar was greater for the rare flower
426 type because it received most of its visits during the period with 40% sucrose and very
427 few visits in the period with 10% sucrose. Therefore this scenario is only realistic if rare
428 plant species are able to invest a higher amount of resources into floral rewards than abun-
429 dant plant species. In addition, to achieve maximum benefit from a temporary increase
430 of nectar sugar the increase needs to occur synchronously in all plant individuals. Such
431 a synchronous change could be genetically determined or triggered by an environmental
432 cue.

433 Conclusions

434 Overall, the results of this experiment suggest that rare plant species pollinated by *B.*
435 *terrestris* experience a slight disadvantage with respect to the quantity of flower visits,
436 coupled with a strong disadvantage regarding the quality of pollination. Offering a higher
437 nectar sugar concentration, even if only temporarily, would be a way to alleviate this
438 rarity disadvantage, but that requires that rare plant species invest additional resources
439 into nectar production, which may not be possible under all circumstances. Future studies
440 should therefore aim to understand how the costs and benefits of floral reward production
441 vary depending on environmental conditions. This knowledge will help to preserve plant
442 populations that are experiencing strong shifts in relative abundance as a result of global
443 change.

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452 **Conflicts of interest/Competing interests**

453 The authors declare that they have no conflict of interest.

454 **Ethics approval**

455 Ethics approval was not required for this study according to local legislation.

456 **Availability of data and material**

457 The datasets used and/or analysed during the current study are available from the corre-
458 sponding author on reasonable request.

459 **Code availability**

460 R code of the analyses in this paper is available from the corresponding author on rea-
461 sonable request.

⁴⁶² **Authors' contributions**

⁴⁶³ GB, RJJG and JL designed the experiment. RK and GB performed the experiment and
⁴⁶⁴ analyzed the data. GB wrote the first draft of the manuscript. All authors contributed
⁴⁶⁵ critically to the revisions.

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610 **Figure legends**

611 **Fig. 1** a) Relative visitation rate and b) relative expected pollination success of the rare
612 flower colour for each combination of treatment and sucrose concentration in the rare
613 flowers. A relative visitation rate or expected pollination success of 0.5 indicates that the
614 rare flower colour received the same number of visits or conspecific pollen grains per flower
615 as the abundant flower colour. Values below 0.5 indicate a rare-colour disadvantage, values
616 above 0.5 a rare-colour advantage. Expected pollination success was calculated based on
617 the bumblebee's visitation sequence and a geometric decay model of pollen carryover.
618 Circles are data points with area proportional to the number of flower visits (minimum
619 43, maximum 433 visits), squares indicate weighted mean values.

620 **Fig. 2** a) Relative visitation rate and b) relative expected pollination success of the rare
621 flower colour as a function of reward scenario and treatment. In the “constant” reward
622 scenario both flower colours contained 25% sucrose solution, while in the “variable” reward
623 scenario the rare flower colour's sucrose concentration was first increased to 40%, then
624 decreased to 10% (see Fig. 1). Squares and error bars show the mean and 95% credible
625 interval of the expected value of the posterior predictive distribution, calculated from 100
626 samples. Circles are data points with area proportional to the number of flower visits
627 (minimum 43, maximum 781 visits).

Table 1 Number of replicates (bee individuals) per treatment (number of flowers of the rare colour and number of simultaneously foraging bumblebees), rare colour and colony.

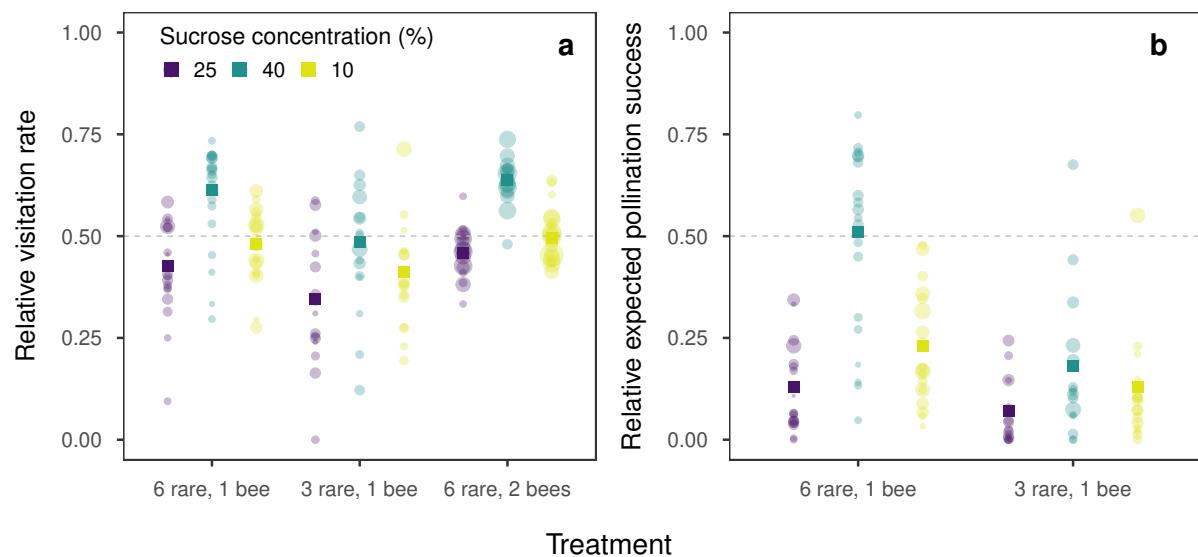
| Treatment | 3 rare flowers, 1 bee | | 6 rare flowers, 1 bee | | 6 rare flowers, 2 bees | |
|-------------|-----------------------|------|-----------------------|------|------------------------|------|
| Rare colour | yellow | blue | yellow | blue | yellow | blue |
| Colony A | 4 | 4 | 4 | 5 | 5 | 3 |
| Colony B | 4 | 4 | 5 | 5 | 4 | 4 |

Table 2 Results of a weighted zero-inflated beta regression of the rare flower colour's relative visitation rate ($N = 102$, 1 zero). Both the mean (location) of non-zero values and the zero-inflation parameter were modelled as a function of all predictors, with the number of flower visits of each visitation sequence as weights. The intercept is the estimated value for the “constant” reward scenario, treatment “6 rare, 1 bee”, colony “A” and rare colour “blue”. The table shows the mean value of each parameter's posterior distribution on the link (logit) scale together with its 95% credible interval (CI). Parameter values whose CI does not overlap zero are in bold. The estimated standard deviation for the group-level (random) effect of bumblebee individual was 0.35 [0.29, 0.43] (mean posterior estimate, 95% CI in square brackets) for the mean of non-zero values and 5.07 [3.00, 8.27] for the zero-inflation parameter. The scale parameter of the beta distribution of non-zero values was estimated as 124.66 [122.61, 126.74].

| Predictor level | Mean estimate | 2.5% | 97.5% |
|--|---------------|--------|-------|
| Effect on mean of non-zero values | | | |
| Intercept | -0.54 | -0.75 | -0.32 |
| Reward “variable” | 0.54 | 0.53 | 0.55 |
| Treatment “3 rare, 1 bee” | -0.34 | -0.57 | -0.11 |
| Treatment “6 rare, 2 bees” | 0.19 | -0.06 | 0.42 |
| Rare colour “yellow” | 0.20 | 0.01 | 0.40 |
| Colony “B” | 0.18 | -0.01 | 0.38 |
| Reward variable x “Treatment 3 rare, 1 bee” | -0.03 | -0.04 | -0.02 |
| Reward variable x “Treatment 6 rare, 2 bees” | -0.05 | -0.06 | -0.04 |
| Effect on zero-inflation parameter | | | |
| Intercept | -19.88 | -36.61 | -9.85 |
| Reward “variable” | -1.03 | -18.63 | 15.57 |
| Treatment “3 rare, 1 bee” | 8.17 | -1.52 | 24.11 |
| Treatment “6 rare, 2 bees” | -1.76 | -21.27 | 16.68 |
| Rare colour “yellow” | 4.10 | -2.69 | 12.14 |
| Colony “B” | -4.05 | -11.84 | 2.68 |
| Reward “variable” x Treatment “3 rare, 1 bee” | -17.29 | -40.55 | 2.88 |
| Reward “variable” x Treatment “6 rare, 2 bees” | 0.31 | -24.34 | 26.35 |

Table 3 Results of a weighted zero-inflated beta regression of the rare flower colour's relative expected pollination success ($N = 70$, 1 zero). The estimated standard deviation for the group-level (random) effect of bumblebee individual was 0.82 [0.64, 1.06] (mean posterior estimate, 95% CI in square brackets) for the mean of non-zero values and 5.34 [3.06, 9.02] for the zero-inflation parameter. The scale parameter of the beta distribution of non-zero values was estimated as 39.40 [38.57, 40.29]. See the legend of Table 2 for further details.

| Predictor level | Mean estimate | 2.5% | 97.5% |
|---|---------------|--------|-------|
| Effect on mean of non-zero values | | | |
| Intercept | -2.91 | -3.47 | -2.37 |
| Reward "variable" | 1.61 | 1.59 | 1.63 |
| Treatment "3 rare, 1 bee" | -1.13 | -1.69 | -0.58 |
| Rare colour "yellow" | 0.62 | 0.07 | 1.18 |
| Colony "B" | 0.59 | 0.04 | 1.17 |
| Reward variable x "Treatment 3 rare, 1 bee" | -0.15 | -0.18 | -0.11 |
| Effect on zero-inflation parameter | | | |
| Intercept | -17.02 | -29.07 | -8.88 |
| Reward "variable" | -1.06 | -12.33 | 9.77 |
| Treatment "3 rare, 1 bee" | 5.93 | -2.53 | 17.37 |
| Rare colour "yellow" | 4.05 | -3.05 | 12.01 |
| Colony "B" | -4.30 | -12.48 | 2.81 |
| Reward "variable" x Treatment "3 rare, 1 bee" | -15.18 | -30.81 | -2.37 |



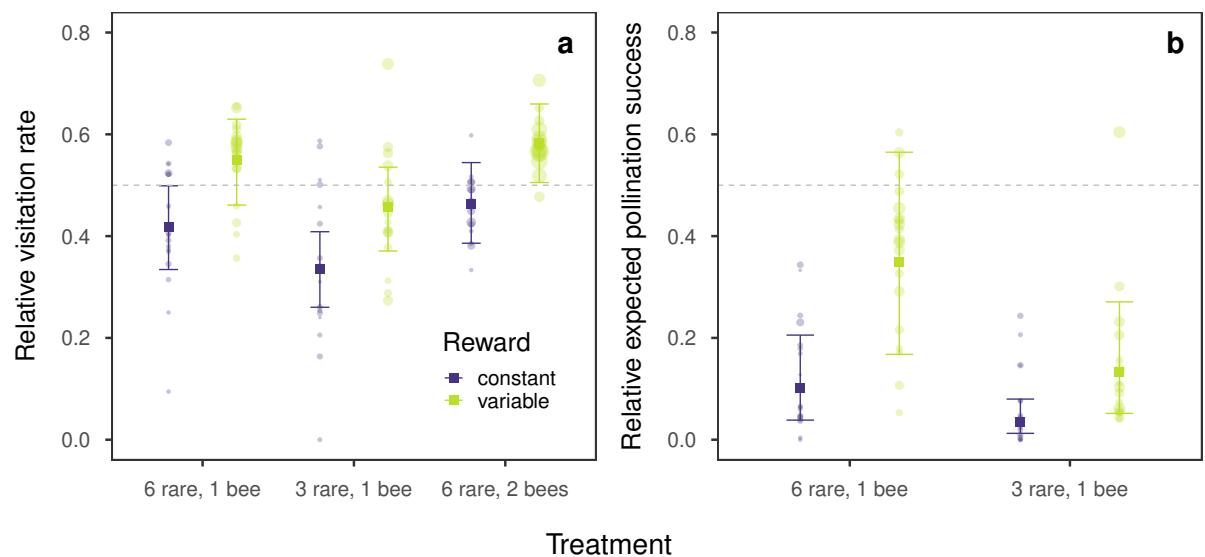


Fig. 2

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